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Original Article

Polycyclic aromatic hydrocarbons (PAHs) in sediment and two tilapia fish species with the health risk of their consumption of the Shatt Al-Arab River, Basrah, Iraq

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Abstract: PAHs are toxic pollutants that endanger human health and the environment. This study aimed to assess the PAH levels in sediment and two tilapia species of *Oreochromis niloticus* and *Coptodon zillii* in the Shatt Al-Arab River along the Basrah City, southern Iraq, from May to October 2021. In addition, the risk to human health from fish was calculated using dietary daily intake and the carcinogenic potencies of PAH concentrations. Sixteen PAH congeners were found in sediment and fish samples. The total PAH concentrations in sediment and fish samples ranged from 37.46 to 76.33 µg/g dry weight and 23.55 to 55.81 µg/g wet weight. The total concentration pattern of PAHs was as follows: Sediment > *O. niloticus* > *C. zillii*. PAH levels in the fish's dietary intake were 0.00866 mg/kg body weight/day for 8 PAHs and 0.01288 mg/kg body weight/day for 16 PAHs, respectively. The TEQ (0.0025888 mg/kg body weight/day) exceeded the SV (0.677 ng/g wet weight) of the USEPA.

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Introduction

Polycyclic aromatic hydrocarbons (PAHs) are common in nature and have a negative impact on the environment. The extraction and exploration of crude oil and gas resources is a major source of PAHs. Even though the petroleum industry has improved its practices, accidents continue to occur, resulting in hydrocarbon pollution of the environment, both land and water, in most oil-producing countries, including Iraq (Al-Hijaj et al., 2019). Oil from the petroleum industry enters the aquatic environment through leaching from oil tanks, seepage from offshore vessels, erosion and runoff from crude oil-contaminated land, refinery effluents, ruptures from poorly maintained flowlines, and gas flaring (Patel et al., 2020; Wang et al., 2021). Vandalism, inadequate maintenance, and design flaws could all be reasons for oil leaks into the environment. When the component enters the aquatic environment and mixes with water or sinks into sediment, it causes severe damage to benthic organisms. Fish, crustaceans, and mollusks are less valuable and appealing on the market due to the unpleasant odor or flavor caused by hydrocarbon pollution.

he main routes of PAH exposure in fish are water diffusion through their gills and skin and consuming contaminated foods (Santana et al., 2018). Because of their remarkable chemical stability and lipophilic nature, PAHs can easily permeate biological membranes and accumulate in organismal adipose tissues after ingestion. When fish and other aquatic life are exposed to pollution, they rapidly absorb PAHs and accumulate to levels above the ambient level (Zhao et al., 2014; Wang et al., 2015; Awe et al., 2020). The physiological burden of PAHs is determined by the organisms' ability to biotransform the chemicals, whereas its bioaccumulation pattern in aquatic organisms varies according to the trophic level (Advaiti et al., 2013). Fish are the most vulnerable to toxicant concentrations in aquatic environments (Okpashi et al., 2017).

PAHs are classified into two categories based on their physical and biological properties and the number of fused aromatic rings in their structural

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makeup. Light PAHs (LMW) have two to three aromatic rings, whereas heavy PAHs (HMW) have four to six rings (Keith, 2015; Jesus et al., 2020). HMW can persist in aquatic environments and bioaccumulate in aquatic species such as fish, and it is more carcinogenic than LMW due to its increased stability and stubbornness (less easily biodegraded by native microbes) (Honda and Suzuki, 2020; Jesus et al., 2020). Although less cancer-causing, LMW PAHs threaten many aquatic creatures (Rose, 2012). The chemical composition, chemical arrangement, and physical and chemical properties of aromatic rings all influence the stability and distribution of PAHs in the natural environment (Muthukumar et al., 2013; Patel et al., 2020). The distribution of PAHs in the aquatic ecosystem, including water, fish, and sediment, can provide information about the source of their emissions. While PAHs emitted from combustion processes (pyrolytic origin) frequently contain elevated concentrations of HMW and fewer LMW PAHs, higher concentrations of LMW PAHs are typically related to naturally occurring PAHs, either petrogenic or pyrogenic in origin (Ravindra et al., 2008; Lee et al., 2022).

PAHs have gained attention due to their potential harm to human health and the environment. PAHs have also been shown to harm aquatic species, causing DNA damage, decreased growth, altered endocrine function, malformed embryos and larvae, and impaired growth and development (Collier et al., 2008; Honda and Suzuki, 2020). Aquatic foods are highly nutritious and appealing due to their high protein and low fat content. Consumers are drawn to these foods because of their health benefits, widespread availability, and low cost, although hazardous chemical exposure has long been a concern. Fish is one of the most common aquatic species available for consumption in Iraqi markets (Coad, 2010; Uyar, 2020; Çiçek et al., 2023). PAHs and other contaminants have been linked to human exposure via food. Because some aquatic organisms are caught in hydrocarbon-contaminated waters, PAH contamination of fish species that humans frequently consume could have serious health consequences (Dhananjayan and Muralidharan, 2012; Akinsanya et al., 2020). Therefore, the current study aims to measure PAH levels in sediment samples and two economic fish species, *Oreochromis niloticus* and *Coptodon zillii*, in the Shatt Al-Arab River in southern Iraq. The study also intends to assess the health risks associated with consuming fish in the study area.

Materials and Methods

The Shatt Al-Arab River delta in Basrah governorate covers an area of approximately 969059 km^2. In southern Iraq's Garmat Ali region, the Shatt Al-Arab River is formed when the Tigris and Euphrates rivers merge. The Karon River is the river's only tributary. The Shatt Al-Arab River is approximately 175 km long and has a width ranging from 0.4 to 1.5 km. The Shatt Al-Arab River's depth frequently rises as it approaches the Persian Gulf, reaching a peak of 12.2 meters (Farid, 2017). Its water level is affected by the Persian Gulf's high and low tides, which have an average tidal range of about 1.7 m. The Shatt Al-Arab River is completely mixed at a restricted temperature and chlorinates vertical stratification (Saad and Kell, 1975; Hug et al., 1978). The discharge of this river, which extends 5 km into the Persian Gulf (Farid et al., 2020), floods the Kuwaiti Gulf. The annual precipitation totals 140 mm, starting with a monthly average of less than 1 mm in October and peaking at 29.3 mm in December (Al-Saad et al., 2019).

