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Research Article

Risk assessment of aromatic hydrocarbon transfers cyclic through fish consumption (case study: *Mesopotamichthys sharpeyi* of Huralazim wetland in Iran)

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Abstract

The present study aimed to analyze the concentration of aromatic hydrocarbon transfer cyclic through consumption of Binni fish (*Mesopotamichthys sharpeyi*) at Huralazim wetland and estimates the risk of consumption at 7 stations of Huralazim wetland in spring 2018 (one season). A statistic populations included sediment (n=21), water (n=21) and fish (n=21). The concentration of aromatic hydrocarbons ranged from 178.45 to 1386.26 mg/kg. Acenaphthene and Pyrenees had the highest concentration of hydrocarbon compounds in sediments. In water samples of 7 stations, the concentration of aromatic hydrocarbons ranged from 17.12 to 651.79 mg/ L, and Acenaphthene and Naphthylene with the mean concentrations of 42 and 28.83 mg/L, respectively, which were the most abundant aromatic compounds (p<0.05). The results indicate the highest and lowest concentrations of total aromatic hydrocarbons in fish caught at station 7 (43±2 mg/kg) and station 1 (9.52±3.06 mg/kg), respectively. The highest carcinogenesis rate (1.13) and mutation rate (23.49) were found at stations 7 and station 5, respectively. The gradual carcinogenesis rate of Benz [a] pyrene ranged from 0.00003 to 0.0029, and its mean rate in Huralazim wetland reached 0.0027. The gradual mutation risk assessment for Benz [a] pyrene was estimated to be 0.055 (which ranges from 0.034 to 0.061). Most of the hydrocarbon compounds in the sediments have originated from pyrolytic and fossil fuels. According to mutant and carcinogenic standards, the daily consumption of fish in this wetland increases the risk of cancer and mutation incidences. Measures should be taken to reduce the consumption of fish at risk in Huralazim wetland, thus minimizing the risk of gradual cancer or mutation.

Keywords: Risk assessment, Transfer cyclic, *Mesopotamichthys sharpeyi*, Huralazim Wetland.

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Introduction

In recent years, aromatic hydrocarbons have received much attention due to their adverse effects on health and the ecosystem. The main way for aromatic hydrocarbons to enter into the aquatic ecosystems is flowing domestic and industrial wastewater to waters and leakage of crude oil containing these compounds. Disposal of household waste systems, agriculture, and groundwater streams leads to the flow of large amounts of aromatic hydrocarbons and heavy metals into the environment (de Mora *et al*., 2004; Yang *et al*., 2019). Global oil demand is predicted to increase from 84.7 million barrels per day in 2008 to more than 150 million barrels per day in 2030 (IEO, 2014), which increases the importance of the study of pollution causing from aromatic hydrocarbons in oil (Amankwaa *et al*., 2021).

Not only the toxicity of hydrocarbons but also the other properties such as bioaccumulation and biomagnification in the food chain are serious threats to the ecosystems and their organisms (Lucke *et al*., 2019). Being directed into the aquatic ecosystems, these pollutants may directly harm aquatic organisms and can be stored in the food chain through the effects of bioaccumulation and biomagnification on the tissues and organs of aquatic organisms. Thereafter, fish consumption is ultimately a threat to human health (Mhadhbi and Boumaiza, 2012; Thomas and Bu-Olayan, 2020).

Sediments are the main site of accumulation of pollutants in aquatic environments, playing a pivotal role in the accumulation of some heavy metals and hydrocarbon pollutants in benthic invertebrates (Kosari *et al*., 2021) and their transfer to higher food levels such as plants and fishes (Dudhagara *et al*., 2016). Fish (Usydus *et al*., 2009) increase the risk of cancer in consumers due to exposure to petroleum products in environments and feeding on lower food chains containing contaminants (Ohiozebau *et al*., 2017; Baharvand *et al*., 2021). Some Polycyclic aromatic hydrocarbons (such as chrysene benzo [a] pyrene-1, 2, 3 and benzo [b] fluoranthene) have been identified as carcinogens.

These substances and heavy metals cause mutations and have genetic effects on the examined animals (Thyssen *et al*., 1981; Lakshmanan *et al*., 2019). Huralazim wetland, a wetland with 5 reservoirs and full of petroleum products, is one of the leading centers of oil extraction in Iran, which spread the pollution in this water area. Concerning that, oil pollution is one of the main threats to ecosystems (Orji *et al*., 2012), determining the concentration and assessing the pollution levels can determine the trend of change in this region. Regarding the severe pollution, which endangers human life because of feeding on this chain (Hosani and Anouti, 2014; Pradit *et al*., 2018) as well as destruction and degeneration of the biological chain, necessary measures should be taken to reduce pollution, so that the concentration of metals does not exceed the allowable limit.

