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Original Article

Bioaccumulation of hydrocarbon compounds in the muscle of three aquatic birds in Um Alnaaj Marsh, Iraq

Firas Sabeeh Idan, Salih Hassan Jazza*

Department of Biology, College of Science, University of Misan, Misan, Iraq.

Abstract: The current study was conducted to determine the concentrations and origins of total petroleum hydrocarbon TPHs and polycyclic aromatic hydrocarbons PAHs in three bird species of *Anas platyrhynchos*, *A. crecca* and *A. acuta* collected from November 2020 to April 2021 in Um Alnaaj Marsh, Iraq. Based on the results, the TPHs (g/g.dry weight) in muscle tissues were 13.79 and 16.74 in *A. platyrhynchos*, 40.84 and 43.0 in *A. crecca*, and 10.08 and 11.18 in *A. acuta* during winter and autumn, respectively. The PAHs (ng/g.dw) in muscle tissues were 41.22 and 146.86 in *A. platyrhynchos* and 31.17 and 42.98 in *A. crecca* during winter and autumn, respectively, whereas it was 24.41 to 75.51 in *A. acuta* during autumn and winter, respectively. The lower molecular weight PAHs was less than higher molecular weight PAHs in all bird species throughout the period study. The origins of PAHs in muscles tissues of the studied bird species were estimated according to the ratio of LPAHs/HPAHs, Fluo/Pyr, Phe/Ant, Inpy/(Inpy+BghiP), Ant/(Ant+Phe), and BaA/(BaA+chr) and based on the findings, they were mostly pyrogenic and few petrogenic. The results also showed that the bird species have the ability to accumulate these compounds in their muscles according to values of BCF which is between 2.29 and 5.81 in *A. crecca* and *A. platyrhynchos*, respectively. These bird species were contaminated with petroleum compounds and the consumption of their meat may pose public health hazards.

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Introduction

The marshes are unique ecosystems because of their biodiversity (UNEP, 2006; Bedair et al., 2006; Saleh et al., 2020). The Mesopotamian marshes are important wetlands in the Middle East as resting places and passages for many migratory birds (Al-Handal et al., 2016). Pollution is one of the main problems threatening all living organisms, particularly in the marshes (NRC, 2003). Crude oil spills are a major problem that often occurs during oil drilling and result in the pollution of water bodies (Oshienemen et al., 2018; Carpenter, 2019), leading to serious environmental concerns worldwide. Crude oil spreads over the water to form a thin layer and prevents the access of atmospheric oxygen to aquatic biota. It also prevents photosynthesis and leads to disturbances in the food chain (Frank and Boisa, 2018). Environmental disasters resulting from oil production and transportation are growing (Jernelov, 2010; Eckle et al., 2012) and their recovery takes 2-10 years, with long-term effects generally limited to changes in communities (Mendelssohn et al., 2012). One of the oil pollution problems is polycyclic aromatic hydrocarbons (PAHs), which impact water quality, sediments, and living organisms in the aquatic ecosystem (Zhang et al., 2015; Kuppusamy et al., 2020; Jazza and Khwadem, 2021).

Waterfowl are sensitive to petroleum pollution, especially PAHs, which enter their tissues through water and food, having significant behavioral and physiological effects (Shore et al., 1999). Exposure to PAHs leads to many carcinogenic effects in adult birds (Malcolm and Shore, 2003). Albers (2006) and Giese et al. (2000) pointed out that high molecular

*Correspondence: Salih Hassan Jazza
E-mail: salih-jazza@uomisan.edu.iq
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weight PAHs consisting of 4-6 rings are the most toxic to birds, especially to embryos and young birds, while in adult birds, they lead to a decrease in egg production and hatching and their effects on the production of sex cells; thus lead to a reduction in reproductive levels. Pereiara et al. (2009) discovered that the PAHs compounds accumulated in eggs of golden eagles in high concentrations. Kwok et al. (2013) observed that eggs of waterfowl and egrets in Jiangsu Province (Central China) contain high amounts of these compounds. Birds, like other vertebrates, contain highly oxidized p450 enzymes and, therefore, can rapidly metabolize and excrete most PAHs readily (Verbrugge et al., 2001; Troisi et al., 2006). The present study aimed to investigate the levels and origins of petroleum compounds in three bird species viz. *Anas platyrhynchos*, *A. crecca* and *A. acuta* in Um Alnaaj Marsh, Iraq.

