Possible Health Assessment For Heavy Metal Concentration in Water, Sediment, and Fish Species and Pregnant Women's Biomonitoring in Miankaleh Peninsula, Iran

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Abstract

A human bio monitoring study, investigating most consumed fish species exposure to heavy metals was done in northern part of Iran, Miankale Peninsula, in winter 2019. Metals levels were evaluated in various tissues of fish, as well as Turkmen pregnant women's biological indicators. For this purpose, 20 water and sediment, 14 fish and 16 human samples were collected. Concentration of Cr, Co, Cu, As, Hg and Pb metals were determined by ICP-MS device. The highest mean concentration of Cu and Cr in water (93.35 and 80.91 µg/l respectively), Hg and Pb in sediment (7.4 µg/g for both), Cu and Pb in liver (27 and 18.9 µg/g for *C. carpio*; 1414 and 31.7 µg/g for *L. auratus*), muscle (10 and 18.8 for *C. carpio*; 37.2 and 8.27 µg/g for *L. auratus*), and skin (26.4 and 9.9 for *C. carpio*; 10.8 and 11.74 µg/g for *L. auratus*) of both fish species, and Cu in blood (2.53 mg/l), hair (8.87 µg/g), fingernail (36.46 µg/g), and toenail (29.04 µg/g) samples were observed, while Co had the lowest concentration in all samples. Liver in fish samples and fingernail in pregnant women were the tissues with the highest heavy metals accumulation, whereas the lowest concentrations of heavy metals were observed in the muscles of fish species and pregnant women's blood sample. Hg concentration in water and sediments, the muscle of fish, and pregnant women's blood and hair samples were higher than the values suggested by various organizations. This study showed that the level of heavy metals, especially Hg, in water, sediments and fish is a threat to human health. Therefore, it is recommended that the necessary information about the consumption of seafood with high amounts of Hg should be provided in this area.

Introduction

In the recent decades, industrial and urban development and efforts to improve living conditions and access to energy have led to the release of chemical pollutants, resulting in major environmental concerns (Ezemonye, Adebayo et al. 2019). Water is one of the important parts of the environment the quality of which has been affected by various human activities (Coffie 2015). Among various pollutants, heavy metals pose a serious threat in ecosystem health due to their high stability (Vrhovnik, Arrebola et al. 2013; Ogbomida, Nakayama et al. 2018). Although these metals enter the aquatic environment naturally through rocks weathering, the main source of metals in aquatic environment is industrial, mine, and agricultural activities, as well as, municipal wastewater (Walker, Hopkin et al. 2001; Asante 2015); therefore, lakes are the main place for restoring heavy metals directly and indirectly (Elkady, Sweet et al. 2015). Heavy metals can accumulate through the food chain in marine organisms due to their non-degradability and stability in water and sediment samples (Javed and Usmani 2016; Ullah, Maksud et al. 2017; Fuentes-Gandara and Herrera-Herrera et al. 2018).

Metals concentration in water and sediment samples play an important role in the bioavailability of heavy metals, but do not provide enough information about the state of the ecosystem ; therefore, the use of biomarkers is essential. Fish are at the highest level of the marine food chain, and they are reliable biomarkers for monitoring heavy metal pollution in aquatic ecosystems (Varol and Sünbül 2019). So the concentration of metals in fish tissue can be much higher than their concentration in water (Usero, Izquierdo et al. 2004). On the one hand, a large amounts of heavy metals accumulate in the various

organs and tissues of fish through suspended particles in water, food, ion exchange in lipophilic membranes (e.g. gills) over time, which can be harmful to human health (Dadar, Adel et al. 2016; Ezemonye, Adebayo et al. 2019; Varol and Sünbül 2019). On the other hand, fish is considered as an animal source of protein for human (37% of total animal protein) (Food 2018), which is highly recommended due to its high levels of fatty acids, especially omega-3, protein, vitamins and minerals (Adel, Dadar et al. 2016; Basim and Khoshnood 2016; Fuentes-Gandara, Herrera-Herrera et al. 2018). Eating fish, at least twice a week, can reduce the risk of heart attack (Kris Etherton et al. 2002; Fallah, Saei-Dehkordi et al. 2011). Evidences also show that fish consumption is increasing, with global per capita consumption rising from 9.9 kg in 1960 to 14.4 kg in 1990 and 19.7 kg in 2013 (Bianchi, Chopin et al. 2014). The amount of metals in the edible parts of fish is important for human health, because of the consumption of contaminated fish (Varol and Sünbül 2020). Besides, the overuse of this beneficial nutrient is a major path of heavy metals transmission to human body (Ahmed, Baki et al. 2016, Basim and Khoshnood 2016).

Diet is the most important route for heavy metals to enter human body (Zhuang, McBride et al. 2009). Despite the importance of fish consumption, it can be a serious threat to human health owing to the possibility of high heavy metals concentration (Varol, Kaya et al. 2017), hence, the potential risk assessment of metals in fish consumption is necessary (Garnero, de los Angeles Bistoni et al. 2020). Since heavy metals can have carcinogenic and non-carcinogenic effects on humans (Varol, Kaya et al. 2017), there are several ways to assess the health risk of carcinogens and non-carcinogens due to the exposure to toxic metals by fish (Saha, Mollah et al. 2016, Griboff, Wunderlin et al. 2017). The target hazard quotient (THQ) and Carcinogenic Risk (CR) are suggested by the US Environmental Protection Agency to assess the potential non-carcinogenic and carcinogenic risk associated with the use of heavy metal-contaminated fish (Basim and Khoshnood 2016). Many researchers have used these methods to investigate the carcinogenic and non-carcinogenic effects of heavy metals (Atique Ullah, Akter et al. 2019, Esilaba, Moturi et al. 2020, Garnero, de los Angeles Bistoni et al. 2020, Kortei, Heymann et al. 2020, Sadeghi, Loghmani et al. 2020).

Typically, human health risk in terms of chemicals is estimated by using default methods of exposure and measuring pollutants in various sources. These methods may not be accurate enough and estimate the amounts less or more than the real exposure. For example, it may examine only one of the sources or pathways of the contaminants entering the body. In this case, the estimated amount is less than the actual amount. Nevertheless, human biological monitoring (HBM) includes all the pollutants entering from different sources and different pathways and provides a better estimation of the possible amount of the pollutants in the human body (Cherrie, Semple et al. 2006). Therefore, human biomonitoring assessment contributes to the calculation of the total amount of heavy metals entering from different sources and routes, the environmental conditions of the region, distribution of exposure to different population groups, identification of vulnerable groups, identification of highly infected areas as well as a better estimation of health assessment (Cherrie, Semple et al. 2006, Angerer, Ewers et al. 2007, Clewell, Tan et al. 2008, Angerer, Aylward et al. 2011, Louro, Heinälä et al. 2019, Faure, Noisel et al. 2020). Bio monitoring is a direct measurement of chemicals in biological samples, such as blood, serum, urine, hair, nails, and saliva

of an organism which have been exposed to contaminants (LaKind, Aylward et al. 2008, Hays and Aylward 2009, Lemos, Novaes et al. 2009, Angerer, Aylward et al. 2011). Studies have shown that various factors, such as age, sex, body weight, geographical factors, time of exposure, workplace, diet, smoking, sanitation, health conditions, or the consumption of vitamin and mineral supplements affect the bioaccumulation of heavy metals in human body (Christensen 1995, Marrugo-Negrete, Ruiz-Guzmán et al. 2013, Alrobaian and Arida 2019, Al-Saleh 2020). However, the main focus is on pregnant women, because women are highly vulnerable during pregnancy period; in addition, developing children and fetuses are extremely sensitive to pollutants (Vahter, Berglund et al. 2002).

