Forum geografic. Studii și cercetări de geografie și protecția mediului
Volume **XVIII**, Issue **2** (**December** 2019), pp. **159-166**
http://dx.doi.org/**10.5775/fg.2019.061.d**

Risk assessment of consuming aromatic hydrocarbons (The case study: Mesopotamichthys sharpeyi of Huralazim Wetland in Iran)

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Received on 16-09-2019, reviewed on 30-09-2019, accepted on 30-10-2019

Abstract

Background and goal: Aromatic hydrocarbons are one of the most important environmental pollutants in the environment. These compounds, even at very low concentrations, have carcinogenic and mutagenic properties and are quantitatively and qualitatively known as compounds that have entered all parts of the environment due to human activities. This research studies the concentration of aromatic hydrocarbons in the predominantly fish (Mesopotamichthys sharpeyi) in Huralazim wetland and calculates the risk of consuming this fish by a human.
Materials and Methods: In the spring of 2018, 210 fish were collected from 4 reservoirs (7 stations). The analysis of 16 hydrocarbon compounds was carried out using the GC-FID (Shimadzn-14A) equipped with a capillary column (RTX-5).
Results: The highest concentration of oil-based hydrocarbons was in fish of station 7 (43.93 ± 2.70 mg/kg) and its lowest concentration was at station 1 (9.52 ± 3.06 mg/kg). The highest carcinogenesis incidence rate was 1.13 at station 7 and the highest mutation rate was 23.49 at station 5. The gradual carcinogenesis rate of Benz [a] pyrene was 0.00003 to 0.0029 and n in general, through Huralazim Wetland it was 0.0027. The gradual mutation risk assessment for Benz [a] pyrene was estimated as 0.055 (which ranges from 0.034 to 0.061).
Conclusion: According to the standard of mutant and carcinogenic compounds [5], it can be said that the daily consumption of fish in this wetland increases the risk of cancer and mutagen in the consumer population. Measures have to be taken to reduce the consumption of caught fish which are exposed to pollution in Huralazim Wetland, in order to minimize the risk of gradual cancer or mutagen, especially in the natives of that area.

Keywords: *risk of consumption, aromatic hydrocarbons, Bani fish, Huralazim Wetland*

Rezumat. Evaluarea riscului consumului de hidrocarburi aromatice (studiu de caz: Mesopotamichthys sharpeyi din zona umedă Huralazim, Iran)

Scop: hidrocarburile aromatice reprezintă unul dintre cei mai importanți poluanți ai mediului. Acești compuși, chiar în concentrații foarte mici, au caracteristici cancerigene și sunt cunoscuți din punct de vedere cantitativ și calitativ drept compuși care afectează toate componentele mediului datorită activităților umane. Studiul de față analizează concentrația hidrocarburilor aromatice în ihtiofauna (Mesopotamichthys sharpeyi) din zona umedă Huralazim, calculând riscul la care se expune populația consumând acest pește.
Material și metodă. În toamna anului 2018, 210 pești au fost colectați din 4 rezervoare (7 stații). Analiza celor 16 compuși ai hidrocarburilor s-a făcut cu ajutorul GC-FID (Shimadzn-14A), echipat cu o coloană capilară (RTX-5).
Rezultate: Cea mai mare concentrație a hidrocarburilor pe bază de ulei a fost înregistrată la stația 7 (43.93 ± 2.70 mg/kg), iar cea mai mică concentrație la stația 1 (9.52 ± 3.06 mg/kg). Cea mai ridicată rată a incidenței cancerigene a fost de 1,13 la stația 7, iar cea mai mare rată de mutație, 23,49, la stația 5. Rata cancerigenă graduală a Benzo [a] pirenei a variat între 0,00003 and 0,0029 și n în general, pe tot cuprinsul zonei umede Huralazim fiind de 0,0027. Riscul mutației gradule pentru benzo [a] pirena a fost evaluat la 0,055 (variind între 0,034 și 0,061).
Concluzii. Conform standardelor compușilor cancerigeni și cu potențial pentru mutații genetice, se poate afirma că un consum zilnic de pește provenit din această zonă umedă crește riscul de cancer pentru populație. Trebuie luate măsuri pnetru a reduce consumul de pește pescuit aici, întrucât acesta este expus unei poluări considerabile din zona umedă Huralazim, astfel ncât să se limiteze riscul de îmbolnăviri în rândul populației autohtone.

