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IMPACT OF GAS OIL ON IONIC REGULATION, CHLORIDE CELLS AND HISTOPATHOLOGICAL ALTERATIONS IN GILLS OF MUGILID *Liza abu* (Heckel) JUVENILES

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ABSTRACT

To exhibit effect of long term exposure to gas oil on ionic regulation, chloride cells (CC),total epithelial cells (EC) and histopathological alterations in gills of *Liza abu* (Heckel) juveniles. Several laboratory experiments were conducted under two temperatures, warm (27±2.3ºC) and cold (15±1.5ºC). Fishes were exposed to 0.5, 0.25 and 0.1 cm/l V/V of gas oil under high temperature and to 0.25, 0.1 and 0.05 cm/l under low temperature. Hydrocarbons concentrations in exposure solutions and blood plasma were measured at 5th and 14th day of treatment. A notable decline in levels of petroleum hydrocarbons in exposure solutions during both 5th and 14th days was noticed simultaneously its levels in blood plasma synchronized with those exposure solutions. A significant reduction ($P< 0.01$) of Na^+ , K^+ levels in blood plasma. Rise in number and percentage of chloride cells under both temperatures were also observed. It ranged under warm conditions from 5.12-3.64x10^6cells/gm tissue compared to control treatment (1.9x 10^6 cells/gm tissue) and 5.8-4.6 x 10^6 cells/gm tissue under cold conditions. The increase in number of chloride cells coincided with distinguished multiplication in number of branchial epithelial cells under both selected temperatures. However, the histopathological investigations of gills in fish exposed to gas oil conc. 0.25 and 0.5 cm/l for 14 days revealed many histological changes mainly curling, hyperplasia , fusion ,and necrosis in secondary lamellae and lifting in epithelial layers along with terminal hypertrophy of the gill filament.

INTRODUCTION

Transportation of oil derivatives via Shatt Al-Arab River and Basrah canal causes large quantities of oil spilled into aquatic environment.

Petroleum generally is a complex chemical mixture, which impact biology and physiology of fish when it spilled to the aquatic environment. Hydrocarbons are lipophilic compounds. It penetrate via the lived membranes which composed essentially of lipids (Roy *et al*., 1996) and accumulate in fish tissues so the fish tainted and its economic value would be reduced (Hellou *et al*., 1994; Zhou *et al*., 1997). Al-Saad and Al-Asadi (1989) revealed the positive correlation between petroleum compounds concentration and lipid content in fish tissues.

Ionic regulation affected largely by hydrocarbon compounds, this was documented in several studies (Thurberg *et al*., 1978; Engelhardt *et al*, 1981; Stickle *et al*, 1982; Al-Kindi *et al*.,1996 ; Brauner *et al.,* 1999) .

Usually ions flexes in fish gills were related with rich- mitochondria cells called chloride cells (CC) which contain Na^+,K^+-ATPase coenzyme within its membranes which is responsible for ions active transport (Eddy , 1982). Engelhardt *et al*. (1981) recorded an increasing in CC number vacuolated in its shape in rainbow trout *Salmo gairdneri* exposed to crude oil emulsion, as well as Haensly *et al*. (1982) observed an increasing in other kinds of branchial epithelial cells, particularly mucous cells in *Pleuronectes plattessa* exposed to crude oil in field study.

Because the gills represent the respiratory and osmoregulatory organ in fish so that it confronts environmental pollutants which may affect its functions and alter histological construction. The histological alterations considered as biomarkers for environmental pollution (Tricklebank , 2001).

The local studies about effects of oil pollution on physiological aspects targeted on effects on osmoregulation in some crustaecea (Hashim and Ahmed, 1998; Ahmed *et al*. ,2000), also Al-Obaidi (2000) studied the effect of the toxicity of Al- Dourah Refinary wastes on some aquatic invertebrates, Nasir (2000) studied the accumulation of Basrah crude oil in goldfish *Carassius auratus* and biochemical parameters affected by oil, also Al-Khafaji(2000, 2001) studied the effects of Al- Dourah Refinary wastes on survival rates and some haematological parameters and hatching success in some fish species of Dijlah River.

So there is no local studies about effects of short and long term exposure to oil derivative Gas oil which spill or discharge to the aquatic environment from the oil derivatives tankers and machinery boats in Shatt Al-Arab River and Basrah Canal so this study aim to determine the effects of the long term exposure of sublethal concentrations of gas oil on

ionoregulation (Na^+, K^+), chloride cells and histological alterations in khishni gills and the influnces of various temperature degrees on them and to know if these variables are suitable as biomarkers for monitoring and early predicting of oil pollution .