The US National Academies of Sciences, Engineering, and Medicine in recent report, entitled “Using the 21st Century Science to Improve Risk-Related Evaluations” (USNAS 2017), considers human biological monitoring as an essential tool for human exposure to chemical substances (<http://foodmetabolome.org>). In addition, Under the authority of the European Union (EU), the Horizon2020 European Joint Program HBM4EU (2017–2021) established more accurate evidence of human exposure to chemicals with the aim of minimizing human health risk involves evaluating human biological monitoring (Ganzleben, Antignac et al. 2017). In Canada, a human bio monitoring program has been established in collaboration with Canadian Health Measures Survey (CHMS) and the Public Health Agency of Canada, measuring 250 chemical contaminants in biological monitoring components, such as blood, urine, and hair over the ages of 3 to 79 (Canada 2010, Canada 2013, Canada 2017, Haines, Saravanabhavan et al. 2017, Eykelbosh, Werry et al. 2018).

Miankaleh International Wetland is a unique habitat for wintering migratory birds and aquatic reproduction. In addition, it plays an important role in feeding local people. Despite the high use of seafood by the locals in the area, there is no basic knowledge about the levels of heavy metals in various human tissues. The aim of this study is monitoring Cr, Co, Cu, As, Hg, and Pb metals in water and sediment samples as well as measuring heavy metals concentration in *C. carpio* and *L. auratus* fish species to estimate the health risks of heavy metals in relation to fish consumption. Moreover, for the first time, biological monitoring of heavy metals in pregnant women's hair, blood, fingernail, and toenail is performed in two areas named Chapaqli and Qaraesou around the wetland. we also tried to investigate socio demographic and lifestyle factors which may have changed the levels of these heavy metals in pregnant women's organs.

Material And Methods

Study area

Miankaleh Peninsula, which is known as a Wildlife Refuge and Biosphere Reserve, is situated in the southeastern region of the Caspian Sea with geographical coordinates of 36°50'N 053°17'E and surface area of 96,678.5 hectares. Miankaleh was determined a Ramsar site in 1975. It includes two parts, water and land environment, and as a result of the Caspian Sea water retreat, it has been separated from the bay and the sea in the form of a sandy and saline lands. It is nearly 60 km long with an average width of

2.5 km and 6.5 km in its downstream and upstream sections, respectively. The mean elevation is about 28 – 10 meters below the sea level, the annual rainfall is approximately 700 mm, and the temperature is about 17 degrees Celsius. Due to its unique features, it is a worthy place for migratory birds and breeding aquatic animals to spend the winter, consequently, about half a million migratory birds migrate from Siberia to this region every year. 47 species of birds, 24 species of fish, 27 genera of phytoplankton, and 5 genera of benthos have been identified in this area. Presence of food, chemical, metal, and non-metallic industrial units around the wetland as well as the location of oil reservoirs and hydrocarbon activities have affected the wetland and caused physical, chemical, and biological pollution of the wetlands and surrounding rivers(Ramsar 1975, UNESCO 1976).

Field sampling

10 water and sediment sampling sites of study area were chosen including Qalepayan (S1), Miankaleh center (S2), Galougah(S3), Bandargaz(S4), Esmailsay(S5), Qaraesou (S6), Bandartorkman(S7), Chapaqli (S8),Khozeini channel (S9) and Ashourade(S10) (Fig. 1). Water and sediment sampling was taken at each station with three replications in the winter 2019. Ruttner Water and Ekman-Grab sampler were applied for gathering surface water and sediment samples respectively. Water samples were fixed with 5 ml 70% nitric acid and stored in an iced cool box so as to analyze metals and sediment samples were preserved in plastic bags for drying and analysis.14 fish samples were bought from 9 fish markets of Ashourade, Bandar Torkman, Chapaqli, Qaraesou, Qhalepayan, Galougah, Esmailsay, Khozeini channel, and Miankale center. Furthermore, Human biomonitoring was performed based on the protocol specified by the Medical Ethical Committee of Iran in collaboration with the staff of the health center (*Ethical Committee of Golestan University of Medical Sciences.(IR.GOUMS.REC.2019.126)*). Ethical principles and privacy were fully observed, and a written consent was received from the participants while completing the questionnaire. Blood, hair, fingernail, and toenail samples were taken from 16 Turkmen pregnant women from two sites including Chapaqli and Qaraesou.

Sample preparation for heavy metal analysis

Water and sediment sample preparation was done according to the modified EPA digestion method (*EPA 3005a and EPA 3050B respectively*). First, suspended particulate matters were removed from water samples by using a Whatman no.1 (0.45 mm) filter paper. 10 mL HNO₃ (65.0%) was added into 100 ml of the water sample and then heated on a hot plate at 90 to 95°C until the volume reached 15-20ml. Then, samples were moved in a 100 ml volumetric flask and smoothed through Whatman glass filter paper (pore size 110 mm) prior to the analysis. Sediment samples were dried in an oven at 105°C for 24 hours. Afterwards, 0.5 g of each sample was digested by the combination of nitric acid (69%), hydrochloric acid (37%), and hydrogen peroxide (30%) in a ratio of 1: 1: 2 with continual heating. After dilution, the samples were passed through Whatman No. 41 filter paper.

Purchased fish were transferred to the laboratory in cold flask, their species were identified, and autopsies were carried out to determine the metals concentration in liver, muscle, and skin tissues of fish samples. One gram of samples was put into microwave digestion tubes and 5 ml HNO₃ (65%) and 1 ml H₂O₂were

added to each tube (U.S. EPA-3052, 1996). Thermal decomposition was used to determine the concentration of total mercury (Hg) in fish tissues following the U.S. Environmental Protection Agency recommendations- EPA 7473 (1998).

For human bio monitoring, Turkmen pregnant women's sampling was performed in two villages, Chapaqli and Qaraesou. Hair samples from the occipital area of the scalp and fingernail and toenail samples were picked up with a pair of sterilized stainless steel scissors. All the samples were placed in plastic bags prior to analysis. Furthermore, 5 mL blood samples were taken by nonmetal needles and test tubes based on specific protocols. For analysis, all specimens were transferred to the laboratory in containers labeled with the subject identification number and date. Hair (3g) and Nail (1g) samples were kept in an oven at 110 °C for 1 hour and 550 °C for 4 hour respectively and finally maintained in a desiccator pending analysis. The produced ash was digested with 10ml of 6:1 mixture of concentrated nitric acid and perchloric acid; the mixture was warmed up for perfect vaporization to gain a water transparent solution. Each digested samples was moved into a 100 mL volumetric flask and reached the specified volume with distilled water (Abdulrahman, Akan et al. 2012). 4 mL of HNO₃ (69%) and 2 mL of H₂O₂ (35%w/v) were used to digest blood samples in the microwave digestion system in 200 °C for 10 minutes (Alrobaian and Arida 2019). Finally, the analysis of metals (Cr, Co, Cu, As, Hg and Pb) in surface water, sediments, fish, and human tissues was done by ICP-MS device (HP-4500 model) equipped with Asx-520 auto-sampler.

Human health risk assessment

Target Hazard Quotients

Target Hazard Quotients provided by the US Environmental Protection Agency was used to calculate the risk for the people with non-carcinogenic diseases according to Formula1(EPA 2004, IRIS 2010).THQ higher and lower than 1 indicates a high probability and non-probability of a potential risk. The Total Target Hazard Quotients (TTHQ) is obtained from the sum of all metals, according to Formula 2(Chien, Hung et al. 2002).

$$THQ = \frac{EF * ED * IR * C}{BW * RfD * AT} * 10^{-3} \quad (A.1)$$

$$TTHQ = \sum_{i=1}^n THQ_i \quad (A.2)$$

THQ: Target Hazard Quotients;

EF: Frequency of exposure (365 days/year);

ED: Total exposure time (70 years);

IR: Daily consumption rate of fish; 33.15 g/day based on (Iran Fisheries Organization 1398);

C: Concentration of metal in muscle tissue of consumed fish (mg/kg wet weight);

Bw: The average of adults body weight (70 kg);

AT: Average exposure days (365 days/year*70year = 2550 days);

RfD: (mg/kg-day); The oral reference dose for Cr, Co, Cu, As, Hg and Pb are 0.003, 0.03, 0.04, 0.0003, 0.0001 and 0.0035 mg/kg BW/day respectively (USEPA 2011, Finley, Monnot et al. 2012, USEPA 2012, USEPA 2019).

