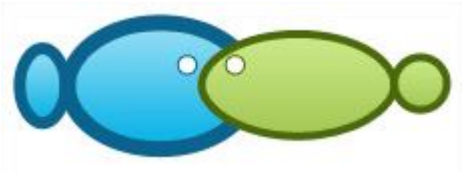

Uptake, tainting and depuration in common carp (*Cyprinus carpio*) exposed to the water soluble fraction (WSF) of crude oil

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Abstract. In short-term experiments, common carp (*Cyprinus carpio*) was subjected to a water-soluble fraction (WSF) of light crude oil (Nahran-Omar) to verify whether it could lead to fish tainting. The WSF was prepared by mixing the oil with cold river water by stirring. The main constituents of WAF were aromatics with low boiling points, although these compounds only made up a small portion of crude oil. Fish with a lipid content of 3% (550 g) and 9% (1750 g) wet weight were exposed to concentrations (0.5, 0.9, and 1.6 ppm) of WSF for 8 hours. The taint sensory panel assessment was achieved and the evaluation results were compared with the hydrocarbons analysis data obtained by capillary gas chromatography (GC). Although the fish exposure time was very short, the fish flavor was statistically different ($p < 0.01$) from control, even if the WSF concentration (0.5 ppm) was low. The uptake of hydrocarbons by fish tissues was closely related to the lipid content. High-lipid fish (55 ppm) uptake more hydrocarbons than low-lipid fish (32 ppm). Naphthalene and toluene were the main hydrocarbons that accumulated in fish tissues. High-lipid fish (i.e. 9% wet weight) need at least 12 days to depurate and their depuration rate was slow. High molecular weight aromatics were slower depurated in fish than low molecular weight compounds. The accumulation of hydrocarbons was great in the belly flap muscle (53 ppm), followed by red (52 ppm), lower flank (50 ppm) and white (30 ppm) muscles respectively. Even after 30 days of depuration, the fish muscles were not hydrocarbon free.
Key Words: fish tainting, WSF, hydrocarbons, oil pollution.

Introduction. Hydrocarbons are generally provided to the aquatic environment through three main sources: synthesis by organisms (biosynthesis), geochemical processes and human (anthropogenic) inputs (Turki 2016). Consequently, the hydrocarbons in aquatic ecosystems are either biogenic or abiogenic (pollutants) origin. Hydrocarbon pollutants are introduced into the aquatic environment from a variety of sources including: direct atmospheric fallout, chronic leakage of industrial waste and sewage, accidental discharge during transport operations, use and disposal of refinery products, natural oil spills, and runoff from the land and water, etc. (Parinos et al 2013; Inyang et al 2018). Adzigbli & Yuewen (2018) reported that between 4 and 6 million tons of oil may be introduced into the aquatic environment annually from non-biogenic sources.

The study of the hydrocarbon pollutants effects on aquatic ecosystems in recent years has received much attention. The overall effects of petroleum hydrocarbons on aquatic organisms have been studied in diverse ways (Jiang et al 2012; Incardona et al 2014; Langangen et al 2017). Beyer et al (2016) demonstrated important variables to consider when assessing the effects of petroleum hydrocarbons on aquatic organisms experimentally. These are the levels at which hydrocarbons accumulate, the period of the remaining hydrocarbons in the organisms, and the composition of hydrocarbons in water and organisms. Many researchers have focused on studying hydrocarbons accumulation, depuration and retention in aquatic organisms (Ololade et al 2011; Almeda et al 2013; Sørensen et al 2019), due to their great commitment to understanding hydrocarbons pollution problems. Various aquatic organisms, including fish and other species, have been used in studies of toxicity, accumulation, and depuration of hydrocarbon

contaminants (Butler et al 2016; Akinsanya et al 2018). Crude oil, water soluble fraction (WSF) of crude oil and individual hydrocarbons such as benzene, toluene, xylene, naphthalene, etc. can be used as ideal contaminants (Bamidele & Eshagberi 2015; Hodson 2017).

Several authors (Meador et al 2010; Maurya et al 2018, 2019) have also conducted extensive investigations of environmentally persistent organic pollutants such as pesticides and polychlorinated biphenyls (PCBs), etc. Significant differences in specific species have been found in the uptake, accumulation, depuration and retention rates of these pollutants by aquatic organisms (Uekusa et al 2017; Maurya et al 2019).

It has been found that lipids are the biological factor that plays a major role in the behavior of organic pollutants in the tissues of aquatic organisms. The higher the lipid content in tissues, the more likely it is to accumulate and maintain pollutants in the tissues (Akinsanya et al 2018; Ambrosio et al 2018). Studies have shown that the main pathway for the entry of organic pollutants into aquatic organisms is by the partitioning of pollutants between water and body lipids (Schäfer et al 2015; Ko et al 2018). Although there are many studies on organic pollutants in aquatic organisms, the role of lipids in tissues in controlling the uptake, depuration and retention of pollutants in organisms is still not entirely clear. Therefore, basic studies on the distribution and isolation of lipids from the tissues of aquatic organisms are an important step in fully understanding the interactions between pollutants and organisms (Parrish 2013).

Common carp (*Cyprinus carpio*) is an important fish in fisheries and aquaculture in some of the world's freshwater areas. It is a fatty fish that usually contains 2-10% of total lipid (wet weight) (Ljubojević et al 2017). These factors stimulate the choice of this fish in the current study as a good candidate for studying the role of lipids in the process of uptaking and retaining petroleum hydrocarbons.