Sampling was conducted in four sampling sites along the Shatt Al-Arab River delta that displayed various urbanization-related disturbances (Fig. 1): Basrah Center (30°33'00.0"N, 47°47'10.0"E), Garmat Ali (30°48'10.6"N, 47°45'03.8"E), Abu Al-Khasib (30°27'44.5"N, 48°00'06.0"E), and Al-Siba (30°20'16.5"N, 48°15'34.5"E). The most significant industries in Basrah are oil and gas extraction, production, and export; shipping; railroads; agriculture; the food industry; heavy industries such as iron, steel, fertilizers, and petrochemicals; and fisheries. As a result, anthropogenic activities such as oil production, transportation, dwellings, workshops (such as carpentry and mechanics), sales of various items, and petroleum product handling facilities

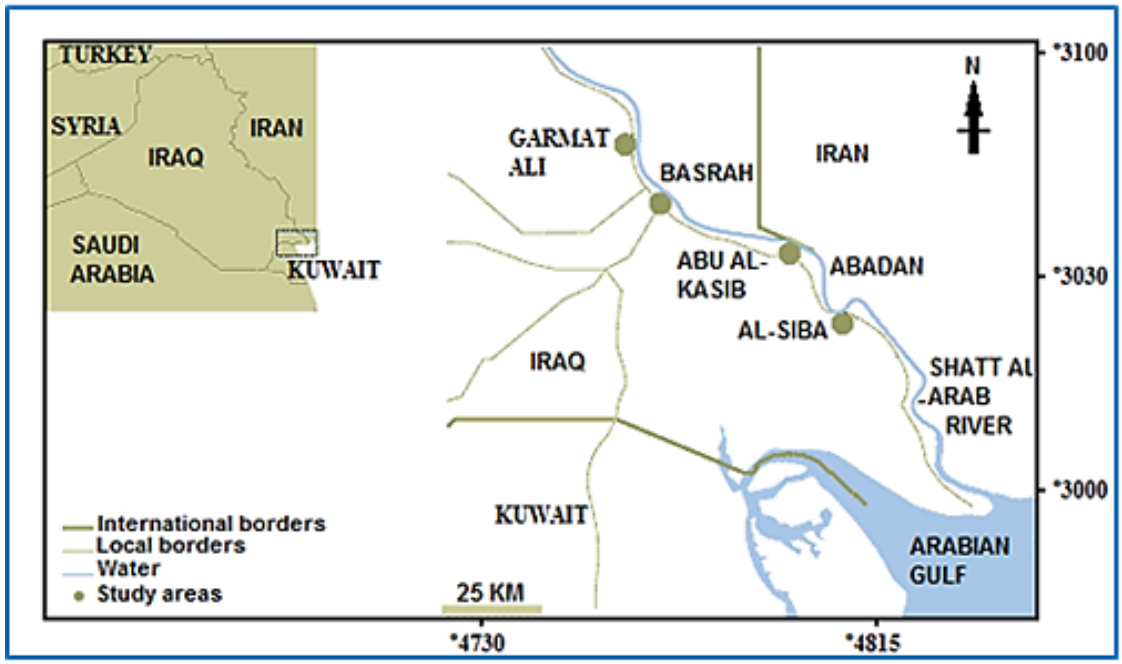

Figure 1. Map of study areas and sampling sites.

significantly impact its water. In addition to the numerous agricultural lands and fields that surround them, numerous industrial and service facilities are located in the Garmat Ali area, including a thermal power station, a paper mill, Basrah International Airport, a water injection plant for the city's oil wells, an Iraqi date factory, and Basrah University. Many fishing boats can be seen in the local waters. These activities had a significant impact on the area's water. The Abu Al-Khasib area is home to one of Iraq's major ports, Abu Floss, and a chemical fertilizer manufacturing company. The water at Abu Al-Khasib is thought to be only slightly contaminated. Al-Siba has a tourist park and a gas field. Abadan, an Iranian port, is close by. As a result, it is expected that various human activities and chemicals discharged by Iranian petrochemical and oil refinery factories in Abadan will have a relative impact on Al-Siba's water.

Between May and October 2021, fish and sediment samples were collected from the four locations mentioned above. After removing scales and large debris from the sediment sample, a portion of the top 5 cm was scooped into sterile 50 ml bottles with the sediment collector. The covered, filled sediment bottles were stored in an icebox. Sediment samples were kept in the lab's freezer at 4°C before examination. After several days of air drying, stones and other debris were removed from sediment samples. The samples were ground and passed through sieves with 2 and 75-mm mesh sizes to remove unwanted items. Before extraction, the materials were sieved with a 0.5 mm mesh sieve and stored in foil sheets.

The current experiments used 319 Nile tilapia (*O. niloticus*) (Linnaeus, 1758) and 198 redbelly tilapia (*C. zillii*) (Gervais, 1848) (Perciformes, Cichlidae) with average length and weight of 11-20.5 cm and 75-305 g, and 9-16 cm and 90-165 g, respectively. A hand net was used to collect fish samples. Live fish were transported to the lab in cold, sterile boxes filled with river water. Before dissecting and removing the flesh and other sections of the fish, the scales were removed, and the samples were rinsed with running water and then distilled water. The samples were placed in sample bottles, weighed (wet weight), and then homogenized with anhydrous sodium sulfate in a mortar and pestle. The combination was labeled and wrapped in aluminum foil for analysis.

The highest-purity analytical-grade chemicals, solvents, and reagents were used and sourced from Fluka, Merck, Sigma Aldrich, Supleco (Germany),

and Himedia (India). AccuStandard (USA) provided internal standard solutions for 16 PAHs and deuterated PAHs. Glassware was washed with water after 24 hours of soaking in the cleaning solution. After 24 hours of immersion in 10% hydrochloric acid, it was thoroughly cleaned with tap and distilled water. The glassware was oven-dried at 250ºC after each acetone wash. After drying, it was stored until it was ready to use. Before being used, the glassware was washed with n-hexane.

The surrogate standard was added to the samples before extraction and used as an internal quantification standard. Stock solutions created working standards for the calibration and spiking studies. PAHs were extracted from sediment samples using the Soxhlet extraction method (AOAC, 2005). Five grams of sediment were weighed, and 5 g of anhydrous sodium sulfate was mixed into the sample. The samples were placed in a cellulose thimble and extracted with 150 ml of dichloromethane in a Soxhlet extractor for 24 hours. The extract was concentrated by evaporation overnight in a fume cupboard while covered with perforated aluminum foil. After homogenizing with anhydrous sodium sulfate, the fish sample (5 g) was placed in 100 ml beakers. 40 ml of n-hexane and dichloromethane (1:1 vol/vol) were used to extract solvents. For approximately 25 minutes, the beaker's contents were shaken with a magnetic stirrer. After decanting the extract into a clean conical flask, 20 ml of solvent was added, and the procedure was repeated. After combining the extracts, a thin layer of anhydrous sodium sulfate was placed over a glass wool plug in a small glass funnel, and the mixture was filtered into a conical flask for receiving. The extracts were concentrated in a fume cupboard overnight, covered in perforated aluminum foil.

The USEPA's (1996) technique was used to clean sediments and fish samples. A 600 × 19 mm clean-up column was made. The opening was plugged with glass wool, 3 g of activated silica gel (60 mesh) was added, and the column was topped with anhydrous sodium sulphate. The column was washed with 20 ml n-hexane before being discarded. The concentrated extract was loaded onto the prepared column, and the elution process involved 50 ml of n-hexane. The eluates were then reduced to 1 ml using a rotary evaporator and a gentle stream of pure nitrogen. Before being subjected to gas chromatography mass spectrometry analysis, one milliliter of the extract was transferred to a clearly labeled vial and kept at 4ºC.