Cuvinte-cheie: *risc de consum, hidrocarburi aromatice, peștele bani, zona umedă Huralazim*

Introduction

Wetlands and their related areas are special displays of water resources. A local wetland is a natural manifestation that, in the process of its formation, its soil is saturated with surface and underground water. Under normal and environmental conditions, wetlands are formed gradually and during a long period of time. They have biological sequences. Hence, the population growth, as well as the industrial and agricultural developments has quickly infected these valuable areas (Christensen and Bzdusek. 2005, Moon et al., 2006). Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds with two or more non-volatile or semi-volatile aromatic rings (Singh et al., 2016) that are produced naturally or by human activities. Polycyclic aromatic hydrocarbons are formed during incomplete combustion of organic materials and are found in oil

reserves in large amounts and are released during the extraction, transport, or processing of oil (Pampanin and Sydnes, 2013). These compounds have a very low solubility in water and are lipophilic so they are quickly stored in fat tissue (Veiga et al., 2014).

It is predicted that in 2030, the global demand for oil from 84.7 million barrels per day in 2008 reaches to more than 105 million barrels per day. As a result, most of these compounds will be found in nature (International Energy Outlook 2013). In aquatic environments, sediments of rivers are the main place of receiving and storing pollutants. Besides, they play an important role in the accumulation of petroleum and its derivatives in benthic macro-invertebrates (benthos) and transferring them to higher nutritional levels such as plants, invertebrate, and fish (Mendi and Uluozlii, 2006). Fish are one of the most important extracted material in the wetlands (Usydus et al., 2009), which due to exposure to petroleum in these environments as well as feeding on lower nutritional chains that have stored pollutants increase the risk of cancer in the consumers (Ohiozebau et al., 2017). Among the hydrocarbon compounds, some polycyclic aromatic hydrocarbons (PAHs) such as Benzo [a] pyrene, chrysene, indeno (1,2,3-c, d) pyrene and benzo (b) fluoranthene are known as the causes of cancer or mutation which have also genetic effects on laboratory animals (Deutsch-Wenzel 1983; Thyssen et al., 1981).

Conventional crude oil production method is not able to meet the increasing demand for oil- based hydrocarbons energy. This has led to the significant growth of oil extraction from the bed of wetlands and seas (Parajulee and Wania, 2014). This type of extraction can be seen in Khuzestan province and Huralazim wetland. In the oil-rich province of the Khuzestan, contamination with polycyclic aromatic hydrocarbons is one of the most common pollutions, which is increasing in Huralazim wetland due to extensive oil extraction from the fields (Jena'ale and Buazar, 2014). Huralazim wetland is vulnerable and has contamination since it was dried during the years of the war between Iran and Iraq as well as the oil extraction to a high level. It made it difficult to assess the level of pollution in the fish caught from these areas. In this research, the accumulation of polycyclic aromatic hydrocarbons (PAHs) in muscle tissue of mesopotamichthys sharpeyi, as one of the herbivorous and saprophagous predominant species, with a frequency of 24.6% in Huralazim wetland, and the degree of carcinogenesis and mutagenesis effect of this species are studied.

Methodology

Sampling was carried out in spring in 2018 and at the stations specified in the Penta-reservoirs of the Huralazim wetland (Figure 1 and in accordance with Table 1). With regard to the volume of water, the area and the rate of activity of exploration, extraction, and development of oilfields located in the wetland, the stations were selected as follows:

The reservoir 1, in which undeveloped oil reserves of Sohrab has located, includes two stations (station 1 and station 2). Reservoir 2 located in the North Azadegan oil field and it includes two stations (station 3 and station 4). Reservoir 3 located in the South Azadegan oil field and North Yaran oil field, and it includes two stations (station 5 and station 6). Reservoir 4 located in the South Azadegan oil field and it includes one station (station 7) and reservoir 5 located in the North Yaran oil field and includes one station (station 8) that this reservoir is eliminated due to dryness of reservoir 5.

