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IMPACT OF TOLUENE ON IONIC REGULATION AND OXYGEN CONSUMPTION OF FRESHWATER CRAB *Sesarma boulengeri* (Calman)

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Summary

The present study has been conducted to explain the effect of sub lethal concentrations of toluene on some physiological aspects of freshwater crab *S. boulenger*i including oxygen consumption rate, water and ionic regulation, bioaccumulation and histopathological effects. Three sub lethal concentrations (2.5, 5.5 and 10.5 ppm) of toluene were used to study their effects during long term exposure (14 days) under 28° C. The results showed that there was a positive correlation between oxygen consumption rate and toluene concentration. Oxygen consumption rate reached to 0.223 mg O2/g/h in 10.5 ppm toluene. Body water content showed an increase to 71.17% in 10.5 ppm toluene. Ionic regulation also affected by toluene. A significant decrease ($P<0.01$) was recorded in the level of Na^+ in the haemolymph, as it reached to 245.5 mmol/l in 10.5 ppm toluene. While K^+ concentration in the lymph showed a significant increase ($P<0.01$) to 12.41 mmol/l comparing with the control. Bioaccumulation of toluene in the gills in 5.5 ppm toluene reached to 2.98 mg/g dry wt. during the 3rd day of exposure. While it decreased to 1.34 mg/g dry wt. during the 11th day of exposure. Histopathological changes include swelling, hyperplasia, and hypertrophy in gill filaments. Recovery data after 4 days of transfer to clean water showed that the physiological parameters studied (oxygen consumption rate, water and ionic regulation) did not return to the normal levels.

INTRODUCTION

Transportation of oil derivatives via Shatt Al-Arab River and Basra channel causes large quantities of oil spills into aquatic environment.

Toluene is one of petroleum derivatives. It is a mono-aromatic hydrocarbon which when enter aquatic environment impact many physiological and biological activities of aquatic animals.

Ionic regulation affected largely by hydrocarbon compounds (Thurberg *et al*., 1978; Engelhardt *et al*., 1981; Brauner *et al*., 1999).

It has been found that toluene affect ionic regulation of many aquatic animals such as fish (Faddaq *et al*., 2004; El-Sayed *et al*.,, 1995; Buikema, 1980) and aquatic invertebrates (Hashim and Ahmed, 1998; Bakke and Skjoldal, 1979).

Gill of Crustaceans represents the respiratory and osmoregulatory organ. It is in direct contact with the pollutants in the water. This will affect its function and alter histological construction. This histological alternation can be considered as biomarkers for environmental pollution (Tricklebak, 2001).

There are many local studies concerning petroleum hydrocarbons pollution and their effects on aquatic animals (Ahmed *et.al*.,2001; Hashim and Ahmed,1997; Al-Saad, 1990; Al-Obaidi, 2000), the levels of hydrocarbons in fish and sediment (Al-Saad, 1995, Al-Saad *et al*.,1997; Al-Saad and Al-Timari,1993; Al-Khafaji, 2000, 2001). However, there are no local studies concerning toluene pollution and its sub lethal physiological effects. Due to increase the level of hydrocarbons pollution in land water especially Shatt Al-Arab Estuary, and due to the lack of information of toluene pollution, this study was conducted to show the physiological impacts of sub lethal concentration of toluene on freshwater crab *Sesarma boulengeri*, which is an important species of freshwater crustacean that disappeared from large area of Shatt Al-Arab Estuary banks due to increasing activity of transportation of oil derivatives via Shatt al-Arab estuary. So this species were chosen to study the impact of toluene on oxygen consumption rate, water and ionic regulation and histopathological effects on the gills.

Materials and Methods

Collecting and acclimating of fresh water Crab *Sesarma boulengeri*: Fresh water crab *S. boulenge*ri was collected from Shatt Al-Basra channel during March 2004 to October 2004 at low tide from intertidal zone by hands. Kept in plastic containers (10 L) with some water from the same environment. The animals were brought to the laboratory, put in glass aquariums (30 X 30 X 60 cm) each aquarium contain 3L of de chlorinated tap water.

Acclimation was continued for 7 days on laboratory condition. Animals were fed a commercial diet (26% protein). Feeding was stopped before 24 hours of starting the experiments.

Preparation of stock toluene (saturated solution)

Five liters of toluene were purchased from GPR (General Purpose Reagent) with the following properties:
Atomic wt. 0.860 – 0.866
Boiling degree 110 – 111° C
Purity: 92.12 %
Refracting index: 1.493 – 1.497.

The saturated solution of toluene was prepared by dissolving 535mg toluene in distilled water making up to 1 liter (NAS, 1975). The stocked toluene was transfer to separating funnel, shacked for 20 min., then left to settle for 10 min. The upper layer was dissenting; remaining solution is the saturated toluene. Kept in close bottle in 10° C for at least 48h before use.

Preparation of exposure solutions:

Three sub lethal concentration of toluene (2.5, 5.5, 10.5 ppm) from stock toluene were prepared. Each transfer to a glass aquarium (30 X 30 X 60 cm) containing 10 liter dechlorinated tap water.

Two replicates for each concentration were used with twelve individuals for each treatment and control treatment.

Water temperature, salinity, pH were determined daily during the experimental period which continued for 14 days.

Opaque plastic cover was used to cover the aquarium to reduce stress from the experimental animals.

Physiological measurements:

Sampling:

Two to three individuals of crabs were taken from each toluene concentration during the 1st, 5^{th}, 10^{th} and 14^{th} day. The weight of the crabs was taken and it was ranged between 10.4 to 20.5 g. The haemolymph were taken using microsyring (100 µl) by puncturing the podium membrane between the 4^{th} and 5^{th} walking legs. Lymph volume was ranged between 10 – 50 µl, putting in plastic vials (12ml) dilute to 50 to 100 times using deionized distilled water., then kept under freezing (-12° C) for later assay of Na^+ and K^+.