Determination of Estimated daily intake

The estimated daily intake (EDI) depends on metal and food concentration, and body weight. Two hypotheses have been considered to estimate heavy metals risks caused by fish consumption; cooking has no effect on metals concentration (Cooper, Doyle et al. 1991), the eaten dose is equal to the absorbed dose of the contaminant (USEPA 1989). The estimated amount of daily intake was calculated based on a method proposed by USEPA using formula3(Ikem and Egiebor 2005, Christophoridis, Kosma et al. 2019).

$$EDI = \frac{C * IR_D}{BW} \quad (A.3)$$

EDI: Estimation of Daily intake of metals through fish consumption (mg/kg body weight/day);

C: Concentration of metal in muscle tissue of the consumed fish (mg/kg wet weight);

IR_D: (intake rate) Daily consumption rate of fish; 33.15 g/day based on (Iran Fisheries Organization 1398);

Bw: The average of adults' body weight (70 kg).

Carcinogenic Risk

According to EPA standards, the life expectancy or number of years that a person is exposed to dangerous pollutants is equal to 70, which is used in the calculations related to carcinogenic and non-carcinogenic effects (Jeszka-Skowron, Zgoła-Grześkowiak et al. 2017). In order to assess the risk of cancer in a person, it is necessary to take all the factors into account. To evaluate the carcinogenic effects of the exposure to carcinogenic heavy metals studied by swallowing, CR was calculated based on EPA and through the Eq. 4. In this equation, CR is Carcinogenic Risk and CFS shows Carcinogenic Slope Factors for the substance (ATSDR 2010, Feed 2013, Wei, Wang et al. 2020). Carcinogenic risk index between 10^{-6} (risk of carcinogenic during human life 1 in 1,000,000) and 10^{-4} (risk of carcinogenic during human life 1 in 10,000) indicates a range of potential risks predicted for the causes of cancer. Therefore, chemicals with a risk factor of less than 10^{-6} are not considered as the chemicals of concern (Demirezen and Aksoy 2006, Liu, Liang et al. 2006, USEPA 2015). Relevant calculations were performed based on the standard

assumptions proposed by USEPA. According to this standard, the amount of CSF for the Cr, As, and Pb was equal to 0.5, 1.5, and 0.0085 mg/kg/day, respectively (Demirezen and Aksoy 2006).

$$CR = EDI * CSF$$

Statistical Analysis

Statistical test analysis was carried out using R software. First, descriptive statistics of heavy metal concentration in water, sediment, fish, and human samples were determined; furthermore, an independent sample T test, one-way analysis of variance (ANOVA), and Pearson tests were used to evaluate the significant differences in metal concentration and correlations in the data.

Results And Discussion

Heavy metals concentration in water and sediment

Water and sediment samples were collected at 10 stations to determine the concentration of heavy metals chromium, Cobalt, Copper, Arsenic, Mercury, and lead. The overall mean concentration of the metals in the surface water were $44.56 \pm 5.9 \mu\text{g/L}$ Cr, $10 \pm 0.17 \mu\text{g/L}$ Co, $69.03 \pm 13.37 \mu\text{g/L}$ Cu, $8.79 \pm 1.16 \mu\text{g/L}$ As, $6.52 \pm 0.91 \mu\text{g/L}$ Hg, and $1.71 \pm 0.15 \mu\text{g/L}$ Pb (Table 1). Cu and Cr had the highest mean concentration and Pb had the lowest mean concentration, the average metals concentration in the region was as follows: $\text{Cu} > \text{Cr} > \text{Co} > \text{As} > \text{Hg} > \text{Pb}$. Like this study, high concentration of Cu has been observed in other studies due to anthropogenic activities (Gimeno-García, Andreu et al. 1996, Akindele, Omisakin et al. 2020). The highest concentration of Cu, Co and As was observed in Chapaqli station, Hg and Pb in Esmailsay and Cr in Qaleh Payan station, and the lowest concentration of metals was observed in Galugah. In the same area, Abadi et al. (2018) observed higher levels of Hg and Pb and lower levels of Co, Cu and As in southern Caspian sea coasts than those observed in this study. Concentration of Co, Cu, Pb, and As in water was higher in this study than those in the results of other researches (Humbatov, Ahmadov et al. 2015, Fan, Chen et al. 2020, Pandiyan, Mahboob et al. 2021). However, only the concentration of Hg was higher than the standard value set by WHO and USEPA, and the concentration of Cr was close to the standard value of WHO (Table 2).

Table 1
Concentration of heavy metals in water and sediments at different stations

	Water (µg/l)						Sediment (µg/g dry weight)					
Station	Cr	Co	Cu	As	Hg	Pb	Cr	Co	Cu	As	Hg	Pb
S1	80.91	4.08	93.35	7.75	14.85	1.61	3.7	1.28	2.9	4.2	7.4	7.4
S2	61.15	8.45	27.08	6.47	6.29	1	4	1.43	4.5	6	17.7	11.7
S3	2.2	2.73	1.58	5.25	3.04	1.45	3.6	1.63	3.7	4.8	7.7	9.3
S4	112.6	3.55	62.45	11.33	3	1.19	3.2	1.28	3.2	6.4	51.2	8.9
S5	49.94	8.41	52.1	6.29	15.87	3.12	3.5	1.29	2.7	1.5	7.3	9
S6	35.24	9.59	16.73	5.56	6.5	2.94	2.8	1	2	2	6.8	4.5
S7	35.18	11.4	19.8	4.86	2.41	0.76	3	1	2.5	2.9	629	7.2
S8	20.8	36	205.3	26.67	4.96	1.83	3.4	1.2	2.6	4.9	6.6	6.5
S9	31.82	5.36	197	7.32	1.67	1.64	2.7	0.9	2.1	3.3	8.8	7.7
S10	14.49	10.7	14.9	6.42	6.56	1.62	2.9	0.86	10	6.9	390	7.7
Mean	44.56	10	69.03	8.79	6.52	1.71	3.2	1.2	3.6	4.3	113	8

Table 2
Comparison of heavy metal concentration with different standards and other literature

Location		Cr	Co	Cu	As	Hg	Pb	References
southeastern Caspian Sea	Water(µg/l)	44.56	10	69.03	8.79	6.52	1.71	This study
	Sediment (µg/g)	3.2	1.2	3.6	4.3	113	8	
WPV ^a	WHO	50		3000	10	1	10	(WHO 2017)
	USEPA	100		1000	10	2	15	(USEPA 2017)
SQG _S ^b	TEL ^c	42.3		124	7.24	0.13	30.2	(CCME 1999)
	PEL ^d	160		271	41.6	0.7	112	
	ERL ^e	81		150	8.2	0.15	46.7	(NOAA 2012)
	ERM ^f	370		410	70	0.71	218	
Caspian Sea	Water		1.06	5.53	1.26	1.42	1.92	(Abadi, Zamani et al. 2018)
Caspian Sea	Water		0.04	0.81	1.51		0.24	(Humbatov, Ahmadv et al. 2015)
South Caspian Sea	Sediment (winter)			8.1	2.6		10.6	(Ghorbanzadeh Zaferani, Machinchian Moradi et al. 2016)
Southern Caspian Sea	Sediment		15	19	11		13	(Agah, Hashtroudi et al. 2011)
South Caspian Sea	Sediment	95	16.3	31			20	(Alizadeh Ketek Lahijani, Naderi Beni et al. 2018)
Sheyang River	Water	–		3.12	1.83	0.025	0.55	(Zhao, Xu et al. 2018)
	Sediment	37.19		23.51	12.85	0.020	16.87	
Yangtze River	Water	0.279		1.47	1.94	36.3	0.829	(Fan, Chen et al. 2020)
	sediment	34.4		19.7	8.80	0.065	25.8	
Point Calimere Wildlife Sanctuary	Water	2.9	0.4	0.1		1.5	1.4	(Pandiyani, Mahboob et al. 2021)
	sediment	0.8	1.8	0.3		26	2.5	

Location		Cr	Co	Cu	As	Hg	Pb	References
Shadegan Wetland	Water	ND	ND	ND	ND	ND	1.11	(Ashayeri and Keshavarzi 2019)
	sediment	54.62	10.77	20.42	3.40	0.07	16.89	
<i>a: Water Permissible Value;</i>								
<i>b: Sediment Quality Guideline;</i>								
<i>c: Threshold Effect Level;</i>								
<i>d: Probable Effect Level;</i>								
<i>e: Effect Range Low;</i>								
<i>f: Effect Range Medium.</i>								