MATERIAL AND METHODS

Fish acclimation

Khishni *Liza abu* (Heckel) juveniles (weight 6-8 gm) were obtained from fish farm of Marine Science Center in University of Basrah in Garmat Ali location, and transferred to the laboratory in plastic containers (15l volume). The fishes were acclimated on laboratory conditions using plastic containers (50 l volume) filled with aged tap water, The acclimation continued for one week before the beginning of the experiment. The fish were fed once a day (2% of fish weight) with commerce foodstuff (protein 17%). The nutrition stopped 24 hours before the beginning of the experiment, quarter water volume replaced daily.

Gas Oil properties

Gas oil is one of oil derivatives which created of refining the crude oil. Gas oil composed of C_{12}-C_{20} , density 0.81-0.85 gm/cm^3, boiling degree 200- 360°C, composed of 20% aromatic compounds, 1-3% olefins, the other rest components were saturated compounds (nephthens and paraffins) and 1% sulfur (Ministry of Oil ,2000).

Preparation of exposure solutions: -

Five liters of gas oil was brought from Shuaiba Refinery in Basrah Govrnerate. After series of toxicity tests, the selected doses of gas oil –in – water emulsion were (0.5, 0.25 and 0.1) cm/l V/V which are equivalent to (415, 207.5 and 83) mg/l W/V in warm conditions and (0.25, 0.1 and 0.05) cm/l which are equivalent to (0.25, 0.1 and 41.5) mg/l in cold conditions, they added to glass aquarium 60x30x30 cm, two replicates for each concentration with 10 individuals each.

Measurement of hydrocarbons in exposure solutions and blood plasma:

Unesco (1977) method was used for hydrocarbons extraction from water. Then the reading recorded directly in mg/l using Oil Content Analyzer (Model OCMA – 310 HORIBA Ltd) made in Japan 1995. The same method was used for measuring hydrocarbons in blood plasma.

Measurement of Na^+ and K^+ levels in exposure solutions and blood plasma:

Three fishes every day were sacrificed to each variable parameter by blow on the head, caudal fin was cut, blood was collected by heparinized capillary tubes (volume 1x75 mm), blood plasma was separated using microcenterfuge (MSE model HJ- 488A), the separating plasma diluted with deionized distilled water and put in deep freezing conditions(-12°C) for later assay .

Separation, staining and counting chloride cells and total epithelial cells in gills:

Separation of chloride cells (CC) from the gills, were carriedout according to Sargent *et al*., (1978). To separate four left branchial arches were cut, put in % 0.9 sodium chloride at 4°C in order to remove the blood and attached matters, dried by filter paper, weighed and scraped the branchial epithelial layer, put in modified Hanks solution (Salman and Eddy, 1987). CC separation from epithelial cells was done by plastic medical syringe volume 5 Cm^3 using light pressure, two drops of 0.04% Neutral Red dye to cells suspension, incubated at 37°C for 5-10 minutes to stain chloride cells, two drops of 0.5% Methyl Blue were added for 2-3 minutes at laboratory temperature to stain other epithelial cells (EC) (Bancroft, 1975). Total number and percentage of CC and TEC were counted with in 1gm of tissue of gills using haematocytometer and compound microscope power 40x.

Preparation of gill sections:

The second and third branchial arches removed from the right side of fish head currently killed. The gill arches fixed in Bouin's fluid for 24 hours, after series of gradient conc. of ethylene alcohol cleaned, infltrated and embeded in paraffin wax then sectioned by rotary microtone to 5-7µm thickness and stained with Haematoxyn and Eosin dyes (Humason, 1979), finally examined by compound microscope.

RESULT

Hydrocarbons levels in exposure solutions

Hydrocarbons concentrations in exposure solutions (Table 1) revealed a notable reduction during the fifth and 14th days at both warm and cold conditions .The concentrations reduced to6.6, 6.2 , 6.5 % in (0.5, 0.25 and 0.1) cm/l which equivalent to conc. (415 , 207.5 , 83) mg/l respectively under warm conditions . While it reached to 11 , 9.8 , 10.5 % in (0.25, 0.1 and 0.05) cm/l which equivalent to conc.(207.5 , 83 , 41.5) mg/l respectively under cold conditions .

