Levels of polycyclic aromatic hydrocarbons (PAHs) and certain chemical elements in the tissues of two types of freshwater turtles from Al-Hammar marsh, Southern Iraq.

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Abstract

The fresh water turtles, Euphrates Softshell Turtle, Rafetus euphraticus (Family Trionychidae), and natural turtle, caught from two stations 1). Hareer, and 2). Al-Barga located in Southern Iraqi Al-Hammar marsh, were investigated for levels of polycyclic aromatic hydrocarbons (PAH) and Chemical elements Cd, Co, Cu, Fe, Mg, Mn, Ni, and Pb in the tissues Muscles, Liver, Ovary, Kidney, and Heart. PAHs were determined by HPLC technique, and chemical elements by atomic absorption technique. Concentrations of PAHs reported were higher in all tissues of Rafetus Euphraticus from both stations, and an extreme levels were reported in the heart of Rafetus Euphraticus and PAHs were highly accumulated in the kidney of both species. Levels of investigated chemical elements were higher in the tissues of Rafetus Euphraticus than thr normal turtle. Higher levels reported for Fe and lower were for Cd and Pb for both studied species. The most effected tissues in both species were ovary followed by liver then by muscles. Concentrations of Cd and Pb could not distinguish between studied species or sites due to their low values.

Key Wards: Turtle, Rafetus Euphraticus, Polycyclic Aromatic Hydrocarbons, Chemical Elements, HPLC, Atomic Absorption Spectrophotometry.

Introduction

Marsh lands of Iraq with 4500 km^2 area (Al-Shabeeb, 2015) at the Southern Iraq stretches between three Governorates, Basrah, Missan, and Dhe Qar, it receives fresh water via Tigris and Euphrates rivers inside Iraq and Karkha river from Iran (Richardson and Husain,2006). They have economic, social, and biodiversity value (Al-Gburi, et al., 2017).

Aquatic lives of the marshes are effected by different pollutants among which are the petroleum fuel spills resulting from damage , transportation, accidents and various industrial and mining activities (Saadon, 2015).

All softshell turtles are highly aquatic and restricted to freshwater rivers and lakes at low to moderate altitudes; a few species occasionally venture into brackish or saline coastal waters. Most

softshell turtles prefer slow-moving streams and rivers with muddy or sandy bottoms, but they can also be found in ponds, marshes, and lakes (Toas Diagen, (2016).

The geographic range of Rafetus Euphraticus Softshell Turtle is confined to the Euphrates and Tigris basins in Turkey, Syria, Iraq and Iran, its range in Iran is limited to Khuzestan Province in the Southern West of Iran adjacent to the Eastern South Border of Iraq (Ghaffary et al., 2008).

In the aquatic ecosystem, an increased impact by environmental pollution due to anthropogenic changes in the planet including overfishing, agricultural runoff and marine emerging infectious diseases. Fresh water turtles were investigated for heavy metals pollution around the world, concentrations of zinc, cadmium, copper, nickel, selenium, manganese, mercury and lead in blood of 22 clinically healthy, loggerhead turtles (Caretta caretta), captured for several reasons in Puerto López Mateos, Baja California Sur, Mexico were determined and set as baseline for heavy metal pollutants (Ley-Quiñónez,et al., 2011). Zinc was the most prevalent metal in blood (41.89 µg g−1), followed by Selenium (10.92 µg g−1). The mean concentration of toxic metal Cadmium was 6.12 µg g−1 and 1.01 µg g−1 respectively. Mean concentrations of metals followed this pattern: Zn > Se > Ni > Cu > Mn > Cd > Pb and Hg.

Relevant heavy metals in the tissues blood, kidney, and liver of two highly endangered sea turtle species (Caretta caretta and Chelonia mydas) from the important nesting area on the Northeast Mediterranean Sea were investigated (Yipel, 2017). The highest mean concentration was of Fe, while Hg and Pb were lowest. All tissue concentrations of Al, As, Fe and Mn were significantly different between the species. In particular, As, Cd, Cu, Mn, Ni, Se, Zn concentrations were lower in Caretta caretta and Cd, Hg, Mn, Zn concentrations were lower in Chelonia mydas than those reported in other parts of the world.

A large number of loggerhead sea turtles (Caretta caretta) from a nesting colony from Cape Verde, West Africa were sampled to establish the blood levels of 11 elements (Cu, Mn, Pb, Zn, Cd, Ni, Cr, As, Al, Hg, and Se) (Camachoa, et al., 2013). Zn and Se exhibited the highest concentrations (median values as high as 6.05 and 2.28 µg/g, respectively). The median concentrations of the most toxic compounds, As, Cd, Pb, and Hg, were relatively low (0.38, 0.24, 0.06, and 0.03 µg/g, respectively).

Methods

Due to the lake of distribution of different types of turtles, two stations at the Eastern site of Al-Hammar Marsh within Basrah Governorate: 1) Harrer, and 2) Al-Barga 30°42'6.99" N, 47°35'3.43" E (Figure 1) were investigated for the existence of turtles. Caught turtles took over month of time following their movements and transfer as well as taking in consideration their suitable habitat for nesting water ways in which it should has alluvial soil and sandy banks. Rafetus Euphraticus turtle prefer shallow and calm water in which Eastern Al-Hammar marsh is characterized by these conditions.

