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Fate of Oil Hydrocarbons in Fish and Shrimp after Major Oil Spills in the Arabian Gulf

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Pollution of the marine environment with crude oil represents one of the most serious environmental problems that confront Saudi Arabia and other Gulf states. Oil pollution in the Arabian Gulf environment may affect the inhabitants through 1) human health hazard resulting from the consumption of contaminated sea food 2) loss of food due to alteration of species productivity or elimination of some species, and 3) deterioration of recreation areas. Moreover, the problem of oil spill may be more severe in this part of the world. This is mainly because the source of drinking water in various Gulf states depends largely on sea water from which desalinated water is produced. Contamination of sea water with crude oil may adversely affect the quality of desalinated water and may badly damage desalination plants.

During the last twelve years, the Arabian Gulf has been affected by two major oil spills. The first spill occurred on February 4, 1983 during the Iraq-Iran War, where about 4,000 barrels per day of crude oil from the Nowruz oil field were introduced into the Gulf for several months (Fayad 1986). The spill has caused a severe environmental problem in the area.

The second major oil spill occurred during the 1991 Gulf War. It has been estimated that about 10.8 million barrels of crude oil were introduced into the Gulf (Tawfiq 1993). The oil was released from several sources including the export terminals in Kuwait and many other sunken oil tankers in the war zone. Moreover, it is estimated that about six million barrels of crude oil and 100 million m^3 of gas were burning every day (El-Desouky and Abdulrahmeem 1991).These quantities were reduced with time as more oil wells were capped (Environmental Protection Council 1991). The fall-out from the unburned oil has worsened the oil pollution problem in the Gulf. The large quantities of spilled oil were expected to induce a serious environmental problem. The shallow, high temperature, high salinity water and poor circulation of the Gulf may also increase the vulnerability of the Gulf environment to degradation by spilled oil.

Although the Arabian Gulf has suffered from many oil spill incidents in the past, there is very limited published information about the levels of oil hydrocarbons in edible fish (Al-Yakoob et al., 1993; Krahn et al., 1993; Fowler et al., 1993; Readman et al., 1992; Douabdul et al., 1987).

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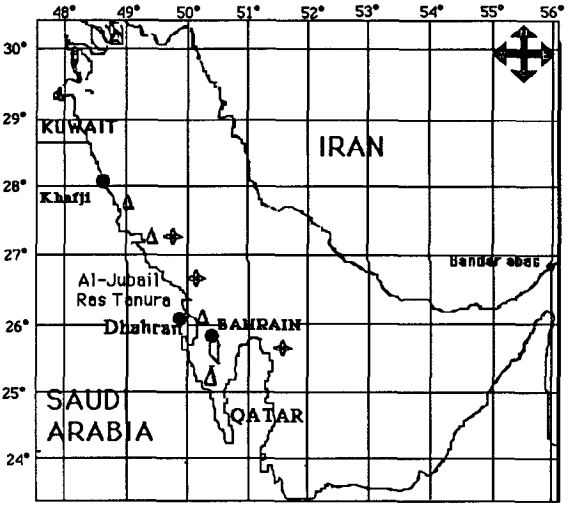

Figure 1. A map of the Saudi coastline of the Arabian Gulf showing the 1983 (⊕) and the 1991 (Δ) sampling sites.

Following the 1983 Nowruz oil spill, fishing in the Arabian Gulf was banned by the Saudi authorities for some time, and a comprehensive study was carried out to assess the effect of the spilled oil on fish from the Gulf. A similar study was conducted after the 1991 oil spill that occurred during the Gulf War. This paper summarizes the results of both studies which were carried out to assess the extent of contamination of various fish species of commercial value from the Arabian Gulf with oil hydrocarbons.

MATERIALS AND METHODS

During the first study carried out in 1983, five fish species were collected from the fishing ground in the Arabian Gulf. Shrimps (*Penaeus semisulcatus*) were collected from a shrimp fishing ground near Safaniyah. Following the 1991 oil spill fish were collected from several locations along the Saudi Gulf coast on three occasions. Samples were collected in February 1991 (about one month after the Gulf War), August 1991, and February 1992. Five species of fish of commercial value were also collected in this study. Sampling locations for both studies are shown in Figure 1.