Materials and Methods

Study area: Um Alnaaj Marsh is one of the Al-Hawizeh Marshes, extending along the Iraqi-Iranian border, with a length of about 30 km and a width of about 25 km (Fig. 1) (Yuonis et al., 2011; Hassan et al., 2012). It is fed with water from outside Iraq, such as Al-Dwiridj, Nissan, Karkheh and Al-Kfagia rivers, and inside of Iraq from Al-Mshrah and Al-Kahlaa rivers (Taylor et al., 2011). The climate in the marsh is dry and subtropical, with less than 200 mm annual rainfall and low humidity (Al-Khatib, 2008; Hashim et al., 2019).

Collecting samples: The birds were collected from November 2020 to June 2021 using the netting (douche), and after anesthesia, they were placed in the icebox until reaching the laboratory. Three bird species of *A. platyrhynchos*, *A. crecca* and *A. acuta* were sampled. The specimens were washed with distilled water and after removing their feathers, the muscles were cut into small parts, then dried, grounded, and sieved using the metal sieve of 63 μm and placed in clean glass to prepare for analysis.

Bioconcentration factor (BCF): BCF was calculated according to Mccarty (1986) using the equation of BCF with PAHs in water = CB / CWD, where CB is the concentration of PAHs in the organism and CWD = concentration of PAHs in water.

Measurement of PAHs in muscle tissues: PAHs were extracted from muscle tissues based on Grimalt and Oliver (1993). For this, a five g of sample was removed, and then a mixture of methanol: benzene (1:1) was added to it and the extraction process was carried out for 24-36 hours at 40°C. Then saponification process was performed by adding an aqueous solution of KOH for 2 hours at 40°C. The extract was left to cool and transferred to a separating funnel, and 50 ml of n-hexane was added. The sample was shaken vigorously, left to settle, and separated into two layers i.e. a soaped and an unsoaped layer containing hydrocarbons dissolved in hexane. The sample was passed through a chromatographic column consisting of glass wool at the bottom, topped with a layer of silica gel and a layer of alumina and a layer of anhydrous sodium sulfate (Na_2SO_4) to obtain the aliphatic fraction. Then 30 ml benzene was added to extract the aromatic fractions and placed in a vial for reading by Spectrofluorometer in the Marine Science center laboratory to determine TPHs and Gas chromatography at Nihran Aomr laboratories to measure PAHs.

Origins of PAHs: The following parameters were calculated to determine the origins of PAHs. The ratio of LPAHs/HPAHs: If the value is more than one, the origin of PAHs is considered petrogenic, and the values less than one show the pyrogenic origin (Vrana et al., 2001). The ratio of Phe/Ant: If the value is greater than ten, the origin of PAHs is considered petrogenic, whereas the value less than ten indicates its origin pyrogenic (Doong and Lin, 2004). Fluo/Pyr: If the ratio value is greater than one, the origin of PAHs is considered pyrogenic. The values less than one show the origin of PAHs petrogenic (Zakaria et al., 2002). The ratio Inpy/(Inpy + BghiP): If the ratio is less than 0.2, the origin of PAHs would be petrogenic, and the ratio between 0.2-0.5 indicates mixed origins. In a ratio higher than 0.5, the origin of PAHs is pyrogenic