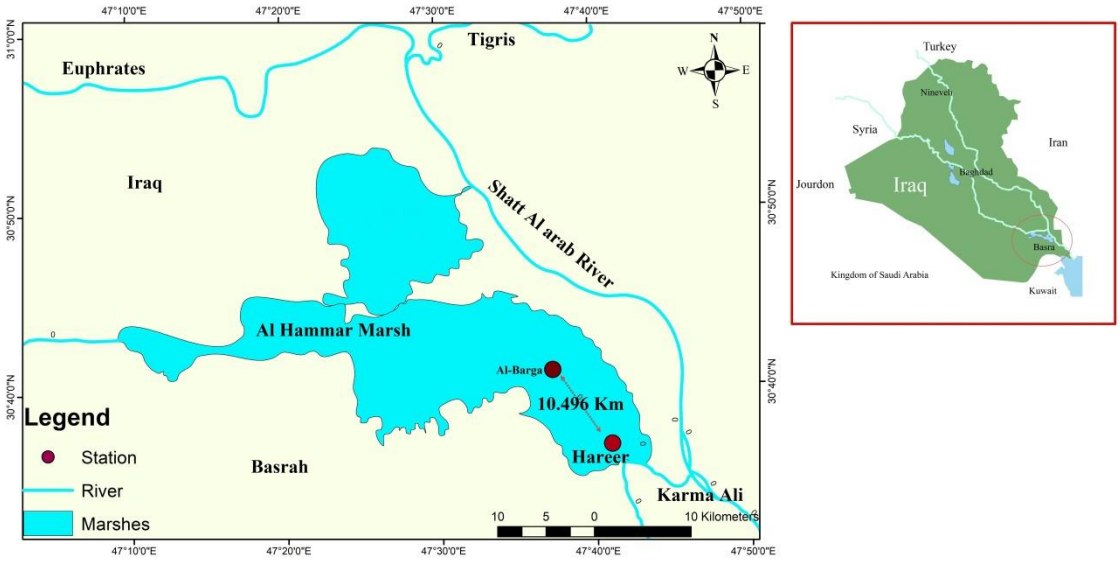

Figure 1. Location map for Southern Iraqi Marshlands, showing the sampling stations, 1)Hareer, and 2) Al-Barga.

Within this study two types of turtles were caught from sites 1 and 2. The fresh water turtle and Rafetus Euphraticus softshell turtle, Figure 2. Samples transferred to the lab and cut to excrete their inner tissues, Kidney, Ovary, Liver, Muscles, and Heart. Polycyclic Aromatic Hydrocarbons in each tissue was soxhlet Extracted for 8 h with a mixture (50/50, v/v, spectroscopic grades) of hexane and dichloro methylene. Extracts were concentrated and reduced in volume under gentle nitrogen flow, then solvent exchanged to pure hexane and stored prior to determination of petroleum hydrocarbons by HPLC, while heavy metals were digested in Teflon beakers in acid mixture of HCl and HNO_3 . After treatments, samples were kept in fridge prior to analysis. Heavy metals were extracted according to the method explained by ROPME (1982), and measurements were conducted by Flame Atomic Absorption Spectrophotometer Model PG, AA500.

Fresh water turtle

Rafetus Euphraticus

Figure 2. Types of Turtles exist in Southern Iraqi Marshland.

Results

The concentrations of polycyclic aromatic hydrocarbons and heavy metals in the tissues of the two studied turtles from selected stations within Al-Hammar Marsh, Southern Iraq are presented in tables 1, and 2 respectively.

Table 1. Concentrations of petroleum hydrocarbons (PAH in µg/g) in the tissues of two aquatic turtles from Al-Hammar marsh, Southern Iraq.

	Station 1, Hareer		Station 2, Barga	
Tissues	Fresh Water Turtle	Rafetus Euphraticus	Fresh Water Turtle	Rafetus Euphraticus
Kidney	15.19	17.21	9.24	10.53
Ovary	2.94	3.92	3.17	3.11
Heart	-	8.05	-	0.33
Muscle	8.16	12.17	2.43	1.13
Liver	4.57	6.08	1.70	2.23

Distribution of PAHs in the tissues of studied turtles seems to be higher in the kidney followed by muscles liver and ovary for samples from station 1 (Hareer), while for samples from station 2 (Barga) the trend as follows: kidney>overy>muscle>liver, this could be explain on the bases of different diet habitates. On the other hand, levels recorded in Rafetus Euphraticus were higher than in common turtles, this could be explain on the bases of skin absorption for the softshell Rafetus Euphraticus. For Rafetus Euphraticus, heart is a main target for PAHs, it reported a certain levels compared to nil for common turtle from both sites.

Table 2. Concentrations of heavy metals (in µg/g) in the tissues of two aquatic turtles from Al-Hammar marsh, Southern Iraq.