The WSF of crude oil were used as pollutants in this study because most of the major effects of oil spill incidents come from contact of water-soluble hydrocarbons with aquatic organisms more than the effects of oil slick or dispersed oil (Olaifa 2012; Lee et al 2015). The WSF includes a wide range of volatile or low-volatile hydrocarbons that are mainly rich in aromatic compounds from benzene to alkyl naphthalenes (Rodrigues et al 2010; Perrichon et al 2016). These hydrocarbons are toxic, carcinogenic and soluble in water, which can accumulate in the tissues of aquatic organisms when a petroleum spill occurs (Sharanagouda & Karegoudar 2001). It is expected that there will be a difference in the concentration of crude oil WSF in the aquatic environment. High concentrations of WSF can be found near oil spills. However, the concentrations of WSF in organisms or water gradually decrease to reach background levels in a specific period of time, unless the oil continues to be released from spills (Lee et al 2015).

Common carp, like most fish in a natural aquatic environment, can avoid pollution with WSF of crude oil (Bukola et al 2015), but on farms, it cannot avoid this type of pollution. The natural conditions these fish may encounter are high levels of WSF for short periods of time or low levels for long periods. The first possibility is, in fact, the situation in which common carp stocks are exposed for a few days to a specific oil spill, which can certainly happen on Iraqi common carp farms located in the Shatt Al-Arab River near the movement of oil tankers where the opportunities for oil spills increased. Exposure to a low level of WSF along the river may also occur as a result of normal vessel activity or where hydrocarbons are spilled into natural drainage systems. To understand the effects of WSF crude oil on common carp under these two conditions, a set of fish exposures to WSF must be performed to uncover key elements that control the behavior of these pollutants. While, there are some studies which have been done with the toxic effects of petroleum hydrocarbons on common carp behavior and physiology (Nasir & Hantoush 2010; Farid et al 2016), there was no data available in scientific literature on the tainting of these high-lipid fish by the WSF of petroleum. The main objective of our research was to conduct a set of short-term exposure experiments with common carp fish using different concentrations of WSF of crude oil to detect threshold levels that could taint the fish. As well as studying the rates of uptake and depuration of hydrocarbons in fish tissues and determining the relationship between lipid content and whole muscle and different sections of muscle tissue.

Material and Method. Nahran-Omar crude oil (light-API gravity > 34), obtained from the Iraqi South Oil Company (ISOC), was used to prepare the water soluble fraction (WSF) in recent experiments. The crude oil had the characteristics described in Table 1, according to Ali et al (2013). The oil was stirred with filtered cold river water (obtained from Shatt Al-Arab River with a salinity of about 5.3 ‰) at a ratio of 1:99 v:v for 1 day into a stainless steel mixing vessel (500 L) with a vigorous mechanical stirrer and a drain on the bottom. The mix was allowed to settle for 2 days, then the WSF was kept cool. The stock solution of WSF was prepared promptly prior to use in the tests.

Table 1

Properties of Nahran-Omar crude oil

Index	*Value*
Density by 20°C, kg m^{-3}	856
Sulfur content, % of mass in crude oil	0.73
Fraction (i.b.t.-180°C)	0.029
Fraction (180-360°C)	0.64
Water content, % of mass	Absence
Content of mechanical impurities, % of mass	Absence
Concentration of chloride salts, mg dm^{-3}	23.01
Content of paraffin in crude oil, % mass	3.0
Temperature, °C freezing point of kerosene fraction	- 59°C
Pour point of diesel fraction	- 12°C
Content of fractions boiling, % mass	
Up to 200°C	34.35
Up to 350°C	59.87

The current experiments were carried out at the environmental pollution laboratory, University of Basrah, in fiberglass exposure aquariums (2000 L) with plastic caps and overflow system. The aquariums were filled with diluted stock solution of WSF and the fish were subsequently cautiously put into the aquariums. The aquariums were well covered during the experiment to maintain the stability of hydrocarbons profile in the exposure water. Steady concentrations of oxygen and WSF in the exposure water were maintained by the proportionate and continuous addition of oxygenated river water and the stock solution of WSF from the aquariums top at a flow rate of 255 and 475 mL min^{-1} respectively and an oxygen level of 80-100% saturation. Samples of WSF stock solution were taken at a beginning and end of the experiment and analyzed to determine the WSF concentration. The oxygen level and the flow rates of diluent river water and WSF stock solution were monitored every hour during the exposure period and a suitable amendment was done if needful.

Live fish common carp were obtained from the ponds of Marine Science Center of Basrah University. Fish have been held in aerated filtered river water and fed in the laboratory for 9 weeks, from June to August, 2019. A groups of 15 fish with an average weight of 550 g and muscle lipid content of 3% wet weight were placed in three aquariums and subjected to different concentrations (0.5, 0.9 and 1.6 ppm) of WSF for 8 hours. Other groups of fish (15) with different muscle lipid content of 3% and 9% (1750 g) wet weight were only exposed to 1.6 ppm of WSF for 8 hours and then depurated at different intervals: 1, 4, 12, 19 and 32 days in clean river water. The fish were starved for 1 day before exposure. The water temperature was 20.5-21°C and the photoperiod was adjusted to 12 hours light/12 hours dark. The tests were performed with control treatment by placing the fish in a clean river water (without WSF) aquarium and with three replicates.

At the end of the experiments, the fish was killed by hitting it on the head and then washed well with water. The fish guts and skin were removed, then filleted and frozen at -45°C for sensory assessment and analysis of hydrocarbons by gas chromatography (GC), taking samples of liver, whole muscle, belly flap, lower flank (the flank part below the lateral line and above the belly flap), red and white muscles. For fish

with 3% lipid content, the hydrocarbons were determined only in liver and whole muscle tissues.