PAH analysis of fish and sediment samples was carried out using GC-MS equipment. The capillary column HP-1 (methyl siloxane) from Agilent Technologies in Palo Alto, California, USA, was used to separate target analytes (30 m length, 0.25 mm i.d., and 0.25 m film thickness). The carrier gas used was high-purity helium (99.99%) at a 1.2 ml/minute flow rate. Sample injector temperatures were set to 250 and 300°C, and samples were injected in splitless mode with a volume of 1 ml. A 60°C initial column temperature was maintained for 1 minute before ramping up to 200°C at 10°C/minute for 2 minutes and finally to 300°C at 10°C/minute for 6 minutes. The mass spectrometry parameters were specified as follows: Store mass range: m/z 30-600 m; ionization source: electron ionization at -70 eV; ion source temperature: 250°C. Individual PAHs were identified by comparing the retention times of the samples to standard solutions.

Analytical lab blanks were used for quality control and treated the same as real samples. The PAHs found in the appropriate blanks were deducted from the sample extracts. The lower detection limit, 3.3 SD of the blank, was calculated as 0.01-0.04 µg/g. The average recovery, measured by including 100 g of PAH standards before extraction, was close to 100% for all standards.

The toxicological risks associated with PAH concentrations in fish samples were assessed by comparing the reported values to regulatory limits and guidelines. The dietary daily intake concentrations of 8 and 16 PAHs from contaminated fish species were calculated to estimate the risks to human health from PAH exposure via contaminated fish consumption. The daily dietary intake of PAHs from contaminated fish by adults was assessed. The daily intake of PAHs from fish consumption was calculated by multiplying the individual PAH content in each fish by the adult's

average weight consumption rate. This study's fish consumption averaged 2.4 km/year. Data on fish consumption were obtained from https://www.fbsa.gov.iq/ar/view/1560 (2011-2017).

The risk of developing cancer from consuming fish containing PAHs was assessed. To determine each PAH's carcinogenic potential, the individual toxicity equivalency factor (TEF) was multiplied by the PAH concentrations in the sample (Martorell *et al*., 2010). The toxicity equivalency (teq) factor estimates the relative toxicity of individual PAH fractions to BaP. Toxic equivalence factors have been used to regulate substances with similar modes of action (such as PAHs). The TEFs developed by Larsen and Larsen (1998) and the USEPA (2002) were applied to a typical adult weighing 70 kg, and these values were used to calculate PAH as BaP equivalents. The TEQ was calculated by adding the carcinogenicity of each PAH (i.e., TEQ = Σ BaPteq). To assess the health risk of PAHs associated with fish consumption, the screening value (SV) was calculated using the Nozar *et al*. (2013) method: SV = ((RL / CSF) BW) / CONR and compared to the estimated TEQ value. The SV is the chemical concentration in edible tissue that poses a risk to consumers. BW is the body weight (70 kg), RL is the highest acceptable risk level (10-5), CSF is the oral cancer slope factor (7.3 mg/kg/day), and CONR is the rate of fish consumption.

Fish samples were dissected to remove the desired organs. Fish liver, fillet, and gills were each placed in their own clearly labeled bottle, fixed in 5% formalin for at least 48 hours, and then transferred to a rack of sampling bottles. The tissue was prepared using standard techniques for histopathological examinations (Eagderi et al., 2013). Following removal from the fixative and a five-minute rinse in tap water, the samples were dehydrated in escalating ethanol concentrations (70, 80, and 90% alcohol) for at least two minutes each. The dehydrated tissues were washed for two minutes in xylene, a wax-miscible agent before being set in paraffin. A microtome (Leica, Germany) was used to prepare about 5 µm slides. Each cut sample was mounted on a glass slide after being dried in a hot air oven to remove moisture. The sections were dewaxed in a wax-miscible agent for at least two minutes before rehydration in progressive ethanol concentrations (90, 80, and 70% alcohol). The tissues were stained with hematoxylin and eosin and then placed in an aqueous eosin and hematoxylin solution for three minutes each. Afterward, they were placed on a slide, labeled, and covered with a cover slip. The tissues were examined and microphotographed using an Olympus light microscope (Philippines).

A one-way analysis of variance (ANOVA) was used to compare the variation in acquired values across sites and sample types. Means were compared using Duncan's multiple range test (DMRT), and $P<0.05$ was considered significant. The calculations were performed using the SPSS (Statistics Package for Social Sciences) version 16 for Windows program (IBM-USA). The mean and standard deviation were calculated, and regression analysis was performed to identify relationships. The graphs were created using Microsoft Excel 2007.

Results

The sediment samples contained 16 PAH compounds, including naphthalene (NAP), acenaphthene (ACE), acenaphthylene (ACN), fluorine (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLO), pyrene (PYR), benzo (a) anthracene (BaA), chrysene (CHR), benzo (b) fluoranthene (BbF), benzo (k) fluoranthene, benzo (a) pyrene (BaP), dibenzo (a,h) anthracene (DahA), benzo (g,h,i) perylene (BghiP), and indeno (1,2,3-cd) pyrene (IcdP) (Table 1). The total PAHs concentration ranged from 37.46 to 76.33 µg/g dry weight. The Basrah Center sampling site had the highest concentration of PAHs, while the Abu Al-Khasib site had the lowest one. ANT had the lowest total concentrations (4.80 µg/g dry weight), and PYR had the highest (24.94 µg/g dry weight). The current study found that 31% of PAHs had two to three rings, 43% had four rings, and 26% had five to six rings (Fig. 2). The PHE/ANT ratio was 9.73, 0.51, 2.17, and 9.44 for sediment samples collected from Basrah Center, Garmat Ali, Abu Al-Khasib, and Al-Siba, respectively. The sampling sites in Basrah Center,

Table 1. Concentration of PAHs (µg/g dry weight) in Shatt Al-Arab River sediment areas.

PAHs	Basrah center	Garmat Ali	Abu Al-Khasib	Al-Siba	Total
NAP	3.45±0.57^{a}	1.57±0.49	1.54±0.57^{c}	2.45±0.57^{ab}	9.01
ACE	2.55±0.57^{a}	1.83±0.57^{a}	1.74±0.57^{a}	1.78±0.57^{a}	7.90
ACN	2.73±0.57^{ab}	1.43±0.10^{ab}	1.23±0.14^{ab}	2.83±0.57^{a}	8.22
FLU	2.83±1.15^{ab}	1.89±0.57^{ab}	1.87±0.57^{ab}	2.32±0.57^{ab}	8.91
PHE	4.77±0.57^{a}	1.24±0.57^{bc}	2.92±0.57^{c}	5.41±0.57^{a}	14.34
ANT	0.49±0.17^{ab}	2.43±1.15^{a}	1.34±0.57^{ab}	0.54±0.02^{b}	4.80
FLO	4.27±1.15^{a}	4.66±1.15^{a}	3.64±1.15^{a}	5.67±0.57^{a}	18.24
PYR	9.87±0.57^{a}	3.72±0.57^{b}	2.44±0.57^{b}	8.91±1.15^{a}	24.94
BaA	4.08±0.88^{a}	3.28±0.57^{a}	3.67±0.57^{a}	3.27±1.15^{a}	14.30
CHR	7.56±1.15^{ab}	4.69±1.15^{bc}	3.21±0.57^{c}	8.78±1.73^{a}	24.24
BbF	7.83±0.57^{a}	3.32±1.73^{bc}	2.34±1.15^{c}	5.92±0.57^{ab}	19.41
BkF	5.91±1.15^{ab}	2.76±0.57^{cd}	2.65±1.15^{c}	4.25±0.57^{abcd}	15.57
BaP	5.25±0.57^{a}	3.78±1.15^{a}	2.43±0.57^{a}	5.61±1.73^{a}	17.07
DahA	4.37±0.57^{ab}	2.23±0.57^{c}	2.55±0.57^{c}	4.51±0.58^{ab}	13.66
BghiP	5.86±0.02^{abc}	2.81±0.57^{bcd}	1.63±0.57^{d}	5.55±1.73^{ab}	15.85
IcdP	4.51±0.56^{ab}	2.46±1.15^{b}	2.26±1.15^{b}	3.95±0.58^{ab}	13.18
Total	76.33	44.10	37.46	71.75	229.64