Table (1): Reduction percentages of total HC conc. W/V in exposure solutions

Warm conditions				Cold conditions			
Conc mg/L	**Percentage %**			**Conc mg/L**	**Percentage %**		
	Time 0	**Fifth day**	**14th day**		**Time 0**	**Fifth day**	**14th day**
415	%100	14.6%	6.6%	207.5	%100	20%	11%
207.5	%100	15%	6.2%	83	%100	225%	9.8%
83	%100	13.9%	6.5%	41.5	%100	22.5%	10.5%

Hydrocarbons levels in blood plasma

The results showed a decreasing in HCs conc. in blood plasma exposed to gas oil under warm conditions (0.5, 0.25 and 0.1cm/l) at 14th day , it reached 17.5 , 16.4 and 16 mg/l respectively compared with its levels in fifth day of treatment (36 , 29 and 24 mg/l). While under cold conditions HCs levels decreased to 23.2 , 12.5 and 10.5 mg/L in 14th day of exposure to doses (0.25, 0.1 and 0.05) cm/l respectively. (Figure 1)

Na^+ and K^+ levels in exposure solutions

Na^+ and K^+ conc. in the media increased with the rise of gas oil dose in the exposure solutions. Its levels reached under warm conditions (0.5 cm/l treatment) to 13.2 and 0.9 mmol/l respectively compared with control (8 and 0.78 mmol/l) in the same order.

Also under cold conditions they reached in (0.25 cm/l) treat. to 12 and 0.86 mmol/l respectively.(Table2)

Table (2): Na^+ and K^+ conc. in exposure solutions after 14 days

Warm conditions			Cold conditions		
Gas oil additive volume cm/l	**Na^+ mmol/l**	**K^+ mmol/l**	**Gas oil additive volume cm/l**	**Na^+ mmol/l**	**K^+ mmol/l**
Control	8	0.78	Control	8	0.76
0.1	9.5	0.8	0.05	9.8	0.78
0.25	11	0.82	0.1	11.2	0.81
0.5	13.2	0.9	0.25	12	0.86

Na^+ and K^+Levels in blood plasma

Na^+ levels in blood plasma of Khishni juvenile exposed to gas oil doses (0.5 and 0.25) cm/l under warm conditions reached (112.5±6.8), (118.66±3.2) and (119±4.3) mmol/l compared with control (127.83±2.92) mmol/l. While under cold conditions Na^+ reduced significantly ($P<0.01$) in 14th day of exposure to dose 0.25 and 83 cm/l it reached to 109.33 ±3.05 and (116.33±1.52) mmol/L compared with control (125.5±4.92) mmol/l. The results also showed that there was a significant difference in Na^+ conc. under different temperature degrees.(Figure2)

K^+ levels in blood plasma of khishni juveniles exposed to gas oil. Exposure of fish to different doses of gas oil (0.5 and 0.25) cm/l under warm conditions caused a significant reduction ($P<0.01$) in K^+ conc. as it reached to (8 and 7) mmol/L respectively in the 14th day of exposure compared with control (10 mmol/L). However under cold conditions K^+ reduced significantly ($P<0.01$) to (7.96±0.25) and (8.13±0.32) mmol/l compared with control (10.88±0.25) mmol/l while it reduced significantly ($P<0.05$) to (8.53 ±0.55)mmol/l in the lowest dose treatment (0.05 cm/l). Also there was significant reduction under different temperature degrees.(Figure3)

Number and percentage of chloride cells and total epithelial cells

The results revealed increasing number of chloride cells (CC) and total epithelial cells in branchial tissue under warm conditions in the three treatments, the number of CC reached in 14th day of exposure to $5.12x10^6$, 4.48 $x10^6$ and 3.64 $x10^6$ cell/gm tissue respectively compared with the control (1.9 x 10^6 cells/ gm tissue).

Percentage of CC under high temperature at 14th day 5.53 – 4.81% compared with control 3.11%. While in cold conditions CC number increased at the 14th day 5.8 - 4.6x 10^6 cells/ gm tissue compared with control (2.14 $x10^6$ cells /gm tissue) and EC also highly increased at the same conditions, therefore percentage of CC under low temperature degrees showed increasing (5.63 – 5.45%)compared with control (3.6%). CC and epithelial cells (EC) responded with gas oil concentrations.

Histopathological study of the gills

Microscopic examination to longitudinal gill sections of fish exposed to gas oil 0.25 cm/l for 14 days showed (A) control. (B)curling in secondary lamellae and lifting in filament epithelial layer.