In the present study, the accumulation of polycyclic aromatic

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hydrocarbons (PAHs) in sediments, water, and *M. sharpeyi* muscle were measured. This species is one of the dominant species of indigenous fish in Huralazim (frequency 24.6%). This fish consume a large part of the food chain as they are Saprophagous and herbivorous (Rezaei and Papahn, 2013). Moreover, the degree of carcinogenesis and mutagenesis effect of this species was studied.

Materials and methods

The present study was performed in Huralazim wetland in the west of Khuzestan province, Iran (47°57' E, 47° 16' W, 31° 47' N, and 41° S). It was conducted in spring 2018 at the identified stations in four reservoirs of Huralazim wetland. The studied variables included the concentration of aromatic hydrocarbons in sediment, water, and the *M. sharpeyi* (Binni) muscle tissue in Huralazim wetland. A total of 63 samples (sediment, water and fish) were collected (Table 1 and Fig. 1).

Table 1: Huralazim wetland reservoir stations.

	Station	UTM			DMS	
Sohrab undeveloped oil field	1	R38	765764	3501658	313709.8	474805.9
	2	R38	765221	3501841	313716.2	474745.4
North Azadegan oil field	3	R38	766565	3486920	312911.0	474821.9
	4	R38	766124	3477671	312411.3	474756.3
South Azadegan and North Yaran oil fields	5	R38	761738	3473771	312208.4	474506.7
	6	R38	764854	3468623	311918.8	474659.6
South Azadegan oil fields	7	R38	765441	3461467	311526.2	474713.9

Collection and sampling methods

Sediment sampling and measurement of petroleum hydrocarbons

Sediment samples were collected using Van Veen Grab with a cross-section of 0.025 m^2 and then transported to the laboratory in aluminum containers with ice (Ropme, 1999). 100 g (Sartreus, Germany) of sediment and 300g of sodium anhydride sulfate were mixed at high speed in a heater-stirrer for 3 min (IKA RHBACIC 2, Germany). Sediment hydrocarbons were extracted with solvent (50:50) of dichloromethane and n-hexane by Soxhlet for 12 h (Standard Soxhlet Assembly Complete 600mL 600RDSX, England). After separating the solvent with a rotary evaporator (Rotary Evaporator, RV 10 DS99, USA), the sample volume increased to 15 mL; then reduced to 4 mL by passing nitrogen gas, transferring into the special vials for injection into the Chromatography (model: Agilent Technologies-7890B GC) connected to a mass spectrometer (model: Agilent Technologies-5975C) with HP-5MS capillary column (length 30 m× outer diameter 0.25 mm × 25 µm inner diameter).

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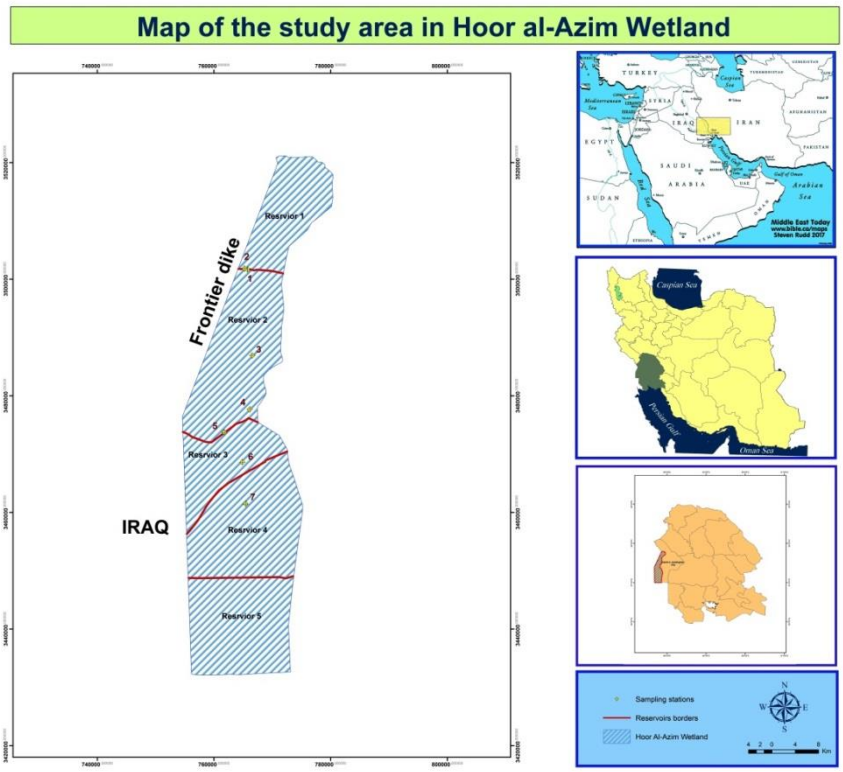