Hydrocarbons were extracted from fish tissue by the stream distillation method of Ackman & Noble (1973). Distilled water (80 mL) were placed in an extraction flask (250 mL) and boiled. After collecting 20 mL of water condensate, the flask was cooled and the water condensate was removed. Fifty g of thawed and minced fish tissue were appended to the flask. Dichloromethane (1 mL) was then added. The distillation continued until 20 mL of condensate was collected. The condensate was poured into a centrifuge tube (50 mL) ice-cooled. Dichloromethane (200 µL) containing heneicosane (internal standard) was then added. The mixture was vortexed for 1 minute and centrifuged at 1500 rpm for 5 minutes. Finally, the dichloromethane layer was removed from the tube and concentrated to 5 µL for hydrocarbons analysis. The distillation was done in triplicate. Control tissues were distilled in the same procedures of tainting tissue samples.

Extraction or WSF from water exposure samples was performed using the procedure of Sørensen et al (2019). The WSF (985 mL) was put in the extraction flask. One mL of hexane was then added and the flask was closed. The mixture was shaken for 1 minute and allowed to settle for 15 minutes at 5°C. Two µL of hexane layer was removed by Hamilton syringe (10 µL) containing 0.2 µL of hexane and 1 µL of heneicosane. The contents were then injected into a device for hydrocarbon analysis.

The hydrocarbons analyses were accomplished with an Allegent capillary gas chromatography (GC) (Agilent, USA) with flame ionization detector (FID) (Agilent, USA) and a split injection system. The chromatography was conducted on Agilent US2463233H DB-petro methyl silicone fused silica capillary column (100 m × 250 µm ID × 0.5 µm film thickness). The operating temperatures for FID and injector were 300°C and 320°C, respectively. The initial temperature of column was 60°C held for 4 minutes while the final temperature was 280°C held for 30 minutes and the rate was 4°C minutes^{-1}. The carrier gas was helium (99.999%) with a flow rate of 1.5 mL minute^{-1}.

GC-MS analyses were achieved using a Shimadzu gas chromatography 15A-Mass Spectrometry QP-1000A (Shimadzu Scientific Instruments, Kyoto, Japan) provided with data processor and library search system LSS-20 for spectral data. The chromatography was carried out on a DP-5MS fused silica capillary column (30 m × 0.25 mm ID × 0.25 µm film thickness). Injector temperature was 250°C and the injected volume was 1 µL. Helium gas was used as carrier gas at a constant flow of 1.5 mL minute^{-1}. The column temperature was programmed at 45°C for 16 minutes and then at a rate of 12°C minute^{-1} to 280°C for 24 minutes. The MS operating conditions were: Electron impact (EI) ionization, ion source 230°C, electron energy 70eV, interface temperature 280°C. The mass range scanned was from 50 to 550 amu. The compounds were identified using the spectra of reference compounds from mass spectra libraries.

Thirteen hydrocarbon standards (99% purity) were used to evaluate the recovery efficiency of hydrocarbons from fish tissues by technique of stream distillation. The hydrocarbons concentrations in tissue samples were calculated according to the internal standard, the recovery efficiency and the response factors of GC. Table 2 illustrates the recovery efficiency of the hydrocarbon standards spiked to fish tissue by steam distillation technique. All spiked hydrocarbons were recovered with the highest recovery for isopropylbenzene, and the lowest recovery for 2-methylnaphthalene. Quantitative analysis of WSF components in the river water samples was based on the internal standard and WSF concentrations in the sample, which were calculated based on the correction factors of Ernst et al (1989). The procedural blank was performed daily and all solvents were distilled.

Lipids were extracted from fish tissues (muscle and liver) according to the method of Vikøren et al (2017). The tissues were finely cut and placed in a centrifuge tubes (15 mL). The tissues were then extracted using 10 mL of a mixture of solvents (chloroform, methanol and water) (2:2:1.5 v:v:v). The tubes were then vigorously vortexed for 5 minutes, placed in the refrigerator for 1 day and then vortexed again for 5 minutes. After that, the tubes were centrifuged at 2000 rpm for 10 minutes. Two distinctly separate layers of solvents were observed with residual tissues remaining in the top layer. The clear chloroform layer was removed and concentrated for analysis.

Table 2

Hydrocarbons recovery from fish muscle (see Table 3 for hydrocarbons symbol)

Hydrocarbons	*Recovery correction factor (100% hydrocarbon recovery from stream distillation method)*	*Final correction factor (recovery correction factor/GC response correction factor)*
2	7.65	7.14
5	8.34	8.14
6	4.67	4.34
7	7.73	7.42
8	5.56	5.22
9	8.63	8.35
10	6.78	6.42
12	5.81	5.36
14	4.50	4.15
15	3.60	3.20
17	5.62	5.16
18	1.87	1.33
19	2.63	2.27

Lipids analysis was performed using a Iatroscan TH-I0 analyzer MK-III with a flame ionization detector (FID) (Iatron Laboratories Inc., Japan). The detector used hydrogen and air flow rates of 160 mL minute^{-1} and 2 L minute^{-1} respectively. The scan speed was 0.42 cm second^{-1}. Chromarods-SIII (silica gel) was used for TLC separations. Chloroform extract was concentrated and spotted on Chromarods. The Chromarods were then developed in hexane, chloroform, isopropanol and formic acid (80:14:1:0.2 v:v:v) for 50 minutes. Lipids were quantified based on the calibration of Chromarods with authentic standards.

Sensory assessment of taint was conducted by staff of the Fisheries Department at Basrah University, where they had experience in tasting fish samples. Fish was thawed and minced. Exactly 50 g of fish meat were cooked. Four meat samples were provided for each taster. The first was the control fish meat and the others were the fish meat exposed to WSF concentrations. Afterwards, the respondents were asked about the smell and taste of meat samples to inquire about the differences in comparison with the reference meat sample. The results were then analyzed statistically by ANOVA (one-way analysis of variance) to test the significant difference between means. Means differences were deemed significant at the 1% significance level. Significant means were isolated by LSD (least significant difference) test. All data were treated by SPSS (statistics package for social sciences) version-20 for Windows-program (IBM-USA). A correlation analysis was sometimes performed to assess the relationship between some criteria using Microsoft-Excel.