Table (3): Number and percentage of chloride cells and total epithelial cells

Warm conditions				**Cold conditions**			
Gas oil additive volume cm/l	Number of CCx10^6	Number of EC x10^7	Percentage of CC %	Gas oil additive volume cm/l	Number of CCx10^6	Number of EC x10^7	Percentage of CC %
Control	1.9	6.09	3.11%	Control	2.14	5.89	3.63%
0.1	3.64	7.56	4.81%	0.05	4.6	8.8	5.63%
0.25	4.48	8.85	5.06%	0.1	5.1	9.35	5.45%
0.5	5.12	9.25	5.53%	0.25	5.8	10.3	5.22%

In fish gills exposed to 0.5 cm/l(C) there was hyperplasia in many secondary lamellae,fusion and necrosis.(D)lifting in epithelial layer of gill filaments. (E) complete fusion in secondary lamellae also was showed (F) hyperplasia and terminal hypertrophy.

DISCUSSION

The persistence of petroleum compounds in water is extensively affected by temperature degree.This study showed that the conc. of HCs in the test solutions decreased by 93.6% in the conditions of high temperature (27±2.3)° C and by 92.6% in the conditions of low temperature (15±1.5) ° C. A similar result was recorded by Korn *et al*. (1979) and Short and Harris (1995, 1996) and Duxbury and Duxbury 1997. This decrease in HCs conc. in the water is due to volatilization of light compounds (which includes Mono Aromatic Hydrocarbons and light Alkanes less than 10 carbon atoms), evaporation and biodegradation (Ramsino *et al*., 1984; Moles *et al*.,1979). Also artificial aeration enhances evaporation of HCs (Anderson *et al*.,1974) .

This study showed that the conc. of HCs in blood plasma was correlated with the conc. of exposure media, as high conc. of HCs in the blood plasma coincided with high conc. of gas oil in the exposure media. The rout of influx of HCs to the blood is via gill membranes (Heras *et al*.,1992) as soluble HCs is connected with lipoproteins in the blood and conveyed through the circulatory system to the vital organs such as liver and flesh.

Movement of HCs depend on its conc. ratio of tissue to water , on diffusion and flow rate of blood through tissues, HCs oxidized by cytochrome P450 system and produce more polar metabolites easily

excreted by gills or bile (Ackman *et al*.,1996). This process continues until equilibrium point (Zhou *et al*.,1997).The study also revealed that HCs conc. in the blood plasma was higher under cold conditions comparing with its conc. under warm conditions because the exposure temperature affects uptake and elimination processes of HCs in case of lipid content was similar (Petersen and Kristensen , 1998).

The ionic regulation was highly affected by gas oil. This was clear from the conc. of Na^+ and K^+ in the blood plasma. Na^+ and K^+ conc. in the blood plasma decreased in all HCs conc. this means that the permeability of gill membranes to monovalent ions is highly affected by HCs and diffused out of the blood into hypoosmotic environment (Zbanyszek and Smith, 1984). McKeown and March (1978) found a slight decrease in Na^+ in the blood of fresh water acclimated rainbow trout *Salmo gairdneri* exposed to 200 mg/l of Bunker C oil. A similar result was reported by Brauner *et al*.(1999) in gobid fish *Hoplosternum littorale* exposed to crude oil and by Engelhardt *et al*. (1981) in rainbow trout exposed to 200 ml/l of water soluble fraction of crude oil.

It is thought that the disturbance in osmoregulation was related to the damage of the gills and destroy of the chloride cells which are a specialized cells of the active transport of ions and because of Na^+ , K^+ - ATPase inhibition by adverse effect of HCs (Engelhardt *et al*.,1981).

However, the exposure to gas oil caused an increase in the number and percentage of chloride cells (CC) in the branchial epithelia. This explained as a stress response to pollutants (Wendelaar Bonga *et al*.1990). A similar result was recorded in rainbow trout exposed to crude oil emulsion (Engelhardt *et al*.,1981). It is thought that the source of increased number of CC may be of multiple mitosis divisions in epithelial cells (EC) of gills (Thompson and Sargent ,1978) or it belong to conversion of undifferentiated EC to active CC (Ahmed,1996) for purpose of maintain the ionic balance. Also the total epithelial cells TEC of the gill membranes was also increased through exposure to gas oil which was recorded in *Pleuronectes platessa* exposed to oil spill in Amoco Cadiz (Haensly *et al*.,1982) .