Figure 1: The study area in Huralazim wetland

Water sampling and measurement of petroleum hydrocarbons in the water

Water samples were taken using a Ruttner bottle from a depth of 20 cm and then transported to the laboratory with ice (Ropme, 1999). Organic compounds were extracted from the water by the liquid-liquid extraction method. Before extraction, 50 µL of five internal standards (Naphthalene-d8 phenannterene-d10, Terphenyl d14, Chrysene-d12, Perylene-d12) were added to the sample to measure the accuracy and calculate the sample recycling. One liter of the filtered water sample was transferred into a separatory funnel (DURAN, Germany) and extracted with 100 mL of saturated sodium chloride salt solvent and 100 mL of dichloromethane solvent for three times (APHA, 1992). Then, 7-8 grams of activated copper were added to the samples and rested overnight to remove sulfur compounds.

To separate and purify aromatic hydrocarbons, samples were passed through columns one and two by chromatography. Column 1 of chromatography contained inactive silica gel (0.5 water) used to separate polar compounds. The output samples were reduced to 1 mL from column 1 of chromatography and passed through column 2 which contained active silica gel. Aromatic hydrocarbon compounds were separated by column 2 eluted with hexane/dichloromethane solvent. Finally, the sample volume was reduced with pure nitrogen gas to a nearly dry phase, and 150 µL of isooctane was

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dissolved and transferred to the special vials for injection to chromatography device (Zakaria *et al*., 2002).

Fish sampling and measurement of petroleum hydrocarbons

Fishing gill nets (with a 5 cm mesh size) were used to catch Binni (*M. sharpeyi*) and the fish was identified using code recognition keys (Hafez *et al*., 2017). For the current study, 21 fish were collected from seven stations. The samples were washed with distilled water and transferred to the laboratory (Hodson, 2017). Fish muscle tissues were separated from the mid-back of the body, placed in an aluminum foil, labeled, and then frozen at -18°C (Frena *et al*., 2016).

The samples were dried entirely in the preparation stage since PAHs aqueous compounds can be harmful because of volatility. To reach this stage, the samples were placed in a freeze dryer (Model FD- 10V) for 72 hours at a temperature of -50°C under vacuum conditions until the complete water exited. Dried samples (0.2 g) were poured into a Transform 600 Microwave Digestion, and 4 mL of potassium hydroxide saturated solution in alcohol and 10 mL n-hexane were added to the sample. After placing the cells inside the digestion system, extraction was carried out at 129°C for 17 minutes. After cooling the solution, 6 mL of the organic phase was mixed in the centrifugal tube (DOMEL Centric 250IVD) with 3000 rpm for 3 minutes. The extract was evaporated and concentrated by a rotary evaporator (Senco RV 8-VC) until its volume reached 0.5 mL. The extracted solution was filtrated using silica sheets activated with 4 mL of dichloromethane solution and then again with 4 mL of dichloromethane-hexane (with a volume ratio 1:1). The material was washed with 4 mL of dichloromethane-hexane and again, evaporated to reach 0.5 mL by rotary evaporator. One mL of acetonitrile was added, and the mixture was concentrated to reach a volume of 50 mL. The extract was transferred to a volumetric flask with 2 mL capacity, including 0.5 mL of ultra-pure water, and the solution was filtrated using a 0.22-micron filter paper (Merck, Germany). Ultimately, 20 µL of the resulted solution was injected into a GC-FID (Shimadzu-14A, Japan) equipped with Rtx-5 capillary columns (Ropme, 1999).

Toxicity Factors

Two Toxic Equivalence Factors (TEFs) (Ashiru and Ogundare, 2014) (carcinogenesis) and the Mutagenic Equivalency Factor (MEF) (Kofi *et al*., 2018) were used. Mutagenesis was used to express the relative toxicity of hydrocarbon compounds of individual fish. The toxicity levels were calculated for the existing hydrocarbon coefficients and summed these coefficients from the Toxic Equivalent (TEQ) and meta-genetic (MEQ) equations as follows:

$TEQ = \sum TEFi \times Ci)$

$MEQ = \sum (MEFi \times Ci)$

TEF defines the toxic potency of individual PAHs that include Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene,

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Benzo[k]fluoranthene, Benz[a]pyrene, Indeno[1,2,3-cd] pyrene and Dibenzo[a,h]anthracene. Ci is considered the concentration of cyclic hydrocarbons.