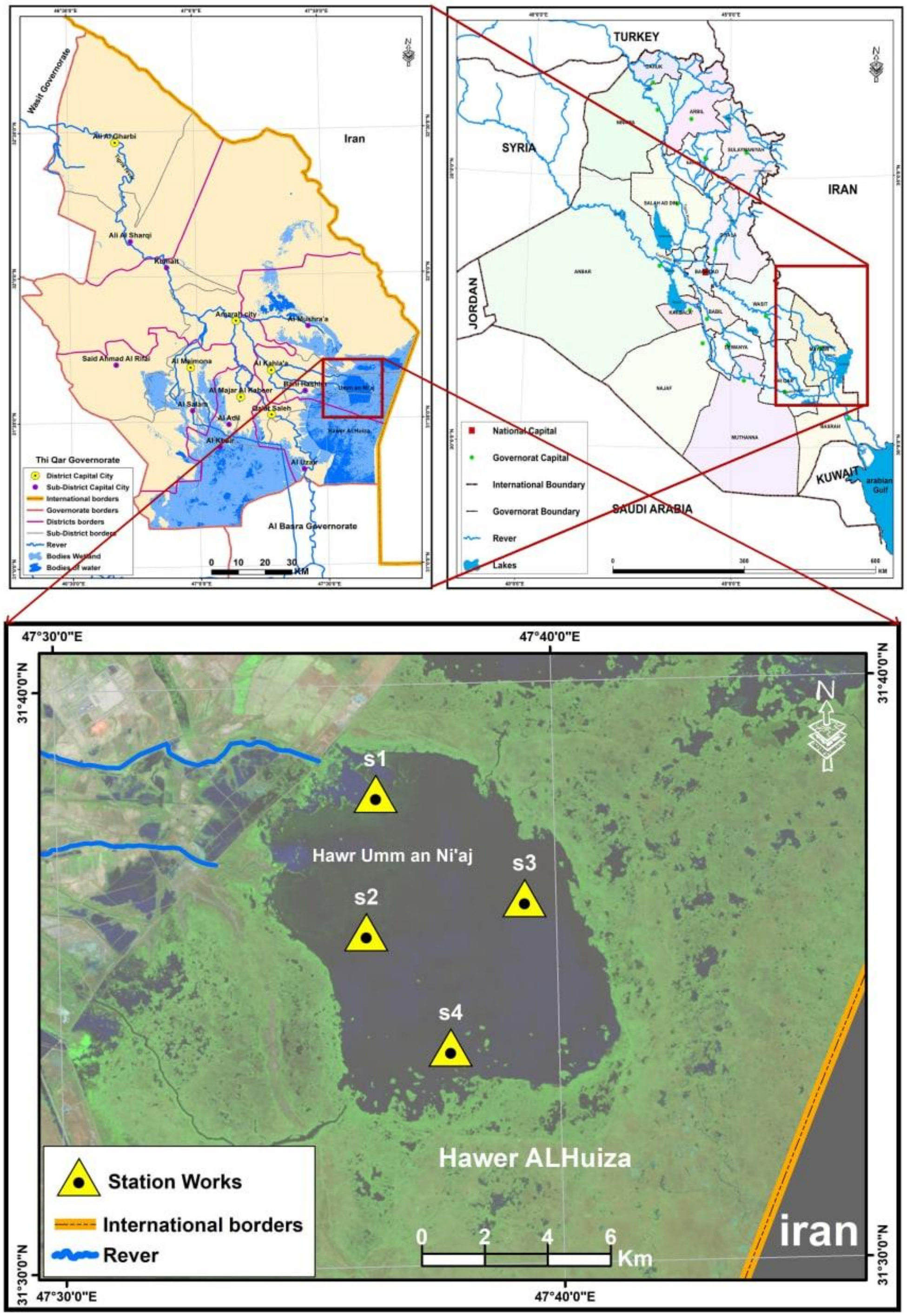

Figure 1. The sampling stations in Um Alnaaj Marsh.

(Tolosa et al., 2004; Guo et al., 2007). The ratio (Ant/(Ant+Phe): The ratio less than 0.1 indicates the sources of PAHs from petroleum, while a ratio more than 0.1 indicates the sources of PAHs pyrogenic (Guo et al., 2007). The ratio BaA/(BaA+Chr): A ratio less than 0.2 indicates petrogenic origins, 0.2 to 0.35 mixed origins, and greater than 0.35 indicates pyrogenic origins (Guo et al., 2007).

Results and Discussion

The results showed that the concentrations TPHs range 40.84-43 μg/g dw in *A. crecca* and 10.08 to 11.18 in *A. acut*a during autumn and winter,

Table 1. Seasonal variation in the fat content of the studied aquatic birds.

Species	Seasons	Fat contents %	Average
A. platyrhynchos	Autumn Winter	1.75 1.58	1.66
A. crecca	Autumn Winter	1.76 1.4	1.58
A. acuta	Autumn Winter	1.62 1.76	1.69

Table 2. Seasonal variations of PAHs levels (ng/g dw) in muscles tissues (ND: Not detected).