The histological study revealed that there were severe histopathological alterations during exposure to gas oil. The cell damage and necrosis occure under highly toxic condition Al-Sayed *et al.* 1995. Curling of lamellae was suggested to cause a decrease in blood hydrostatic pressure with in the pillar cell system (Engelhardt *et al*.,1981).

The lifting in epithelial layers, the hyperplasia and fusion in secondary lamellae was also recorded by El-Sayed *et al*.(1995) who explained lifting as a physiological response to increase the distance between the pollutant

and circulatory system, hyperplasia and fussion may serve as protective function by decreasing the amount of vulnerable gill surface area. Hyperplasia in gill sections promoted the result of increasing number of EC above.

In conclusion, this study showed that *Liza abu* juveniles is sensitive species to gas oil pollution so it is suitable as indicator species. The temperature degrees play a great role in the accumulation of hydrocarbon compounds in the blood and tissues. Besides ionic regulation, chloride cells (CC), branchial epithelial cells (EC) and the histopathological alteration are suitable biomarker to oil pollution in inland water.

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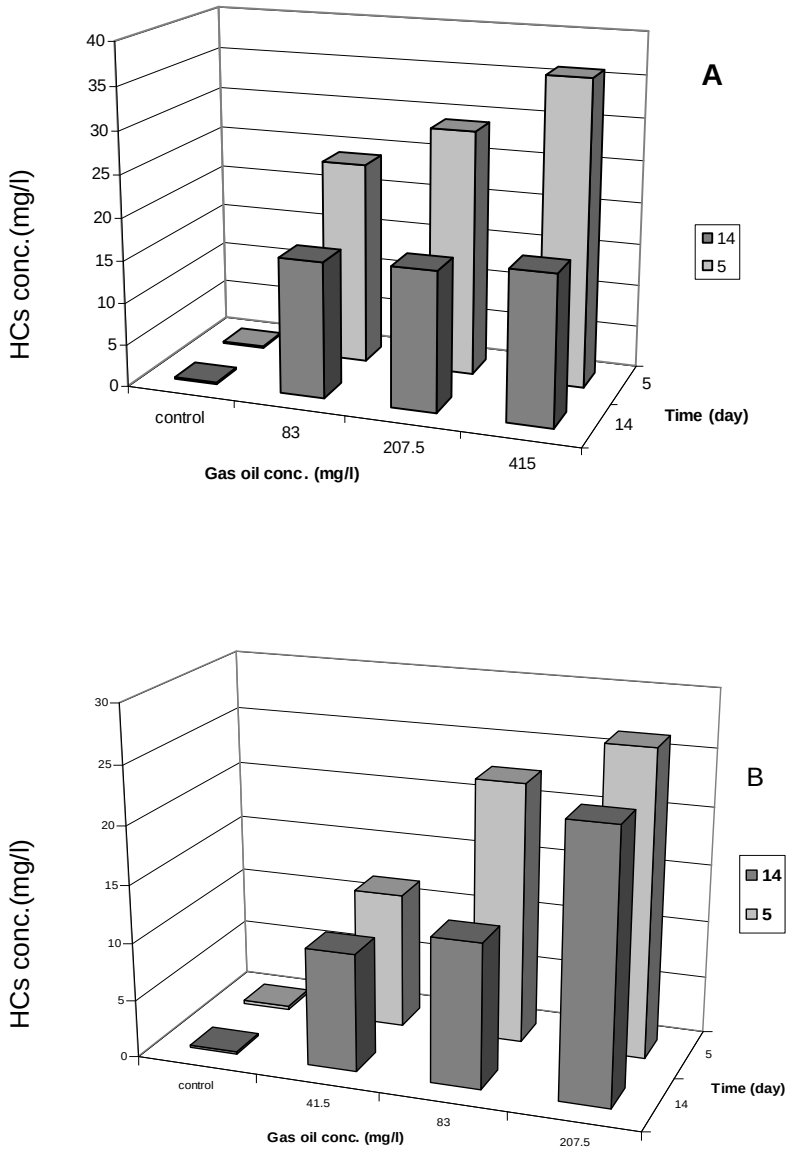
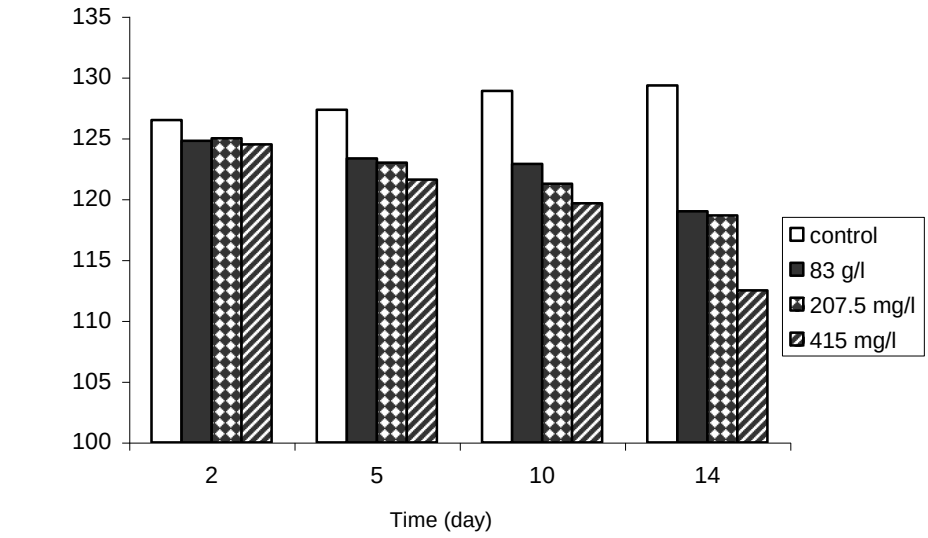