Water content of the body:

The water content of the body was estimated by taking the wet weight of the sampled individuals then taking dry weight after drying the animals in electric oven under 105° C for 24 hours (Ahmed, 1996).

Na^+ and K^+ concentration measurement:

The concentration of ions (Na^+ and K^+) in the haemolymph was determined by flame photometer (ANA-10AL) after calibrating the apparatus using standard solution of NaCl (2 ,1,0.5,0.25 mmol/l) and KCl (0.25 , 0.15, 0.1, 0.05 mmol/l) (Ahmed,1996).

Oxygen consumption Rate:

Oxygen consumption rate of *S. boulanger*i exposed to sub lethal concentration of toluene (2.5, 5.5, 10.5 ppm) were measured. Four individuals were taken (weighted 15.3-20.3g). Each one kept in 1 liter conical flask containing dechlorinated tap water. The flasks were closed firmly. Aeration was used by using plastic tube pass through the plastic stopper. The flasks were covered with opaque plastic cover to reduce stress (Sumich *et al*., 1996). Acclimation on this enclosed environment was continued for 24h. The experiment began by stopping the aeration and adds three volumes of toluene to each flask as follow (2.5, 5.5, 10.5 ppm toluene. The 4^{th} flask was kept as control.

Oxygen concentration in each flask was determined by using DO meter (MPS55) through intervals (30, 60,120,180 min) after stopping aeration.

Oxygen consumption rate was calculated as mg O_2/g /h.

Hydrocarbon concentration in the gills:

Hydrocarbon concentration was determined in the gills of *S. boulenger*i exposed to 5.5 ppm toluene.

Three glass aquariums (30X30X60cm) were used. Each contain 10 liter of water containing 5.5 ppm toluene twelve individuals were kept in each aquarium. Using plastic opaque cover to minimize evaporation. The experiment continued for 11 days. Samples of crabs were taken during the 3^{rd}, 7^{th}, 11^{th} days of exposure.

Extraction of Hydrocarbons from the gills:

The method of UNEP (1992) was used for extraction of hydrocarbons. After drying the gills of crabs that exposed to toluene using freeze-drying method for 48h. The gill samples were crush and kept in vials. The extraction was done using methyl alcohol in soxhlet apparatus.

The extraction continued using mixture of methanol: Benzene (1:1) for 24 hours. The extracted aromatic portion is dissolve in 5ml hexane, and then the concentration of toluene was determined using UV Cintra 5GBC Scientific Equipment on wave length 484 nm.

Depuration:

Recovery experiment was done for the individidual remaining from the experiment of sub lethal exposure for 14 days.

Five individuals were taken from each concentration (2.5,5.5,10.5 ppm toluene) (weighed 4.84-10.88 g) wash with clean water and transfer to glass aquarium (30 X30 X60 cm) each contain 3 liter of clean tap water. The individuals were kept for 4 days. At the end of the experiment the following parameter were taken:

Na^+ and K^+ concentration in the lymph.

Water content of the whole body.

Hydrocarbons concentration in the gills.

Histopathological study:

Gill samples were taken from individuals that exposed to 5.5 ppm toluene for 14 days. Fixed in Bouin's solution for 24h, dehydrated using ethyl alcohol. Sectioning was curried out using rotating microtome to 5-7 microns. Staining was done by using Haematoxyline-Eosing Method (Humason, 1979).

Results:

Concentration of toluene in the exposure solution:

Fig 1 showed the concentration of toluene in the experimental solution (2.5, 5.5, 10, 5 ppm) in the 5^{th} an 14^{th} day of exposure. There was a decrease in toluene concentration in the 5^{th} day from 0.82, 1.21, 2.11 ppm in the exposure concentration to 0.21, 0.25, 0.34 ppm respectively.

Effect of toluene on oxygen consumption rate:

Fig. 2 showed oxygen consumption rate of *S. boulangeri* exposed to sub lethal concentration of toluene (2.5, 5.5, 10.5 ppm) for 24 hr.

The experiment showed an increase in the oxygen consumption rate with increasing toluene concentration.

Oxygen consumption rate reached to 0.223 mg O_2/g/h in 10.5 ppm toluene comparing with the control (0.163 mg O_2/g/h).