The overall mean concentration of the metals in the sediment was 3.2 ± 0.16 µg/g Cr, 1.2 ± 0.07 µg /g Co, 3.6 ± 0.66 µg /g Cu, 4.3 ± 0.46 µg /g As, 113 ± 70.54 µg /g Hg, and 8 ± 0.60 µg /g Pb (Table 1). Unlike water samples, Hg and Pb had the highest concentration in sediment samples, and Co had the lowest concentration; the concentration sequence of heavy metals in sediment samples was as follows: Hg > Pb > As > Cu > Cr > Co. The highest concentration of Co, Cr and Pb was observed in Miankaleh center, whereas the highest concentration of Cu, As and Hg was perceived in Ashouradeh station .Lahijani et al.(2018)and Agah et al. (2011)showed lower Cr, As, Cu and Pb sediment concentration in Southern Caspian Sea than the levels obtained in this study. Cr, Cu, and Pb sediment concentration observed in this study is lower than the values obtained in Yangtze River (Fan, Chen et al. 2020); however, higher concentration of these metals was observed in Point Calimere Wildlife Sanctuary(Pandiyan, Mahboob et al. 2021).Only Hg concentration was higher than the permissible standards set by Canadian sediment quality guidelines for the protection of aquatic life and National Oceanic and Atmospheric Administration, so it was approximately 160 times higher than PEL and ERM values and 560 times higher than TEL and ERL values (Table 2).Existence of agricultural lands and using fertilizers and pesticides by farmers can be some of the main reasons for the accumulation of Pb and Hg in sediments (Hashmi, Malik et al. 2013, Pandiyan, Mahboob et al. 2021). A comparison between the heavy metal concentration in this study with the other similar studies is presented in Table 2. A similar result was obtained in the study of water and sediment samples of The Point Calimere Wildlife Sanctuary by Pandiyan et al.(2021);the highest concentration of Cr was in water samples and the highest concentration of Hg and Pb was observed in sediment samples. In addition, high concentration of Hg and Pb in sediment samples and high concentration of Cu in water samples in BS region were observed by Tian et al.(2020).

In order to identify and interpret the source of heavy metals more accurately, principal component analysis (PCA) and correlation analysis were used. A correlation matrix map of six heavy metals in water and sediments is demonstrated in Fig. 2. Relationships among metals in the surface water demonstrate significant positive correlations between Co and Cu ($r = 0.9$, $p < 0.000$), Cu and As ($r = 0.68$, $P < 0.000$), and Co and Pb ($r = 0.47$, $p < 0.03$); besides, correlations between metals in the sediment display significant positive correlations between Co and Cr ($r = 0.85$, $p < 0.002$), Cr and Pb ($r = 0.68$, $P = 0.03$), Cu and As ($r =$

0.66, $p = 0.03$). Correlation of heavy metals has been observed in many studies in water and sediment samples (Islam, Das et al. 2020, Muhammad and Ahmad 2020).

PCA results showed that in water samples with eigen values > 1 , Co, As and Cu are in the same group and were correlated with station eight. Pb and Hg are also in the same group and were correlated with the station number five. Cr did not form any clusters with other metals but was associated with the first station (Fig. 3). The first component (Co, As and Cu) was 45%, the second component (Pb and Hg) was 26%, and the third variable (Cr) included 19% of the component. PCA for water showed that three components had 90% of the variance. The main source of metals in PC1 could be municipal wastewater which entered the wetland without treatment. The source of Pb and Hg metals in the PC2 could also be human activities (agricultural activities and transportation). Many studies have shown that Pb and Hg are the marker elements of atmospheric deposition, primarily due to the long-distance atmospheric transport of anthropogenic Pb and Hg (Chaturvedi et al., 2018; Chen et al., 2019; Lin et al., 2013; Xiao et al., 2017; Yamaguchi et al., 2003). In addition, agricultural effluents are other sources of these metals entry into aquatic environments (Xiao, Jian et al. 2017, Chaturvedi, Bhattacharjee et al. 2018, Chen, Liang et al. 2019). The PC3, which contained only Cr, could be attributed to natural sources and geogenic sources. The natural origin of Cr has been observed in many studies (Micó, Recatalá et al. 2006, Xu, Wang et al. 2014, Deng, Yang et al. 2020, Lin, Li et al. 2020, Tian, Wu et al. 2020). In sediment, Cr, Co, and Pb were in one group, and Cu and As were in the other group (Fig. 3). PC1 (Cr, Co and Pb) included 46% and PC2 (Cu and As) included 32% of the variance. Principal component analysis for sediment showed that these two components included 0.78% of the variance. Heavy metal concentration in sediment was not only affected by the current concentration in the surface water but also caused changes in metal concentration owing to settling, accumulation, and binding (Schertzinger, Ruchter et al. 2018). Therefore, determining the source of the metal in the sediment is more complex than in water. Metals in PC2 were probably affected by human activities, especially municipal wastewater, while the origin of metals in PC1 could be both human due to agricultural and transportation activities (for Pb and Co) and natural (for Cr). Pearson correlation of Cr with Co and Pb as well as As and Cu in sediment and the high correlation of Cu with Co and As in water samples were consistent with PCA results for both sediment and water, indicating the common sources of each heavy metal group.

Heavy Metals Concentration in fish species

The concentration of heavy metals, Cr, Co, Cu, As, Hg, and Pb in the liver, muscle, and skin of *C. carpio* and *L. auratus* fish species demonstrates in Table 3. The distribution of the heavy metal concentration in the liver, muscle, and skin in *C. carpio* was as follows: Cu $>$ Pb $>$ Hg $>$ Cr $>$ As $>$ Co, Pb $>$ Cu $>$ Hg $>$ Cr $>$ As $>$ Co and Cu $>$ Pb $>$ Hg $>$ As $>$ Cr $>$ Co, whereas in *L. auratus*, it was Cu $>$ Pb $>$ As $>$ Hg $>$ Cr $>$ Co, Cu $>$ Pb $>$ As $>$ Hg $>$ Cr $>$ Co and Pb $>$ Cu $>$ As $>$ Cr $>$ Hg $>$ Co respectively. Cu and Pb had the highest concentration, and Co had the lowest concentration in liver, muscle, and skin tissues in both fish species. High levels of Cu and Pb compared to other heavy metals in different species of fish were observed in Cauvery delta region, India by Dhanakumar et al.(2015). Regarding the analysis of the elements between the different species for each tissue, no significant differences for all metals was observed in the muscle of fish species. The concentration of Hg and Pb showed no significant differences in the liver and skin of fish species, while