Assessing the Gradual Risk of Carcinogenesis and Mutagenesis

Maximum exposure of humans with a mean lifespan of 70 years with different doses of PAH in a diet (mg kg-1 BWd-1) (carcinogenic and mutagenic) is calculated by the following formula:

$$\text{Carcinogenic (mutagenic)} \quad PAH = \frac{(TEQorMEQ) \times IR \times CF}{BW}$$

This equation shows the maximum exposure level based on the EPA guideline (USEPA, 1993). In the equation, IR is the mean of fish consumption per year (IFSY, 2014), and the CF denotes carcinogenesis factor (0.001 mg μg^{-1}) and BW is the body weight, which was considered as an average of 70 kg.

Results

Table 2 shows the measurement of oil-based hydrocarbons in fish muscle in the reservoirs of Huralazim Wetland. The highest and lowest hydrocarbon concentrations were observed in station 7 (43± 2 mg/kg) and station 1 (9 ± 3 mg/kg), respectively. The highest concentration of PAH was related to acenaphthene (30.67 mg/kg).

Table 2: The results of measurement for oil-based hydrocarbons of the fish muscle in reservoirs of Huralazin wetland (mg/kg)

Compound	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Total
Naphthylene (Nap)	12.85 ± 0.64a	14.20± 0.31a	17.27 ± 0.81a	21.14± 0.50a	27.89± 0.96a	37.04± 0.08a	43.93± 2.71a	24.90
Acenaphthylene (Acel)	11.01 ± 0.50a	11.75 ± 0.09a	18.79 ± 0.42a	23.37 ± 0.24a	23.84 ± 0.19a	29.33 ± 0.19a	30.96± 0.53a	21.29
Acenaphthene (Ace)	15.43 ± 0.21a	16.56 ± 0.18a	22.39 ± 0.24a	26.10 ± 0.06a	39.68 ± 2.28a	47.24 ± 0.06a	47.33± 0.72a	30.67
Fluorene (Flu)	<10	<10	<10	<10	<10	<10	<10	
Phenanthrene (Phe)	12.55 ± 0.22a	14.60 ± 0.25a	15.36 ± 0.08a	16.21 ± 0.11a	19.66 ± 0.41a	18.44 ± 0.27a	19.46± 0.14a	16.61
Anthracene (Ant)	<10	<10	<10	<10	<10	<10	<10	
Fluoranthene (Flt)	<10	<10	<10	<10	<10	<10	<10	
Pyrene (Pyr)	<10	<10	21.69 ± 1.93a	<10	<10	18.09 ± 0.27a	17.89± 0.62a	15.75
Benz[a]anthracene (BaA)	<10	<10	<10	<10	<10	<10	<10	
Chrysene (Chr)	<10	<10	<10	<10	<10	<10	<10	
Benzo[b]fluoranthene (BbF)	<10	<10	<10	<10	<10	<10	<10	

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Tabel 2 (continued):

Compound	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Total
Benzo[k]fluoranthene (BkF)	<10	<10	<10	<10	<10	<10	<10	
Benz[a] pyrene (BaP)	14.28 ± 0.12a	13.31 ± 0.11a	17.61 ± 0.21a	18.38 ± 0.26a	23.49 ± 2.24a	19.46 ± 0.19a	19.32± 0.59a	17.98
Indeno[1,2,3-cd] pyrene (IcP)	<10	<10	<10	<10	<10	10.45 ±0.22a	11.17 ±0.04a	10.23
Dibenzo[a,h] anthracene (DhA)	<10	<10	<10	<10	<10	<10	<10	
Benzo[g,h,i]perylene (BgP)	<10	<10	<10	<10	<10	<10	<10	
Total	9.52 ± 3.06a	14.20± 0.31a	17.27 ± 0.81a	21.14± 0.50a	27.89 ± 0.96a	37.04± 0.08a	43.93 ± 2.70a	

The same small letters by the same number in indices of two mean values denote statistically significant difference between means at =0.05 level.

According to the hierarchical cluster analysis (HCA), stations 6 and 7 showed less similarity than the other stations. Stations 1, 2, 3 and 4 were each in a cluster or at least a distance in comparison (Fig. 2).

