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Mudskippers a good bioindicator for polluted soils in the mudflat region of southern Iraq

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Abstract Mudskippers are good biological guide to marine pollution because they are endemic to the coastal areas of water that considered to be the original environment of life. Therefore, the area of Iraq's mudflats are affected by pollutants that reaching a narrow zone of Iraq and neighboring countries which regarded as oil activities area that directly affecting fishes due to increasing the likelihood of hydrocarbon accumulation. Besides, there are large ports of loading platforms nearby as well as this area regarded as a fishing ground. In the current study, hydrocarbons were analyzed using the mudskipper samples that were divided into three groups according on their length, which ranged from 11-16 cm, 17.5-19.5 cm and 20-23 cm, respectively, and the fishes were divided into three groups of weight; 7.13-17.33 g, 18.6-25.33 g and 26.7-36.6 g. Pollutant accumulation is a very reliable sign of environmental contamination. the ratio of Anthracene/(Anthracene+Phenanthrene) measured more than 0.1 (0.52, 0.62, 1.57) is in agreement with the combustion origin, Fluoranthene/(Fluoranthene+Pyrene) measured more than 0.35 which is in agreement with the prospect of petroleum input, and finally, the ratio of BaA/(BaA+Chry) measured about 0.16 in the third group which is attributable to cool combustion.

نطاط الماء كمؤشر بيولوجي لتلوث التربة بالهيدروكاربونات في منطقة المسطحات الطينية جنوب العراق

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المستخلص - تعد اسماك نطاط الماء دليلا بيولوجيا جيدا للتلوث البحري كونها مستوطنة في المناطق الساحلية التي تعتبر بيئتها الاصلية وتتأثر منطقة المسطحات الطينية في العراق بالملوثات التي تصل إلى المنطقة الساحلية الضيقة التي تقع ضمن العراق والبلدان المجاورة ذات الانشطة النفطية التي تؤثر مباشرة على الأسماك الموجودة فيها بسبب زيادة احتمال تراكم الهيدروكربونات. وبالإضافة إلى ذلك، توجد موانئ كبيرة مثل منصات التحميل القريبة، فضلا عن ذلك تعتبر منطقة مصائد للاسماك البحرية التي يعتمدها سكان البصرة في غذائهم. وقد تم تحليل الهيدروكربونات في الدراسة الحالية باستخدام عينات من الأسماك التي قسمت إلى ثلاث مجموعات وفقاً للطول والتي تراوحت بين 11-16 سم و 17.5-19.5 سم و 20-23 سم، على التوالي، وقسمت الأسماك أيضاً إلى ثلاث مجموعات حسب أوزانها إذ تراوحت بين 7.13-17.33 غم و 18.6-25.33 غم و 26.7-36.6 غم. يعتبر تراكم الملوثات في انسجة الاسماك مؤشراً على التلوث البيئي. ان نسبة (Anthracene + Phenanthrene) / Anthracene قد سجلت قيمة اكبر من 0.01 وكانت القيم كالتالي (0.25, 0.62, 1.57) والتي تشير الى ان مصدرها من عمليات الاحتراق، بينما النسبة بين (Fluoranthene + Pyrine) / Fluoranthene فقد سجلت تقريبا 0.35 قد يكون مصدر هذه الهيدروكاربونات الحلقية من اصل نفطي، وأخيراً فان نسبة الـ BaA/(BaA+Chry) كانت حوالي 0.16 في المجموعة الثالثة والتي تشير الى مصدرها من عمليات الاحتراق المختلفة.

الكلمات المفتاحية: نطاط الماء، المسطحات الطينية، الهيدروكربونات، التراكم الحيوي، مركبات الهيدروكربونات الأروماتية متعددة الحلقات.

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Introduction:

Mudflat location with deposit of oil refinery from neighboring oil industrial activities, represent, the high level of polycyclic aromatic hydrocarbon (PAHs). The fish species known as mudskipper is the most prevalent in Iraq's intertidal zone and can be used as a bio-indicator for environmental pollution. *Boleophthalmus dentatus* (Valenciennes) described by Cuvier and Valenciennes (1837), with dentition and first and second dorsal fins (Rathod, 2005).

Pormansyah *et. al*. (2019) studied the distribution of the species of mudskippers in Indonesian water, they recorded 24 species of mudskippers in several Indonesian islands. Loi *et al.* (2020) investigated the behavior that mudskipper follow in feeding by special modified apparatus they were examined by macroscopic and microscopic dissection, micro-computed topography scanning, and scanning electron microscopy, and found that the fish transport the diatom cell from the soil to their digestive canal with few mud particles, which was done by horizontaling of the lower jaw teeth. *Boleopthalmus dussumieri* is a mudflat amphibious estuarine fish which live in Ulhas River estuary in Thane.