Lowercase and uppercase letters indicate that there are statistically significant differences between PAHs concentrations at $P<0.05$.

Table 2. PAHs ratios for the surface sediments of the Shatt Al-Arab River areas.

PAHs ratios	Basrah center	Garmat Ali	Abu Al-Khasib	Al-Siba
PHE/ANT	9.73	0.51	2.17	9.44
FLO/PYR	0.43	1.25	1.49	0.63

Garmat Ali, Abu Al-Khasib, and Al-Siba had FLO/PYR ratios of 0.43, 1.25, 1.49, and 0.63, respectively (Table 2). Sediment analysis of PAH concentrations showed significant differences ($P<0.05$) between compounds and sites.

Table 3 shows the PAH concentrations in *O. niloticus* and *C. zillii* samples. The samples contained a total of sixteen PAH compounds. The total PAH concentration in *O. niloticus* ranged from 30.59 to 55.81 µg/g dry weight. BbF exhibited the highest total concentration (17.34 µg/g wet weight), while NAP had the lowest (3.06 µg/g wet weight). *Coptodon zillii* had total PAH concentrations ranging from 23.55 to 55.81 µg/g wet weight. BbF had the highest total concentration of individual PAH compounds (14.35 µg/g wet weight), while NAP had the lowest (2.78%). In the current study, 24 and 30% of PAHs in *O. niloticus* and *C. zillii* samples had two to three rings, 43 and 42% had four rings, and 32 and 29% had five to six rings (Figs. 3, 4). The results of PAH in fish revealed significant ($P<0.05$) differences in PAH concentrations between compounds, sites, and fish.

Table 4 summarizes the results of the daily PAH intake for Shatt Al-Arab River fish in adult humans.

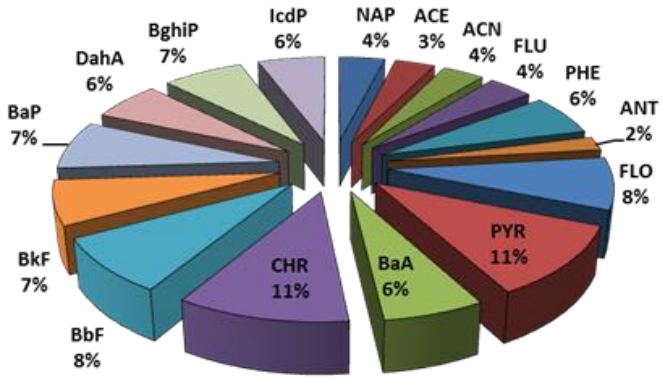

Figure 2. The percentage of each PAH in the surface sediments of the Shatt Al-Arab River.

Dietary intake for 8 and 16 PAHs was investigated, and total dietary intake was expressed in mg/kg body weight/day. Adult human total dietary intake of 8 PAHs and 16 PAHs in Shatt Al-Arab River fish was 0.00866 and 0.01288 mg/kg body weight/day, respectively. The results showed significant differences ($P<0.05$) in an adult human's daily PAH intake for each fish and among them. Table 5 shows the dietary intake calculation for eight PAHs from Shatt Al-Arab River fish and BaPteq. For fish, an estimated SV of 0.013 mg/kg was found. The total TEQ value from the sampled fish was 0.0025888

Table 3. The concentration of PAHs (µg/g wet weight) in *Oreochromis niloticus* and *Coptodon zillii* from Shatt Al-Arab River areas.

PAHs	*O. niloticus*					*C. zillii*				
	Basrah center	Garmat Ali	Abu Al-Khasib	Al-Siba	Total	Basrah center	Garmat Ali	Abu Al-Khasib	Al-Siba	Total
NAP	1.46±0.01^{ab}	0.57±0.01^{cd}	0.87±0.01^{bc}	0.16±0.01^{d}	3.06	1.33±0.01^{ab}	0.26±0.10^{c}	0.63±0.02^{bc}	0.56±0.01^{bc}	2.78
ACE	1.73±0.01^{ab}	0.83±0.02^{cd}	0.93±0.02^{cd}	1.37±0. 57^{bc}	4.86	1.43±0.02^{b}	0.66±0.01^{c}	0.43±0.0^{d}	1.73±0.10^{a}	4.25
ACN	2.33±0.01^{a}	0.22±0.02^{d}	0.15±0.01^{d}	0.73±0.01^{cd}	3.43	1.72±0.02^{a}	0.74±0.01^{bc}	0.58±0.01^{bc}	1.00±0.57^{abc}	4.04
FLU	1.72±0.57^{a}	0.04±0.02^{c}	1.27±0.57^{ab}	1.88±0.01^{a}	4.91	1.46±0.57^{a}	0.82±0.01^{ab}	0.09±0.01^{b}	1.67±0.01^{a}	4.04
PHE	3.89±0.57^{a}	2.11±0.57^{bc}	1.05±0.57^{c}	1.90±0.01^{bc}	8.95	2.83±0.57^{a}	2.47±1.15^{a}	1.57±0.01^{ab}	1.98±0.57^{ab}	8.85
ANT	3.11±0.57^{a}	1.89±0.57^{ab}	1.00±0.57^{b}	2.03±0.57^{ab}	8.03	3.09±1.73^{a}	1.58±0.01^{a}	1.26±0.01^{a}	2.25±0.57^{a}	8.18
FLO	3.36±0.01^{ab}	1.36±0.57^{cd}	2.26±0.57^{bcd}	2.45±0.01^{bc}	9.43	3.82±0.01^{a}	1.92±0.01^{ab}	1.38±0.57^{a}	2.17±1.15^{ab}	9.29
PYR	4.21±0.57^{ab}	3.83±1.15^{ab}	2.64±0.01^{b}	3.66±0.01^{ab}	14.34	3.27±0.57^{a}	2.00±1.15^{a}	1.48±0.57^{a}	3.88±0.01^{a}	10.63
BaA	3.64±0.02^{abc}	2.26±0.01^{c}	3.23±0.01^{bc}	4.10±0.57^{ab}	13.23	3.55±0.57^{a}	2.63±0.57^{a}	1.94±0.01^{a}	3.44±0.01^{a}	11.56
CHR	3.38±0.57^{a}	3.85±0.01^{a}	2.38±0.01^{b}	3.00±0.01^{ab}	12.61	3.75±1.37^{a}	2.84±0.57^{a}	2.26±1.15^{a}	3.28±1.37^{a}	12.13
BbF	5.82±1.15^{ab}	4.66±0.57^{bc}	2.33±0.57^{c}	4.53±1.15^{bc}	17.34	5.11±0.01^{ab}	2.70±0.57^{b}	2.78±1.15^{b}	3.76±0.01^{bc}	14.35
BkF	3.65±1.15^{ab}	3.05±0.57^{ab}	2.94±0.02^{ab}	4.82±1.15^{ab}	14.46	3.32±0.57^{a}	2.32±0.01^{a}	2.03±0.01^{a}	2.64±0.01^{a}	10.31
BaP	5.42±0.57^{ab}	3.36±0.57^{b}	3.01±0.01^{b}	3.27±0.57^{b}	15.06	4.33±0.01^{abc}	2.44±0.01^{cd}	1.77±0.57^{d}	2.36±0.57^{cd}	10.9
DahA	4.63±0.01^{ab}	3.87±1.15^{abc}	2.00±0.57^{c}	4.78±0.57^{ab}	15.28	3.00±0.57^{a}	2.56±1.15^{a}	2.52±1.15^{a}	4.21±0.57^{a}	12.29
BghiP	3.37±0.57^{a}	3.21±0.01^{a}	2.47±1.15^{a}	4.03±0.02^{a}	13.08	3.24±1.73^{ab}	2.78±1.15^{ab}	1.22±0.01^{b}	3.61±0.01^{ab}	10.85
IcdP	4.09±1.15^{a}	2.76±0.01^{a}	2.06±0.57^{a}	4.39±0.57^{a}	13.3	2.47±0.01^{a}	2.93±0.01^{a}	1.61±0.01^{a}	3.11±0.01^{a}	10.12
Total	55.81	37.87	30.59	47.10	171.37	47.72	31.65	23.55	41.65	144.57