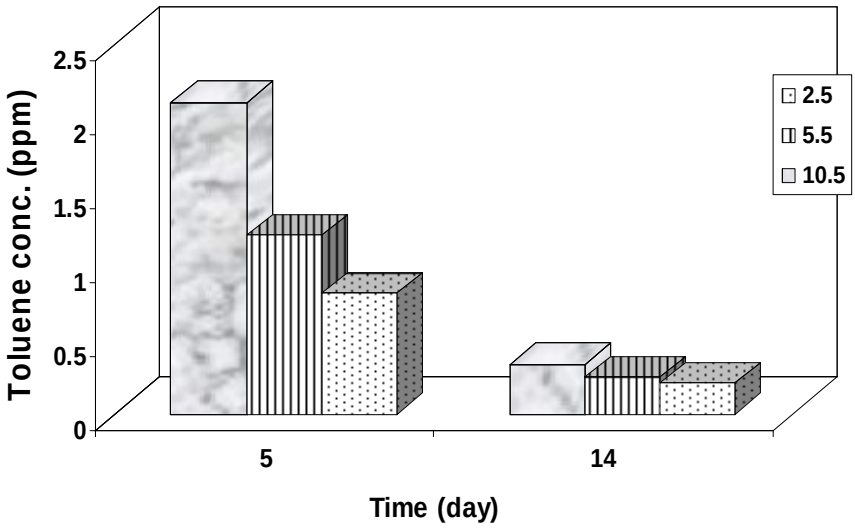

Fig 1: Toluene concentration (ppm) in the exposure media

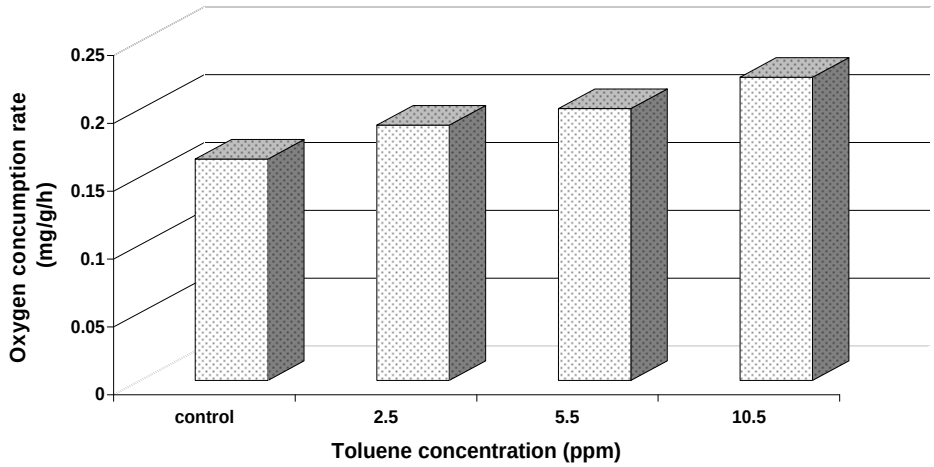

Fig.2: Oxygen consumption rate in *S boulengeri* after 24h of exposure to different levels of toluene.

Water content of the body:

Fig.3 show the water content of the body of *S. bolengeri* exposed to 2.5, 5.5, 10.5 ppm toluene. The result showed that there was a significant ($P<0.01$) increase in water content of the body after 14 days of exposure to 10.5 ppm toluene. As water content reached to 71.17% ± 8.41 comparing with the control (66.9% ± 0.8). While there was a non-significant ($P>0.01$) increase in water content in the 2.5 ppm toluene after 10 days of exposure, as it reached to 68.9% ± 1.06 comparing with the control.

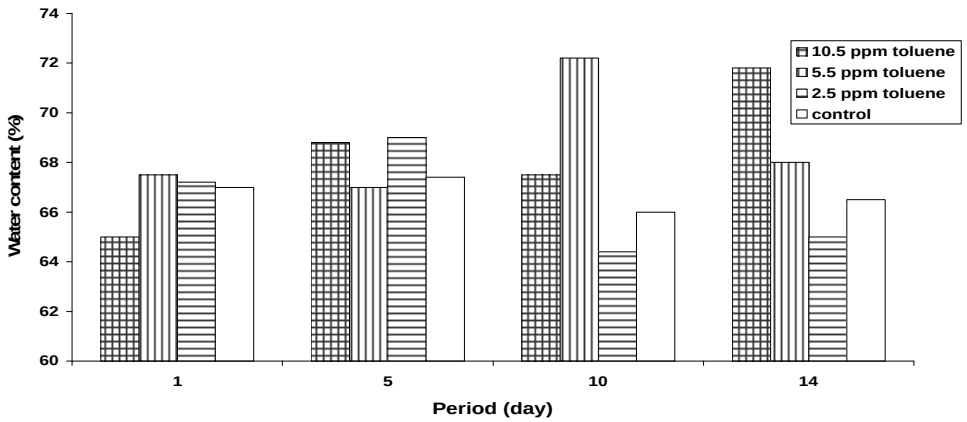

Fig.3: Water content (%) in the body of *S.boulengeri* exposed to different levels of toluene.

Ionic concentration of the haemolymph (Na^+, K^+):

Fig 4 showed the concentration of Na+ in the lymph of *S.boulengeri* exposed to sublethal concentration of toluene (2.5, 5.5, 10.5 ppm).

The exposure caused a significant ($P<0.01$) decrease in the Na+ level during the 1st day of exposure in all concentrations. The significant decrease ($P<0.01$) continued till the 10th day of exposure in 10.5ppm toluene comparing with the control.

K^+ concentration show disturbance (fig 5) in all toluene concentration, while in the 14th day there was a significant increase ($P<0.01$) in all toluene concentration, comparing with the control (7.29 mmol/l).

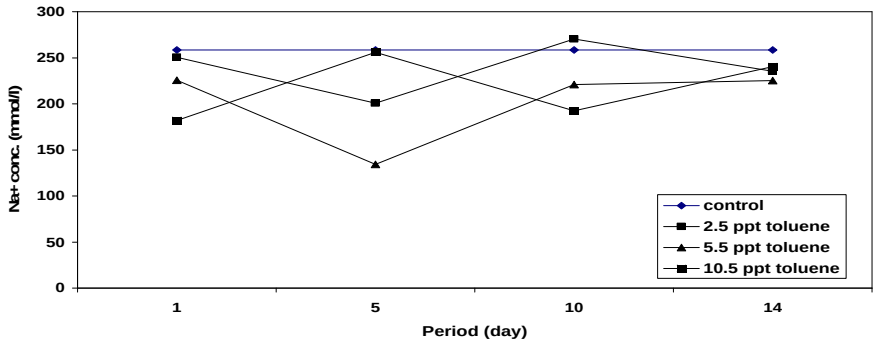

Fig.4: Sodium concentration (mmol/l) in the haemolymph of *S. boulengeri* exposed to different levels of toluene for 14 days.