Figure 1: HCs concentration (mg/l) in the blood plasma of *L. abu* at (A)27º C and (B) 15º C.



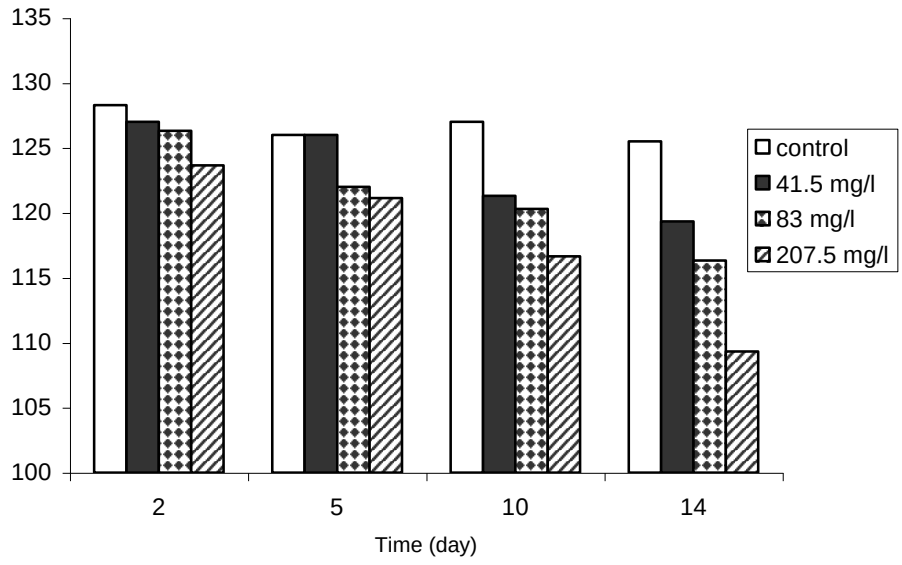
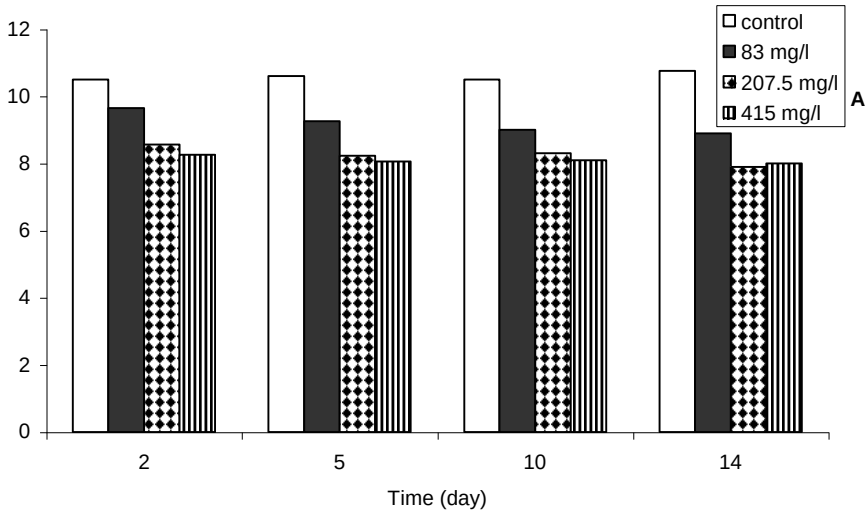
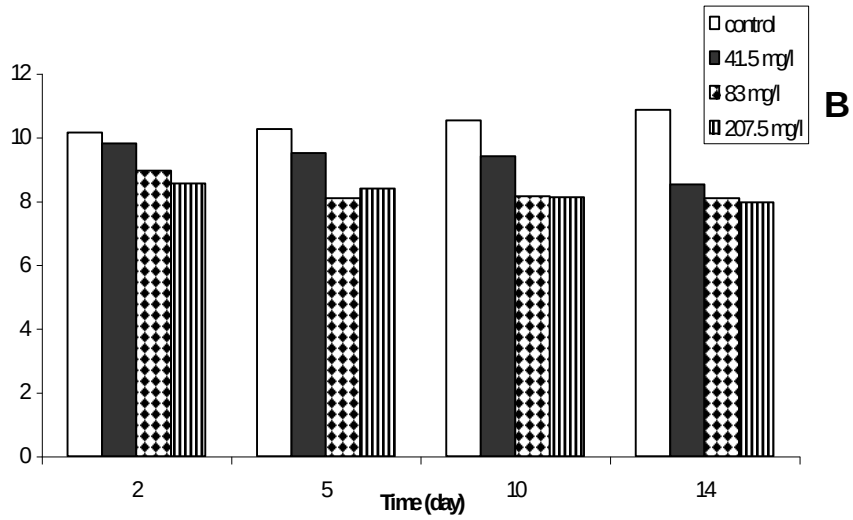
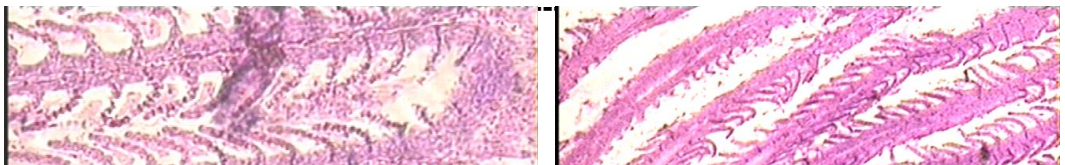