Results and Discussion. The GC analysis showed that low-boiling aromatic hydrocarbons were the main components of the WSF (about 65%), which represented small components of initially crude oil (Nahran-Omar). Aliphatic hydrocarbons formed low concentrations in these fractions. The compounds, toluene, ethylbenzene, m+p-xylene, o-xylene, 1-ethy-3-methylbenzene, 1-ethyl-2-methylbenzene, 1,2,4-methylbenzene, and 1,2,3-methylbenzene were the dominant element in WSF. Other aromatic compounds within the WSF profile include naphthalene and its substitutes (2-methyl naphthalene, 1-methyl naphthalene, ethyl naphthalene, and dimethyl naphthalene). Among the less common aromatics in the composition of WSF were benzene, isopropylbenzene, propylbenzene, 1,3,5-trimethylbenzene, and C4 alkylbenzenes (Several tetramethyl-and propyl-methyl-benzenes). The WSF also demonstrated levels of 2-methylhexane and methylcyclohexane (Figure 1 and Table 3). The hydrocarbons of WSF from Nahran-Omar crude oil showed a model similar to that previously mentioned for WSF components of other crude oils (Rodrigues et al 2010; Olaifa 2012; Sandoval 2016). They were predominantly with monocyclic aromatic hydrocarbons such as benzene, toluene and

xylene. These compounds are highly soluble in water and cause tainting and toxicity to fish (Dighiesh et al 2019). Alkanes are often missing in WSF because they are insoluble in water. Alkane compounds usually have no odor or taste and are not attributed to tainting (Kakkar et al 2011).

No deaths were recorded among fish exposed to WSF for 8 hours. Fish behavior was normal. The fish were only disordered by persons who closely monitored aquariums. Concentrations of hydrocarbons in aquariums decreased significantly during testing. The rate of decline was about 50% of which some are stuck in the walls of aquariums, and other parts can be vaporized.

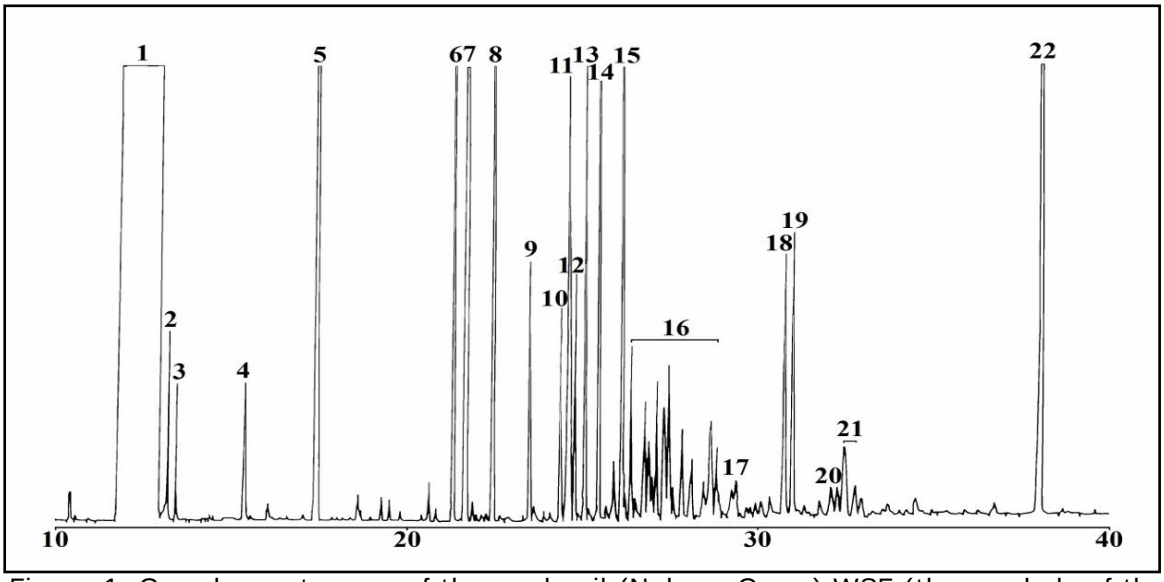

Figure 1. Gas chromatogram of the crude oil (Nahran-Omar) WSF (the symbols of the hydrocarbons identified are shown in Table 3).

Table 3

Hydrocarbons identified in the WSF of Nahran-Omar crude oil

Symbol	*Hydrocarbons*	*Symbol*	*Hydrocarbons*
1	Hexane (solvent)	12	1,3,5-Trimethylbenzene
2	Benzene	13	1-Ethyl-2-methylbenzene
3	2-Methylhexane	14	1,2,4-Trimethylbenzene
4	Methylcyclohexane	15	1,2,3-Trimethylbenzene
5	Toluene	16	C_4 Alkylbenzenes
6	Ethylbenzene	17	Naphthalene
7	2-Methylnaphthalene	18	m+p-Xylene
8	1-Methylnaphthalene	19	o-Xylene
9	Ethylnaphthalene	20	Isopropylbenzene
10	Dimethylnaphthalenes	21	Propylbenzene
11	Heneicosane (Internal standard)	22	1-Ethyl-3-methylbenzene

The sensory evaluation of the taint showed that all fish samples in all tests were tainted by 1% level of significance. The level of significance between fish samples exposed to 0.5 ppm and concentrations of 0.9 ppm and 1.6 ppm was 1%. It was also 1% between the concentration of 0.9 ppm and 1.6 ppm (Table 4). In spite of the duration of exposure was very short (8 hours), significant differences in odor and tainting between WSF-exposed fish and control fish were observed. Fish with low WSF concentration (0.5 ppm) were less tainted than those exposed to high concentrations (0.9 ppm and 1.6 ppm). However, connoisseurs were able to detect tainting at a concentration of 0.5 ppm ($p > 0.01$) which was significantly different from concentrations of 0.9 ppm and 1.6 ppm. These results were considerably associated with GC analysis of hydrocarbons (Table 5). Figure 2

illustrates the relation between the data of sensory evaluation and the WSF concentrations in water and fish muscle. The threshold concentration of WSF that tainted the fish was less than 0.5 ppm. It was found that the threshold concentration of the WSF that tainted fish depends on factors such as exposure time and concentration (Lockhart et al 2002; Tierney et al 2013).