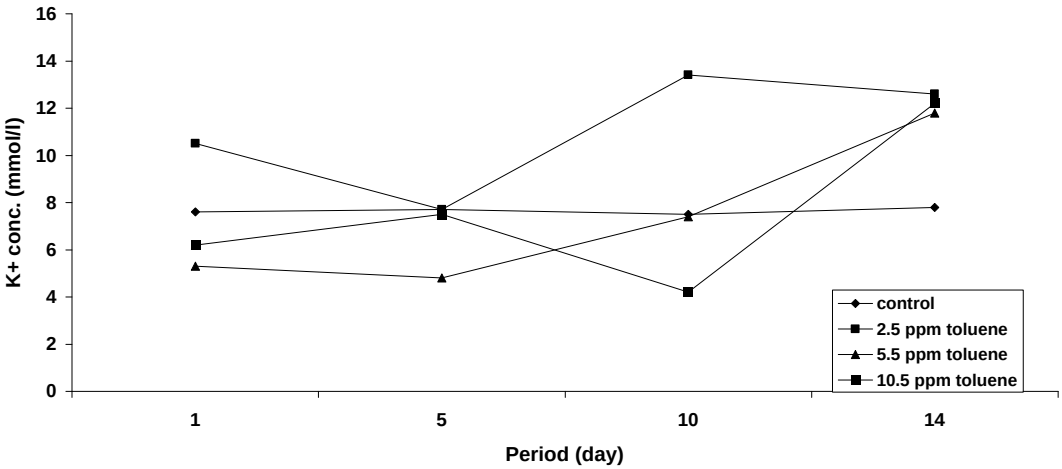

Fig.5:Potassium concentration (mmol/l) in the haemolymph of *S. boulengeri* exposed to different levels of toluene for 14 days.

Bioaccumulation of toluene in the gills:

Table 1 show the concentration of toluene in the gills of *S. boulengeri* exposed to 5.5 ppm toluene for 11 days. There was an increase in the toluene level as it reached to 2.98µg/g dry wt. after 3 days of exposure, and then it decreased to 2.25µg/g dry wt. after 7 days of exposure. During the 11^{th} day of exposure the concentration reached to 1.34 µg/g dry wt.

Table 1: Toluene accumulation in the gills of *S. boulengeri* exposed to sub lethal concentration of toluene (5.5 ppm) for 11 days.

Day	Toluene conc. µg/g dry wt.)
Control	**0.04**
3^{rd} day	**2.98**
7^{th} day	**2.25**
11^{th} day	**1.34**

Depuration:

The recovery results showed that physiological parameters did not return to the normal levels as in control and as follow:

Water content of the body:

Fig 6 show no significant (P>0.01) changes in water content in the body after 4 days of transfer to clean water as it reached to 67.28% , 69.67%, 69.25% comparing with the level during the 14^{th} days of exposure to toluene (2.5, 5.5, 10.5 ppm respectively).

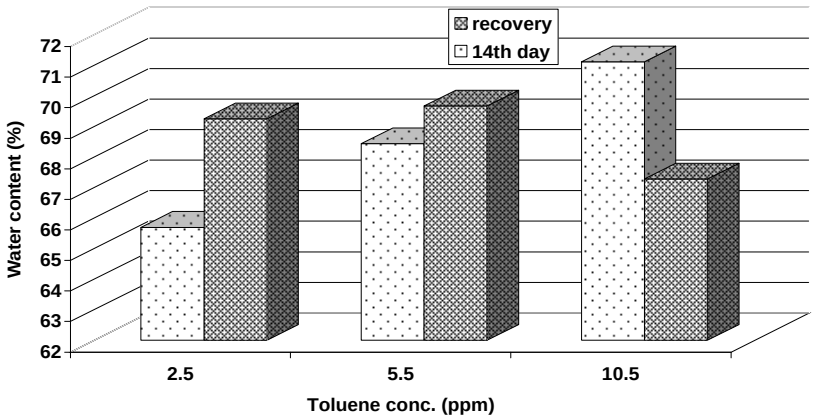

Fig.6: Water content (%) in *S. boulengeri* after 4 days of transfer to clean water comparing with the 14th day of exposure to toluene.

Ionic concentration in the haemolymph:

Fig 7. showed the concentration of Na^+ in the lymph after 4 days of transfer to clean water. The results showed that there was a significant increase ($P<0.01$) in Na^+ level in 2.5, 5.5 ppm toluene, While there was no significant ($P>0.01$) increase of Na^+ in 10.5 ppm toluene comparing with the concentration in the 14^{th} day of exposure to toluene.

Fig 8. also shows the concentration of K^+ in the lymph after 4 days of transfer to clean water. It showed that there was a significant ($P<0.01$) decrease in K^+ level (6.76, 5.0, 3.5 mmol/l) in all concentration of toluene (2.5, 5.5 10.5 ppm respectively) comparing with K^+ concentration during the 14^{th} day of exposure to toluene.

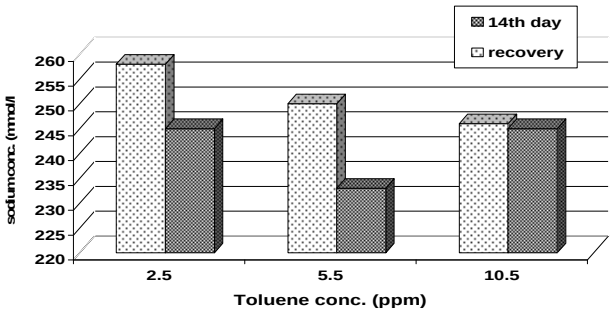

Fig.7: Sodium concentration (mmol/l) in the haemolymph of *S. boulengeri* after 4 days of transfer to clean water comparing with the 14th day of exposure to toluene.