During the study Rathod (2005) found that the health of this fish was affected by pollutants of an origin anthropogenic activities. The low glycogen and high fat content were occurred in the monsoon because of the reduce of salinity stress through the rainy season. The anthropogenic activities such as solid waste disposal, refinery, ship movement, etc. are very clear in the estuarine environment (Athalye *et al.,* 2003).

The estuary makes a large rich area of mudflat deposited from the river sediments. Many animals like mudskippers, crabs …ect may be used the sediments as place to live. Al-Behbehani and Ebrahim (2010) evaluated the parasites in sample of mud-skippers during the summer season under different environmental marine factors, no external or internal parasites were recorded in this study. Kuwait Bay is a good environment, for mudskipper to colonize because the mud particles are very sticky, unlike sands, therefore it can be a bioindicator for this area.

The mudskippers *Boleophthalmus dussumieri* were found in Tiab and Bandar e Pol bays located in the east and west of Bandar Abbas, Arab Gulf. The length and weight of the fish were studied both with algometric relationship (Sana *et al.,* 2017). In vitro experiment the polycyclic aromatic hydrocarbon (PAH) affected the erythrocyte membrane stability in mudskippers because of changes in the building of membrane (Sinaei, 2013).

There are evidences that the mudskippers are under the exposure of many pollutants such as ammonia toxicity in the gills and liver that affected the metabolism of protein and amino acids causing reduced of regulation (You, 2018). However, Ansari *et al.* (2014) measured the response of mudskippers in three different stations in Bushehr coasts of the Arabian Gulf, for 5 biomarkers were high in some stations which exposed to oil. Acute exposure to crude oil causes changing in enzyme activities as well as possible effects on the liver activity (Pan *et al.,* 2022). Their study on acute exposure to crude oil within 48 hours revealed changing in glutathione peroxidase (GPx), superoxide desmotase (SOD), and catalase (CAS) activity.

They have assessed biotoxicity by integrated biomarkers (IBR) response signs which were later developed. As a result, the liver has showed that SOD, CAT and GST activity were higher than the maximum induction complications of SOD, CAT and GPx in the liver first arose before doing so in the gills, and vice versa. SOD and GPx were activity increased with low levels of pollution and decreased at high levels of pollution concentrations.

As typical biomarkers of mudskippers is preferable to choose SOD and GPx in the gills and CAT and GST in the liver (Dong *et al.,* 2016). In Kuwait and the northern portion of the Hormuz Strait (Arabian Gulf), *Periophthalmus waltoni* is present, whereas *P. cantonensis* is frequently found in southern China and southeast Asia (Amin *et al.,* 2003; Clayton, 1993).

The amphibious lifestyle of the mudskippers, which may directly exposed them to numerous toxins in coastal waters, may need an explanation. Eco-toxicological substance and aquaculture both regularly employ changing in water quality measures (Dabruzzi *et al.*, 2011).

Iraq's mudflats is influenced by the oil industries and the resulting fallout that deposits on the region. Besides, the mud bounce fish breathe air and move through the mud by developing fins which are strong and enable them to walk on the mud. This work aimed to evaluate the accumulation of polyaromatic hydrocarbons (PAHs) in mudskipper as a bioindicator for mudflat area in southern Iraq.

Materials and Methods:

Taxonomy of Mudskipper Fish:

Systematic position of *Boleopthalmus dussumieri* (Cuvier & Valencienne, 1837) is as follows:

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Gobiidae
Subfamily: Oxudercinae
Genus: *Periophthalmus*
Species: *Periophthalmus barbarous*

Other species of mudskippers found in the region include *Periophthalmodon schlosseri*, *Oleophthalmus boddarti* and *Scartelaos histophorus*, among others. Taxonomy is constantly evolving as new research and data become available, so the classification of mudskippers and other organisms may change in the future as new information emerges.

Sample Collection:

All samples were collected from the mudflat region where the mudskipper fish are populated. The samples were placed in ice container then transported to the laboratory. Afterward, the fish were washed with distilled water. However, the fins, tail and internal organs were removed by metal knife and muscles kept in the refrigerator until measurement. The samples were divided into three groups according to lengths, weight (Table 1).

Table 1. Show the partition of the sample according to the length and weight of the fish.