Table 4

Sensory assessment of fish samples exposed for 8 hours to crude oil WSF concentrations

Details	*0.5 ppm*	*0.9 ppm*	*1.6 ppm*
Connoisseurs number	20	20	20
Odd sample identification (%)	100	100	100
Significance level (%)	1	1	1

Table 5

Hydrocarbons in tissues of fish exposed for 8 hours to crude oil WSF concentrations

WSF	*Tissue*	*0.5 ppm*	*0.9 ppm*	*1.6 ppm*	*Control*
Hydrocarbons in tissues (ppm)	Muscle	15.65	27.37	32.22	1.00
	Liver	16.85	31.13	39.69	2.00
Total accumulation (hydrocarbons concentration ratio in tissue to water)	Muscle	31.30	30.41	20.14	---
	Liver	33.70	34.58	24.80	---

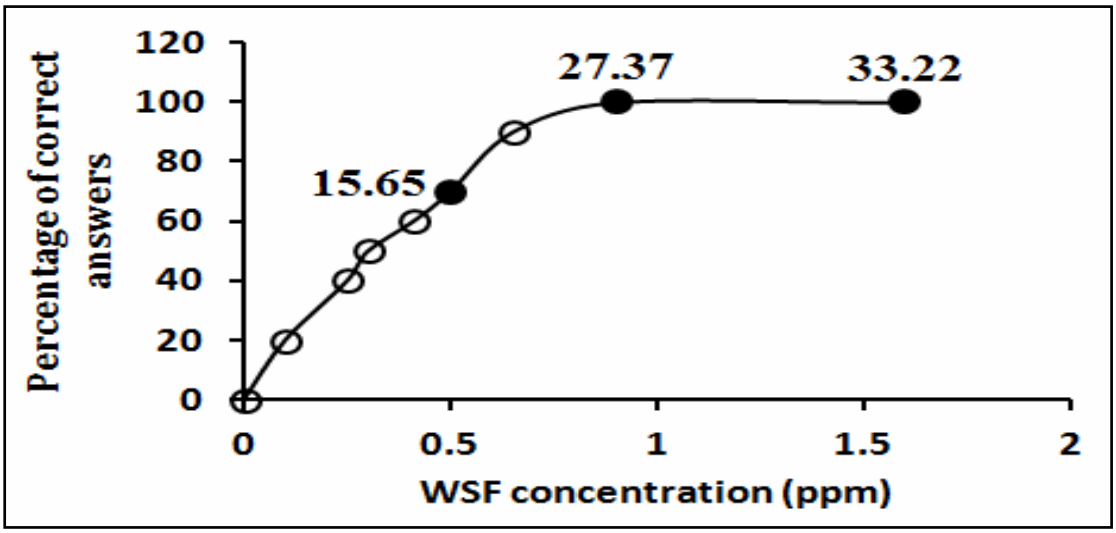

Figure 2. The relation between the sensory evaluation results and WSF concentrations in water and fish muscle.

The levels of hydrocarbons in fish muscle exposed to different concentrations of WSF are shown in (Figure 3). GC analysis showed that the type of hydrocarbons in the fish tissues analyzed was similar to those in WSF, although hydrocarbons are concentrated in tissues at different levels (Table 6). These concentrations in fish do not necessarily reflect the relative concentrations of hydrocarbons in the natural environment (Tierney et al 2013). The analysis by GC complements the sensory assessment of the taint. The tainting can only be assessed by sensory tools such as smell and taste, while GC analysis can provide a detailed composition of tainting hydrocarbon compounds. This can help to determine whether fish tissue is tainted or not. However, the GC is not strained, always available (24 hours a day), and allows comparison of laboratory data. The non-polluted river water used in this study was tested to determine the background levels of hydrocarbons. Hydrocarbons are not detected in water samples. Fish control showed total hydrocarbon concentrations in muscle (1 ppm) and liver (2 ppm) (Table 5), which were subtracted when the total hydrocarbon levels in tainting fish were calculated.