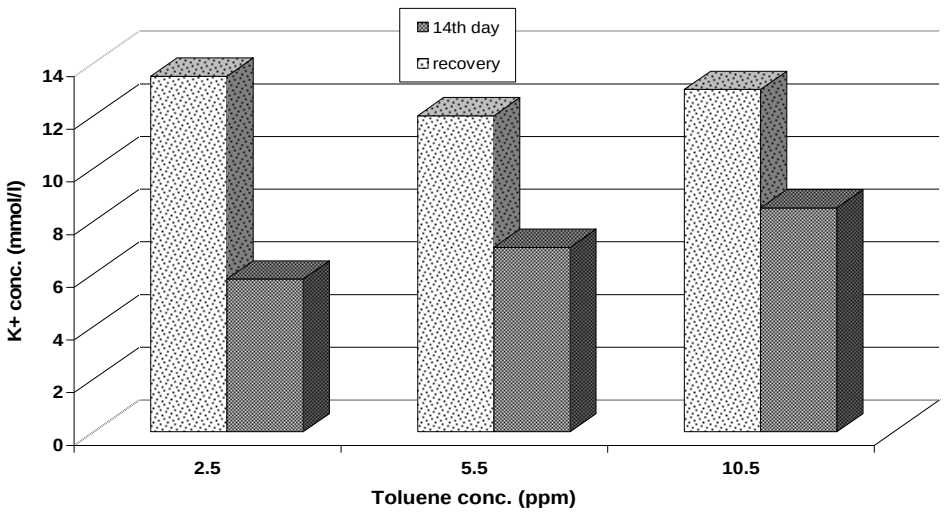

Fig.8: Potassium concentration (mmol/l) in the haemolymph of *S. boulengeri* after 4 days of transfer to clean water comparing with the 14th day of exposure to toluene.

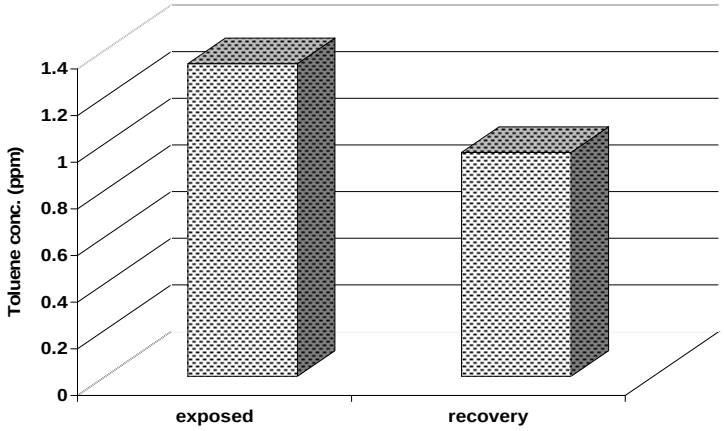

Fig.9:Toluene concentration in the gills of *S.boulengeri* exposed to 5.5 ppm toluene for 11 days and after 4 days of transfer to clean water.

Bioaccumulation of toluene in the gills:

After 4 days of transfer to clean water there was a decrease in toluene concentration in the gills which reached to 0.96µg/g dry wt comparing with the 14th day of exposure to 5.5 ppm toluene (1.34 µg/g dry wt) (fig 9).

The histopathological effect:

Many histopathoogical changes occurred on the gills during the exposure to 5.5 ppm toluene for 14 days. These changes include swelling in many region of the gill filament (plate2), hypertrophy in some filaments and hyperplasia, which caused fusing of many gill filaments (plate1, 2, 3). Necrosis in some places of the epithelia was noticed.

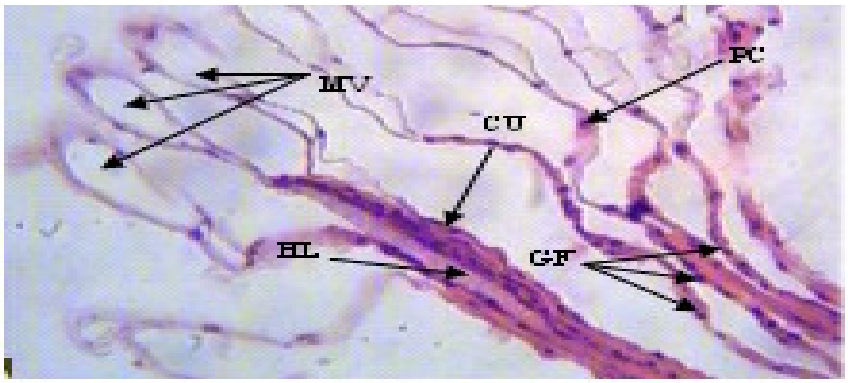

Plate 1: Longitudinal section in the gill of *S. boulengeri* show the marginal vessel (Mv), Cuticle (Cu), Pillar cells (PC), Haemolymph lacuna (HI) and gill filament (Gf). (400X).

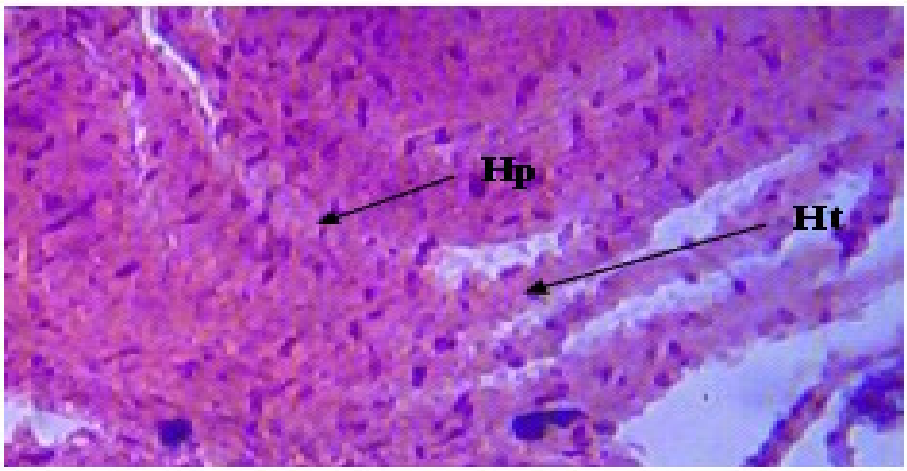

Plate 2: Longitudinal suction in the gill of *S. boulengeri* exposed to 5.5 ppm toluene for 14 days. Show Hypertrophy (Ht), Hyperplasia (Hp) in the gill filaments. 400X