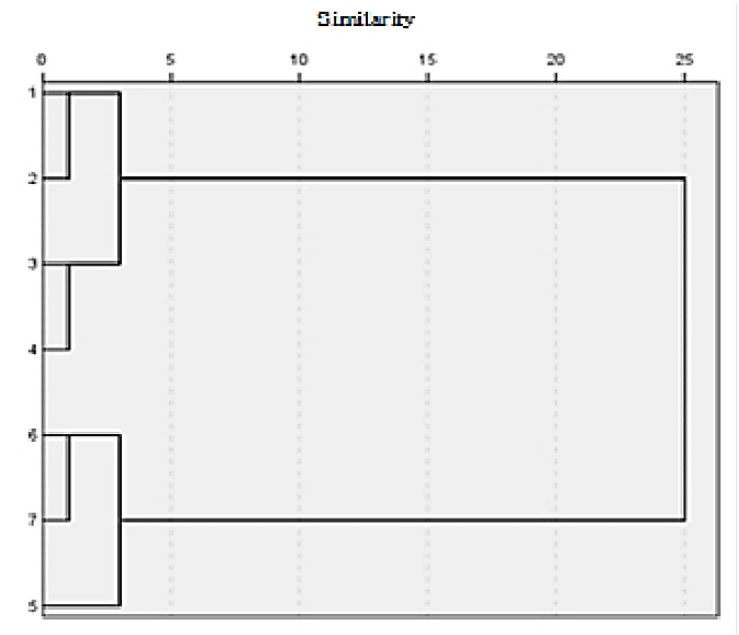

Figure 2: Hierarchical cluster analysis (HCA) for categorizing the PAHs in fish muscles- (1) Station 1, (2) Station 2, (3) Station 3, (4) Station 4, (5) Station 5, (6) Station 6 and (7) Station 7.

Comparison of 2 and 3 ring hydrocarbons with 4, 5, 6 and 7 rings showed that the concentrations were significantly higher (p<0.05) (Fig. 3).

Two Toxic Equivalence Factors (TEFs) and Mutagenic Equivalency Factor (MEF) indicate cancer and the cause of mutation (Table 3). According to the values of TEFs and MEFs indicators, the rate of mutation caused by fish consumption was higher than that of cancer incidence. The highest mutation rate at station 5 and the highest carcinogenicity rate at station 7 were

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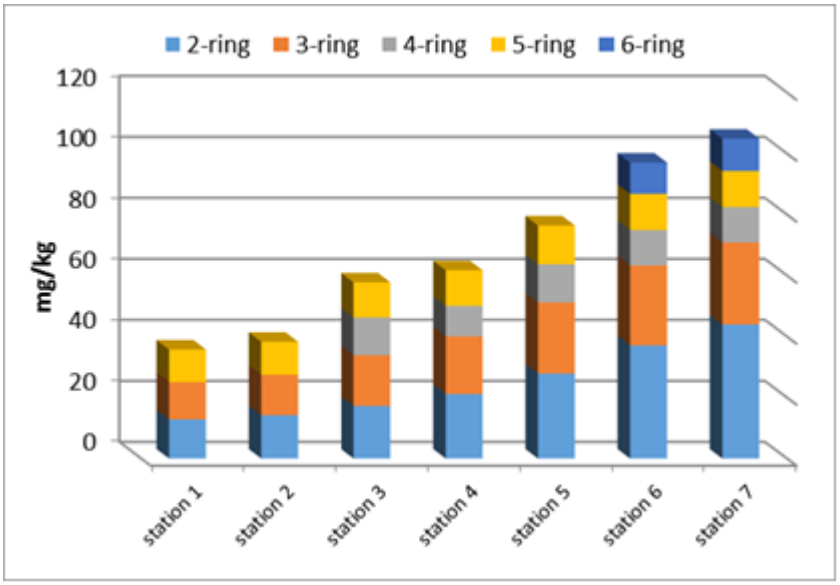

Figure 3: Comparison of hydrocarbons levels based on the fish muscle hydrocarbon rings in different stations.

Table 3: The results of calculation for Toxicity Equivalence Factors (TEFs) and Mutagenic Equivalence Factor (MEF)

Gradual risk assessment	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Total
TEQ	0.013	0.014	0.017	0.018	0.023	1.06	1. 13	1.04
MEQ	13.31	14.28	17.61	18.38	23.49	22.69	22.78	21.15

Table 4 displays the evaluation of carcinogenicity and mutagenicity based on the values of carcinogenicity indices and mutation occurrence based on per capita consumption of fish recorded by the Iranian Fishery Statistical Reports. The highest incidence of cancer and mutation occurred at station 7.

Table 4: Carcinogenic risk assessment based on the values of carcinogenicity indices and mutation occurrence

Gradual risk assessment	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Total risk
Toxicant	0.00003	0.00003	0.00004	0.00004	0.00005	0.0027	0.0027	0.0027
Mutagenic	0.034	0.037	0.045	0.034	0.061	0.059	0.059	0.055

In the water of Huralazim wetland, Acenaphthene (M=42 mg/ L) and Naphthylene (28.83 mg/ L) have the highest amount of hydrocarbon compounds. Other compounds (Benzo [g, h, i] perylene, Indeno [1, 2 3-cd] pyrene and Dibenzo [a, h] anthracene) had the lowest concentration (with values of 4.34, 4.36 and 6.14 mg / l) (p<0.05). The highest concentration of 16 petroleum hydrocarbons was measured at station 7 (p<0.05). Distribution of aromatic hydrocarbons in the water of Huralazim wetland (Figure