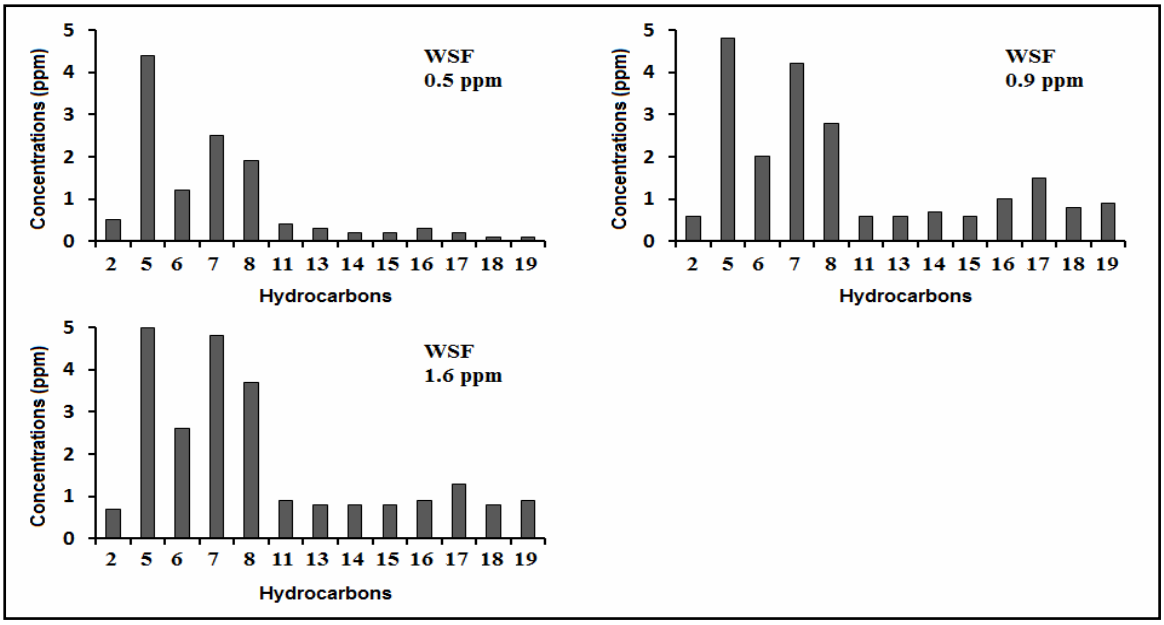

Figure 3. The main hydrocarbon concentrations in fish muscle (see Table 3 for hydrocarbons symbol).

Table 6

Hydrocarbon bioaccumulation* in tissues of fish exposed for 8 hours to crude oil WSF concentrations (see Table 3 for hydrocarbons symbol)

Hydrocarbons	*0.5 ppm*		*0.9 ppm*		*1.6 ppm*	
	Muscle	*Liver*	*Muscle*	*Liver*	*Muscle*	*Liver*
5	7.2	3.9	4.8	3.2	5.8	3.3
7+8	0.7	0.3	3.2	1.9	2.4	1.6
16	3.1	5.0	1.4	2.4	2.5	2.0
17	5.4	9.0	8.9	6.1	7.8	6.8
18+19	1.0	1.5	3.1	3.2	1.4	2.4
21	0.2	3.1	2.2	2.0	0.2	1.1

* Bioaccumulation = individual hydrocarbon concentration ratio to the total hydrocarbons concentration in tissue/ equivalent ratio in water

The bioaccumulation in the present fish is indicated by the presence of levels of aromatic hydrocarbons such as toluene, xylene, alkylbenzene and naphthalene in its tissues (muscle and liver). These compounds are known to cause unpleasant odor in fish (Rose et al 2012; Kjølholt et al 2014). Toluene and naphthalene hydrocarbons were the highest bioaccumulation in fish tissues. Naphthalene was generally more easily accumulated than toluene (Table 6). These results are consistent with other investigator (Tierney et al 2013). Akinsanya et al (2019) reported that toluene is the hydrocarbons answerable for the taint, and noted that its accumulation in tissues occurred through the blood via the gills. This mechanism can also be assumed in this study.

The bioaccumulation of hydrocarbons in common carp is higher than other fish species (Rimayi & Chimuka 2019). This may be due to the fact that the common carp contains a high percentage of muscle lipid compared to the low percentage of lipid in the muscles of these fish. For example, Ernst et al (1987) reported that the uptake of hydrocarbons in cod *Gadus morhua* (muscle lipid by 0.75% wet weight) exposed to a concentration of 3 ppm of WSF for 8 hours was 0.7 ppm (20-30 times less than the fish in this study containing 3.27% wet weight of muscle lipid (Table 7)). This apparent difference in lipid between the muscles of cod and common carp may explain the low uptake of hydrocarbons in cod. Moreover, triacylglycerols, which may be accountable for the hydrocarbons accumulation, are higher in current fish than the others. In general, it is known that hydrocarbons dissolve considerably in adipocytes (Zhou et al 1997), and therefore the greater the amount of lipid in animals, the greater the likelihood of

contamination with hydrocarbons (Schlechtriem et al 2012; Koranteng-Addo et al 2018). The uptake of hydrocarbons by fish also depends on their behavior. The main ways in which hydrocarbons are made available to fish are gills or drinking water (Enuneku et al 2015; Akinsanya et al 2018). Fish drinking water rates depend on the requirement to preserve a stable osmotic balance, so fish such as common carp drink excessively to recompense for the loss of water from the gills. In this regard, common carp may be more able to uptake hydrocarbons than other fish, because it is a very active fish in swimming. Another important factor in bioaccumulation is the rate of circulation of blood in fish. It is known that contaminants increase the movement of water on the gills (i.e., ventilation rates in fish), which will change the blood flow pattern in the gills (Tierney et al 2013). Hydrocarbons are likely to be transmitted through fish's blood by triglyceride-rich lipoproteins, which are present in sufficient quantities in the plasma to transport hydrocarbons to different tissues of the fish body (Zhou et al 1997).