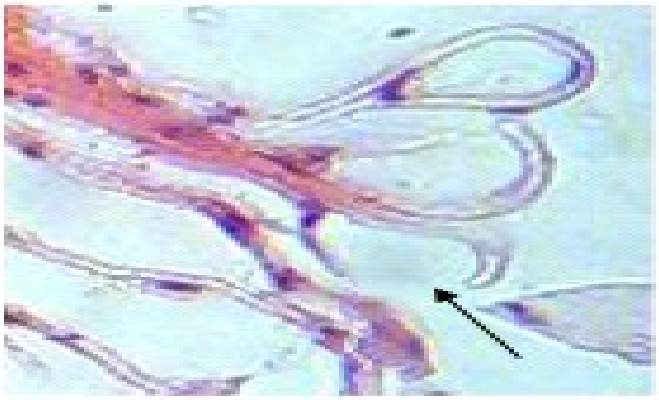

Plate 3: Longitudinal suction in the gills of *S. boulengeri* exposed to 5.5 ppm toluene for 14 days show Necrosis (N) in the Marginal vessels of the gill filaments.

Discussion:

The persistence of petroleum compounds in water is extensively affected by temperature. Present study showed that the concentrations of Hcs in the test solutions decreased sharply at the end of the exposure experiment. Similar result was recorded by Korn *et al*., (1979); Short and Harris (1995); Duxbury and Duxbury (1997) and Faddaq *et al*., (2004). The decrease in HCs conc. in water is due to volatilization of light compounds (which include Mono Aromatic Hydrocarbons and light Alkenes less than 10 carbon atoms), evaporation and biodegradations (Moles *et al*., 1979; Ramusino *et al*., 1984).

The results showed that freshwater crab *S. boulengeri* exposed to sub lethal concentration of toluene suffer from several physiological alternations concerning ionic and water regulation.

The results showed an increase in the oxygen consumption rate during exposure, ranged between 0.613-0.223 mg O_2/g/h. This increase also was reported for Amphipod *Pulex pulex* exposed to benzene (Hashim, 1984). The increase is related to accumulation of hydrocarbon in the tissues (Hashim, 1984). O2 consumption rate also showed an increase in isopod *Cirolana borealis* exposed to toluene (0.125, 1.25, 5.7 ppm,) as O_2 consumption rate reached to 184.84, 210.4, 166.62 µg/g/h respectively comparing with control (181 µg/g/h (Bakke and Skjoldal, 1979).

This increase is related to increasing the energy demand under stress of oil pollution (Faddaq *et al.*, 2004). Besides the damage of the gill epithelia

may explain this increase (Engelhardt *et al*., 1981). Also the increase in Oxygen consumption rate is accompanied with increase in temperature and increase the time of exposure to the pollutants (Smith and Hargreaves, 1983).

Toluene exposure caused a disturbance in water content of the body. This result was recorded also by Ahmed *et al*., (2001) in *S. boulangeri* exposed to crude oil. This is because of increasing the permeability of the gill membrane during exposure to crude oil. This increase in water content also recorded in the gills of *Potamon floviatile* (euryhaline Crab) exposed to benzene (Hashim and Ahmed, 1998).

The present study also reported disturbance in ionic regulation (Na^+ and K^+) in the haemolyph. The disturbance also recorded for *S. boulengeri* exposed to crude oil (Ahmed *et.al*.,2001), as the concentration of Na^+ and K^+ showed a decrease comparing with the control, and in *Potamon fluviatil*e exposed to benzene (Hashim and Ahmed,1998). This disturbance is related to the damage of the gill epithelia and the chloride cells which is responsible for active transport of the ions (Lignot and Charmantier, 2001). It was reported that pollutants disturb the transportation of ions to the haemolymph through the gill membrane (Anger and Charmatier, 2000).

The present study showed that the conc. of HCs in the gills was correlated with the concentration of exposure media, as high concentration of HCs in the gills coincided with high concentration of toluene in the exposure media. The route of influx of HCs to the blood is via gill membranes (Heras *et al*., 1992). Movement of HCs depends on its concentration ratio of tissue to water, on diffusion and flow rate of blood through tissues (Ackman *et al*., 1996).

In this study the damage of the gill epithelia is the main result of disturbing the active transport process of the ions though the gill membrane. The increase of toluene concentration in the gills of *S. boulengeri* in the beginning of exposure then its decrease in the end of exposure time was recorded also by Lee *et al*, (1972) in the muscles of *Mytilus californianus* exposed to hydrocarbons.

It is well known that aquatic animals can bioaccumulate aromatic hydrocarbons which are dissolving easily in water and with higher concentration comparing with other hydrocarbons (GESAMP, 1993).

The result of recovery after 4 days of transfer to clean water showed that the physiological parameters (Na^+, K^+ level, water content) did not return to the normal level. Ahmed *et. al*.,(2001) found that the water content and Na^+ level in the body of *S. boulengeri* exposed to crude oil return to it's normal level after 5 days of transfer to clean water. That was evident also from the concentration of toluene in the gills after 4 days of transfer to clean water, as

toluene concentration was 0.96 µg/g dry wt., while in control it was only 0.04 µg/g dry wt.