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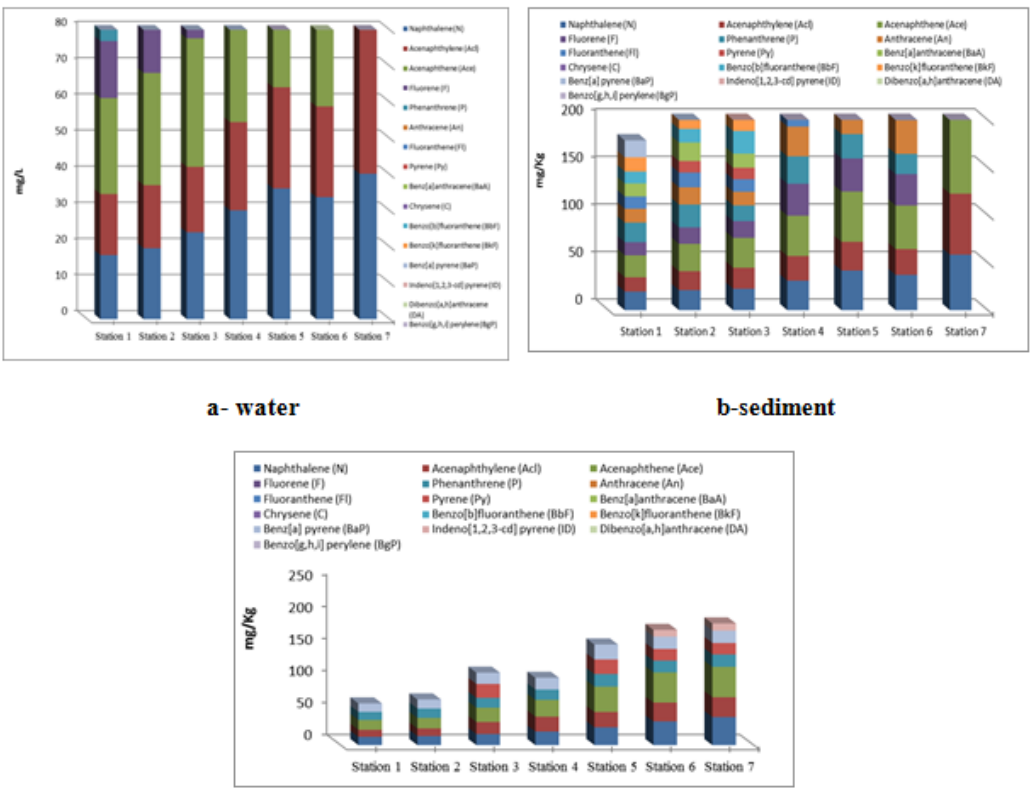
4-a) showed the increasing trend of pollution towards stations 6 and 7, and station 1 had the lowest pollution (p<0.05).

In the sediments of Huralazim wetland, the hydrocarbon compounds (Pyrene, Chrysene, Indeno [1, 2, 3-cd] pyrene, Dibenzo [a, h] anthracene, and Benzo [g, h, i] perylene) in all stations were less than the detection limit of the device. In general, the changing trend in the concentration of aromatic hydrocarbons increased from station 1 to station 7 and it was from 178.45 mg/kg in station 1 to 1386.26 mg/kg (p<0.05) (Fig. 4- b).

Aromatic hydrocarbons measured in the fish muscle of the Huralazim wetland at various stations are shown in Figure 4-c. Stations 6 and 7 with 7 hydrocarbon compounds had the highest number of hydrocarbon compounds. Furthermore, stations 1 and 2 have the lowest level of hydrocarbons, and the level of pollution has increased moving towards for the last stations (reservoir 3 and 4). Five hydrocarbons (including Naphthylene, Acenaphthylene, Acenaphthene, Phenanthrene, and Benz [a] pyrene), Pyrene and Indeno[1,2,3-cd] pyrene were measured in all 7 stations, 4 stations and 2 stations, respectively. Acenaphthene was the most abundant hydrocarbon compound at all stations (p<0.05).



a- water b-sediment

c- *M. sharpeyi* (Binni fish)

Figure 4: Comparison of aromatic hydrocarbon pollution in Huralazim ecosystem based on selected stations.