Table 1: Sampling Stations

Station	X	Y
1	765730.75	3501747.01
2	765260.91	3501806.43
3	766565.51	3486920.11
4	766124.16	3477671.14
5	761845	3473681
6	764854	3468623
7	765441	3461467

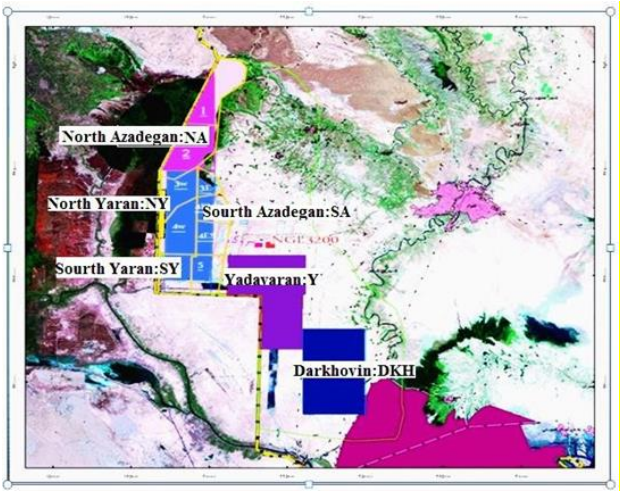

Fig. 1: The studied area in Huralazim wetland (Hawizeh Marshes)

Collection and preparation of sample

Gill fishing nets (with a 5cm source) were used to catch Binni (Mesopotamichthys sharpeyi) (Rezaei and Papahn, 2013) and this kind of fish was identified using code recognition keys (Coad, 2010) and Abdoli (2000). 210 fish were collected from seven stations. The samples were washed with the distilled water and then were transferred to the laboratory (Jazza et al., 2015). The muscular samples were separated from

the mid-back of the body of fish. Afterwards, it was placed in an aluminum foil, labeled, and then frozen at -18 ° C (Ohiozebau et al., 2017). In the preparation stage, the samples were completely dried, because aqueous compounds are intrusive in the PAHs analysis. To reach this stage, the samples were placed in a Freeze Dryer Model FD- 10V for a period of 72 hours at a temperature of -50 ° C and under vacuum conditions until the water was completely dried (Jazza et al., 2015). Fish samples were prepared for injection into gas chromatography using Pena method (2006). Therefore, 0.2 g of each dried specimen was poured into a Transform 600 Microwave Digestion, and 4 ml of potassium hydroxide saturated solution in alcohol and 10 ml N-hexane are added to the sample. After placing the cells inside the digestion system, extraction was carried out at 129 °C and in a period of 17 minutes. After cooling the solution, 6 ml of the organic phase contained in it 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 to 0.5 ml. Then, the extracted solution was filtrated using silica sheets which had been activated with 4 ml of dichloromethane solution and then again with 4ml of dichloromethane-hexane (with a volume ratio 1:1). The material was washed with 4ml of dichloromethane hexane and again it was concentrated to 0.5 ml by the evaporator. 1 ml of acetonitrile was added to it and the mixture was again concentrated to reach a concentration of 0.5ml. The obtained extract was transferred to a volumetric flask with 2 ml volume, which includes 0.5 ml of ultra-pure water, and the solution was filtrated using a 0.22-micron mesh. Ultimately, 20 µl of the resulted solution was injected into a GC-FID (Shimadzn-14A) device equipped with Rtx-5 capillary columns. The concentration of 16 aromatic hydrocarbon derivatives was then calculated through a reference material (Certified Reference Material).

Toxicity factors

Two Toxic Equivalence Factors (TEFs) (Nisbet and LaGoy, 1992) (carcinogenesis) and the Mutagenic Equivalence Factor (MEF) (Durant et al., 1996; 1999) (mutagenesis) were used in order to express the relative toxicity of hydrocarbon compounds of fish for individuals. In these two formulas, the toxicity levels were calculated for the existing hydrocarbon coefficients and summed these coefficients from the toxicity equation (TEQ) and meta-genetic equation (MEQ).

$$TEQ = \sum(TEF_i \times C_i)\ MEQ = \sum(MEF_i \times C_i)$$

C_i is the measurement of 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 ($mgkg^{-1}\ BWd^{-1}$) (carcinogenic and mutagenic) is calculated by the following formula:
carcinogenic (mutagenic)

$$PAH = \frac{(TEQ \text{ or } MEQ) \times IR \times CF}{BW}$$

This equation shows the maximum exposure level based on the EPA Guide (USEPA, 1993). In the equation, IR is the mean of fish consumption per year (Iranian Fisheries Statistical Yearbook, 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