Lowercase and uppercase letters indicate that there are statistically significant differences among PAHs concentrations at P<0.05.

Table 4. PAHs' daily intakes of Shatt Al-Arab River fish for adult humans.

PAHs	*O. niloticus*	*C. zillii*	Total mg/kg of body weight/day
	mg/kg of body weight/day	mg/kg of body weight/day	
NAP	0.00009±0.00001^{b}	0.00008±0.00001^{b}	0.00025
ACE	0.00012±0.00001^{c}	0.00010±0.00001^{c}	0.00031
CAN	0.00013±0.00002^{c}	0.00008±0.00001^{c}	0.00030
FLU	0.00013±0.00001^{c}	0.00011±0.00002^{c}	0.00036
PHE	0.00024±0.00002^{d}	0.00019±0.00001^{d}	0.00061
ANT	0.00018±0.00002^{d}	0.00021±0.00001^{d}	0.00063
FLO	0.00024±0.00002^{c}	0.00024±0.00001^{c}	0.00071
PYR	0.00040±0.00001^{d}	0.00029±0.00001^{d}	0.00105
BaA	0.00037±0.00001^{c}	0.00035±0.00001^{c}	0.00108
CHR	0.00032^{b} ±0.00001	0.00033^{b} ±0.00001	0.00100
BbF	0.00047^{d} ±0.00001	0.00041^{d} ±0.00001	0.00133
BkF	0.00039^{d} ±0.00001	0.00032^{d} ±0.00001	0.00106
BaP	0.00042^{d} ±0.00001	0.00035^{d} ±0.00001	0.00113
DahA	0.00038^{c} ±0.00001	0.00035^{c} ±0.00002	0.00109
BghiP	0.00034^{b} ±0.00001	0.00032^{b} ±0.00001	0.00101
IcdP	0.00035^{d} ±0.00005	0.00029^{d} ±0.00001	0.00096
Total 8 PAHs	0.00304	0.00272	0.00866
Total 16 PAHs	0.00457	0.00402	0.01288

Lowercase letters indicate that there are statistically significant differences among PAHs daily intakes at P<0.05.

mg/kg body weight/day.

Based on the histological examinations, the primary lamellae of *O. niloticus* and the secondary lamellae of *C. zillii* showed significant hypertrophy and hyperplasia. *Oreochromis niloticus* showed epithelial cell hyperplasia, whereas *C. zillii* showed secondary lamellar shortening and fusion. The muscle bundles of *C. zillii* and *O. niloticus* were split and atrophy, but only *O. niloticus* had muscle bundle necrosis (Fig. 5a-c). *Coptodon zillii* and *O. niloticus* livers exhibited necrosis, cellular degeneration, and hepatopancreas necrosis (Fig. 6a-d).

Discussions

The predominance of LMW and HMW PAHs in the sediments suggests the presence of substantial amounts of combustion products from pyrolytic processes and/or petrogenic sources (Wu et al., 2001). A sediment quality guideline of 1000 ng/g dry weight total PAHs was developed to protect estuarine fish

Table 5. Fish dietary intakes of 8 PAHs, BaPteq and TEQ (mg/kg of bodyweight/day) for adult humans (70 Kg).

PAHs	TEF	*O. niloticus*	*C. zillii*	Total BaPteq
		BaPteq	BaPteq	
BaA	0.1	0.000037	0.000035	0.000108
CHR	0.001	0.00000032	0.00000033	0.000001
BbF	0.1	0.000047	0.000041	0.000133
BkF	0.01	0.0000039	0.0000032	0.0000106
BaP	1	0.00042	0.00035	0.00113
DahA	1	0.00038	0.00035	0.00109
BghiP	0.02	0.0000068	0.0000064	0.0000202
IcdP	0.1	0.000035	0.000029	0.000096
Total TEQ		0.00093002	0.00081493	0.0025888

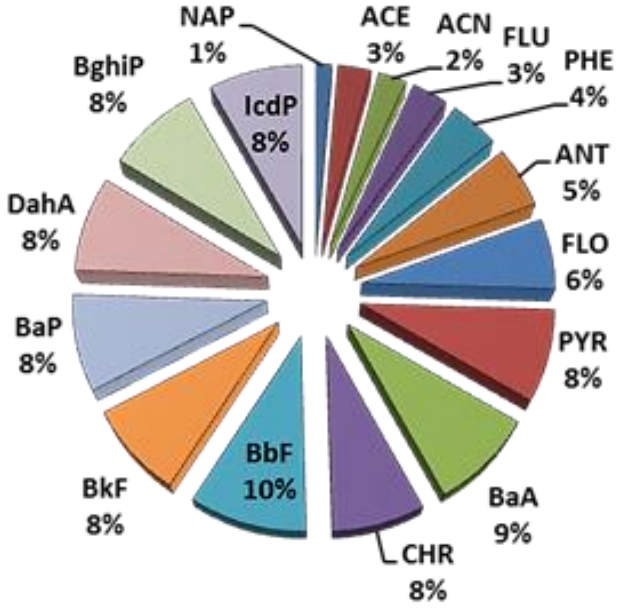

Figure 3. The percentage of each PAH in *Oreochromis niloticus* from the Shatt Al-Arab River.