Figure 2: Na^+ concentration (mg/l) in the blood plasma of *L. abu* at (A)27º C and (B) 15º C.





]Figure 3: K^+ concentration (mmol/l) in the blood plasma of *L. abu* at (A) 27° C and (B) 15° C



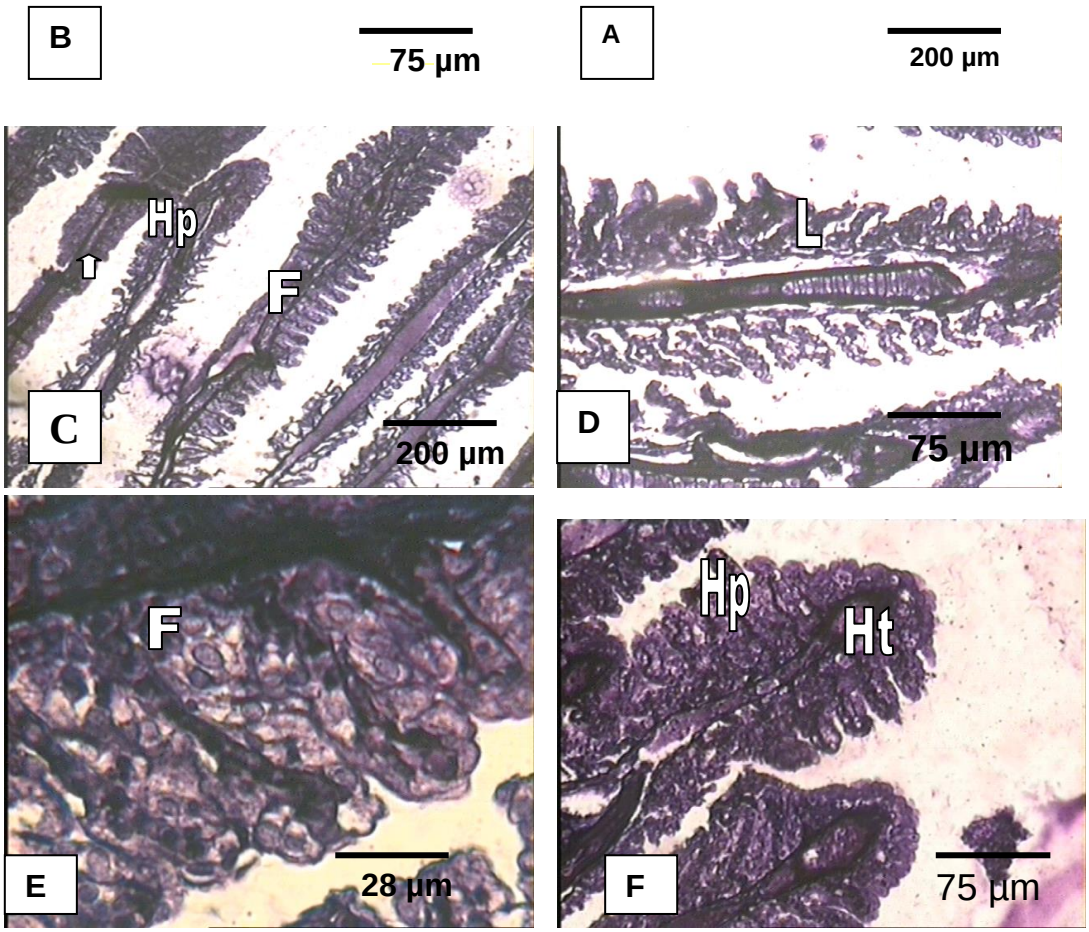


Plate (1): Longitudinal sections in gills of *L. abu* exposed to 0.25 and 0.5 cm^3/l a.control. b.Gills exposed to o.25 cm^3/l : (C) curling and (L) lifting in epithelial layer. Gills exposed to 0.5 cm^3/l: c. (Hp)hyperplasia,(F) fusion and necrosis. d. (L) lifting and necrosis. e. (F) fusion. f. (Hp) and (Ht).

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الخلاصة

لمعرفة تأثير التعرض طويل الأمد لزيت الغاز على التنظيم الأيوني و اعداد خلايا الكلوريد و الخلايا الطلائية الكلية و التغيرات النسيجية المرضية في غلاصم يافعات سمكة الخشني *Liza abu* (Heckel) .أجريت عدة تجارب مختبرية تحت درجات حرارة مختلفة , الدافئة (27+2,3) والباردة (15+1.5) . عرضت الأسماك الى التراكيز (415 و5 .207 و83) ملغم / لتر من زيت الغاز في درجات الحرارة العالية و الى التراكيز (207.5 و 83 و 41.5)ملغم/ لتر تحت درجات الحرارة الواطئة . لوحظت