



RESEARCH PAPER

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Histopathological changes of water soluble fraction of Iranian crude oil in muscle of yellow fin sea bream (*Acanthopagrus latus*)

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Abstract

Increasing amount of industrial and commercial chemical discharge into the aquatic environment has led to pathological side effects on aquatic organism. Water soluble fraction (WSF) of crude oil can pollute water and cause histopathological alterations in fish. Therefore, sea water pollution with crude oil could detrimentally impact the fish health. The aim of the present study was to assess the effect of different concentrations of WSF on muscle of yellow fin sea bream. The fish were exposed to 0% (control), 2%, 4%, 8% and 16% of WSF for a period of 21 days. The histopathological alterations were examined in four categories of circulatory, degenerative, proliferative and inflammatory. Inflammatory changes in control group was lower than that in 8% and 16% WSF exposed groups ($p < 0.01$), and in 2% and 4% WSF exposed groups was lower than that in 8% and 16% WSF exposed groups ($p < 0.01$). In conclusion, the present study showed the effect of concentration and period of exposure to WSF on yellow fin sea bream. Iran is one of oldest Middle East producers of crude oil, so monitoring adverse histopathological effect of pollution could help keep yellow fin sea bream from waterborne pollution and prepare the ground for further studies.

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Introduction

Introduction

Many studies investigated the effect of water quality changes on the cellular changes and monitoring histopathological changes can help to evaluation of pathological side effects of water born pollution and assessment histopathological alteration of fish in response to organic trace pollution has provided information to biomonitoring plan designed for various aspects of environmental risk assessment. Fish health monitoring is useful for assessment of water toxicity and pollution and provides quantitative data as well as valuable information of ecological relevance to the chronic adverse effects by waterborne pollution (Oost *et al.*, 2003; Tayel *et al.* 2013; Afifi *et al.* 2014).

In many studies, release of crude oil was a major threat to marine environment. At the previous studies, marine pollution was striking and toxic to fish and environment and might have a negative impact on marine ecosystem and fisheries resource (Anderson *et al.*, 1974; Lockhart *et al.*, 1996; Jordi *et al.*, 2001).

Crude oil is a mixture of chemical material with aliphatic, aromatic and polar components, which could generate intoxication by toxic components namely xylene, naphthalene, benzene and toluene. (Garrett *et al.*, 1998; Edema *et al.*, 2007; Kim *et al.*, 2013).

Notwithstanding that water and oil are insoluble, crude oil included a very small soluble section, called water soluble fraction (WSF) (Edema, 2012). Fish have a very high capability to accumulate WSF (Collier *et al.* 1995). Water accommodated fractions for three reasons could be the cause of the aquatic toxicity: first chemical toxicity associated with dissolved materials (from oil or dispersant), second physical effects associated with contact with oil droplets and third effect associated with enhanced uptake resulting from direct animal oil contact (Singer *et al.*, 1998).

WSF extracted from crude oil have detrimental effects on water body population and decrease fish population and prepare the ground for the establishing histopathological alteration and with interact with cellular and tissue processes in different ways to produce toxicity (Anderson *et al.*, 1974; Solangi and Overstreet, 1985; Gunderson *et al.*, 1996).

Many studies investigated the impact of the environmental pollution on histopathology of muscle fiber and confirmed histopathological effect on muscle and proposed monitoring the histopathological pollutant effect on muscle fiber might be bioindicator. (Tayel *et al.* 2007; Jehaeshadevi *et al.* 2012; Nouh and Selim, 2013).

Iran is the most ancient Middle East producer of crude oil and one of the area exposed the oil pollution (Helio International 2006; Namdari *et al.*, 2012). The yellow fin sea bream is one of the valuable important species throughout the world on the other hand; yellow fin sea bream lives in estuaries and brackish water and one of the important food resources for local consumption (Gwo, 1994; Hu *et al.*, 2007; Savari *et al.*, 2013).