Cr, Co, Cu, and As in the liver and Cu in the skin were significantly different. Liver was the tissue which accumulated these elements in *C. carpio* and *L. auratus* except for As and Cr in *C. carpio*; furthermore, metals in *L. auratus* liver were more than *C. carpio* liver except for Hg. Cr, Hg and Pb which were accumulated in *C. carpio* muscle more than *L. auratus* muscle, while Co, Cu, and As were most accumulated in the *L. auratus* muscle. Additionally, metals were accumulated in *C. carpio* skin more than *L. auratus* skin except for Pb. Metallothionein protein content and metabolic activity of the liver lead to higher accumulation of heavy metals in the liver tissue than other fish organs (Kargın and Çoğun 1999, Çoğun, Yüzereroğlu et al. 2005, Nabavi, Nabavi et al. 2012); in addition, the liver acts as a filter to detoxify metals, which leads to higher accumulation of metals (Jezierska and Witeska 2006). In this study, as in other studies, the concentration of heavy metals in the liver was higher than other organs (Yılmaz, Özdemir et al. 2007, Keshavarzi, Hassanaghaei et al. 2018, Aytekin, Kargın et al. 2019). In general, the concentration of heavy metals in the muscle was less compared to the liver. fish muscles are the main edible parts, But they are not the tissue in which heavy metals are accumulated. Low concentration of heavy metals in muscle relative to other organs has also been shown in other studies (Yılmaz, Özdemir et al. 2007, Rajeshkumar and Li 2018, Aytekin, Kargın et al. 2019). Maurya et al.(2019) showed that heavy metals concentration in the seven species of fish studied in River Ganga basin was as follows: liver > gill > muscle. Also, the trend of heavy metals concentration in different species of fish was as follows: Zn > Cu > Pb > Cd > Cr. The results of ANOVA test in the *C. carpio* species showed that there was a considerable difference between the amount of Cr and As in liver and skin tissues. In addition, a significant difference was observed between liver and muscle in relation to Co and As. In the case of *L. auratus* species, significant difference was clear between liver and skin for Co, Cu, As, and Hg metals. Furthermore, liver and skin had remarkable differences in terms of Co, Cu, and As metals (Fig. 4). Rajeshkumar et al.(2018) showed that the concentration of heavy metals in the liver of *C. carpio* was as follows, Pb > Cu > Cr > Cd; also, higher concentration in liver than muscle and a significant difference in the concentration of heavy metals in different tissues of *P. fluvidraco* and *C. carpio* species was observed. The results of this study were somewhat similar to the results of our study. As it can be seen in Table 4, mean values of Pb, Hg, As, and Cu were above the recommended limits of FAO/WHO/EU in muscle tissue of *C. carpio* and *L. auratus*. Although, mean Cu in muscle tissue of *C. carpio* and *L. auratus* was higher than the amount suggested by WHO\EU, the levels of Cu in muscle tissue of *C. carpio* were very close to FAO recommended value. Moreover, while Cr value in muscle tissue of *C. carpio* and *L. auratus* exceeded the maximum limit recommended by EU, Cr mean value in muscle tissue of *C. carpio* and *L. auratus* was below the upper limits given by WHO. The heavy metal levels measured for muscles of the two fish species from Miankaleh wetland in this study and in other literatures are represented in Table 4. Heavy metals concentration in the existing literatures indicated that metal contents in the fish muscles vary depending on where and which species were caught. Even the average of the two fish species studied in the same area in other studies showed lower and higher values than the obtained values.

Table 3
Mean metal concentration (µg/g) in the liver, muscles, skin of two fish species

organ	species	Cr	Co	Cu	As	Hg	Pb
liver	<i>C. carpio</i>	3.65 ± 1.03 ^a	1.2 ± 0.34 ^a	27 ± 7.50 ^a	2 ± 0.3 ^a	17.4 ± 5.64	18.9 ± 4.19
	<i>L. auratus</i>	7.35 ± 1.41 ^b	5.4 ± 1.49 ^b	1414 ± 629 ^b	28.2 ± 7.1 ^b	11.1 ± 3.41	31.7 ± 1.19
muscle	<i>C. carpio</i>	5.5 ± 1.44	0.2 ± 0.02	10 ± 1.11	4 ± 0.74	9.3 ± 2.02	18.8 ± 5.03
	<i>L. auratus</i>	4 ± 0.96	0.29 ± 0.07	37.2 ± 17.3	5.8 ± 1.2	4.98 ± 1.89	8.27 ± 3.61
skin	<i>C. carpio</i>	8.87 ± 1.67	1.1 ± 0.32	26.4 ± 4.85 ^a	9.17 ± 1.92	5.02 ± 2.39	9.9 ± 4.57
	<i>L. auratus</i>	5.72 ± 1.66	0.8 ± 0.32	10.8 ± 2.29 ^b	5.98 ± 1.15	1.7 ± 0.57	11.74 ± 5.27

Table 4

Comparison of heavy metal concentration in the muscles of two examined fish with different standards and other literature

		Cr	Co	Cu	As	Hg	Pb	References
WHO ^a		50		30			2	(WHO 1989)
FAO ^b				10			0.5	(FAO 1983)
EU ^c		0.15		30	0.1	0.5	0.5	(GB2762 2017)
southeastern Caspian Sea	<i>C. carpio</i>	5.5	0.2	10	4	9.3	18.8	This study
	<i>L. auratus</i>	4	0.29	37.2	5.8	4.98	8.27	
Gorgan Bay	<i>C. carpio</i>	6.4					0.43	(Alipour and Banagar 2018)
	<i>L. auratus</i>	0.93					8.6	
Beyşehir Lake	<i>C. carpio</i>	12.11					2.84	(Özparlak, Arslan et al. 2012)
Caspian Sea	<i>C. carpio</i>	74.6					139.9	(Saravi, Karami et al. 2012)
	<i>M. auratus</i>	31.27					77.67	
Caspian Sea	<i>C. carpio</i>	37.82					139.3	(Tabari, Saravi et al. 2010)
	<i>M. auratus</i>	83.95					75.12	
Caspian Sea	<i>C. carpio</i>		0.92	4.41			0.77	(Solgi, Alipour et al. 2018)
Tigris River	<i>C. regium</i>	1.05	0.04	0.05	0.02		0.11	(Töre, Ustaoglu et al. 2021)
	<i>C. trutta</i>	1.14	0.03	0.77	0.25		0.19	

a: World Health Organization

b: Food and Agriculture Organization

c: European Union

		Cr	Co	Cu	As	Hg	Pb	References
Liuzhou	<i>wild fish</i>	0.796		0.656	0.028	0.012	0.009	(Miao, Hao et al. 2021)
a: World Health Organization								
b: Food and Agriculture Organization								
c: European Union								

Human Health risks posed by heavy metals in fish species

Heavy metals level in the muscle of the two fish species was used to assess health risk by EDI, THQ and CR values (Sharafi, Nodehi et al. 2019, Abd-Elghany, Zaher et al. 2020). Figure 5 provides the estimated THQ for each heavy metal from the two fish species consumption. The estimated target hazard quotients (THQ) for metals were reduced for *C. carpio* and *L. Auratus* species respectively: Hg > As > Pb > Cr > Cu > Co, and Hg > As > Cr > Pb > Cu > Co. For all metals, THQ values were less than 1. This indicated that people would not encounter significant non-carcinogenic health risks from the ingestion of metals through fish consumption. The lowest THQ value for the two species was perceived for Co (2.6E-06 and 4.1E-06 for *C. carpio* and *L. auratus* respectively), while the highest mean THQ value was for Hg (4E-02 and 2.1E-02 for *C. carpio* and *L. auratus* respectively), which still demonstrates no potential health risk. The two species under the current study were found invulnerable for consumption, however, if people are exposed to more than one heavy metal at the same time, they can encounter blended or interactive effects (Loaiza, De Troch et al. 2018). Therefore, the TTHQ was evaluated to find out the additive effects of various heavy metals on human body. Our results demonstrated that the combined impact of all heavy metal levels was still lower than the acceptable limit of 1 for TTHQ (Fig. 5), but consumption of *C. carpio* has a higher risk than *L. auratus* (4.9E-02 versus 3.1E-02).

EDI of Pb, Cd, Cr, As, and Hg through the consumption of *C. carpio* and *L. auratus* fish species was summarized in Table 5. EDI values were compared with the respectively recommended daily dietary allowance of each individual metal proposed by several references, such as Joint FAO/WHO Expert Committee on Food Additive (JECFA) As, Cu, and Pb (JECFA,1982, 1989, 2000), World Health Organization (WHO) for Co (WHO, 1996, 2006), and National Research Council (NRC) for Cr (NRC, 1989). It was found that EDI results for all metals were less than the recommended levels, indicating that there is no risk to human health in relation to the consumption of heavy metals studied through the consumption of the selected fish species. The results depicted that the lowest value of EDI was perceived for Co through the utilization of the two selected fish species (7.71E-05 and 8.29E-05 for *C. carpio* and *L. auratus* respectively), while the highest was for Cu in *L. auratus*(1.06E-02) and Pb in *C. carpio*(5.38E-03).