The results of the measurement of oil-based hydrocarbons in fish muscle in the reservoirs of Huralazin wetland are given in Table 2. The highest concentration of hydrocarbon was observed in station 7 (43.93 ± 2.70 mg/kg) and its lowest concentration at station 1 (9.52 ± 3.06 mg/kg). 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)

Cycle	Compound	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Total
2	Naphthylene (Nap)	a 12.85 ±0.64	a 14.20 ±0.31	a 17.27 ±0.81	a 21.14 ±0.50	a 27.89 ±0.96	a 37.04 ±0.08	a 43.93 ±2.71	24.90
3	Acenaphthylene (Acel)	a 11.01 ±0.50	a 11.75 ±0.09	a 18.79 ±0.42	a 23.37 ±0.24	a 23.84 ±0.19	a 29.33 ±0.19	a 30.96 ±0.53	21.29

3	Acenaphthene (Ace)	a 15.43 ±0.21	a 16.56 ±0.18	a 22.39 ±0.24	a 26.10 ±0.06	a 39.68 ±2.28	a 47.24 ±0.06	a 47.33 ±0.72	30.67
3	Fluorene (Flu)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
3	Phenanthrene (Phe)	a 12.55 ±0.22	a 14.60 ±0.25	a 15.36 ±0.08	a 16.21 ±0.11	a 19.66 ±0.41	a 18.44 ±0.27	a 19.46 ±0.14	16.61
4	Anthracene (Ant)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
4	Fluoranthene (Flt)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
4	Pyrene (Pyr)	< 10	< 10	a 21.69 ±1.93	< 10	< 10	a 18.09 ±0.27	a 17.89 ±0.62	15.75
4	Benz[a]anthracene (BaA)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
4	Chrysene (Chr)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
5	Benzo[b]fluoranthene (BbF)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
5	Benzo[k]fluoranthene (BkF)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
5	Benz[a] pyrene (BaP)	a 14.28 ±0.12	a 13.31 ±0.11	a 17.61 ±0.21	a 18.38 ±0.26	a 23.49 ±2.24	a 19.46 ±0.19	a 19.32 ±0.59	17.98
6	Indeno[1,2,3-cd] pyrene (IcP)	< 10	< 10	< 10	< 10	< 10	a 10.45 ±0.22	a 11.17 ±0.04	10.23
5	Dibenzo[a,h]anthracene (DhA)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
6	Benzo[g,h,i]perylene (BgP)	< 10	< 10	< 10	< 10	< 10	< 10	< 10	-
	Total	a 9.52 ±3.06	a 14.20 ±0.31	a 17.27 ±0.81	a 21.14 ±0.50	a 27.89 ±0.96	a 37.04 ±0.08	a 43.93 ±2.70	-

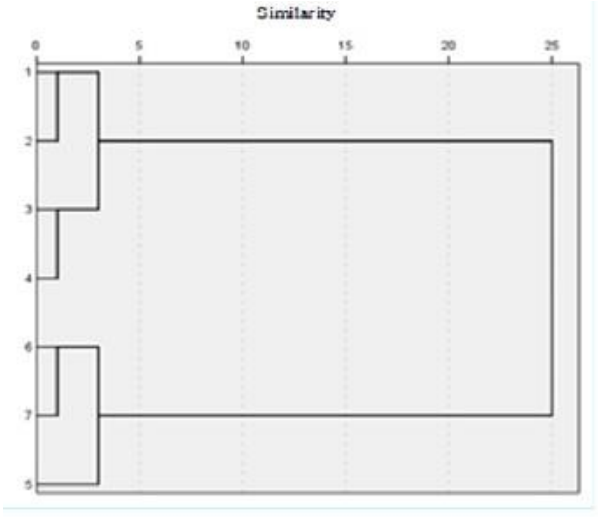

Fig. 2: Hierarchical cluster analysis (HCA) for categorizing the PAHs of the muscle in the fish- (1) Station 1, (2) Station 2, (3) Station 3, (4) Station 4, (5) Station 5, (6) Station 6 and (7) Station 7

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

Comparison of hydrocarbons (2 and 3) showed that the concentration of this hydrocarbon group was higher in stations 4, 5, 6 and 7 as compared with the initial stations (P <0.05).