PAHs individuals	*A. platyrhynchos*		*A. crecca*		*A. acuta*	
	autumn	winter	autumn	winter	autumn	winter
Ace	ND	ND	ND	ND	ND	ND
Phe	2.22	ND	ND	ND	ND	ND
Ant	3.31	ND	1.98	ND	ND	ND
fluo	6.99	2.08	5.54	ND	3.13	7.89
pyr	8.12	2.50	7.87	ND	ND	2.10
chr	69.41	9.31	ND	4.91	ND	16.30
BaA	19.79	ND	1.66	ND	ND	3.16
BbF	11.61	1.16	1.16	ND	ND	1.35
BkF	16.54	2.47	7.28	4.01	3.75	6.80
BaP	5.99	23.70	2.89	22.25	10.04	34.67
InP+DahA	ND	ND	ND	ND	ND	ND
BghiP	2.88	ND	14.60	ND	7.49	3.24
∑PAHs	146.86	41.22	42.98	31.17	24.41	75.51
LPAHs	5.53	-	1.98	-	-	-
HPAHs	141.33	41.22	43	31.17	24.41	75.51
LPAHs/HPAHs	0.03	-	0.04	-	-	-
Phe/Ant	0.67	-	-	-	-	-
Fluo/Pyr	0.86	0.83	0.70	-	-	3.75
Inpy/(Inpy+BghiP)	-	-	-	-	-	-
Ant/(Ant+Phe)	0.59	-	1	-	-	-
BaA/(BaA+CHR	0.22	-	1	-	-	0.44

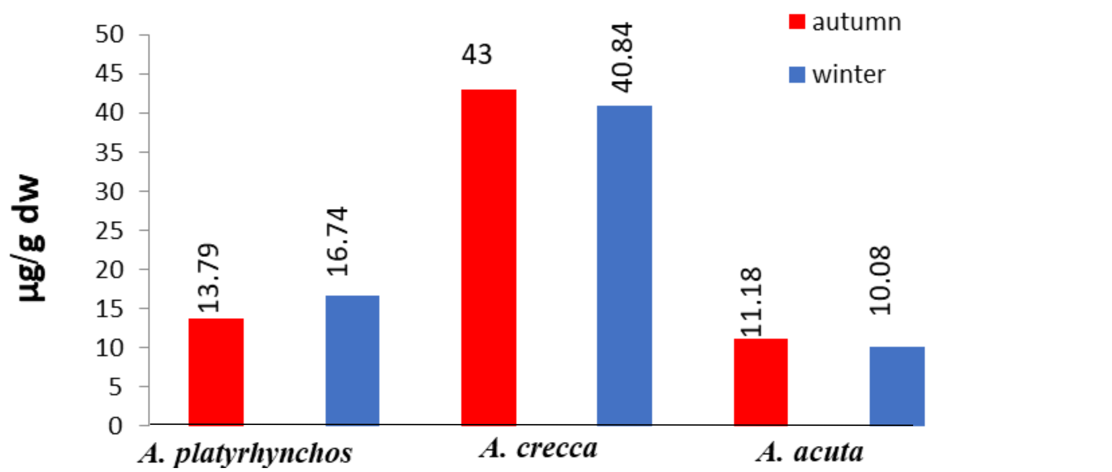

Figure 2. Seasonal variation of TPHs levels in the studied bird species.

respectively, whereas it ranged from 13.79 to 16.74 in *A. platyrhynchos* in winter and autumn, respectively (Fig. 2). The ability of the birds to accumulate TPHs varies and this may be due to different seasons, lipid content, feeding habits, age, and sex of birds. The highest levels of TPHs in the

Table 2. Origin of PAHs in the studied aquatic bird species.

Indices	*A.platyrhynchos*	*A. crecca*	*A. acuta*
LPAHs/HPAHs	Pyrogenic	Pyrogenic	-
Phe/Ant	Pyrogenic	-	-
Flu/Pyr	Petrogenic	Petrogenic	Pyrogenic
Inpy/(Inpy+BghiP)	-	Pyrogenic	-
Ant/(Ant+Phe)	Pyrogenic	Pyrogenic	-
BaA/(BaA+Chr)	mixed	Pyrogenic	Pyrogenic

Table 3. Bioconcentration factor (BCF) in the studied aquatic bird species.

Species	Concentrations of PAHs in birds	Concentration of PAHs in water	BCF
A. platyrhynchos	94.04	16.17	5.81
A. acuta	49.96	16.17	3.08
A. crecca	37.07	16.17	2.29

A. crecca, during autumn, maybe due to its feeding habits on aquatic organisms such as plant material and invertebrates (Sterry et al., 2001), because the aquatic plants accumulate petroleum hydrocarbons in their tissues higher than in the aquatic environment (Al-Saad, 1995). In addition, different species of waterfowl feed different prey types, which accumulate petroleum hydrocarbons in their tissues because of missing mixed-function oxidase systems (Lawrence and Weber, 1984; Jazza, 2015).