As regard yellow fin sea bream is frequent in Persian Gulf and Musa Estuary and considering contamination from the Persian Gulf transferred into the Musa estuary and the fact that this estuary is located around many small industries and the biggest Iranian petrochemical complex, this site developed to the risk of marine ecosystem pollution. (Scott, 1995). The study aimed to investigate the histopathological alteration in muscle of yellow fin sea bream after exposure to WSF prepared from Iranian light crude oil.

Material and methods

Fish and acclimatization conditions

The yellow fin sea bream specimens with 111.68 ± 1.52 gr body weight and 18.27 ± 0.23 cm standard length were collected during 2013 from Musa estuary in the head of the Persian gulf using baited traps an

acclimated to laboratory condition in south of Iran aquaculture research center for 20 days before the start the first experiment. During this period, fish were maintained in 5 numbers of 1000 (40fish per tank) liter polystyrene tanks filled with seawater from the capture site and equipped whit filter and oxygenation system under a natural photoperiod and fed every two days with commercial food (chineh). No mortality was seen during acclimatization period.

Chemicals

Light Iranian crude oil (API=36.70) was donated by petroleum industry from Abadan oil refining company. The crude oil barrels were kept and protected in a safe and convenient place before preparing the stock solution.

Preparation of water soluble fraction

The WSF was prepared according to the method described by Anderson et.al., (1974). For preparation of WSF, a part of crude oil was added to nine part of water in container. The mixture container was capped and covered with aluminum foil to reduce the evaporation and avoid light. Then the solution was stirred using a magnetic agitator with slow speed for 24 h at room temperature and allowed to settle 1 h to separate the water and oil phases. The solution below the oil phase siphoned off from the bottom of the mixing container and this stock solution was lenis to make up the nominal concentration for exposure. Only the liquid phase of the WSF of crude was used for the study. New solution was prepared daily.

Experimental treatment

For the entire duration of experiment a week before creation of the contamination after acclimation period fish were randomly transferred to stock tank to 15 number of 300-liter polystyrene tanks (15fish per tank), which had static system and continuous aeration and fish were held in temperature-controlled room on natural photoperiod with 13 h light and 11 h darkness at equable $22\pm1^{\circ}\text{C}$ during the three weeks of experimental period and water was continuously monitored for temperature, dissolved oxygen, pH, water salinity and conductivity.

Experimental design

The fish were divided into five groups. Four groups were then exposed to different concentrations of WSF and the control group was not exposed to the pollutant and any group with three replicate per exposure. In the present study each experimental tank contained 300 liter seawater to which 2 (2p), 4(4p), 8(8p) and 16(16p) % of WSF were added. In the control group, sea water was only used. Filtered untreated Seawater was obtained from the musa estuary.

Histopathological examination

The fish were quickly euthanized with (benzocaine, 60mg/liter) and measured for standard length and body weight. Then, the fish muscle section from individual fish of all groups was dissected and immediately fixed in 10% neutral buffered formalin for histopathological examination (Robert. 2001).

The tissue was later washed clean of formalin and passed through a series alcohol concentration to remove the water. The tissue were again passed through a chloroform/alcohol and pure alcohol therefore the muscle sections, 7 μm thick, were cut with semi automate rotatory microtome RM2245 and then stained with hematoxylin and eosin and mounted on glass slide. Images were analyzed by light microscope and recorded with mioticcam 3000 photomicroscope.

Muscle histopathological alteration was assessed semi-quantitatively by assortment the severity of alteration according to bernet *et al.*, (1999) into four significant reaction pattern (rp). In general, four major reaction patterns included circulatory disturbances, degenerative disturbances, proliferative changes and inflammatory changes.