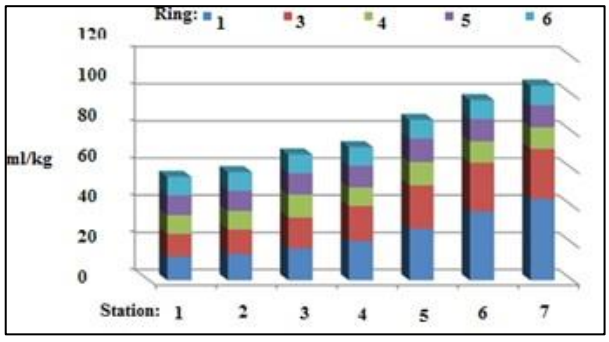

Fig. 3: Comparison of hydrocarbons based on the ring in the fish muscle of the studied stations

Two Toxic Equivalence Factors (TEFs) and Mutagenic Equivalence Factor (MEF), which indicate cancer and the cause of mutation, are shown in Table 3 for hydrocarbon compounds. According to the

values of the two TEFs and MEFs indicators, the rate of mutation caused by this wetland's fish consumption was higher than the rate of cancer formation. The highest mutation rate at station 5 and the highest carcinogenicity rate at station 7 were measured.

Table 3: The results of calculation for toxicity equivalence factors (TEFs) and Mutagenic Equivalence Factor (MEF)

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

The gradual evaluation of carcinogenicity and mutation based on the values of two carcinogenicity indices and the occurrence of mutations based on the per capita consumption of fish recorded in the Iranian Fishery Statistical Journal is shown in Table 4. The highest incidence of cancer and mutation occurred at station 7.

Table 4: Carcinogenic Risk Assessment

Total	Station 7	Station 6	Station 5	Station 4	Station 3	Station 2	Station 1	Gradual risk assess-ment
0.0027	0.0027	0.0027	0.00005	0.00004	0.00004	0.00003	0.00003	Toxic
0.055	0.059	0.059	0.061	0.034	0.045	0.037	0.034	Mutagenic

Discussion

According to Table 1, the total concentration of hydrocarbon compounds in fish caught in the Huralazimm wetland shown for the range 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 shores of the United Arab Emirates 2.7µg/g and 3.40µg/g respectively. Moreover, Jazza et al. (2015) investigated the aromatic hydrocarbon concentrations in the tissues of the two species of "Liza abu" and "Carassius auratus" fish in Iraq, which were 161.61-2.03 ng/g and 0.95-1.875 ng/g of dry weight, respectively. In general, it indicated that the concentration of hydrocarbons in both studies is much lower, compared to the present study. This significant difference was due to the specific position of the Huralazimm 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 ppm) and 2 (14.20 ppm) showed lower hydrocarbon levels compared to other stations, especially station 7 (43.93 ppm). The Huralazimm wetland in the Iranian section consists of five main reservoirs, the reservoir 1 (two stations 1 and 2) has the most natural structure, ecologically. The level of extraction and exploration activities in this reservoir is very limited and this can be confirmed due to the low concentration of oil hydrocarbons in the fish tissue compared to other stations. In addition, the station 7 has the highest level of exploration and extraction activities and the level of hydrocarbons at this station is much higher than other stations. The hierarchical cluster analysis also showed the proximity of stations 6 and 7, which confirmed the findings.

The classification of identified ring hydrocarbons in fish in terms of number of rings showed that the two-and three-ring compounds had a higher frequency than the four –ring compounds (Fig. 2). Furthermore, the concentration of two-ring compounds or lightweight compounds of Naphthylene, Acenaphthylene and Acenaphthene were more abundant in fish caught compared to other hydrocarbon groups. Looking at the results of this study, it can be seen that at most stations, the compounds levels were 16 PAH below the detection limit (<10), which it is more likely due to the decomposition and conversion of these compounds to lower molecular weight compounds (Khairy et al., 2009). In this study, Mesopotamichthys sharpeyi, as an herbivorous and Saprophagous fish, provides a large portion of its nutritional needs from the invertebrate bestial beings. Given that by entering oil-based hydrocarbons into an aqueous medium, these materials are transported across the chain to various parts of planktons, invertebrates, and plants, and, then, they can be transferred to the body of fish and stored in it. Therefore, the amount of contamination in the tissue of Mesopotamichthys sharpeyi can indicate the contamination level in the lower levels of the food chain (Stolyhwo and Sikorski, 2005;

Echeveste et al., 2010; Yakan et al., 2017). 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 (Yim et al., 2004).