Table 5
Calculated estimated daily intake and cancer risk for each heavy metal in fish species

metals	species	EDI ^a (mg/day/person)	Recommended daily dietary (mg/day/person)	CFS ^b	CR ^c
Cr	<i>C. carpio</i>	2.35E-03	0.20 ^e	0.050	1.17E-03
	<i>L. auratus</i>	1.73E-03		0.050	8.66E-04
Co	<i>C. carpio</i>	7.71E-05			
	<i>L. auratus</i>	8.29E-05			
Cu	<i>C. carpio</i>	2.86E-03			
	<i>L. auratus</i>	1.06E-02			
As	<i>C. carpio</i>	1.14E-03	0.13 ^d	1.50	2.58E-03
	<i>L. auratus</i>	1.67E-03		1.50	3.77E-03
Hg	<i>C. carpio</i>	2.67E-03	0.03 ^d		
	<i>L. auratus</i>	1.42E-03			
Pb	<i>C. carpio</i>	5.38E-03	0.21 ^d	0.0085	6.86E-05
	<i>L. auratus</i>	2.36E-03		0.0085	3.014E-05
a: Estimated Daily Intake					
b: Cancer Slope Factor					
c: Cancer Risk					
d: (JECFA, 2009) Evaluations of the Joint FAO/WHO Expert Committee on Food Additives					
e: (RDA, 1989)					

It is noteworthy that exposure to heavy metals is correlated with wide spectrum carcinogenesis and can damage the nervous system seriously),; therefore, CR index was estimated by the use of CFS values for Cr, As, and Pb in two species. In our study, CR for As and Cr in two species were higher than the intolerable range representing the risk of cancer due to exposure to Cd, Cr and, As through fish consumption. The CR values due to the consumption of different metals through *C. carpio* and *L. auratus* is As (2.58E-03) > Cr (1.17E-03) > Pb (6.86E-05) and As (3.77E-03) > Cr (8.66E-04) > Pb (3.014E-05) respectively. Exposure to As because of the consumption of *C. carpio* could cause higher risk for consumers.

Biological monitoring of heavy metals in Turkmen pregnant women's different tissues

Heavy metals concentration in Turkmen pregnant women's blood, fingernail, toenail and hair, considering their place of residence, fishermen families, age and number of dental amalgam fillings is represented in Table 6. Considering the mean heavy metal contents in different organs, higher Cr, Co, Cu, As, and Hg

content were found at fingernail and the highest Pb content was observed at toenail, while the lowest concentration of heavy metals was observed in the blood. Copper had the highest concentration among the studied elements in blood, fingernail, toenail and hair, whereas Co concentration was observed to be lower in all organs.

Table 6

Heavy metals concentration in Turkmen pregnant women based on results from the questionnaire

Organs	Characteristic		N	Cr	Co	Cu	As	Hg	Pb
Blood (mg/l)	Place of residence	Qaraesou	8	0.65	0.008	2.01	0.2	1.04	1.01
		Chapaqli	8	0.16	0.02	3.05	0.3	1.06	1.35
	Fishermen families	Yes	9	0.09	0.02	2.62	0.26	1.38	1.51a
		No	7	0.16	0.01	2.38	0.24	0.5	0.63b
	dental amalgam	Yes	8	0.07	0.01	2.06	0.2	0.74	1.04
		No	8	0.16	0.01	3	0.3	1.35	1.33
	Age	17–27	9	0.07	0.009a	2.03	0.2	0.93	0.98
		27–37	7	0.19	0.02b	3.37	0.34	1.23	1.53
Mean			16	0.11	0.01	2.53	0.25	1.05	1.18
Fingernail (µg/g)	Place of residence	Qaraesou	8	16.8	1.45	42.2	3.19	6.26	6.25
		Chapaqli	8	17.3	1.14	30.7	2.81	5.85	7.01
	Fishermen families	Yes	9	15.45	1.28	39.9	3.32	5.49	6.87
		No	7	19.77	1.31	30.7	3.82	6.99	6.23
	dental amalgam	Yes	8	16	1.72	45	3.87	6.48	3.75a
		No	8	18	0.86	27.8	3.13	5.63	9.51b
	Age	17–27	9	16.28	1.27	38.6	2.84	6.33	5.37
		27–37	7	18.38	1.33	32.8	4.6	5.59	8.77
Mean			16	17.07	1.29	36.46	3.5	6.05	6.63
Toenail (µg/g)	Place of residence	Qaraesou	8	7.03	0.19	28.9	3.28	4.12	19.1
		Chapaqli	8	11.64	0.39	29.1	2.69	2.82	14.04
	Fishermen families	Yes	9	5.65	0.28	25.7	3.5	3.98	16
		No	7	15.5	0.32	34.6	2.03	2.62	17.54
	dental amalgam	Yes	8	8.57	0.28	38.36a	3.68	4.45	20.76
		No	8	10.1	0.31	19.74b	2.29	2.49	12.4
	Age	17–27	9	7.55	0.19a	31.5	3.45	4.21	18.75
		27–37	7	12.3	0.47b	24.9	2.2	2.24	12.97
Mean			16	9.33	0.29		2.98	3.47	16.58

Organs	Characteristic		N	Cr	Co	Cu	As	Hg	Pb
Hair (µg/g)	Place of residence	Qaraesou	8	1.97	0.06	7.4	0.88	3.29	2.32
		Chapaqli	8	1.8	0.12	10.32	1.33	1.42	2.49
	Fishermen families	Yes	9	1.74	0.07	8.58	1.17	2.8	2.34
		No	7	2.13	0.12	9.35	0.99	1.6	2.51
	dental amalgam	Yes	8	1.66	0.06	8.39	0.89	3	2.65
		No	8	2.12	0.12	9.36	1.32	1.72	2.15
	Age	17–27	9	2.19	0.08	10.18	0.95	3.24	2.64
		27–37	7	1.38	0.11	6.7	1.36	0.87	2
	Mean			16	1.89	0.09	8.87	1.11	2.35

The average concentration of Cr, Co, Cu, As, Hg and Pb metals in blood was 0.11 (0.02–0.3), 0.01 (0.006–0.02), 2.53 (0.5–3.9), 0.25 (0.04–0.39), 1.05 (0.08–2.7), and 1.184 (0.3–1.9) mg/l respectively. Although habitat did not have any significant effect on the accumulation of metals in blood, the average of all metals in Chapaqli was higher than Qaraesou except for Cr. The mean concentration of blood heavy metals level was lower in non-fisherman families than in fisherman families except for Cr, so the average concentration of Pb in fisherman families was significantly higher than non-fisherman families. As expected, fish is consumed in fishermen families more than non-fishermen families, so they are more likely to accumulate Hg in these people's body. Prokopowicz et al.(2014) showed that regular fish consumption 2–6 and more than 6 meals a month, increases the amount of Hg in blood by almost 2 and 3 times, respectively. Even small amounts of fish consumption can lead to Hg accumulation in blood (Jedrychowski, Jankowski et al. 2006, Ashrap, Watkins et al. 2020). It has also been found that increasing fish intake during pregnancy increases Hg in the mother's blood and cord blood (Davidson, Myers et al. 2004, Jeong, Ha et al. 2017). The average of the blood metals concentration increased slightly with age, but the effect of age was only significant for Cr metal. Wennberg et al.(2006)also showed as age went up, Pb and Hg concentration in blood increased, but this increase was not notable.

All the blood samples had higher concentration than the standard value for Hg set by USEPA (5.8 µg/L) (Council 2000) and WHO (10 µg/L) (UNIDO 2003). People who had their teeth filled had lower mean metal concentration ; therefore, dental amalgam did not display an association with heavy metal accumulation in blood, however, other studies have shown the effect of dental amalgam on mercury accumulation in blood(Mortada, Sobh et al. 2002, Prokopowicz, Pawlas et al. 2014).The measured levels of Hg and Pb in pregnant women's blood in this study were much higher than the levels of these metals in Korean female population (Lee, Lee et al. 2012), healthy women in their 50s in an urban area of Poland (Prokopowicz, Pawlas et al. 2014), nonsmokers volunteers in Saudi Arabia (Alrobaian and Arida 2019), women in Belgium (40–60 years) (Fierens, Rebolledo et al. 2016), Fez, Morocco, Guiyang, China, Wrocław, Poland, BanskaBystrica, Slovakia (Hrubá, Strömberg et al. 2012), only the amount of Cr was lower than the amount obtained for nonsmokers volunteers in Saudi Arabia (Alrobaian and Arida 2019).