Each reaction pattern divided into several alterations. Circulatory disturbance (rp1) included pathological condition of blood and tissue fluid that was considered in four major parts (hemorrhage, congestion). Degenerative disturbance (rp2) included regressive alteration the lesion that can cause

decreased muscle function or loss of muscle that was considered in four major parts (edema, degeneration of muscle fiber, atrophy of muscle fiber, necrosis, focal hyaline degeneration, increase perimysial space). Proliferative changes (rp3) included alteration leads to an overactive cells or muscle function that was considered in seven major parts (hypertrophy of muscle cells, hyperplasia of muscle cells). Inflammatory changes that related to other reaction pattern were considered in one major part (lymphocyte infiltration of interfascicular space).

Each reaction pattern had importance factor (IF) that according to bernet *et al.*, (1999) was divided into three grades from grade one to grade three. Grade one minimal pathological importance that they are easily reversible as exposure to irritants end, grade two moderate pathological importance that these alteration is reversible in most cases if the stressor is neutralized and grade three marked pathological importance that they are generally irreversible, leading partial or total loss of the organ function.

Every alteration was evaluated using score value (SV) from grade 0 to grade 6 according to bernet *et al.*, (1999), based on the severity of the alteration in the tissue: grade (0) without alteration, grade (2) focal and mild alteration, grade (4) moderate alteration and grade (6) sever alteration. This rating was used to collect an overall evaluation value of histopathological alteration for each muscle of individual fish.

After determination of the SV and IF from the sum of these both.

K_{alt} was obvious from following formula:

$$K_{alt} = \sum alt (cv_{org\ rt\ alt} \times mf_{org\ rt\ alt})$$

And from of the sum of \sum alteration for specific alteration, K_{rt} characterized by the following formula:

$$K_{rt} = \sum alt \sum alt (cv_{org\ rt\ alt} \times mf_{org\ rt\ alt})$$

Eventually g_{org} was determined by the following formula:

$$m_{org} = \sum_{rt} \sum_{alt} (cv_{org\ rt\ alt} \times mf_{org\ rt\ alt})$$

Where (*rt*) is the reaction pattern, (*alt*) is the alteration, ($cv_{org\ rt\ alt}$) is the SV for specific alteration of reaction pattern, (*org*) is the organ that represents muscle, ($mf_{org\ rt\ alt}$) is the IF and (m_{org}) is the muscle index that represents degree of damage of the muscle from individual fish.

The tables included comparison between groups exposed to 2p, 4p, 8p, 16p of WSF and control group that at days 0, 4, 8, 12, 16 and 20 were recorded and after calculation of organ index from comparison average value for each lesion tables for unraveling were complete.

Statistical analysis

Data related to histopathological alterations were analyzed using MIXED procedure. In addition, LSMEANS statement was used to perform multiple comparisons. All analyses were conducted in SAS (SAS, 2008). Data are presented as mean \pm SD. Differences with $P < 0.05$ were co

Results

Mortality

No fish died during the acclimatization period and no obvious mortality rate in 2p, 4p and 8p groups and control group except 2 cases from the 16p group on days 8 and 16 was observed.

Table 1. Index of circulatory disturbance in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Circulatory disturbance		Day of experiment				
Group	0	4	8	12	16	20
2p	2.00 \pm 0.063	2.00 \pm 0.89	2.17 \pm 0.75	2.50 \pm 0.84	2.17 \pm 0.98	1.83 \pm 0.98
4p	2.00 \pm 0.63	2.00 \pm 0.63	2.33 \pm 0.52	1.83 \pm 0.98	2.17 \pm 0.75	1.83 \pm 0.97
8p	2.17 \pm 0.75	1.83 \pm 0.75	1.83 \pm 0.75	1.67 \pm 0.82	2.17 \pm 0.75	1.83 \pm 0.75
16p	2.17 \pm 0.75	2.33 \pm 0.82	2.00 \pm 0.89	1.83 \pm 0.75	2.00 \pm 0.89	2.33 \pm 0.82
control	2.17 \pm 0.75	2.50 \pm 1.05	2.17 \pm 0.75	2.00 \pm 0.63	1.83 \pm 0.75	2.17 \pm 0.98

Histopathological findings

The result of histopathological examination showed in four major type of reaction pattern in Tables 1, 2, 3 and 4. In addition, in Table 5 organ index represent the damage of the muscle.