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Discussion

Analysis of concentration of petroleum hydrocarbons in the sediment of Huralazim wetland

Oil pollution is one of the most critical pollutants entering coastal areas, and cyclic aromatic hydrocarbons are one of the oil's most important hydrocarbon groups (Baharvand *et al*., 2021). The results of studies on molecular weight of hydrocarbon compounds in sediments showed that high molecular weight hydrocarbon compounds (such as Pyrene, Benz [a] pyrene and Benzo [b] fluoranthene) were recognized as one of the main carcinogenic compounds. This issue has been reported in various studies such as Tolosa *et al*. (2005). Hydrocarbons with many benzene rings are less soluble in water and tend to adsorb in sediment due to a higher octanol/water ratio than the compounds with a smaller number of rings (Baumard *et al*., 1998). As the number of benzene rings increases, the more stable molecules and the heavier compounds in the sediments become more stable. In contrast, the concentration of lightweight hydrocarbon compounds decreases with decomposition in sediments (Ashiru and Ogundare, 2019). Therefore, aromatic hydrocarbons with a linear chain structure (such as anthracene decompose) are analyzed easier than the hydrocarbons with a shelf structure (such as Pyrene), which confirms the high concentration of Pyrene in the present study (Simpson *et al*., 2007).

According to the obtained results, the concentrations of all aromatic hydrocarbons in the sediments showed higher values in dry weight, which confirms the higher pollution of the sediments of Huralazim wetland compared to some standards (ERL (4022), ERM (4479), American sediment quality (NOAA) (1684) and Florida Environmental Protection Standards (FDEP) TEL and PEL (19770)).

Analysis of the concentration of petroleum hydrocarbons in the water of Huralazim wetland

The concentration of 14 hydrocarbon compounds in the water of stations ranged from 17.12 to 651.79 mg/L. Some of the 16 hydrocarbon compounds (including Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Benz [a] anthracene, Benzo [b] fluoranthene, and Benzo [k] fluoranthene) were present at all stations. Acenaphthene and Naphthalene had the highest concentrations of hydrocarbon compounds.

Hydrocarbon compounds are slightly soluble in water due to their hydrophobicity, and this solubility decreases with increasing molecular weight and solubility coefficient so that the concentration of the five and six rings compounds with the highest molecular weight and solubility coefficient has a low concentration in seawater and tend to settle in sediments (Walker, 2002). In the present study, 5- and 6-ring hydrocarbons had the lowest concentration in all stations, and 3-ring compounds had the highest

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concentration due to high solubility of these compounds in the water (Sonnefeld *et al*., 1983).

The total concentration of hydrocarbon compounds in fish caught from the Huralazim Wetland ranged from 9.52 mg/kg (station 1) to 47.93 mg/kg (station 7). Tolosa *et al*. (2005) reported the concentrations of hydrocarbons in the fish tissues of *Epinepbelus coioides* and *Lethrinus nebulosus* on the coasts of the United Arab Emirates were 2.7 µg/g and 3.40 µg/g, respectively. Moreover, Jazza *et al*. (2015) found the aromatic hydrocarbon concentrations in the tissues of *Liza abu* (161.61-2.03 ng/g of dry weight) and *Carassius auratus* (0.95-1.875 ng/g of dry weight). In general, the concentration of hydrocarbons in both studies is much lower than that in the present study. This difference is due to the specific position of the Huralazim wetland through the exploration, extraction, and drilling of the well that contributed to the level of contamination in the food chain.

The caught fish from stations 1 (9.52 mg/kg), and 2 (14.20 mg/kg) showed lower hydrocarbon levels than the other stations, especially station 7 (43.93 mg/kg). Huralazimm Wetland in the Iranian side consists of five main reservoirs; reservoir 1 (stations 1 and 2) is cleaner and natural because of the limited level of extraction and exploration activities in this reservoir and low concentration of petroleum hydrocarbons in the fish tissue compared to other stations. Conversely, station 7 has the highest level of exploration and extraction activities. Therefore, the level of hydrocarbons at this station is much higher than the other stations. The hierarchical cluster analysis showed the proximity of stations 6 and 7, which confirmed the findings.

Classification based on hydrocarbon rings showed that the two-and three-ring compounds were more frequent than the four-ring ones. Furthermore, the concentration of two-ring compounds or lightweight compounds of Naphthylene, Acenaphthylene and Acenaphthene were more abundant in fish caught than the other hydrocarbon groups. Regarding the results of the present study, PAHs levels are below the detection limit (<10) due to the decomposition and conversion of these compounds to lower molecular weight compounds (Ashiru and Ogundare, 2019). In this study, *M. sharpeyi*, as an herbivorous and saprophagous fish, consumes a large portion of its nutritional needs from the invertebrate bestial beings. Given that by entering oil-based hydrocarbons into the aqueous medium, these materials are transported across the chain to various parts of planktons, invertebrates, and plants then transferred to the body of fish and stored there. Therefore, the amount of contamination in the tissue of *M. sharpeyi* is indicative of the contamination level in the lower levels of the food chain. In addition, this form of nutrition justifies the higher concentrations of oil-based hydrocarbons due to the higher accumulation of these substances in the body of the invertebrates (Ayoola *et al*.,

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2017; Gospodarek *et al.*, 2019).