The mean concentration of Cr, Co, Cu, As, Hg and Pb metals in hair was 1.89 (0.78–3.09), 0.09 (0.02–0.26), 8.87 (1.76–21.21), 1.11 (0.54–1.76), 2.35 (0.15–4.47) and 2.40(0.5–3.93) $\mu\text{g/g}$ respectively. Place of residence, fishermen families, dental amalgam, and age had no significant effect on the concentration of heavy metals in hair. In addition, no specific trend was observed in the change of metals in relation to the mentioned factors. The concentration of As and Hg in the fishermen families was higher than the non-fishermen families like the results of Okati et al. (2018). As we reported the results of heavy metals concentration in water, sediment and two fish species, it was shown that the amount of Hg was higher than the specified standards, especially for sediment which was much higher than the permissible standards. It can be said that Hg accumulation in water and sediment has led to an increase in the concentration in fish species and consequently in the hair of pregnant women in the region. Marrugo et al. (2013) showed that although there was no significant difference between Hg concentration in relation to the number of days per week with fish consumption (NDWFC), number of meals per day with fish (NMDF), amount of fish consumption weekly (AFCW), a positive correlation between Hg concentration and the mentioned variables was obvious. In addition, the most important source of human exposure to Hg was polluted fish consumption. A significant positive correlations between the amount of fish consumption and Hg concentration in individuals' hair samples around Ayapel swamp, Colombia, was observed by Gracia et al. (2010). Other literatures have indicated that polluted fish is the main source of Hg exposure in communities which consume fish frequently(McDowell, Dillon et al. 2004, Al-Saleh 2020). Based on the guideline of USEPA reference dose (1 $\mu\text{g/g}$ in hair) (USEPA 2005) and WHO "normal" level (2.0 $\mu\text{g/g}$ in hair) for identifying the population at risk because of mercury exposure, when most of the hair samples exceed from the standard values, potential risks estimation for human health is necessary. In our study,75% of the recorded hair samples were higher than the Hg references suggested by USEPA and WHO. This condition can be worrying and should be considered so as to avoid a potential public health crisis due to the polluted fish consumption in the study area. It can be seen that Cr, Cu, As, and Pb levels (0.40–24.56 $\mu\text{g/g dw}$) are higher than in non-liquor users aged between 20–40 (Abdulrahman, Akan et al. 2012)and lower than Cr, As, Hg, and Pb concentration in nonsmokers volunteers (Alrobaian and Arida 2019). In addition, people with filled teeth showed higher levels of Hg and Pb metals; and in the first age group, the average concentration of Pb and Hg was higher. It can be concluded that the increase of age had no influence on metals levels in hair; this result has been obtained in other studies, as well (Okati and Esmaili-sari 2018).

The mean fingernail levels of Cr, Co, Cu, As, Hg and Pb were 17.07 (9.9–23.6), 1.29 (0.11–3.25), 36.46(19.9–54), 3.5(1.44–6.1), 6.05(4.39–7.54) and 6.63(1.63–14.21) $\mu\text{g/g}$ respectively; additionally, the average toenail levels of Cr, Co, Cu, As, Hg, and Pb were 9.33 (1.57–29.6), 0.29 (0.09–0.67), 29.04 (9.24–45.53), 2.98 (1.14–6.15), 3.47 (0.23–5.75) and 16.58 (1.28–23.31) $\mu\text{g/g}$ respectively. The mean concentration of all metals in fingernails was higher than toenails except for Pb, also a significant difference was observed between the concentration of Cr, Co, Hg, and Pb metals in toenails and fingernails. Abdulrahman et al.(2012) also observed notable differences in metal levels in fingernail and toenail in Maiduguri Metropolis, Borno State, Nigeria. Although higher concentration of metals in toenail than fingernail has been observed in a number of studies (Abdulrahman, Akan et al. 2012, Dessie, Melaku

et al. 2020), higher concentration of Cr, Cu, and Pb metals in fingernail has also been seen in other researches (Przybylowicz, Chesy et al. 2012). Compared to hair samples, nail samples showed higher heavy metals accumulation; such differences might be related to the combination of elements with hair keratin structure, which happens by binding to sulfhydryl groups which are available in follicular protein. In this case detergents, such as soap, shampoos, hair pomades, lotions, hair bleaches and dyes can wash away heavy metals from the shaft bulk (Takagi, Matsuda et al. 1986, Abdulrahman, Akan et al. 2012, Koseoglu, Koseoglu et al. 2017). Comparing metals level in fingernail and toenail with various literatures showed that Cr, Co, Cu, As, Hg, and Pb concentration in fingernail and toenail were generally higher than the results in other researches (Abdulrahman, Akan et al. 2012, Marrugo-Negrete, Ruiz-Guzmán et al. 2013, Al-Saleh 2020, Dessie, Melaku et al. 2020), however, mean Cr concentration in fingernail and toenail and Co in toenail were lower than the amount of metals in other literatures (Abdulrahman, Akan et al. 2012, Marrugo-Negrete, Ruiz-Guzmán et al. 2013, Al-Saleh 2020). The average concentration of Cr, Co, Cu and Pb in pregnant women's fingernail in this study were 9.4, 37.9, 4.8, and 6.6 times, and in the toenail were 5.3, 6.2, 5.5, and 14.4 times higher than the healthy women in Poland respectively (Przybylowicz, Chesy et al. 2012). Higher metals concentration can be caused by people's exposure to contaminated sources in the area, so there is a possibility for metals accumulation due to the contact with the contaminants in this area.

ANOVA was conducted to compare human organs; the result indicated that there was no significant difference in relation to heavy metals seen in blood and hair, while a statistically significant difference was observed regarding the heavy metals Cr, Co, Cu, As, and Hg in hair and toenail and the Cr, Cu, As, Hg, and Pb in hair and fingernail. In addition, Cr, Co, Hg, and Pb metals in toenail and fingernail were significantly different from Cr, Co, As, and Pb metals in toenail and hair (Fig. 6). The correlation results of heavy metals in different samples demonstrated that there was a positive correlation between Cr, Co, Cu, and As in blood samples and Cr, Co and Cu metals in hair samples. In addition, positive correlations between Co and As, as well as Hg and Pb were determined in fingernail and toenail samples respectively (Fig. 7)

Conclusion

According to the purpose of this study, the concentration of six heavy metals including cobalt, chromium, copper, arsenic, mercury, and lead in water and sediment samples as well as in muscle, skin and liver of *C. carpio* and *L. auratus* species were measured. In addition, for the first time, biological monitoring of heavy metals in the tissues of Turkmen pregnant women's blood, hair, fingernail, and toenail was performed. The results of metal measurements in water and sediment samples showed that Cu and Cr had the highest mean concentration and Pb had the lowest mean concentration in water samples, whereas in sediment samples, Hg and Pb had the highest concentration, and Co had the lowest one. The concentration of heavy metals in water and sediment samples was as follows: Cu > Cr > Co > As > Hg > Pb for water, Hg > Pb > As > Cu > Cr > Co for sediment. Mean concentration of Cr, Co, Cu, and Pb in the surface water and sediment was lower than the specified standards for water and sediment by various health organizations, while the concentration of Hg in the water and sediments exceeded the standard values. The average

concentration of Hg in water was 6 times higher than WHO standard, while the average Hg in sediments was 160 times higher than PEL and ERM values and 560 times higher than TEL and ERL standards. The higher Hg in water and sediments in the surrounding areas of the wetlands is related to the existence of agricultural lands and use of fertilizers and pesticides by local farmers.

In order to identify and interpret the source of heavy metals more accurately, principal component analysis and correlation analysis were used. Pearson correlation demonstrated that Co, Cu, As, and Pb in water samples and Co, Cr, Cu, As, and Pb in sediment samples had positive correlations. The PCA results for water samples showed that metals are located in three clusters; there are Co, As, and Cu in PC1 which are affected by municipal wastewater, Pb and Hg are in PC2 and their main source is probably agriculture activities and transportation. The PC3, which contains only Cr, can be attributed to natural and geogenic sources. In sediment, metals in PC2 (Cu and As) are probably affected by human activities, especially municipal wastewater, while the origin of metals in PC1 (Cr, Co and Pb) can be both human due to agricultural and transportation activities (for Pb and Co) and natural (for Cr). Pearson correlation of Cr with Co and Pb as well as As and Cu in sediment and the high correlation of Cu with Co and As in water samples were consistent with PCA results for sediment and water, indicating the common sources of each heavy metal group.