It is well known that aquatic animals differ in their ability of recovery after exposure to pollutants, this is depend on the kind of pollutant, concentration of pollutant , exposure time and species (Busdosh,1981) also it depends on the quantity of adipose tissue in the animal body (Kumar *et al*.,1999; Lee,1972)

The histopathological study showed that toluene caused damage and several changes in the gill epithelia represented by swelling, hypertrophy, hyperplasia and necrosis of gill epithelia. These changes was recorded in may aquatic animals as in King Crab *Paralithodes camtscatica* (Smith,1976), in fish *T. zilli* (El-Sayed *et al*.,1995) in freshwater fish *Liza abu* (Faddaq *et al*.,2004) and in some invertebrates (Engelhardt *et al*.,1981). These changes are due to the penetration of petroleum hydrocarbons to the blood through gill membrane which cause damage in the gill epithelia (Engelhardt *et al.,* 1981). Also this may explain as a kind of protection against pollutants which represents by hypertrophy, hyperplasia and fusion of epithelia (El-Sayed *et al*., 1995). Nelson-Smith (1971) referred that petroleum hydrocarbons dissolve in the adipose layer of plasma membrane causing rupturing of this membrane and diffusion of hydrocarbons inside the cells. However Hinton and Lauren (1990) referred that hypertrophy of cells due to effect of pollutants on cell membrane which cause an increasing the permeability of these membrane causing increase the water content of the cells and its organelles.

In conclusion the exposure of freshwater crab *S. boulengeri* to sub lethal level of toluene tend to increase the level of oxygen consumption rate and disturbance in water and ionic regulation and that these parameters did not return to the normal levels even after 4 days of transfer to clean water. Also long exposure to sub lethal concentration of toluene for 14 days caused many histopathological changes in gill epithelia.

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References:

Ahmed, S.M, Al-Saboungi, A.A. and Jassem, A.K. 2001. Effect of Crude Oil on ionic and water balance of freshwater Crab *Sesarma boulengeri* under Lab. Condition. Marina Mesopotamica.16 (2): 453-458.

Ahmed, S.M. 1996. Osmotic and Ionic status in fish inhabiting the water bodies of Basrah, Iraq. PhD. Thesis (In Arabic).Basrah, Univ.145p.

Ackman, R.G.; Heras, H.; and Zhou, S.1996. Salmon lipid storage sites and their role in contamination with water-soluble petroleum materials. Journal of food lipids.3:161-170.

Al-Khafaji, T.Y., 2000. Possible effects of Al-Dourah refinery wastes to some biological aspects in Dijlah river fishes.M.Sc.Thesis –College of Agriculture-Univ.of Baghdad.pp80.

Al-Khafaji, T.Y., 2001. Acute and chronic effects of Al-Dourah refinery wastes to some biological aspects of grass carp *Ctenopharyngodon idella*. 7th Iraqi technical proceeding- Univ. of Mustansyria-Baghdad-Iraq, 8-10 May 2001.pp 565-575.

Al-Obaidi, M.J., 2000. Toxicity of Al-Dourah refinery wastes on some aquatic invertebrate.M.Sc. Thesis –College of Education .Univ. of Baghdad.

Al-Saad, H.T. 1990. Hydrocarbons residues in fishes from Khor Al-Zubair .Northwest Arabian Gulf .Mar.Mesop.5 (1):81-88.

Al-Saad, H.T. 1995. Distribution and sources of hydrocarbons in Shatt Al-Arab estuary and North West Arabian Gulf. PhD. Thesis. Basrah University, Iraq.186p.

Al-Saad, H.T.; Shamshoom, S.M. and Abaychi, J.K. 1997. Hydrocarbons in fish from Shatt Al-Arab estuary and northwest Arabian Gulf following the Gulf oil spill of 1991. Marina Mesopotamica .12(2):213-231.

Al-Saad,H.T. and Al-Timari,A.A. 1993. Sources of hydrocarbons and fatty acids in sediments from Hor Al-Hammar Marsh, Shatt Al-Arab and northwest Arabian Gulf. Marine Pollution Bull.26 (10):559-564.

Anger, K and Charmatier, G. (2000). Ontogeny of osmoregulation and salinity tolerance in a mangrove crab *Sesarma curacaoense* (Decapoda: Grapsidae). J. Exp. Mar. Biol. Ecol. 251: 265-274.

Bakke, F. and Skjoldal, H.R., 1979. Effect of toluene on the survival; respiration and adenylate system of marine Isopoda. J. Mar. Poll. Bull.10 (4):111-115.

Brau; ner, C.T; Ballantyne, C.L.; Vijayan, M.M.and Val, A.L. 1999. Crude oil exposure affects air-breathing frequency, blood phosphate levels and ion regulation in an air-breathing teleost fish *Hoplosternum littoral*. Com.Bioch.Physil.123:127-134.

Buikema, A.L. and Hendricks, A.C.Jr. 1980. Benzene, Xylene and Toluene in aquatic system: A review1. Am.Pet.Inst.Blaacksbury, Verginia. 69p.

Busdosh, M.1981. Long-term of the water-soluble fraction of Prude hoe Bay Crude Oil on survival, movement and food success of Arctic Amphipod *Boeckosmius* (=*Onisimos affinis*). Mar.Envir.Res. 95: 167-180.

El-Sayed, N.K., Moursy, S.A. and Ibrahim, B.M.1995. Acute and chronic toxicity of some aromatic hydrocarbons on *Tilapia zillii*. Bull.Nat.Inst.of Ocengr. And fish.A.R.E. 22(2):613-630.

Duxbury, A.C. and Duxbury, A.B.1997. An introduction to the word's oceans (fifth edition (WCB.MaCraw-Hill.