التغيرات في اليوم الثاني و الخامس و العاشر و الرابع عشر من التعريض بينما قيست تراكيز الهيدروكربونات النفطية في محاليل التعريض و بلازما الدم في اليوم الخامس والرابع عشر من المعاملة . لوحظ أنخفاض ملحوظ في مستويات الهيدروكربونات النفطية في محاليل التعريض خلال اليوم الخامس و الرابع عشر وفي الوقت نفسه فأن مستوياتها توافقت مع تلك التي في محاليل التعريض . وعانت مستويات أيوني الصوديوم و البوتاسيوم أنخفاضا معنويا (احتمالية 0,01) في بلازما الدم . و لوحظ ارتفاع في أعداد ونسب خلايا الكلوريد تحت كلتا درجتي الحرارة . و تراوحت أعدادها من 3,64-5,12 * 10 6 خلية /غم مادة مقشوطة تحت الظروف الدافئة مقارنة مع معاملة السيطرة (1.9 * 10 6 خلية /غم مادة مقشوطة) و 4,6-5,8 * 10 6 خلية /غم مادة مقشوطة تحت الظروف الباردة .ترافقت الزيادة في أعداد خلايا الكلوريد مع زيادة مميزة في أعداد الخلايا الظهارية الكلية في الغلاصم تحت كلا درجتي الحرارة . أظهرت الفحوصات النسيجية لغلاصم الأسماك المعرضة لتراكيز من زيت الغاز (207,5 , 415 ملغم /لتر) لمدة أربعة عشر يوما تغيرات نسيجية مرضية وهي تجعد و فرط تنسج و أندماج و تنخر الصفائح الثانوية و انفصال الطبقات الظهارية للخيوط الغلصمية و تضخم أطرافها .