Table 7

Lipid content in fish tissues

Lipid	*Muscle*	*Liver*
Total lipids (%)	3.27±0.44	4.42±0.55
Triacylglycerol (%)	75.54±5.48	89.86±2.13
Polar lipids (%)	19.21±5.32	4.67±2.14

In common carp, the uptake of hydrocarbons was highly correlated to the muscle lipid content. The total uptake of low-lipid fish (3% wet weight) was 32 ppm, while 55 ppm was in high-lipid fish (9% wet weight) (Figure 4). It is clear that the fish muscle bioaccumulated hydrocarbons in much larger quantities than the surrounding WSF. The relationship between lipid content and the amount of hydrocarbons concentrated in fish tissues was also illustrated by several authors (Schlechtriem et al 2012; Enuneku et al 2015; Koranteng-Addo et al 2018). The main hydrocarbons digested by fish in all conditions were low molecular weight aromatic hydrocarbons containing 1 or 2 rings. High molecular weights aromatic hydrocarbons (higher than dimethylnaphthalenes) were usually missing. Alkanes were also absent due to their low solubility in water. Aromatic hydrocarbons with higher molecular weight are slowly depurated in fish compared to low molecular weight aromatics (benzene, toluene and xylene). This result is consistent with the observations of Jonsson et al (2004). The fish needed at least 12 days to depurate. The depuration rate was slow in high-lipid fish. Tasters were unable to detect any fish tainting after 12 days of depuration. Even after 30 days of depuration, the fish muscles were not hydrocarbons-free.

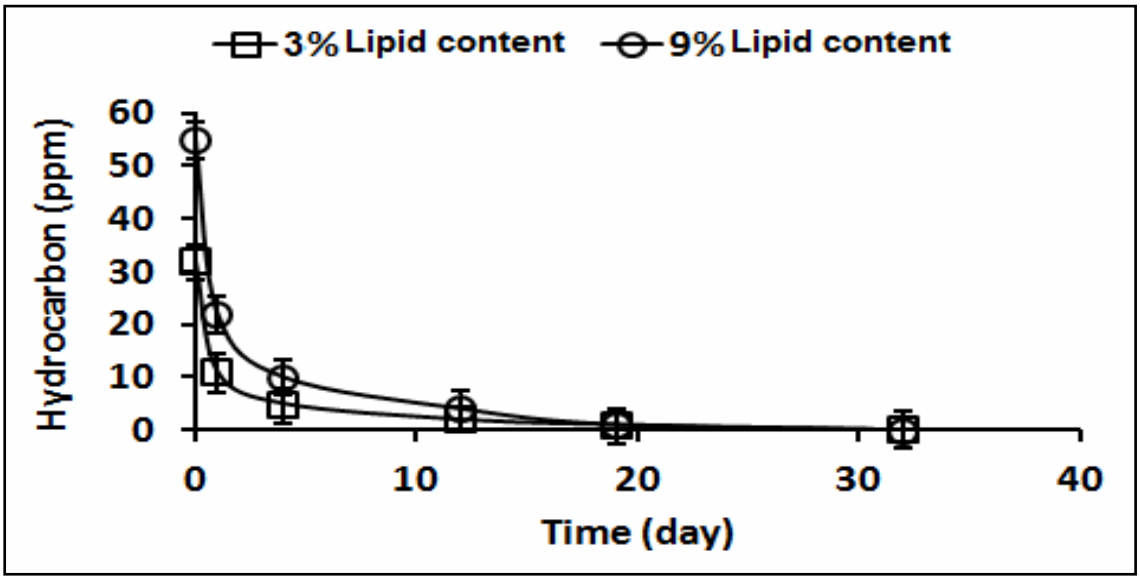

Figure 4. Uptake and depuration of common carp muscle exposed for 8 hours to 1.6 ppm of WSF.

By comparing the rates of uptake and depuration of hydrocarbons in various parts of common carp muscle (9% wet weight lipid content) (Figure 5), the high-lipid muscle concentrated the largest amount of hydrocarbons. This has also been reported by several authors (Nasrollahzadeh Saravi et al 2014; Olyaei et al 2015). Obviously, the lipid distribution is completely dependent on the studied muscle section (Figure 6). The belly flap muscle has the highest lipid content of 30.4%. While, white muscle contains a lower lipid content of 4.0%. Red and lower flank muscles possess 14.6% and 11.9% lipid content respectively. The bioaccumulation of hydrocarbons in various parts of fish muscle has been associated with lipid content. It was significant in belly flap muscle, followed by red muscle, then lower flank and white muscles. The white muscle which has about a quarter of the lipid content in the red and lower flank muscles, contains more than half the hydrocarbons concentration (30 ppm) found in the other two muscles (52 ppm and 50 ppm). There is also a very slight difference in the hydrocarbons uptake between the belly flap muscle (53 ppm) and red muscle (52 ppm), in spite of the belly flap muscle has twice the lipid content in the red and lower flank muscles. This may propose that the hydrocarbons accumulation in fish tissues in the short exposure period (8 hours) may not reach saturation limit. The blood circulation in the belly flap muscle is also likely to be slower than other muscles. The variations in lipid type components may be implicated too. The bulk of the hydrocarbons in the muscles of the fish decreased after 19 days of depuration (Figure 5). The depuration of monocyclic aromatic hydrocarbons was faster than di- and polycyclic aromatic hydrocarbons. This may be due to the high solubility of monocyclic aromatic hydrocarbons in body fluids and their rapid metabolism by enzymes (Butler et al 2016; Sørensen et al 2019). The hydrocarbons held by fish for a longer period were better associated with the lipid content of the tissues. On day 19, there was only 1 ppm, 1.4 ppm, 2 ppm and 2.8 ppm of hydrocarbons in the white, red, lower flank and belly flap muscles respectively. Depuration of the rest polycyclic aromatic hydrocarbons may take very long time. Figure 6 also gives the time required to remove 85% of the hydrocarbons in different parts of the fish muscles. Distinctly, tissues with a high lipid content show a lower depuration rate than those with a low lipid content. Belly flap muscle requires 5.6 days to remove 85% of the hydrocarbons. White muscle requires 1.1 days to reach the same rate of hydrocarbons depuration. Red and lower flank muscles with approximately the same lipid content need 3.4 and 3.9 days respectively to depurate 85% of the hydrocarbons.