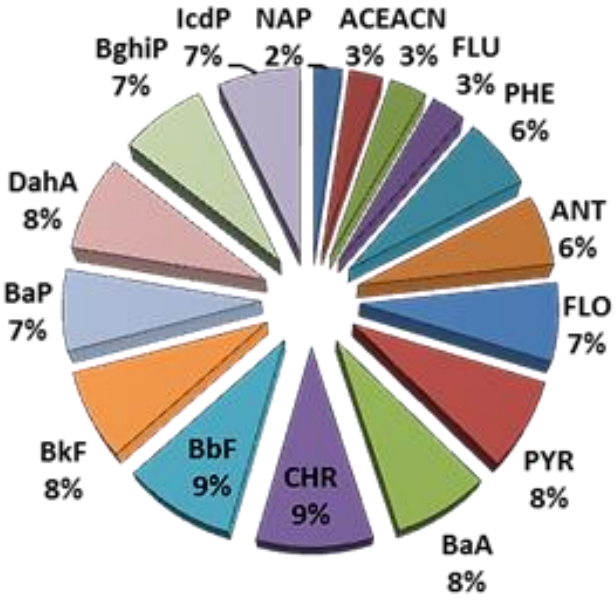

Figure 4. The percentage of each PAH in *Coptodon zillii* from the Shatt Al-Arab River.

from a variety of serious health effects (Olayinka et al., 2019). Using this standard as a guide, the study found that total PAH concentrations in all sampling areas exceeded 1000 ng/g dry weight, indicating that aquatic organisms in the area may be at serious risk to their health and the environment. Polycyclic aromatic hydrocarbon concentrations in soil or sediment can be classified as high risk (>1.0 mg/kg), medium risk (0.001-1.0 mg/kg), or low risk (<0.001 mg/kg) (Nozar et al., 2013). In the current study, the concentration of PAHs in all sampling sites exceeded 1.0 mg/kg, indicating a significant risk. All sediment samples from the study areas exceeded the USEPA's guideline value of 2.5 mg/kg.

Adsorbing PAHs in atmospheric particles can be deposited on the surface of lakes, streams, and oceans by dry or wet deposition, where they can be dispersed by currents and eventually integrated with sediment (Niu et al., 2017; Siudek et al., 2022). PAH deposition in the atmosphere affects sediments in urbanized areas. PAHs can also come from storm and sanitary sewer effluents and roadway runoff (Siudek et al., 2022). PAH concentrations were greater in sediment samples than in water samples (Al-Atbee, 2018; Awe et al., 2020). This could be attributed to their hydrophobic properties and proclivity in adsorbing particles and solid phases. They also settle and become part of the sedimentary record (Wu et al., 2001). Anthropogenic PAHs are primarily produced by the combustion of fossil fuels and the spillage of petroleum products, fuel combustion (pyrolytic), and crude oil (petrogenic) (Wang et al., 2015; Patel et al.,

Table 6. PAH toxicity guidelines (ng/g) for sediment matrices and PAH concentrations in the surface sediments of the Shatt Al-Arab River.

PAHs	Effects range low	Effects range medium	PAHs in Shatt Al-Arab River sediments
NAP	160	2100	2126
ACE	40	640	2120
ACN	20	500	1996
FLU	20	540	2296
PHE	240	1500	3717
ANT	90	1100	1136
FLO	600	5100	4477
PYR	660	2600	6413
BaA	260	1600	4011
CHR	380	2800	6415
BbF	---	---	5140
BkF	---	---	4227
BaP	430	1600	4462
DahA	---	---	3747
BghiP	---	---	4088
IcdP	---	---	3786

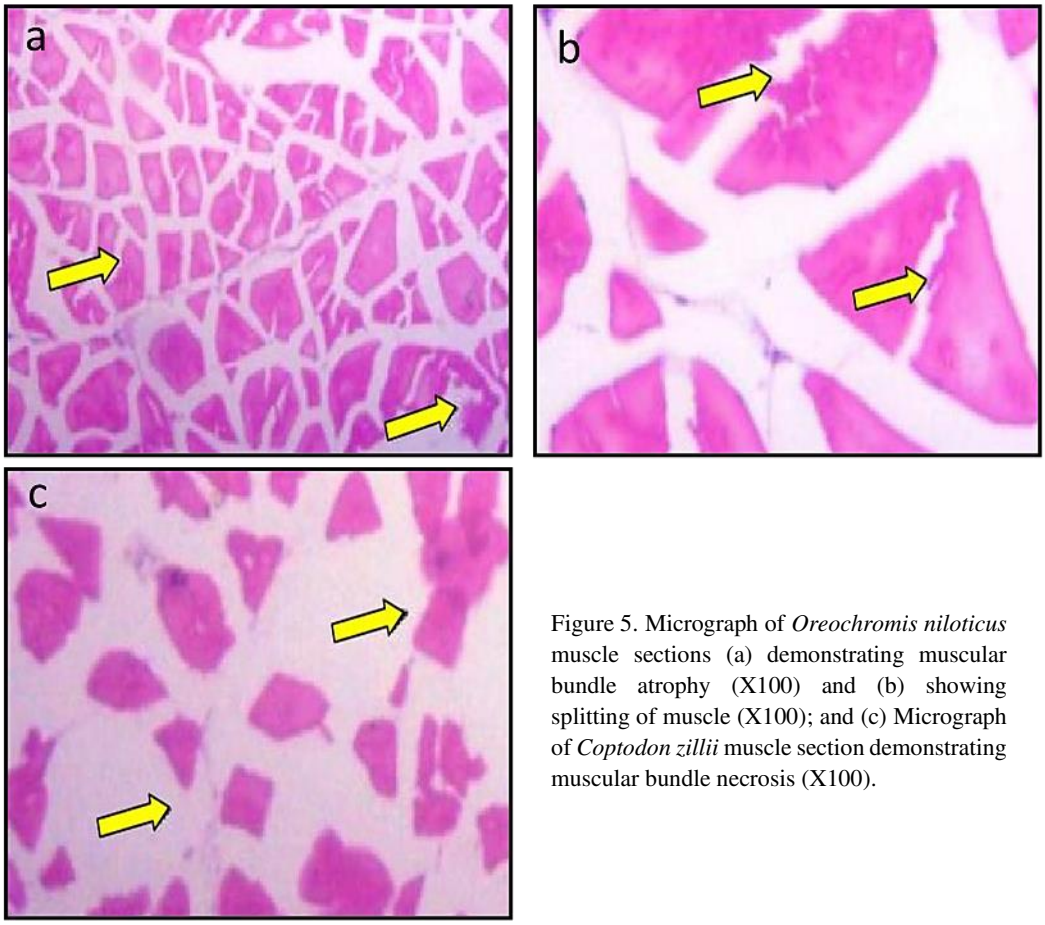

Figure 5. Micrograph of *Oreochromis niloticus* muscle sections (a) demonstrating muscular bundle atrophy (X100) and (b) showing splitting of muscle (X100); and (c) Micrograph of *Coptodon zillii* muscle section demonstrating muscular bundle necrosis (X100).