Parameter	Group (1)	Group (2)	Group (3)
Length (cm)	11-16	17.5-19.5	20-23
Wieght (g)	7.13-17.33	18.6-25.33	26.7-36.6

The Chemical Reagents:

All chemicals and reagents were used of analytical grade as follow: Hexane (purity 99.8%), Dichloromethane, Anhydrous Sodium Sulfate (purity 99%), Alumina and Silica Gel. A PAHs standard mixture include the 16 priority PAHs, particularly, Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benzo[a]Anthracene, Chrysene, Benzo[b] Fluoranthene, Benzo[k]Fluoranthene, Benzo[a]Pyrene, Dibenz[a,h]Anthracene, Indeno[1,2,3-c,d]Pyrene, and Benzo[g,h,i]Perylene. The standard was prepared by mixing solutions containing all the PAHs. then diluted to made a stock solution with acetone and stored at 24uC in darkness to avoid volatilization and photodegradation.

The Preparation of the Sample:

About 50 ml of hexane and dichloromethane (1:1, vol/vol) were added to 10 g of fish muscles then ultrasonicated for 30 minutes at 30ºC. The contents were filtered, and the procedure was repeated three more times on the leftover material, using a brand-new dichloromethane and hexane mixture.

50 ml of a 1:1 (vol/vol) solution of dichloromethane and acetone were used to combine the solvent extracts, pass them through a column filled with anhydrous Na_2SO_4, dry it out with a rotary evaporator, afterward dissolve it in 2 ml of hexane. By using 2 g of aluminum oxide that had been 5% deactivated on top and 2 g of silica gel that had been 5% deactivated on the bottom, the extracts were filtered. Hexane (15 ml), hexane with dichloromethane (9:1) (5 ml), and hexane plus dichloromethane (20 ml) were then used to elute the PAHs (4:1). The combined eluted fractions were evaporated to around 0.5 ml (Chukwjindu *et al.,* 2015).

Chemical Analysis:

A gas chromatograph (GC-MS 2-5975) fitted with an HP5 capillary column (cross-linked PHME siloxane, 0.25-mm film thickness, 0.25 mm by 30 m), and an HP 5973 series mass-selective detector was used to measure, the PAHs in the combined eluted fractions. The mass spectrometer was run in electron impact ionization mode with ionizing energy of 70 eV, scanning at 3.6 scans per second from m/z 50 to 450. The quadrupole and ion source temperatures were 230 and 150 ºC, respectively.

Condition for GC MS:

Agilent 7890 gas chromatography-5975 mass selective detector (USA) system equipped with a fused silica capillary HP-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent, USA). The oven temperature program was as follows: Start (40 ºC), Injection Temp. (290 ºC), Injection Mood: Pulsed Splitless, Flow Control Mode: Constant Flow, Pressure: 9.14 ml/min, total flow: 19.2 ml/min. Column Flow: 1 ml/min, Purge Flow :3 ml/min, Split Ratio 60, Injection Volume: 05 µl. the condition of Mass Spectrometer as fallow: Ione Source Temp.: 320 ºC, Quad Temperature: 150 ºC, Interface Temp.: 290 ºC, Solvent Cut Time: 7 min, End Time: 60 min, ACQ Mode: SIM, Event Time: 0.50 sec. Scan Speed: 1562 (N_2), Start m/z 35, End m/z 650. The work was done in the lab of the oil general Company of Basrah.

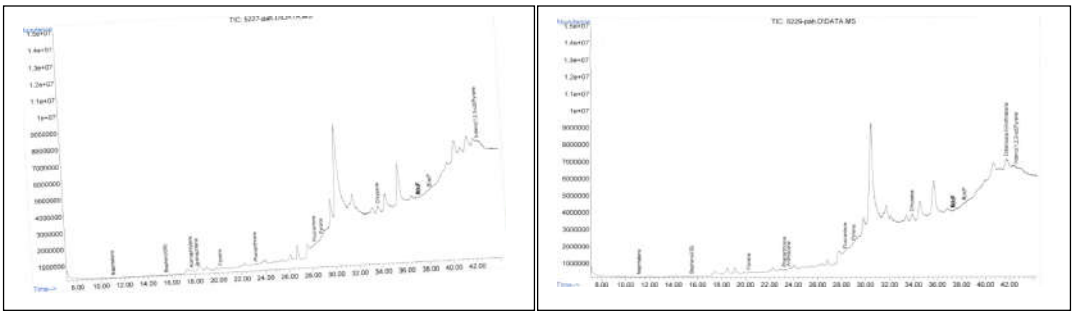
Results and Discussion:

Mudskippers have been the subject of substantial biological and ecotoxicological research, and they are increasingly recognized as promising bio-indicators for environmental monitoring and assessments of coastal waters and tropical or subtropical soft-bottom intertidal systems. Mudskippers are extremely sensitive to their surroundings, therefore further studies on this species, especially those looking at its ecological influence in assessing the level of pollution in coastal water settings, could profit from this ability.