About 30 g of edible muscle tissues were dissected from each fish sample using hexane-rinsed tools. In the case of shrimp (*Penaeus semisculcatus*), several individuals of shrimp were selected at random from each batch and the tissues were removed from the shells and pooled together to make 30 g. Each tissue sample was homogenized with 40 mL of organic free water for about 1 min at 50,000 rpm. The homogenate was then digested in methanolic potassium hydroxide. The tissue digestate was extracted successively with three 50-mL portions of hexane. The combined extract was then filtered through a bed of anhydrous sodium sulfate and concentrated to 1 mL using Kuderna - Danish concentrator.

The hexane concentrate was then passed through a chromatographic column to clean up the sample and to separate the aliphatic and aromatic components. The glass column (30 cm x 0.5 cm I.D.) was filled under hexane with 4.5 g of silica gel and 1.5 g of alumina. The aliphatic fraction was eluted with 15 mL of hexane, after which the aromatic fraction was eluted with 25 mL of hexane-methylene chloride (7:3 v/v). The final solvent for the aromatic fraction was replaced with hexane for GC and GC/MS analysis, and with acetonitrile for HPLC analysis. The detection limit for n-alkanes and PAHs compounds were 0.1 µg/kg and 0.02 µg/kg, respectively. The final volume was made up to 1 mL using a slow stream of nitrogen at room temperature. One method blank and one tissue spiked with both aliphatic and aromatic standards were processed with each set of samples. This spiked sample provided the data necessary to evaluate the recovery and efficiency of the method.

The aliphatic hydrocarbons were analyzed using a Varian 6000 Gas Chromatograph (GC) equipped with a flame ionization detector (FID). The analytical column used was a fused silica capillary column (30 m x 0.25 mm I.D.) coated with 0.25-µm film of SE-54. The polycyclic aromatic hydrocarbons were analyzed using a Waters Model 990 HPLC system equipped with UV and fluorescence detectors. A Vydac 201TP54 HPLC column (25 cm x 4.6 mm I.D.), which is dedicated for PAH analysis was used in this study. The column was eluted with acetonitrile water at a flow rate of 1.5 mL/min. Gradient conditions were programmed from acetonitrile:water (50:50 v/v) for 3 min, then linear gradient elution to 100% acetonitrile over 17 min and to a final hold for 15 min. The system was programmed back to initial conditions over 25 min and equilibrated for 20 min between injections. Polycyclic aromatic hydrocarbon (PAH) standard compounds used for the calibration of the HPLC system were obtained from the National Bureau of Standards (NBS SRM 1647a, USA). Some fish extract samples were also analyzed by GC/MS using a Finnigan OWA-30 GC/MS system. The mass spectrometer in the electron impact mode was scanned from 45 to 450 amu in 1 second. Helium was used as the carrier gas.

RESULTS AND DISCUSSION

Following the 1983 Nowruz oil spill, 94 fish samples were analyzed for oil hydrocarbons. The results of the analyses have shown that aliphatic hydrocarbons of a biogenic origin exist in the muscle tissues of all samples analyzed. However, only limited numbers of samples have produced evidence of contamination by oil hydrocarbons. The concentrations of n-alkanes in the tissues of various fish and shrimp samples ranged from 0.1 to 3,948 µg/kg wet weight and 0.1 to 464 µg/kg wet weight, respectively (Table 1). In general, most of the biota samples had less than 500 µg/kg wet weight of n-alkanes. It has been noticed that concentrations of oil hydrocarbons in fish populations from the Gulf were found to vary according to fish species and sampling location. N-alkane concentrations in the Hamour (*Epinephelus multinotatus*) populations collected from the nearshore fishing grounds between Ras Tanura and Jubail (Figure 1) appear to be about three times higher than the concentrations found in Hamour (*Epinephelus multinotatus*) collected from offshore populations. This is mainly due to the fact that the nearshore area is adjacent to an active offshore oil producing field and thus is impacted by spills originating from drilling platforms as well as tanker traffic in the area. El Samra et al. (1986) have shown that the mean total oil hydrocarbon concentration of 546.4 µg/L in the Gulf waters of the Ras Tanura area compared to 4.14 µg/L measured in other parts of the Saudi Gulf water. Moreover, most of the