2020). Individual PAH compound ratios can be used to detect contamination due to differences in the composition and distribution patterns of PAHs as a function of the emission source (Yang et al., 2009). Ph/An and Fl/Py ratios are commonly used to distinguish between petrogenic and pyrogenic sources of PAHs. PAHs of petrogenic origin are typically associated with Ph/An values > 10, while low ratios < 10 are frequently produced during combustion processes (Zrafi et al., 2013). For the Fl/Py ratio, values > 1 indicate pyrolytic origins, while values < 1 indicate petrogenic sources (Tolosa et al., 2004). The Ph/An and Fl/Py ratios revealed that PAH sources in the Shatt Al-Arab River area could be both pyrolytic

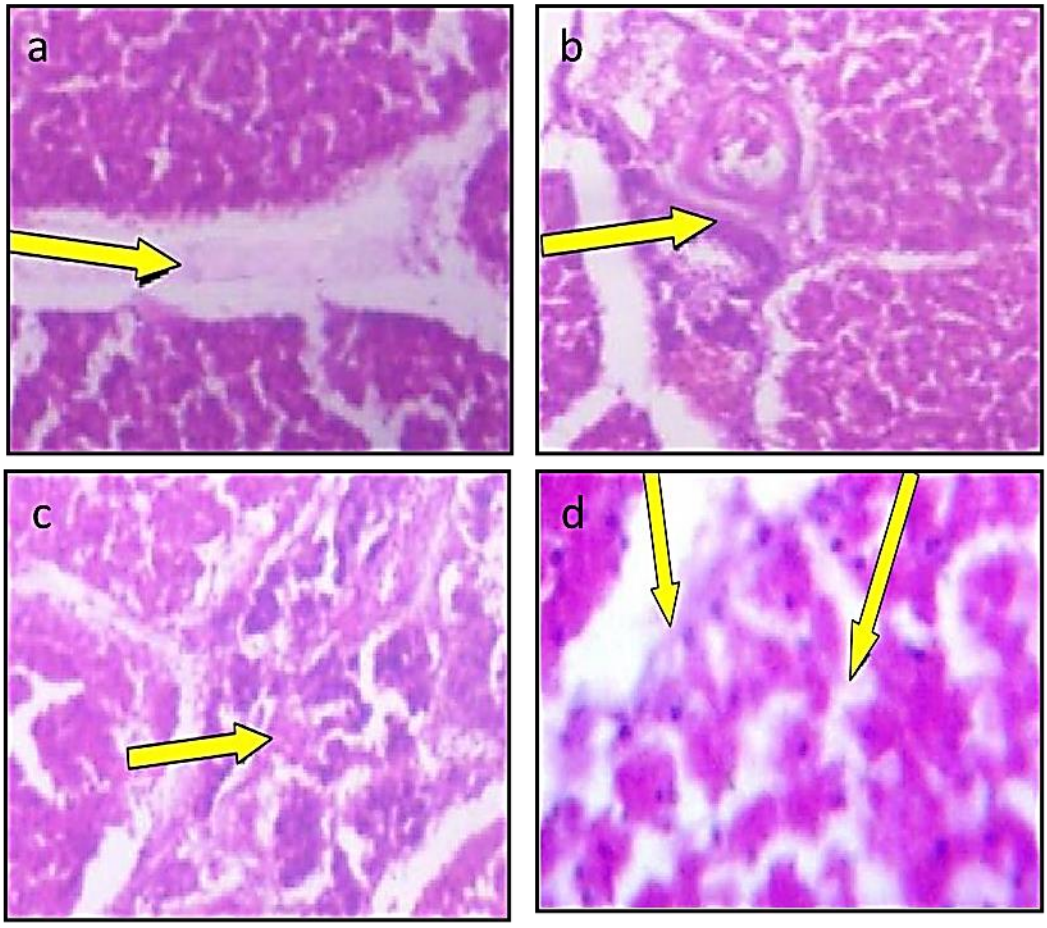

Figure 6. Micrograph of *Oreochromis niloticus* liver sections (a) demonstrating the focal region of hepatic-pancreas necrosis (X100), (b) showing degeneration and cellular degeneration (X100). Micrograph of *Coptodon zillii* liver sections (c) demonstrating the focal region of hepatic-pancreas degeneration (X100), and (d) Micrograph of O. niloticus liver section demonstrating the hepatocytes and nucleus (X100).

and petrogenic.

The potential toxicity of PAHs in sediments was assessed for fish. The sediments' PAH concentration was compared to the US National Oceanic's sediment quality standards (McGrath et al., 2019) (Table 6). Target values for effect range low (ERL) and effect range median (ERM) were used to assess the toxic effects in the sampling sites. A mild toxic effect is expected when PAH concentrations vary between the ERL and ERM values. Furthermore, PAH concentrations below ERL values are unlikely to have adverse effects. Fish in the Shatt Al-Arab River sampling sites are at high risk (Table 6). All PAH compound concentrations in the Shatt Al-Arab River exceeded the ERL and ERM values, indicating the possibility of harmful biological effects such as cancer and reproductive and physiological issues in fish.

The fish samples contained 16 PAH compounds (NAP, ACE, ACN, FLU, PHE, ANT, FLO, PYR, BaA, CHR, BbF, BkF, BaP, DahA, BghiP, and IcdP). In both fish samples, less carcinogenic LMW PAHs and more carcinogenic HMW PAHs were found. Total PAH concentrations varied significantly among fish species and sampling areas. Based on the results of the current work, the percentage composition pattern of PAHs detected in samples is based on the number of rings. The dominance of HMW PAHs in fish samples over LMW PAHs indicates the presence of significant pyrolytic processes (Honda and Suzuki, 2020; Jesus et al., 2020). The present study's findings revealed that the fish samples contained 16 PAHs, eight of which were prioritized by the USEPA. The concentration of PAHs in fish was higher than in the water column; this could be because PAHs are more easily absorbed by fish than other aquatic organisms when exposed to contaminated materials, resulting in elevated levels

compared to those in the surrounding medium (Zelinkova and Wenzl, 2015; Kere et al., 2019). Both fish species had lower total PAH concentrations than crabs in the Olayinka et al. (2019) and shrimp in the Ololade et al. (2008) studies. This could be because fish have been shown to have physiological mechanisms for rapid PAH biotransformation or depuration, which could be influenced by a variety of factors such as chemical exposure route and time, tissue lipid content, environmental factors, exposure to multiple contaminants, and differences in test animal species, age, sex, and health conditions (Olayinka et al., 2019).