There was no effect of group and time on the circulatory disturbances ($P > 0.05$; Table.1).

There was no effect of group and time on the degenerative changes ($P > 0.05$; Table.2).

Table 2. Index of degenerative changes in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Degenerative changes		Day of experiment				
Group	0	4	8	12	16	20
2p	7.33 ± 1.03	7.17 ± 0.98	7.50 ± 1.64	6.67 ± 0.82	7.17 ± 0.75	7.67 ± 0.52
4p	7.67 ± 1.03	7.17 ± 0.98	6.83 ± 0.75	7.17 ± 0.98	7.50 ± 0.84	7.50 ± 0.55
8p	7.33 ± 0.82	7.33 ± 1.03	7.50 ± 0.84	7.00 ± 0.89	6.83 ± 0.98	7.33 ± 0.82
16p	7.00 ± 0.89	7.83 ± 1.33	7.50 ± 0.84	7.33 ± 0.82	7.17 ± 0.98	6.83 ± 0.98
contorol	7.33 ± 0.82	6.67 ± 1.21	7.00 ± 0.89	7.33 ± 1.21	7.83 ± 1.33	6.83 ± 0.75

There was no effect of group and time on the proliferative changes ($P > 0.05$; Table.3).

Inflammatory changes in control group (Fig.1) was lower than that in 8p and 16p groups ($p < 0.01$) and in 2p and 4p groups was lower than that in 8p and 16p groups ($p < 0.01$).

The inflammatory changes on Days 12, 16 and 20 was higher than that on Day 0 ($P < 0.001$). On Day 4 inflammatory changes was lower than that on Days 12, 16 and 20 ($p < 0.05$) and the inflammatory changes on Days 16 and 20 was higher than that Day 8 (Table.4). Increase inflammatory cells infiltration (Fig.2 and Fig. 3).

Table 3. Index of proliferative changes in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Proliferative changes		Day of experiment				
Group	0	4	8	12	16	20
2p	1.83 ± 0.75	2.17 ± 0.75	2.33 ± 0.82	2.00 ± 0.89	2.00 ± 0.89	1.83 ± 0.75
4p	1.50 ± 0.55	2.17 ± 0.75	2.50 ± 0.55	2.33 ± 0.82	1.83 ± 0.75	2.67 ± 0.52
8p	2.17 ± 0.75	2.33 ± 0.82	2.17 ± 0.41	2.33 ± 0.52	2.50 ± 0.55	2.50 ± 0.55
16p	2.33 ± 0.82	1.33 ± 0.52	2.17 ± 0.75	2.50 ± 0.55	2.33 ± 0.82	2.67 ± 0.82
Control	2.50 ± 0.84	2.17 ± 0.75	2.17 ± 0.98	2.17 ± 0.98	1.67 ± 0.52	2.17 ± 0.98

Organ index in the control group was lower than that in the 8p and 16p groups ($p < 0.05$). Organ index in 2p group was lower than that in 16p group ($p < 0.01$) and in 4p group was lower than that in 16p group ($p < 0.05$). The organ index on Days 0 and 4 was lower than that on Days 16 and 20 ($p < 0.01$) and organ index on Days 8 and 12 was lower than that on Day 16 ($p < 0.01$; Table.5).

Discussion

Pollution by crude oil in fish especially in the oil

producing countries has been continually examined (Anderson *et al.*, 1974; brand *et al.*, 2001; Milinkovitch *et al.*, 2011). However, in Iran, less has been studied on native species experimentally. Classification of damage to muscle can be one of the important assessment ways to diagnose contamination with waterborne pollutants. There are different methods and assessment scales for evaluation of histological changes (Bernet *et al.*, 1999; Reddy and rawat, 2013). The aim of this study was to evaluate the histopathological alteration in muscle of

yellow fin sea bream after exposure to water soluble fraction prepared from light Iranian crude oil and to classify this alteration for easy evaluation of damage to muscle of fish.