n-alkanes present in the offshore samples were found to be of a biogenic origin as opposed to those found in nearshore samples. In the present study, the difference between hydrocarbons synthesized by marine organisms and those introduced into the marine environment by human activities was based on the guidelines set by Clark and Brown (1977). These guidelines include the carbon preference index, the presence of the unresolved complex envelope, and the presence of pristane and phytane. Pristane, a branched chain compound, is the most abundant alkane present in plankton and fishes. Phytane, the other branched alkane, is not known to be produced by marine organisms (Clark 1967; Youngblood 1971). A typical example that shows the differences between biogenic and petrogenic oil hydrocarbons in fish tissues is illustrated below. Reconstructed ion chromatogram (RIC) obtained by GC/MS for aliphatic hydrocarbons of a *Hamour* (*Epinephelus multinotatus*) muscle tissue collected from offshore and nearshore areas in the Gulf are shown in Figures 2 and 3, respectively. Figure 2b shows a full scale RIC of the offshore sample, indicating the presence of pentadecane (C-15), heptadecane (C-17), and pristane . Octadecane (C-18) and phytane were absent. Figure 2a shows the expanded RIC for the same sample. Other members of the n-alkane homologs and the unresolved complex mixture were also absent. Figures 3a and 3b obtained for the nearshore sample clearly show the presence of a wide range of n-alkane homologs as well as pristane and phytane, in addition to the unresolved complex mixture (UCM), which gives further evidence of petrogenic contamination with oil hydrocarbons.

Table 1. Concentration of oil hydrocarbons (µg/kg) wet weight found in fish and shrimp sample collected from the Gulf after the 1983 Nowruz oil spill

Fish species	Number of samples	PAHs (µg/kg)	n-Alkane (µg/kg)	N-alkanes and PAH detected
Epinephelus multinotatus (Hamour)	43	0.02 -34,045	0.1 - 1,441	C15 - C28 (Only nearshore samples were contaminated), PAH (Benz(a)anthracene*, pyrene*, Chrysene**.)
Lutjanus malabaricus (Hamrah)	15	0.02 -17,245	0.1 - 3,178	C15 - C28, PAH (Benzo(a)anthracene* and phenanthrene*)
Lethrinus sp. (Shaeri)	18	0.02 - 2,669	0.1 -1,194	C15 - C28, PAH (Benz(a)anthracene*, Benz(e)- pyrene**, Chrysene**, perylene**)
Siganus rivulatus (Safi)	18	0.02 - 2,296	0.1 - 1,134	C12 - C22, PAH (Benz(a)anthracene*, Chrysene** and pyrene**)
Rastrelliger kanagurta (Baagha)	10	0.02 - 4,495	0.1 - 3,948	C12 - C28, PAH (Benz(a)anthracene* and perylene**)
Penaeus semisculcatus (Shrimp)	11	0.02 -1,029	0.1 - 464	C12 - C28, PAH (Benzo(a)anthracene**, Benzo(a)pyrene**, peryl-ene**, and triphenylene)

* *Identification and quantitation based on GC analysis,*
***Identification and quantitation based on GC/MS analysis.*

Sediments in areas where fish were collected were also found to be related to levels of hydrocarbons in the fish. The n-alkane concentrations found in Hamrah and Safi *(Siganus rivulutus*) fish samples collected from areas characterized by sandy sediments were three and eight times higher than those collected from areas with hard rocky substrates, respectively. The lowest concentration of oil hydrocarbons was found in Shaeri (*Lethrinus sp.*) fish.

A box-and-whisker plot obtained for the n-alkane contents of various fish species is shown in Figure 4. The rectangular boxes of the plot cover the middle 50% of the data values between the lower and upper quartiles. The whiskers (straight lines)

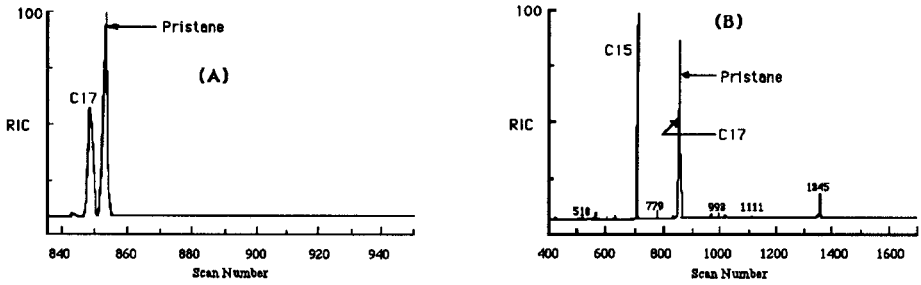

Figure 2. (A) An expanded RIC for the aliphatic hydrocarbon fraction obtained from the muscle tissue of Hamour (*Epinephelus malabaricus*) collected from noncontaminated area from the Gulf. (B) A full scale RIC for the same sample.