	Cd	Co	Cu	Fe	Mg	Mn	Ni	Pb
	Station 1, Hareer							
Tissues	Fresh Water Turtle							
Muscles	2.11	6.31	9.11	40.14	17.14	2.19	8.03	1.11
Liver	1.09	9.22	17.12	62.57	20.06	3.01	11.50	3.15
Ovary	0.09	13.17	13.90	80.81	11.10	1.19	8.11	1.09
	Rafetus Euphraticus							
Muscles	5.41	20.1	7.29	90.90	21.5	1.11	20.09	0.211
Liver	9.16	25.15	11.59	120.51	18.11	2.16	33.77	3.12
Ovary	3.19	18.8	25.77	189.13	20.07	0.91	18.90	1.11
	Station 2, Al-Barga							
Tissues	Fresh Water Turtle							
Muscles	1.60	11.32	3.22	31.39	2.17	6.22	0.821	ND
Liver	ND	15.92	1.15	40.18	5.16	3.19	ND	0,019
Ovary	ND	7.18	4.07	12.70	1.03	1.09	ND	ND

	Rafetus Euphraticus							
Muscles	**ND**	**15.1**	**9.32**	**80.15**	**7.21**	**4.14**	**2.51**	**0.039**
Liver	**1.91**	**6.96**	**5.14**	**111.91**	**9.82**	**2.61**	**1.07**	**0.191**
Ovary	**0.11**	**8.16**	**11.2**	**113.70**	**4.41**	**1.09**	**ND**	**0.231**

Discussion

Common turtles which exist in the aquatic ecosystem of Iraqi Marshland are amphibian reptiles the Caspian terrapin (Mauremys Caspian) and the Rafetus Euphratecus (Euphrates Softshell Turtle) as shown in figure 2 (Garstecki, and Amro, 2013).

The Rafetus Euphratecus is distributed across Iraq, Syria, Turkey, and Iran, although Iraq is thought to contain the largest number of suitable sites for this species, there is a lack of information within the country on this species (Ghaffary et al., 2008). Despite their rapid population decline, individual Rafetus Euphraticus were caught from freshwater Southern Iraqi Al-Hammar Marsh and used throughout this study (Fazaa, et al., 2015).

Oil spills and urban runoff of chemicals fertilizers which contribute to water pollution have serious effect on aquatic lives (flora and fauna). Living organisms such as turtles are effected directly by pollutants or via food they eat.

PAHs and Chemical elements as a pollutants are present globally in aquatic systems, and their potential transfer to environmentaly existing animals can be a serious threat to their health status. Heavy elements Pb, Cd, and Hg were reported in blood and kidney of turtles from different sites overall the world (Cortés-Gómez et al.,2017).

Within this study, PAHs were recorded in all tissues of the studied species, being higher in the tissues of Rafetus Euphraticus compared to common turtle in both stations. Highest concentrations were recorded in kidney followed by muscles, liver then ovary. An exception levels were recorded in the heart of Rafetus Euphraticus with non detactable levels in common turtle.

Aquatic lives in the marsh lands is effected by different pollutants, among which are the petroleum fuel spils resulting from damage , transportation, accidents, and various other activities (Saadon, 2015)

Environmental pollutants, pesticides, metals, fertilizers, polychlorinated biphenyls (PCBs), and hydrocarbons have all been identified as likely contributors to toxicological effects in reptiles, (Sparling, 2010).

For heavy chemical elements, Fe reported as the highest concentrations in all tissues, Co, Cu, Mg, and Ni were reported moderate levels, while Cd, Mn, and Pb were the lowest. As for PAHs, chemical elements reported higher levels in tissues of Rafetus Euphraticus compared to common turtle with few exceptions. High levels of Fe and Mg are due to the reflection of their high concentrations in the sediments of Al-Hammar Marsh (Abdullah, et al., 2015). Concentrations of Cd and Pb could not distinguish between studied species or sitesdue to their low values. The most effected tissues in both studied species were ovary followed by liver then muscles as shown in table 2.

Conclusion

A relatively low informations are available on exposure and effects of PAHs or chemical elements on local turtles in Al-Hammar Marshland of Southern Iraq. Hence, there is no evidence for existing of pollutants in the tissues of available aquatic animals, among which are the turtles, in the study area.

Therefore, PAHs and chemical elements recorded within this study reveals that aquatic animals in the study area are exposed to a certain levels of these pollutants. Pollutants could cause death to aquatic turtles as they accumulate with time, which is in turn led to decrease the population of the aquatic turtles in the studied arAuea. Moreover, due to water pollution , tumer could be developed on turtles (Ghaffari, 2008).

Authors Contribution

Prof. Dr. Faris J. M. Al-Imarah: Team leader, conduct the instrumental analysis and writing the manuscript.

Ass. Prof. Dr. Ghassan Adnan Al-Nagar and Ass. Lect. Zeyad Ak. Meziad conducted the investigation then collecting the samples, transferring them to the lab, clean , cut , separate turtles tissues , then extraction and store prior to analysis by HPLC and AAS.



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