The biotransformation of hydrophobic-containing substances in fish significantly impacts their toxicity, distribution, and excretion (Alani et al., 2012). Most of the world's population depends on seafood, particularly fish, to meet their nutritional requirements. More than 60% of the protein consumed in Iraq comes from fish, which is recognized as an important source of animal protein. Human exposure to a variety of contaminants, including PAHs, has been linked to food intake. Because of their high chemical stability and lipophilic nature, PAHs accumulate in fish fatty tissues following ingestion (Silva et al., 2011; Nwaichi and Ntorgbo, 2016). Fish can be exposed to PAHs through bioconcentration from water across their gills and skin and ingestion of PAH-contaminated particulate matter with food because PAHs readily adsorb onto particulate organic matter, particularly soil sediments (Zhao et al., 2014). Contaminants in fish, such as PAHs, reflect environmental contamination levels (Melo et al., 2022). According to the total PAH concentrations found in fish in this study, the Shatt Al-Arab River is heavily contaminated with PAHs.

The estimated dietary intake values in this study (0.00866 (8 PAHs) and 0.01288 (16 PAHs) mg/kg body weight/day) were significantly higher than values reported in other countries, which were, respectively, 1.77 to 10.7, 626 to 712, 13.8 to 16.7, and 231 ng/day body weight/day for Mumbai, India, Spain, Korea, and Kuwait (Falcó et al., 2006; Dhananjayan and Muralidharan, 2012). The fish, *C. zillii*, had the lowest TEQ value. Tongo et al. (2017) found that the TEQ values for PAHs in *Clarias gariepinus*, *Tilapia zilli*, *Ethmalosa fimbriata*, and *Scomber scombrus* were 0.22, 0.005, 0.30, and 0.03 mg/kg, respectively. Consuming the species with the lowest total mean concentrations of PAHs increases the risk of developing cancer, consistent with the study's findings. The calculated TEQ values agreed with the figures provided by Wu *et al*. (2012). The study's findings contradict those of a previous study of PAH contaminants in *Chrysichthys nigrodigitatus* in Rivers State, Nigeria, in which Effiong et al. (2016) found TEQ values higher than the estimated SV. Furthermore, TEQ values for PAHs in Iranian seafood (fish, crab, and bivalves) were reported to be higher than calculated SV (Nozar et al., 2013), indicating potential health effects. Our results are consistent with reports of lower estimated TEQ values than the SV in studies of PAH concentrations in *feral finfish* from a Hong Kong market (Tongo et al., 2017) and the common eel (*Anguilla anguilla*) from Italy's Tiber River (Patrolecco et al., 2010). However, the present TEQ value is higher than the USEPA SV (0.677 ng/g wet weight) for human fish consumption (USEPA, 2000). According to UNEP (2002) and White and Triplett (2002), the calculated screen value threshold is 0.677 ng/g (wet weight, the potency equivalent concentration) when the carcinogenic risk is 10-5, which is much lower than the wet weight contents of PAHs in fish discovered in this study. As a result, current fish species pose a much higher 10-5 carcinogenic risk.

Based on the results, the primary and secondary lamellae in the studied species showed significant hypertrophy and hyperplasia, epithelial cell hyperplasia, lamellar shortening, and fusion. Histopathological biomarkers are sensitive measures of subcellular stress in organisms exposed to various pollutants over short and long periods (Gupta et al., 2009). As a form of defense, the gills can adapt to toxic substances by increasing the distance of pollutant blood diffusion. The fusion of secondary lamellae caused by hyperplasia, which reduces free gas exchange, jeopardizes the fish's overall health.

Primary lamellar hypertrophy refers to an organ's enlargement or growth in response to an external stressor. This finding is consistent with reports of two species of sturgeons, *Clarias gariepinus* and *O. niloticus*, having a high incidence of hyperplasia in Zimbabwe's Sanyati basin (Fernandes and Mazon, 2003; Mabika and Barson, 2013). This finding suggests that the fish were subjected to stress. Gills are delicate organs that can be damaged by many contaminants, even in small amounts. According to reports, gills perform a variety of important functions (respiration, osmoregulation, and acid-base balance). Fish gills are a target organ for water-borne toxins due to their large surface area in contact with the environment and high sensitivity to chemical and physical changes in the aquatic environment (Evans et al., 2005; Hwang et al., 2011). Because of the harmful compounds in the aquatic environment, changes in organ structures and important gill functions have been observed (Tronosco et al., 2012). The degree of toxicity and exposure duration determine the harm's severity. If fish are exposed to these lesions for an extended period, they may die.

The muscle bundles of the fish examined in this work were split, and atrophy and *O. niloticus* had muscle bundle necrosis. Hexachlorocyclohexane, which can make animals hyperactive and excitable and cause lactic acid to be released, could have been the first stimulus that caused the muscle bundles to separate. As a result, muscle fatigue may have developed. It is possible that exposure to various toxins caused the muscle bundles to atrophy (Shah et al., 2020). Kandasamy (2011) reported that the use of rice pesticides caused changes in the muscular tissue of grass carp (*Ctenopharyngodon idella*). These changes included swelling and necrosis of the muscle bundles. Another study found that PCB and chlorinated pesticide bioaccumulation caused physiological and morphological abnormalities in the muscles of the freshwater fish *Hoplias malabaricus* from Ponta Lake in southern Brazil (Miranda, 2008). Shah et al. (2020) observed isolated areas of necrosis, inflammatory cell aggregation between muscle bundles, and muscle bundle degradation. The current study's findings support previous publications.

Coptodon zillii and *O. niloticus* livers exhibited necrosis, cellular degeneration, and hepatopancreas necrosis based on our findings in the current study. The body's largest organ is the liver, and it serves several critical physiological functions (Safahieh et al., 2012). Although every tissue in the body can metabolize chemicals, the liver is the primary organ for toxin metabolism or transformation, making it a target organ that is heavily influenced by toxins. "Necrosis" is the death of cells in a tissue or organ caused by a disease, an accident, or exposure to toxic or hazardous pollutants. The necrotic cells shrink, and their intercellular connections fail (Hedayati, 2016). Vascular dilatation may be responsible for liver cellular degeneration and necrosis. When the liver is injured, excess blood enters it, clogging the sinusoids. The most common cause of cellular degeneration in the liver is oxygen deprivation caused by gill deterioration (Javed and Usmani, 2019). The findings of this study were consistent with previous studies on the effects of various contaminants on fish liver (Hedayati, 2016; Weber et al., 2020).

Conclusions

This study's sediment samples contained PAHs with low and high molecular weights. The total PAH concentration in the sediment samples exceeded safe limits, implying that aquatic organisms in the Shatt Al-Arab River may pose significant human health and environmental risks. The high ratio of HMW PAHs to LMW PAHs indicates that PAH pollution in the Shatt Al-Arab River may be human-caused. Total PAH levels in fish samples revealed that the Shatt Al-Arab River was highly contaminated with PAHs. The fish had high total PAH levels, indicating that the chemicals had bioaccumulated in their tissues and organs over time. The estimated TEQ levels exceeded the SV of the USEPA, suggesting possible negative impacts on health. According to histopathological analysis, fish were exposed to high levels of PAHs, resulting in changes in the morphologic structure of these organs, muscle bundle necrosis, and cellular deterioration.

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