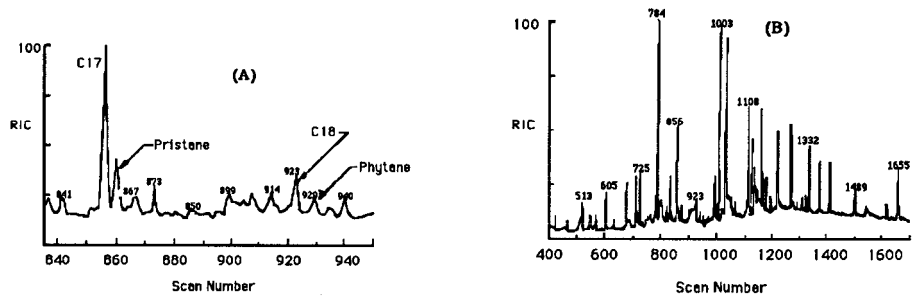

Figure 3. (A) An expanded RIC for the aliphatic hydrocarbon fraction obtained from the muscle tissue of a Hamour (*Epinephelus malabaricus*) from a contaminated area. (B) A full scale RIC for the same sample.

extend out to the minimum and maximum concentration values, while the central line is at the median. The whiskers extend only to those points that are within 1.5 times the inter quartile range. All other outlier values which occur far away from the bulk of the data are plotted as separate circles. As indicated in this figure, the highest median concentration level for n-alkanes was found in Baagha (*Rustrelliger kanagurta*) fish followed by Hamra fish. In general, the median concentrations for n-alkanes were less than 750 µg/kg.

Polycyclic aromatic hydrocarbons (PAHs), which are a strong indicator of petrogenic hydrocarbon contamination, were detected in a limited number of fish samples. The concentration levels of PAHs in various biota samples, as measured

by capillary column gas chromatography, were found to range between 0.02 to 34,045 µg/kg. The high concentration values may be attributed to interferences from many other naturally occurring organic compounds, which remain in the extract after the column chromatography clean-up process. In many cases, GC results obtained for the aromatic extract fractions of the Hamour (*Epinephelus multinotatus*) fish produced extremely complex chromatographic patterns, which makes the identification of PAHs by GC alone an impossible task. Therefore, the use of GC/MS becomes necessary to confirm the results of the analysis. PAHs identified by GC/MS in various samples including chrysene, pyrene, benzo(e)pyrene, benzo(a)anthracene, perylene and triphenylene.

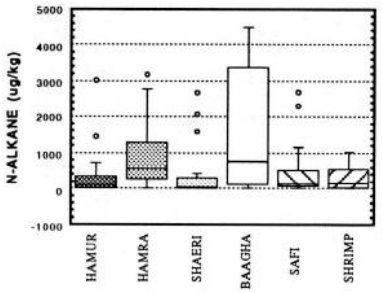

Figure 4. Box and Whisker plot showing n-alkanes concentration in biota samples collected from the Arabian Gulf following the 1983 Nowruz oil spill.

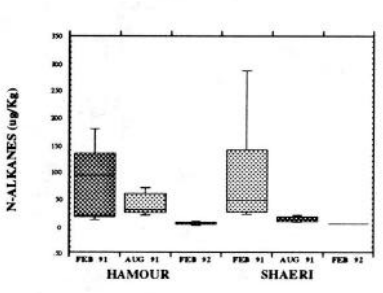

Figure 5. Box and Whisker plot showing the decrease with time of the n-alkanes concentration in muscle tissues of Hamour and Shaeri fish following the1991 Gulf War.

The results of the study indicated that the Nowruz oil spill did not contribute to the concentrations of oil hydrocarbons found in the muscle tissues of fish from the Arabian Gulf. Review of the literature have shown that there is no health hazard standards for oil hydrocarbons in fish. However, comparison between the levels of oil hydrocarbons found in the Gulf fish and those found in fish collected from unpolluted areas, it was concluded that, no human health hazard may occur as a result of consumption of fish from the Gulf.