Marioara *et al.* 2009 after chronic Cadmium intoxication observed miolysis process in peripheral

muscular fibers manifested by contractile apparatus alteration on large area that part of degenerative alteration but in this study degenerative and proliferative changes in all groups were not significant after exposure to WSF. So the severity of alteration could be affected by the time of exposure and severity of the water pollution.

Table 4. Index of inflammatory changes in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Inflammatory changes	Day of experiment					
Group	0	4	8	12	16	20
2p	1.67±0.82	1.83±0.98	1.50±0.55	1.83±0.75	2.50±1.05	2.00±1.10
4p	1.17±0.41	1.50±0.84	1.67±0.82	2.00±0.89	3.00±1.10	2.00±1.10
8p	1.50±0.84	1.50±0.55	2.00±0.89	2.50±0.84	4.00±0.63	2.83±1.17
16p	1.50±0.55	1.67±0.82	2.17±0.98	2.50±0.84	4.00±0.89	3.17±1.17
control	1.17±0.41	1.67±0.82	1.67±0.82	1.83±0.98	1.83±0.98	2.17±1.17

Table 5. Index of muscle in different groups on Days 0, 4, 8, 12, 16 and 20 of experiment.

Gill index	Day of experiment					
Group	0	4	8	12	16	20
2p	12.83±0.75	13.17±1.72	13.50±1.38	13.00±1.10	13.83±1.33	13.33±1.75
4p	12.33±1.63	12.83±2.32	13.50±1.52	13.33±1.21	14.50±1.05	14.00±1.10
8p	13.17±1.33	13.00±2.00	13.50±0.84	13.50±1.87	15.50±1.05	14.50±0.84
16p	13.00±0.63	13.17±2.23	13.83±1.72	14.17±1.47	15.50±1.97	15.00±1.55
control	13.17±0.75	13.00±1.26	13.33±1.63	13.33±0.82	13.17±1.83	13.33±1.63

In this study inflammatory changes confirmed increasing WSF of Iranian crude oil more than 16 present after 16-day exposure could be used as an alarm to start of inflammatory responses. Jeheshadevi *et al.* 2014 observed similar histological alteration on muscle of *Catla catla* exposed to lethal concentration of naphthalene same as mild lymphocyte infiltration. Sharifpour 1997 has also observed inflammatory responses and melanin containing cells after injection of different materials generally around the blood vessel. In this study the same alteration observed in perivascular area in muscle fibers.

In this study, pathological lesions in muscle increased after 16 day exposure to 16% WSF in yellow fin sea bream that following due to infiltration of inflammatory cells and low degenerative disturbance of peripheral muscle fiber and might be as result of accumulated Oil derivatives in tissue that further

studies are needed . Many studies confirmed pathological side effects in muscle of fish after waterborne pollution similar to our observation and purposed the histopathological changes were useful indicator for investigation of waterborne pollution (Marioara *et al.* 2009 ,Al-beiruty *et al.* 2013; Jeheshadevi *et al.* 2014).

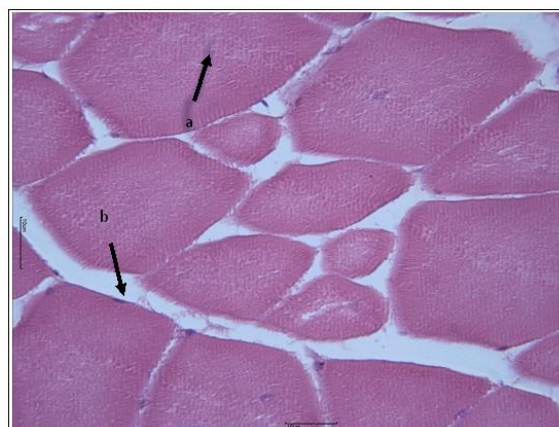


Fig. 1. The muscle of control fish which represents and showed a normal architecture with the muscle cell and muscle fiber (a) and muscle cell nucleus (b).