The distribution of the heavy metal concentration in liver, muscle, and skin in *C. carpio* was $Cu > Pb > Hg > Cr > As > Co$, $Pb > Cu > Hg > Cr > As > Co$ and $Cu > Pb > Hg > As > Cr > Co$, whereas in *L. auratus* $Cu > Pb > As > Hg > Cr > Co$, $Cu > Pb > As > Hg > Cr > Co$ and $Pb > Cu > As > Cr > Hg > Co$ respectively. The level of heavy metals accumulation was approximately similar among the studied species; Cu and Pb had the highest concentration, and Co had the lowest concentration in both fish species in liver, muscle, and skin tissues. Liver was the tissue which accumulated these elements more in *C. carpio* and *L. auratus* except for As and Cr in *C. carpio*; furthermore, metals in *L. auratus* liver was more than *C. carpio* liver except for Hg. Cr, Hg, and Pb were accumulated in *C. carpio* muscle more than *L. auratus* muscle, while Co, Cu, and As were mostly accumulated in *L. Auratus* muscle. Besides, metals were accumulated in *C. carpio* skin more than *L. auratus* skin except for Pb. In general, the concentration of heavy metals in muscle was lower compared to liver; fish muscles are the main edible parts. But muscles are not the tissue in which heavy metals are accumulated. A significant difference was recognized among the liver, muscle, and skin of the fish (*C. carpio* and *L. auratus*) in view of the bioaccumulation of the selected heavy metals. The results of ANOVA test in *C. carpio* species showed that there was a considerable difference between the amount of Cr and As in liver and skin tissues. In addition, a notable difference was observed between liver and muscle in relation to Co and As. In the case of *L. auratus* species, significant difference was clear between liver and skin for Co, Cu, As, and Hg metals; furthermore, liver and skin had remarkable differences in terms of Co, Cu, and As metals. Mean values of Pb, Hg, As, and Cu were above the recommended limits of FAO/WHO/EU in the muscle tissue of *C. carpio* and *L. auratus*. As a result, since these fish are consumed by individuals, people should be cautious about the intake.

THQ, EDI, and CR provided by US Environmental Protection Agency were used to calculate and estimate the risk of exposure to heavy metals as a result of consuming two species of fish. The results of the

indicators showed that EDI and THQ values propose no risk to human in regard with the consumption of the two fish species, but consumption of *C. carpio* has a higher risk than *L. auratus*. However, CR values for As and Cr were greater than 10^{-4} , representing that the cancer risk posed by these metals exposure should not be ignored.

Blood, fingernail, toenail and hair samples were taken from Turkmen pregnant women for biological monitoring. considering the mean heavy metal contents in different organs, higher Cr, Co, Cu, As, and Hg contents were found at fingernail, and the highest Pb content was observed at toenail, while the lowest concentration of heavy metals was observed in blood. Cu had the highest concentration among the studied elements in blood, fingernail, toenail, and hair; whereas the concentration of Co was observed to be lower in all organs. The mean concentration of blood heavy metals level was lower in non-fisherman family than in fisherman family except for Cr, So the average concentration of lead in the fisherman family was significantly higher than non-fisherman families. The average blood metals concentration increased slightly with age, but the effect of age was only notable for cobalt metal. All blood samples had higher concentration than the standard value for Hg set by USEPA and WHO. People who had their teeth filled had lower mean metal concentration; therefore, dental amalgam did not display an association with heavy metal accumulation in blood. Place of residence, fishermen families, dental amalgam, and age had no significant effect on the concentration of heavy metals in hair. In addition, no specific trend was observed regarding metals changes in relation to the mentioned factors. what's more, the concentration of As and Hg in hair samples in the fishermen families was higher than the non-fishermen families. Heavy metals concentration in water, sediment, and two fish species proved that the amount of Hg was higher than the specified standards, especially for sediment which was much higher than the permissible standards. It can be said that Hg accumulation in water and sediment has led to an increase concentration in fish species and consequently in pregnant women's hair in the region. In our study, 75% of the recorded hair samples were higher than Hg references suggested by USEPA and WHO. In addition, people with filled teeth showed higher levels of Hg and Pb in the first age group;; the average concentration of Pb and Hg was higher. It can be concluded that the increase of age had no influence on metals levels of hair. The mean concentration of all metals in fingernail was higher than toenail except for Pb;; also, a significant difference was observed between the concentration of Cr, Co, Hg and Pb metals in toenail and fingernail.

ANOVA was conducted to compare human organs;; the result indicated that there was no significant difference in relation to heavy metals in blood and hair, while there was a statistically huge difference between the heavy metals Cr, Co, Cu, As, and Hg in hair and toenail and between the heavy metals Cr, Cu, As, Hg, and Pb in hair and fingernail. In addition, Cr, Co, Hg, and Pb metals in toenail and fingernail and Cr, Co, As, and Pb metals in toenail and hair had notable differences.

The study showed that the level of heavy metals in water and sediment, especially Hg, are threatening the various fish species which are principle and preferable praise for various migratory shorebirds as well as residents. Therefore, the assessment of wetland habitats, especially Ramsar sites, should be done to understand the current status of heavy metals in major species, including migratory, resident migratory

and resident shorebirds. Also, relevant organizations must control sensitive habitats to mitigate further environmental heavy metal pollution.

US Food and Drug Administration (USFDA 2004) and Environmental Protection Agency (USEPA 2005) recommends pregnant women, women of childbearing age, nursing mothers, and young children, to avoid seafood with high levels of Hg. In this regard, the necessary information should be provided in this area. It is suggested that biological monitoring be performed for different groups, especially young children.

Due to our small sample size, the results may not be valid for all pregnant women; therefore, it is necessary to use more samples to confirm the results and the relationship between the various studied factors and the level of metals in different organs. In general, due to the limitations of this study, however, the results of this study can be used to be compared with other studies.

Although there is no specific organization for pregnant women's biological monitoring in Iran, these results may draw the attention of environmental and health authorities to a national biological monitoring program, especially among vulnerable groups (children and women of childbearing age).

Declarations

Ethical Approval

Human bio monitoring was performed based on the protocol specified by the Medical Ethical Committee of Iran in collaboration with the staff of the health center (***Ethical Committee of Golestan University of Medical Sciences.(IR.GOUMS.REC.2019.126)***). Ethical principles and privacy were fully observed.

Consent to Participate

A written consent was received from the participants while completing the questionnaire.

Turkmen pregnant women from two villages, Qharesou and Chpaqli, living in the northern part of Miankale wetland participate in this study.

Consent to Publish

The authors declare that this paper could be published in your journal, ESPR, after receiving acceptance.

Authors Contributions

All authors contributed in whole writing process are:

Abbas Esmaili-sari as corresponding author, Zahra Shaabani as principle author, Ali MashinchianMoradi, Lobat Taghavi and Forough Farsad as co-authors.

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Competing Interest

The authors declare that they have no competing interest.

Availability of Data and Materials

All data and materials used in the paper are available in reference link or Doi of used papers.

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Figures

Figure 1

Distribution of water and sediment sampling stations in the study area

Figure 2

Correlation test results between heavy metals in water (a) and sediment samples (b)

Figure 3

PCA biplot displaying heavy metals distribution between water (a) and sediment (b) samples

Figure 4

ANOVA test results between different fish tissues at a significant level of 0.05 Different letters indicate a significant difference

Figure 5

THQ and TTHQ values of heavy metals in muscles for each heavy metal in fish species

Figure 6

ANOVA results between different fish organs in Turkmen pregnant women at a significant level of 0.05 (Different letters indicate a significant difference)

Figure 7

Correlation of heavy metals in blood samples (a), hair (b), fingernail (c), and toenail (d)