The analyses of fish and shrimp samples collected from the Arabian Gulf following the Gulf War have produced evidence of contamination of some samples with oil hydrocarbons. The results have shown that most of the samples were contaminated with aliphatic hydrocarbons (n-alkanes). The maximum concentrations for both aliphatic and aromatic hydrocarbons were found in fish samples collected in August 1991 (about 8 mon after the Gulf War). No PAHs were detected in the biota tissues collected in February 1992. In 40% of the Hamour *(Epinephelus multinotatus)* fish samples the n-alkanes have a relatively wide coverage between C 12 to C30; the rest of the samples contained only few n-alkanes such as C15, C17 and pristane. The presence of only odd numbered n-alkanes indicates the biogenic origin of these compounds. Similar results were obtained for Shaeri (*Lethrinus sp.*) fish. Seabream and Sawdah fishes have shown a wide range of n-alkanes distribution in most of the samples which may indicate a petrogenic origin. A box and whisker plot obtained for n-alkanes in Hamour (*Epinephelus multinotatus*) and Shaeri (*Lethrinus sp.*) fish is shown in Figure 5. It can be seen from this figure that the median concentration of n-alkanes in the fish tissues decreased with time following the oil spill.

Polycyclic aromatic hydrocarbons (PAHs) were detected in the muscle tissue of few fish samples after the 1991 Gulf war. PAHs concentration measured in various fish species ranged from 0.02 to 24.4 µg/kg wet weight. The PAHs concentration range found in Shaeri (*Lethrinus sp*.) was 2.7-20.6 µg/kg, while the highest concentration range was found in Hamour (*Epinephelus multinotatus*), 0.02-24.4 µg/kg. The absence of PAHs in the muscle tissues of most fish samples analyzed may be attributed to the ability of the fish to metabolize most PAHs in their livers before they are concentrated in the bile for excretion (Krahn and Malins, 1982; Hellou and Pyne 1987; Varanasi, et al. 1989). Gupta et al. (1993) have shown that, oil hydrocarbon contents of fish samples collected from the Northern Arabian Sea following the 1991 oil spill ranged between 0.47 - 3.67 µg/g wet weight. The average total amount of PAHs in the edible part of the fish reported recently (Al-Yakoob et al., 1993) was 105.3 µg/kg dry weight, with a range of 2.51-563.6 µg/kg dry weight; the highest individual PAHs concentration was for pyrene (79.3 µg/kg dry wt.), whereas the lowest concentration was for chrysene (0.05 µg/kg dry wt.).

Krahn et al., (1993) analyzed subtidal sediments and fish bile from the Arabian Gulf for petroleum-related aromatic compounds. They found that phenanthrene equivalents in the bile composite from Shaeri (*Lethrinus sp.*) displayed a wide range of concentrations (860 - 60,000 µg/kg). Recently, Fowler et al. (1993) have reported that the concentration of oil hydrocarbons measured using fluorometric method as chrysene equivalent in Shaeri fish collected from the Arabian Gulf ranged from 1.8 to 57 µg/g. These concentrations were much higher than those reported in the present study. This may be attributed to the poor selectivity of the fluorometric methods used for measuring PAHs in biota tissues.

The concentrations of n-alkanes in fish samples collected during the 1991 oil spill were in the range of 0.01 to 335 µg/kg wet weight. PAHs concentrations measured by HPLC were in the range of 0.02 to 24.4 µg/kg wet weight. These concentrations were much less than those measured in fish collected following the 1983 oil spill using capillary GC and GC/MS techniques. PAHs identified by GC/MS in fish samples collected in 1983 included chrysene, pyrene, benzo(e)pyrene, benzo(a)anthracene, perylene and triphenylene.The study has also indicated that capillary GC analyses of marine tissues for PAHs suffered from severe interferences from the biogenic organic compounds which remain in the tissue extracts even after extensive clean-up .

It appears that the 1983 and 1991 Gulf oil spills did not affect the edibility of fish caught near the Saudi Arabian coastline of the Arabian Gulf. Generally, the effect of oil spill on fishery in the Gulf seems to be of a short-term, transitory impact. However, the long-term impact of the spill is associated primarily with low energy areas of the shoreline, marshes, mud flats, sandy and rocky beaches, and the shallow subtidal benthic communities such as seagrass beds or soft-bottom areas. These biotopes, which provide the feeding and nursery grounds for the commercially important fish species, may be substantially affected by the Gulf oil spill.

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