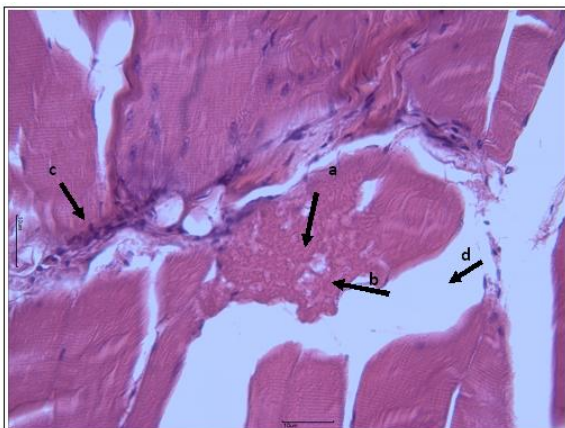


Fig. 2. The muscle of fish exposed to 16% WSF on Day 20 which represents degenerative disturbance of peripheral muscle fiber (a) , necrotic muscle fiber (b), increased in inflammatory cells(c)and increased perimysial space (d).



Fig. 2. The muscle of fish exposed to 16% WSF on Day 20 which represents infiltration of inflammatory cells (a) alongside red blood cells (b) and the melanin deposition within the perivascular area (c).

Al-beiruty *et al* 2013 observed increasing extracellular space between muscle bundles and occasional area of degeneration within individual muscle bundles after 10 days exposure of rainbow trout (*Onchorhynchus mykiss*) to waterborne copper nanoparticle and coppersulphate, histopathological alteration induced by studied indicated the muscle lesion index on muscle after 16 days WSF exposed.

In conclusion, the present study showed the effect of concentration and period of exposure to WSF in yellow fin sea bream. Iran is one of the oldest Middle East producers of crude oil, so monitoring adverse

histopathological effects of oil pollution could help keep yellow fin sea bream from waterborne pollution and prepare the ground for further studies.

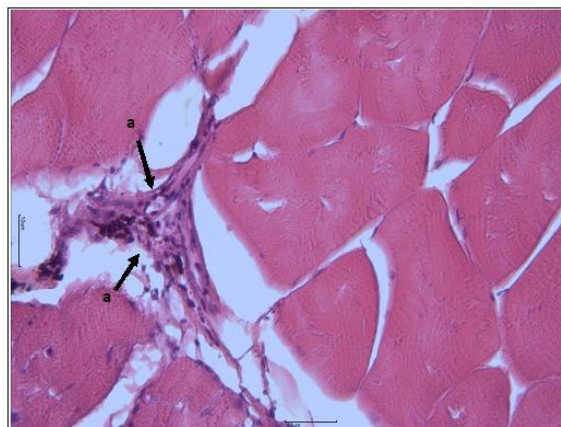


Fig. 3. The muscle of fish exposed to 16% WSF on Day 16 which represents infiltration of inflammatory cells (a) and increased increased perimysial space extracellular.

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Refrence

Affifi M, Alkaladi A, Abu-Zinada O, Zahed NA. 2014. Histopathological and ultrastructural alteration in skin, gills, liver, and muscle of *Siganus canaliculatus* and *Epinephelus morio* caught from Jeddah and Yanbu coast as bio-indicators of oil hydrocarbon pollution. *life science journal* **11(8)**, 880-888.

Al-Beiruty GA, Shaw BJ, Handy RD, Henry TB. 2013. Histopathological effect of waterborne nanoparticles and copper sulphate on the organs of rainbow trout (*Onchorhynchus mykiss*). *Aquatic toxicology* **126**, 104-115.

<http://dx.doi.org/10.1016/j.aquatox.2012.10.005>

Anderson JW, Neff JM, Cox BA, Tatem HE, Hightower GM. 1974. Characteristics of dispersions and water-soluble extracts of crude and refined oils and their toxicity to estuarine crustacean and fish. *Marine Biology* **27**, 75-88.