Comparison of the values obtained by the average rate of compounds 16 PAHs in edible tissue of Binni fish based on the international standards. These standards include the U.S. environmental standard, EPA16PAH (µg/kg.dw 50), the world health organization (WHO) standard, 6 PAHs (µg/kg.dw 20) and the european standard, BaP (µg/kg.dw 8)(Sadatipour et al., 2001) and European commission (OJEU, 835/2011) (Hafez et al., 2017) (2ppb). They determined that the hydrocarbon values available in the tissue of wetland fish of Hawizeh Marshes (Houralazim) were much higher than the standards. IARC (1986) identified six combinations of 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, except of benz[a]pyrene, the amount of other compounds were insignificant. Benz[a]pyrene as a known carcinogenic compound is usually used as an indicator for controlling hydrocarbon compounds in the environment. Hence, in the evaluation of carcinogenic risk or mutagenic risk, it is generally considered as the main combination (Kofi et al., 2018).

Therefore, this combination has the main part in the evaluation of carcinogenic risk or mutagenic risk in Hawizeh Marshes. Indeed, the equivalent toxicity factor (TEQ) and the equivalent mutagenesis factor (MEQ) is related to the toxicity rate B(a) P. TEQBap has a direct correlation with carcinogenicity of compounds and MEQBaP has a direct correlation with mutagenicity of compounds (Zeiger, 2001; Essumang et al., 2013). It means that these non-carcinogenic compounds have destructive effects such as pulmonary diseases, birth defects, sexual dysfunction and reduction of IQ (DeMarini et al., 2004; Essumang et al., 2013). According to the environmental protection agency and the European union, the two combination of indeno (1,2,3- cd)pyrene (IP) and benz[a]pyrene have positive mutagenicity and in terms of carcinogenicity, they have been categorized as a possible causing combination of cancer and carcinogenic composition, respectively. These compounds, alone or in combination with covalence compounds, attaches to the cellular macromolecules such as DNA and by making mistake in DNA replication, provide a background for mutation, so create a tumor and ultimately cause cancer (Orecchio et al., 2009). Other hydrocarbon compounds also have different degrees of carcinogenicity or mutagenicity, which according to the findings of the present study, contained minor amounts. The highest carcinogenicity rate was 13.1 at the station 7 and the highest mutagenicity rate was 23.49, which belonged to station 5. In the study of Kofi et al., (2018), the highest carcinogenicity rate was 3.05 and the highest mutagenicity rate was 4.40. In this study benz[a]pyrene was as the major causative compound of cancer and mutation, but in the study of Kofi et al., (2018), 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 with respect to the station was between 0.00003 to 0.0029 and generally in Hawizeh Marshes was 0.0027 (Table 4). This means that, due to the consumption of these fish during 70 years, out of every 10000, 29 people and out of every 100, 3 people, more likely will be affected by cancer. Compared to the study by Kofi et al., (2018), in the Ghana oysters the carcinogenicity rate was 45 people out of 10000000. Considering the value of threshold of the carcinogenesis intensity 1 × 10-5, which is announced by the USEPA (1993-2009), it 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 the Per capita consumption rate of 9.2 kg (Fishery yearbook, 2015) was 0.055 (range 0.034 - 0.061). It means out of every 100 people, on average, 34 – 61 people are more likely to be exposed to non- cancer-related illnesses. Since the mentioned values are higher than 10-5 USEPA standard, so it can be said that daily consumption of these wetland fishes, increases the mutagenicity risk of the consumers with high consumption. In the study of Kofi et al., (2018), the mutagenicity level of benz[a]pyrene was 9 people out of 1000000 and 38 people out of 1000000 and they expressed that the high consumption of these oysters does not have good results and increases the mutagenicity risk in them. It should be noted that among the two-ring hydrocarbon compounds, naphthylene is a high-dose compound, which according to the American ATSDR, is considered as one of the causes of cancer in humans (US ATSDR, 2005), but it is not included in the calculations. This issue increases the risk of carcinogenicity and it necessitates the need of more attention to prevent from destruction and ruining of Hawizeh Marshes and reduction of human interventions.

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