Comparing the standard of 16 PAHs values of edible tissue of Binni fish from Horelazim wetland with other standards (16 PAHs standards of UUS; EPA, (amounted to 50 µg/kg.dw), 6 PAHs of World Health Organization: WHO (20 µg/kg.dw) and the European 16 PAHs standard, BaP (8 µg/kg.dw) cited by (Ohiozebau *et al*., 2017) and European Commission (OJEU, 835/2011) (OJEU, 2011) (2ppb)) indicate that the hydrocarbon values in the tissue of wetland fish were much higher than that stated in the standards. IARC (1986) identified six combinations (benz [a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benz[a] pyrene, and dibenzo [a,h]anthracene), as mutagenic and carcinogenic compounds, which in the present study, the number of other compounds were insignificant, except for benz[a]pyrene. Benz [a] pyrene is usually an indicator for controlling hydrocarbon compounds in the environment (Orecchio *et al*., 2009). Hence, it is considered as a leading combination in the evaluation of carcinogenic or mutagenic risks in Huralazim wetland

Indeed, the Toxic Equivalent (TEQ) and the M*utagenic* E*quivalents* (MEQ) are related to the toxicity rate B(a) P. TEQBap and MEQBaP had a direct correlation with carcinogenicity of compounds and the mutagenicity of compounds, respectively (Jiao *et al*., 2016). It means that these non-carcinogenic compounds have destructive effects such as pulmonary diseases, congenital disabilities, sexual dysfunction, and reduction of IQ (Sharaf and Shehata, 2015). According to the environmental protection agency and the European Union, the two combinations of indeno (1, 2, 3- cd) pyrene (IP) and benz [a] pyrene have positive mutagenicity and have been categorized as a possible causing combination of cancer and carcinogenic composition, respectively. These compounds either alone or in combination with covalence compounds, attach to the cellular macromolecules such as DNA and in DNA replication, provide a background for mutation, creating a tumor and ultimately cause cancer (Rezaei and Papahn, 2013). Other hydrocarbon compounds have different degrees of carcinogenicity or mutagenicity, which contained minor amounts based on the findings of the present study. The highest carcinogenicity rate (13.1) and mutagenicity rate (23.49) were observed at stations 7 and 5, respectively. Ina previous study, the highest carcinogenicity rate was 3.05, and the highest mutagenicity rate was 4.40 (Kofi *et al*., 2018). In the present study, benz [a] pyrene was the major causative compound of cancer and mutation, but in Kofi *et al*. (2018) stated that, benz [a] anthracene was the most carcinogenic compound, and Indeno [1,2,3- cd] pyrene was the most mutagenic compound.

The rate of gradual carcinogenicity for the combination of benz [a] pyrene ranged 0.00003 to 0.0029 concerning the station, and 0.0027 in Huralazim wetland. It means that the consumption

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of fish would affect 29 out of 10000 or 3 out of 100 people during 70 years. According to Kofi *et al*. (2018), the carcinogenicity rate of Ghana oysters affected 45 of 10000000 people (Ayoola *et al*., 2017). USEPA (2009) stated that the carcinogenesis intensity is 1×10^{-5}, which represents the high carcinogenicity risk of this compound, especially at station 7. The evaluation of gradual mutagenicity risk for the combination of benz [a] pyrene with per capita consumption rate of 9.2 kg (Tolosa *et al*., 2005) was 0.055 (range 0.034 - 0.061). It means that 34- 61 people (per 100 people) are more likely to be exposed to non-cancer-related illnesses. Since the mentioned values are higher than the 10-5 USEPA standard, the daily consumption of this wetland fish increases the mutagenicity risk of the consumers. According to Kofi *et al*. (2018), the mutagenicity level of benz [a] pyrene affected 9 of 1000000, and 38 of 1000000 people, respectively. They expressed that the high consumption of these oysters increases the mutagenicity risk (Ayoola *et al*., 2017). It is worth noting that naphthylene contains a high-dose- hydrocarbon compound among the two-ring hydrocarbon compounds and is one of the causes of cancer in humans (US ATSDR, 2005). This issue increases the risk of carcinogenicity and necessitates more attention to prevent damage to the Huralazim wetland.

As an oil hub and oil extraction and refining activities, the waters of Khuzestan province are exposed to a variety of pollutants, including heavy metals and petroleum products, entering the ecosystem and contaminating the food chain. Therefore, the present study estimated the extent of transport of oil pollutants along the food chain, the percentage of biohazard that threatens this chain, and ultimately humans . According to mutagenic and carcinogenic standards, the daily consumption of fish in this wetland increases the risk of cancer and mutagen for the inhabitants and consumers. Measures have to be taken to reduce the consumption of contaminated fish in Huralazim wetland and minimize the risk of gradual cancer or mutagen, especially those indigenous to the area.

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