<http://dx.doi.org/10.1007/BF00394763>

Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T. 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases* **22**, 25-34.

<http://dx.doi.org/10.1046/j.1365-2761.1999.00134.x>

Brand DG, Fink R, Bengeyfield W, Birtwell IK, Macllister CD. 2001. Salt water-acclimated pink salmon fry (*Oncorhynchus gorbuscha*) develop stress-related visceral lesions after 10-day exposure to sublethal concentrations of the water-soluble fraction. *Toxicological Pathology* **29(5)**, 574-584.

<http://dx.doi.org/10.1080/019262301317226384>

Collier TK, Anulacion BF, Stein JE, Goksoyr A, Varanasi U. 1995. A Field evaluation of cytochrome P450 as a biomarker of contaminant exposure in three species of flatfish. *Environmental Toxicology and Chemistry* **14(1)**, 143-152.

Edema NE. 2012. Effects of crude oil contaminated water on the environment, crude oil_ emulsions composition stability and characterization, Prof. Manar EL-Sayed 2nd Ed. China: In Tech. 230P.

Edema NE, Okoloko GE, Agbogidi O. 2007. Physico-Chemical characteristics of the water-soluble fraction of ogini well-head crude oil and the effects on *Pistia stratiotes* linn (water lettuces). *American-Eurasian J. Agric. & Environ. Sci.* **2(6)**, 633-638.

Garrett RM, Pickering IJ, Haith CE, Prince CR. 1998. Photooxidation of crude oils. *Environmental Science & Technology* **32(23)**, 3719-3723.

<http://dx.doi.org/10.1021/es980201>

Gundersen DT, Kristanato SW, Curtis LR,

Al_Yakoob SN, Matwally MM, Al_Ajmi D. 1996. Subacute toxicity of the water-soluble fractions of kuwait crude oil and partially combusted oil on *Menidia beryllina* and *Palaemonetes pugio*. *Environmental Contamination and Toxicology*. **31**, 1-8.

Gwo JC. 1994. Cryopreservation of yellowfin seabream (*Acanthopagrus latus*) spermatozoa (teleost, perciformes, sparidae). *Theriogenology* **41**, 989-1004.

Helio International. 2006. Energy and sustainable development in Iran. *Sustainable Energy Watch* 2005/2006. 41P.

Hu Y-H, Liu Y-J, Tain L-X, Yang H-J, Liang, Gao W. 2007. Optimal dietary carbohydrate to lipid ratio for juvenile yellowfin seabream (*Sparus latus*). *Aquaculture Nutrition* **13**, 291-297.

Jeheshadavi AK, Ramya TM, Sridhar S, Chandra JH. 2012. Histological alteration on the muscle and intestinal tissue of *Catla Catla* exposed to lethal concentration of naphthalene. *international journal of applied engineering research* **9(2)**, 159-164.

Jordi A, Ferrer MI, Vizoso G, Orfila, Basterretxea G, Casas B, Alvarez A, Roig D, Garau B, Martinez M, Fernandez V, Fornes A, Ruiz M, Fornos JJ, Balguer P, Duarte CM, Rodriguez I, Alvarez A, Onken R, Orfila P, Tintore J. 2006. Scientific management of Mediterranean coastal zone: a hybrid ocean forecasting system for oil spill and search and rescue operations. *Marine Pollution Bulletin* **53**, 361-368.

<http://dx.doi.org/10.16/j.marpolbul.2005.10.008>

Kim M, Hong SH, Won J, Yim UH, Jung JH, Ha SY, An JG, Joo C, Kim E, Han GM, Baek S, Choi HW, Shim WJ. 2013. Petroleum hydrocarbon contaminations in the intertidal seawater after hebi spirit oil spill- effect of tidal cycle characterization of seawater extracts. *Water Research* **47**, 758-768.