The highest levels of PAHs were recorded in *A. platyrhynchos* during winter since they feed plants and protein-rich invertebrates, increasing during spawning i.e. late spring (Gammonley, 1995). If it is time for birds to migrate to other feeding sites, they increase the rates of nutrients and lipid reserves for spring migration, thus, the levels of lipophilic petroleum hydrocarbons can increase (Tidwell, 2010). The lowest PAHs were found in *A. acuta* in winter, the lipid reserves of wintering waterfowl naturally reach their lowest content in mid-late winter which was 1.62 g (Table 1). This result was consistent with the findings of Thompson and Baldassarre (1990).

In *A. platyrhynchos*, PAHs had high molecular weight ranging from 41.22 ng/g dw in winter to 146.86 ng/g dw in autumn (Table 3). The origins of PAHs, according to the ratio of LPAHs / HPAHs was 0.03 less than one, which indicated its source pyrogenic (Fernandes et al., 1997; Vrana et al., 2001; Al-Khatib, 2008), while Phe / Ant ratio was 0.67, less than ten, therefore its source is pyrogenic (Doong and Lin, 2004; Al-Khion, 2012). Flu/Pyr ratio ranged from 0.86-0.83, less than one; thus its origin is petrogenic (Zakaria et al., 2002; Kafilzadeh et al., 2011). Ant/(Ant+Phe) was 0.59, more than 0.1, indicating that its origin is pyrogenic (Yunker et al., 2002; Guo et al., 2007) BaA /(BaA+Chr) was 0.22, indicating its sources are mixed (Jazza and Khwadem, 2021).

In *A. crecca*, PAHs in the muscle tissues ranged from 31.17 to 42.98 ng/g dw in winter and autumn, respectively (Table 3). The highest levels recorded for the two seasons were 25.14 ng/g dw for B(a)P and the lowest 1.16 ng/g dw for B(b)F. The ratio of LPAHs/HPAHs was 0.04, indicating the source of PAHs is pyrogenic (Vrana et al., 2001). The Flu/Pyr ratio was 0.70 showing the origins of PAHs as petrogenic (Qui et al., 2009). Ant/(Ant+Phe) was one indicating the origin of PAHs as pyrogenic (Yunker et al., 2002). The ratio of BaA/(BaA+Chr) was 1 indicating the source of PAHs pyrogenic (Tolosa et al., 2004; Guo et al., 2007).

In *A. acuta,* the most frequent compounds in both seasons were Fluoranthene, B(k)F, B(a)P, and B(g,h,i)P, and ∑PAHs in the muscle tissues was 24.41 ng/g dw in autumn and 75.51 ng/g dw in winter(Table 3). Flu/Pyr ratio was 3.75, indicating the source of PAHs is pyrogenic (Qui et al., 2009; Jazza and Khwadem, 2021). The BaA/(BaA+Chr) ratio was 0.44 in autumn, indicating the sources of PAHs pyrogenic (Guo et al., 2007).

Waterfowl are exposed to PAHs via nutrition, water, inhalation, feather conditioning, and direct ingestion of oil (Fernie et al., 2018). Accumulating PAHs in birds is associated with decreased metabolic capacity and a high-fat percentage (Eisler, 2000). In general, the reason for the differences in the levels of total PAHs in the studied birds during seasons may be due to their metabolism and detoxification capacity (Custer et al., 2001; Roscales et al., 2011) or the fat content that increases the accumulation of PAHs in muscle tissues (Bandowe et al., 2014; Maisano et al., 2016). Also, Jardine et al. (2006) pointed out that pollutant levels can be affected by environmental factors and nutritional status. Birds can eat insects containing different PAH concentrations (Mengelkoch et al., 2004).

The results of the current study revealed that most of the aromatic compounds in all birds have high molecular weight, and few have a low molecular weight because they are high stability and resistant to microbial degradation (Anyakora and Coker, 2007). Based on the results, the origins of PAHs in the studied birds were pyrogenic from human activities and organic wastes (Table 4). The results also revealed that the BCF values ranged from 2.29 to 5.81 in *A. crecca* and *A. platyrhynchos*, respectively (Table 5). The variation of BCF values in different bird species may be attributed to exposure duration, age, sex, habits, fat contents, metabolism process, detoxification and ecological factors (Roscales et al., 2011; Sun et al., 2016).

Conclusion

The results showed high concentrations of PAHs during winter in the studied birds. According to the ratios of LMW-PAHs/HMW-PAHs, BaA/(BaA+ Chr), InP/(InP+BghiP), Phe/Ant, Ant/(Ant+Phe), and Fluo/pyr, the sources of PAHs were mainly pyrogenic and few were petrogenic. The presence of hydrocarbon compounds in the meats of the birds can have risks to human health by their consumption.

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