From the Table (2), the highest concentrations of Fluoranthene, were 6.7, 9.2 and 7.0 ppb/g dry wet., Florene had a concentration record of 3.4, 5.2 and 4.9 ppb/g dry wet., whereas Chrysene had a range of 4.2, 1.5 and 2.1 ppb/g dry wet. for the three groups respectively. Floranthene was the highest value at the three study stations 9.2 ppb/g. Musa *et al.* (2022) recorded the highest value of Floranthene in their survey for estuarine area in Basrah, after which Florene recorded approximately 5.2 ppb/g dry wet./g at the second station, both Pyren, and Chrysen scored at the first station 4.0 and 4.2 ppb/g dry wet./g., respectively. Al-Ali *et al*. (2016) reported the levels of total petroleum hydrocarbons (TPH) in fourteen commercially significant fish species from the Iraqi coastal water in the North West Arabian Gulf.

Table 2. The PAHs concentration in muscles of three groups of mudskipper (*Boleopthalmus dussumieri*)

PAHs Compounds (ppb/g dry wt.)	Phenanthrene	Florene	Acenaphtene	Acenaphtylene	Naphtalene	B(a)P	B(K)F	B(b)F	chrysene	Benzo(a)ant	Pyrene	Fluoranthene	Anthracene	Indeno(1,2,3-cd) Pyrene	Benzo (g,h,i) Perylene	Dibenzo (a,h)Anthracene
group a1	0.11	0.26	0.14	0.11	0.04	0.04	0.23	0.02	0.33	0.01	0.36	0.54	0.22	0.33	0	0.03
group a2	0.17	0.34	0.17	0.16	0.05	0.07	0.21	0.06	0.42	0	0.41	0.67	0.19	0.29	0	0
group a3	0.07	0.27	0.11	0.18	0.01	0.07	0.22	0.02	0.44	0	0.44	0.67	0.17	0.3	0	0
group b1	0.11	0.52	0	0	0.18	0.04	0.06	0.07	0.15	0	0.29	0.92	0.17	0.08	0	0.11
group b2	0.14	0.11	0.01	0.004	0.02	0.04	0.07	0.09	0.19	0	0.17	0.77	0.19	0	0	0.22
group b3	0.11	0.14	0.01	0.01	0.02	0.02	0.04	0.07	0.34	0.01	0.23	0.51	0.2	0.03	0.01	0.19
group c1	0.07	0.18	0.03	0	0.14	0.08	0.24	0.06	0.34	0.07	0.37	0.61	0.04	0.21	0	0.32
group c2	0.1	0.61	0.07	0.17	0.17	0.04	0.16	0.07	0.22	0.08	0.27	0.68	0.09	0.19	0	0.11
group c3	0.04	0.49	0.05	0	0.16	0.05	0.11	0.06	0.21	0.04	0.25	0.71	0.07	0.28	0	0.19



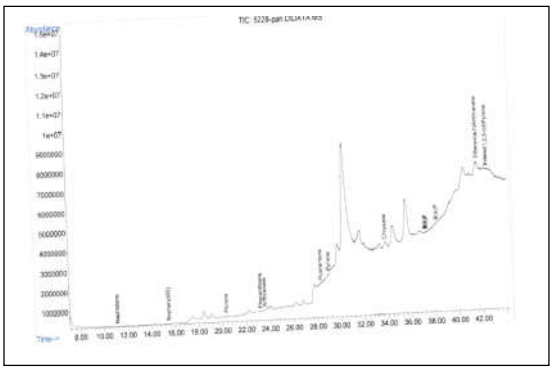


Figure 1. a,b,c the gramatographs of GC-Mass for the PAHs in the Mudskippers (a: group 1, b: group 2, c: group3).

The muscle of the fish had high levels of benzo[a]pyrene, the most cancer-causing polycyclic aromatic hydrocarbon. Previous research has also demonstrated that the main PAHs found in fish tissues include benzo[a]pyrene, naphthalene, and pyrene (Hamad *et al.*, 2014; Al-Saleh and Al-Doush, 2002; Dinh and Nguyen, 2022). Figure (1) shows the chromatograms for the PAHs in the Mudskipper fish at the survey area.