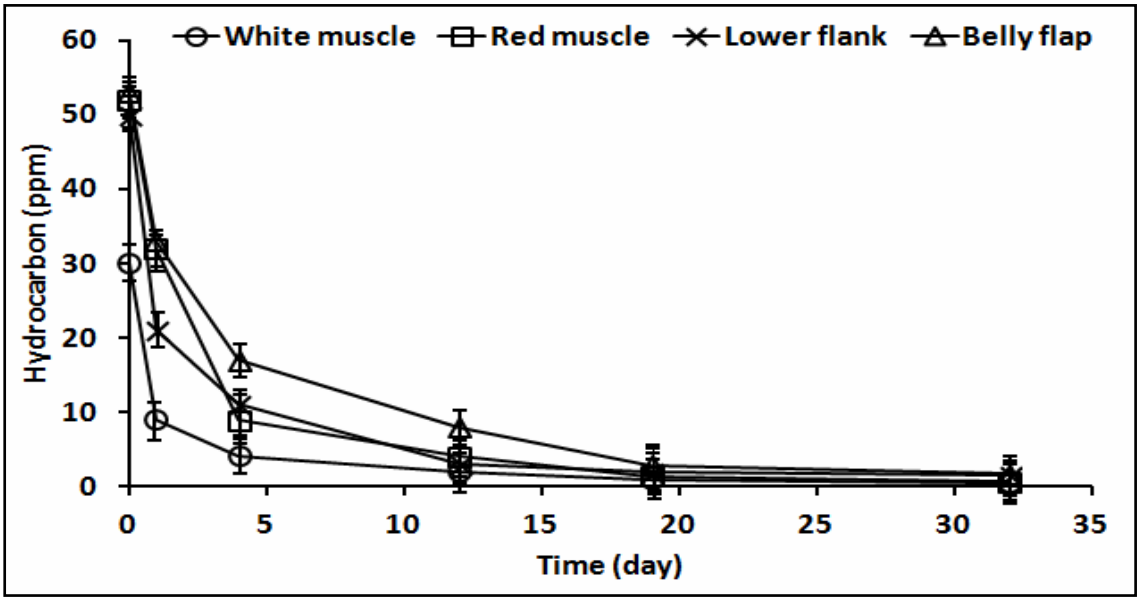

Figure 5. Uptake and depuration of various muscle sections of common carp exposed for 8 hours to 1.6 ppm of WSF.

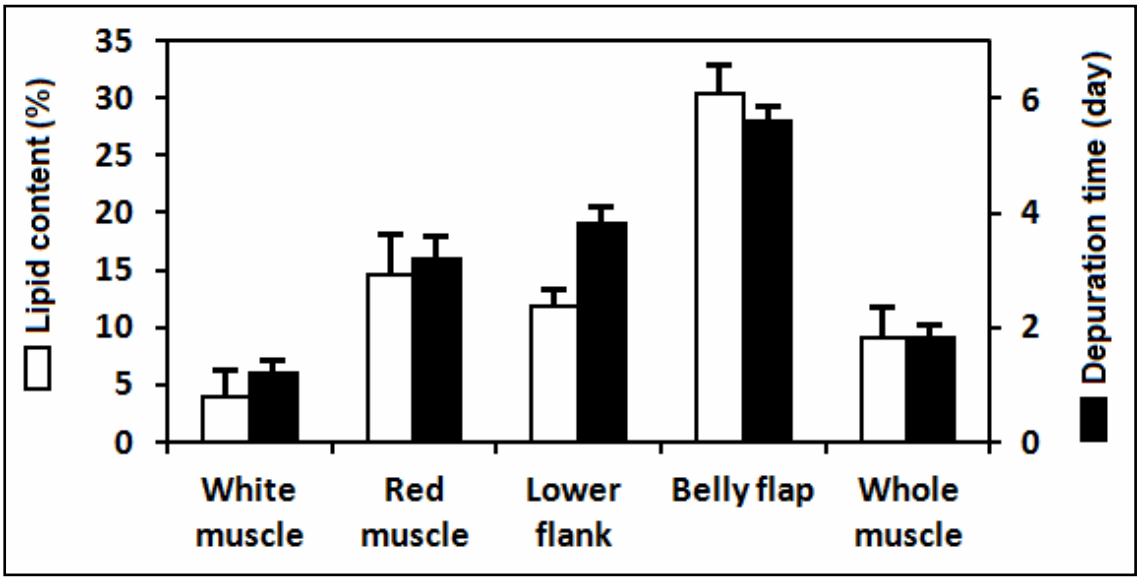

Figure 6. Lipid content in common carp muscle and its various parts and the time required to depurate 85% of hydrocarbons.

Conclusions. The common carp exposed to crude oil WSF dilution for a short period of time is able to uptake significant amounts of hydrocarbons from their environment, so that they become tainted, but without any lethal effects. This is a very important issue in fish farming. Fish may look healthy while they are well tainted. The depuration rates of hydrocarbons in common carp were varied. Some hydrocarbons are depurated fairly quickly, which represent hydrocarbons in fish body fluids. While other hydrocarbons remain for a longer period, those may be trapped in fish's lipid tissue. Although accumulated hydrocarbons are closely related to the lipid content of fish tissues, some differences cannot be explained by this variable. We believe that the difference in the composition of lipid classes and/or the difference in blood circulation in fish tissues may also be involved. The belly flap muscle seem to be the main location for storing hydrocarbons in muscle, which contribute significantly to the retention of the hydrocarbons observed in fish. Therefore, removing the belly flap muscle by the fish consumers would eliminate the main contaminated part of the fish. Research will continue on this species of high-lipid fish in our laboratories to determine other issues such as toxicity, transport, fate of hydrocarbons, etc. This knowledge can also be used to evaluate other biological information.

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