<http://dx.doi.org/10.1016/j.waters.2012.10.050>

- Lockhart WL, Duncan DA, Billeck BN, Danell RA, Rayan MJ.** 1996. Chronic Toxicity of the water-soluble fraction of Norman wells crude oil to juvenile fish. *Spill Science & Technology Bulletin*. **3(4)**, 259-262.
[http://dx.doi.org/10.1016/S1353-2561\(97\)00024-8](http://dx.doi.org/10.1016/S1353-2561(97)00024-8)
- Marioara N, Gabi D, Liliana PC, Duneai B, Maria M, Tapalaga I, Lunca M, Bocaliliana.** 2009. Pathological tissue lesions induced by chronic Cadmium intoxication in silver crucian carp *carassius auratus gibelio*. *Lucrari stiintifice zootehnie si biotehnologii* **42(2)**, 84-90.
- Milinkovitch T, Godefroy J, Theron M, Thomas-Goyan H.** 2011. Toxicity Of Dispersant Application: Biomarkers Responses in Gills of Juvenile Golden Gray Mullet (*Liza aurata*). *Environmental Pollution* **159**, 2921-2928.
<http://dx.doi.org/10.1016/j.envpol.2011.04.035>
- Namdari A, Mazarei Z, Tahernejad T.** 2012. A Comprehensive plan presentation on the generating wastes management in Ahvaz oil production and desalinization plants, on the RCRA basis. *Scholars Research Library* **3(11)**, 5379-5389.
- Nouh WG, Selim AG.** 2013. Toxopathological studies on the effect of formalin and copper sulphate in tilapia as a commonly used disinfectant in aquaculture. *Journal of Applied Environmental and Biological Sciences* **3(6)**, 7-20.
- Oost RVD, Beyer J, Vermeulen NPE.** 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environmental Toxicology and Pharmacology* **13**, 57-149.
- Reddy PB, Rawat SS.** 2013. Assessment of aquatic pollution using histopathology in fish as a protocol. *International Research Journal of Environment Sciences* **2(8)**, 79-82.
- Roberts RJ.** 2001. *Fish Pathology*. U.K.:W.B. Saunders. 472 P.
- Savari A, Hedayati A, Safahieh A, Movahedinia A.** 2010. Determination of some enzymatic indices of yellowfin sea bream (*Acanthopagrus latus*) in Mahshahr creeks (North West Of Persian Gulf). *World Journal of Fish and Marine Sciences* **2(6)**, 475-480.
- Sharifpour I.** 1997. Histology of the inflammatory response of (*Cyprinus carpio* L.) to various stimuli. DVM Thesis, university of Stirling, Scotland. 467 P.
- Scott DA.** 1995. *A Directory of Wetlands in The Middle East*. U.K. Slimbridge. 562P.
- Singer MM, George S, Lee I, Jacobson S, Weetman LL, Blondina G, Tjeerdema RS, Aurand D, Sowboy ML.** 1998. Effects of dispersant treatment on the acute aquatic toxicity of petroleum hydrocarbons. *Environmental Contamination and Toxicology* **34**, 177-187.
- Solangi MA, Overstreet RM.** 1982. Histopathological changes in two estuarine fishes, *Menidia beryllina* (cope) and *Trinectes maculatus* (bloch and Schneider), exposed to crude oil and its water-soluble fractions. *Journal of Fish Diseases* **5**, 13-35.
- Tayel SI.** 2007. Histological and biochemical seasonal changes of *oreochromis niloticus* muscle in relation to water quality at zefta and el-mansoura cities, Damietta branch river Nile, Egypt .J. *Journal of Egyptian Academic Society for Environmental Development* **3(1)**, 97-107.
- Tayel SI, Al_Ebrahim S, Mahmoud SA.** 2013. Histological and muscle composition studies on *Tilapia zillii* in relation to water quality of Lake Qarun, Egypt. *Journal of applied sciences research*. **9(6)**, 3857-3872.