The Sources of PAHs in Fish:

There are several sources of PAHs in the environment, some of them natural and the others are industrial, but it is unclear how they enter the ecosystem or how much of them end up in fish muscle. As is generally known, fish accumulation of pollutants is a great biomarker that represents environmental pollution. Table (3) demonstrates that the ratio of anthracen/(anthracen+phenanthraene), which originates from combustion, was greater than 0.1 (0.52,0.62 and 1.57, respectively), while the Fluorantene/(F+Pyrene) ratio recorded was more than 0.35, indicating that the input is from petroleum. Finally, the third group's ratio of BaA/(BaA+Chry) is approximately 00.16, according to a computation made by Baumard *et al.* (1998). Mudskippers absorb and gather a variety of toxins that are introduced into the coastal environment as a result of industrial, agricultural, domestic, and transportation activities (Polgar *et al.,* 2010).

Table 3. The ratio of (Ant/(Ant+Phe), Fla/(Fla+Pyr), BaA/(BaA+Chry)).

PAHs ratio	Group 1	Group 2	Group 3	Sources
Ant/(Ant+Phe)	0.52	0.62	1.57	Dominance of Combustion
Fla/(Fla+Pyr)	0.62	0.76	0.73	Petroleum Input
BaA/(BaA+Chry)	0.00	0.00	0.16	Cool Combustion

Mudskippers are quite social and active when they are not in the water, feeding and interacting with one another. Since the mud particles are so much stickier than sand, the area close to the Iraqi coast is perfect for burrowing mudskipper and digging crabs frequently create mixed colonies. (Fiddler Crabs-Caridae). Consequently, in the Iraqi mudflats, mudskippers can act as

bioindicators of maritime pollution. However, specific physiological and behavioral changes in bioindicators are used to detect changes in environmental health, a similar result was reached by Al-Behbehani and Ebrahim (2010). The frequency of oil spills in coastal areas has increased, which has worsened intertidal contamination. A complex hydrocarbon called petroleum contains polycyclic aromatic hydrocarbons. (PAHs) are difficult to degrade and have a "tricyclic effect" (Aas *et al*., 2000).

On the flora and fauna of coastal area in Kuwait Bay, regularly released contaminants have immediate detrimental effects. Almost the ecosystems for Iraq and Austria were very similar to each other. PAHs can be converted by biomagnification at different trophic levels, causing contaminants to disperse from their sources and penetrate key areas of human activity, further harming human health, human wellbeing. The intertidal zone is crucial for establishing a connection in the relationship between the ocean and land, which is intimately tied to human life.

After crude oil has contaminated the intertidal sediments, the leftover, continuous oil discharge will occur due to the effect of the tide and infiltrate the thick silt layer or the nearshore ocean, risking ecological surroundings, therefore, researching petroleum's toxicity. The importance of hydrocarbons to intertidal creatures is well known. Analyzing the harm done to the environment by intertidal oil spills. The same species of mudskipper *Boleophthalmus dussumieri* is found in the mud flats of the tidal areas in Iran. Mudskippers are very active when they are outside the water.

Table (4) shows the correlation between the hydrocarbons pollutants (C1) and the same weights of the first group (C2) and the correlation was weak at 0.129. The correlation between the pollutants (C3) and the weight of the fish in the second group (C4) was somewhat high (0.389). A high correlation between the pollutants (C5) and the weight of the fish in the third group (C6) was also recorded (0.557). It is also noted that the highest registered correlation between the third pollutants and the third weight of fish was 0.567. The lowest correlation was between the first pollutants (C1) and the weight of fish for the second group (C4) was 0.093. The amount of pollutants that could be caused by their melting into fat increases with each increase in body mass.

Table 4. The correlation of the pollutants and fish groups.

Rows	C1	C2	C3	C4	C5	C6
C1	1					
C2	0.129201	1				
C3	0.295166	0.424183	1			
C4	0.935583	0.339211	0.389319	1		
C5	0.109826	0.409933	0.401567	0.346423	1	
C6	0.109941	0.807022	0.794233	0.794502	0.557384	1

Conclusion:

The Mudskipper is the best bioindicator of environmental pollution in the mudflat area in the southern of Iraq because these fish are common in the region and are exposed to waste from oil activities and human and industrial wastes, which could increase the chances of PAHs being transported within the food chain and their access to and accumulation in human body.

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