Engelhardt, F.R., Wong, M.P. and Ducy, M.E. 1981. Hydro mineral balance and gill morphology in rainbow trout *Salmo gairdner*i acclimated to fresh and sea water as affected by petroleum exposure. Aquatic toxicology.1:175-186.

Faddagh, M.S., Ahmed, S.M. and Hussein, S.A., 2004. Impact of Gas Oil on ionic regulation, chloride cells and histopathological alternations in gills of Mugilid fish *Liza abu* Juveniles. Marina Mesopotamica. 19(1):36-52.

Geiger, J.G. and Buikema, A.L. 1981. Oxygen consumption and filtering rate of *Daphnia pulex* after exposure to water-soluble fractions of naphthalene, phenanthrene, No.2 fuel oil and coulter creosote. Bull.Env.Conta.Toxi. 27:783-789.

GESAMP,1993. IMO/FAO/UNESCO/WHO/IAE/UN/ENEP. Joint, group of experts on the scientific aspect of marine pollution (GESAMP0). Impact of oil and related chemicals and wasted on the marine environment reports and studies.Imo, London.No.50. 180p.

Hashim, A.A.1984. Effect of hydrocarbons on some functions of freshwater and marine crustacean. Ph.d. Thesis. Dep. Zool. Univ. Swansea.179p.

Hashim, A.A. and Ahmed, S.M.1998.The effect of benzene on ionic and osmotic regulation of fresh water crab *Potamon Fluvujotile*.Marina Mesopotamica.13 (2):262-272.

Heras, H.; Ackman, R.G. and Macpherson, E.1992. Tainting of Atlantic salmon *Salmo salar* by petroleum hydrocarbons during a short term exposure .Marine Pollution Bulletin.24 (6):310-315.

Hinton, D. and Lauren, J.1990.Integrative histopathological approaches to detecting effects of environmental stressors on fishes. Am. Fish. Soc. Sym. 28:51-66.

Humason, G.L.1979. Animal tissue techniques. W.H. Freeman and Company. USA. 661p.

Korn, S., Moles, A. and Rice, S.D.,1979. Effects of temperature on the median tolerance limit of pink salmon and shrimp exposed to toluene, naphthalene and Cook Inlet crude oil. Bulletin of Environmental Contamination and Toxicology.21:521-525.

Kumar, K., Sinha, Y.K. and Pandy, A.K.,1999. Endosulfan and induced hematological alternation in the fresh water air-breathing fish *Anabas testudineus*.J.Natcon.11 (1):125-134.

Lee, R.F., Hirota, J.C., Nevenzel, R., Sauer, H., Benson, A.A. and Lewis. 1972. Lipids in the marine environment. Calif. Mar. Res. Commu. Calcofl. Rep.16:95-102.

Lignot, J.H. and Charmantier, G., 2001. Imunolocalization of Na+, K+-ATPase in the branchial cavity during the early development of the European lobster *Homarus gammarus* (Crustacea, Decapoda).J. Hisotchem. Cytochem. 49:1013-1023.

Lucu, C. and Towle, D.W., 2003. Na+, K+-ATPase in the gills of aquatic crustacean .Comp.Biochem.Physiol.135A:195-214.

N.A.S., 1975. Petroleum in the marine environment. National Academy of Science.Washington, DC.36 (2):41-47.

Moles, A.; Rice, D.D.; and Korn, S.,1979. Sensitivity of Alaskan fresh water and anadromous fishes to Prudhoe Bay crude oil and benzene. Trans.Amer.Fish.Soc.108:408-414.

Nelson-Smith, A.1971. Effect of oil on marine plants and animals. In: Water pollution by oil.Heppel, P. (ed.).Elsevier, New York.273-280.

Ramusino, M.C.; Dellavedova, P. and Zanzottera, D.1984. Effects of Dubai crude oil on *Salmo gairdneri* and *Carassius auratus.* Bull. Environ. Contam. Toxicol. 32: 368-376 oil on *Salmo ga*.

Short, J.W., and Harris, P.M.1995. Petroleum hydrocarbons in near surface sea water of Prince William Sound Alaska, following the Exxon

Short, J.W. and Harris, P.M. 1996. Chemical sampling and analysis of petroleum hydrocarbons in near surface seawater of Prince William Sound after the Exxon Valdez oil spill. Amer.Fish.Soc.Symp.18:17-28.

Smith, M.A.1976.Effect of oil exposure on the ultra structure of king crab gills, antennae and hepatopencrease.Alaska Science Conference. 2:366-376.

Smith, R.L. and Hargreaves, B.R.1983. Respiratory rate in the Mysid Neomysis: Effect of naphthalene, temperature and other factors.U.S.A.477-503.

Sumich, J.L.; Dudley, G.H. and Miller, R.1996. Laboratory and field investigation in marine life.WCB McGraw Hill.U.S.203p

Thurberg, F.P. and Dawsib, M.A. 1978. Some physiological effects of the Argo Merchant Oil on several marine teleosts and bivalve mollusks.

Cited by Kiceniuk, J.W. and Khan, R.A. 1981. Toxicology of chronic crude oil exposure: Sub lethal effects on aquatic organisms. IN: Aquatic Toxicology (Jerome O. (ed.).Niragu John Wiley & Sons. Pp525.

Tricklebank, K.A. 2001. Histological alternations in fish from Sydney reefs: Possible biomarkers for environmental effects? Biomarkers.6 (1):26-32.

أثر التعرض للتولوين على التنظيم ألايوني واستهلاك الأوكسجين في السرطان النهري *Sesarma boulengeri*

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المستخلص

أجريت الدراسة لمعرفة تأثير المستويات تحت القاتلة للتولوين على بعض الجوانب الفسلجية للسرطان النهري *Sesarma boulengeri* والتي شملت معدل استهلاك الأوكسجين والتنظيم ألايوني والمائي والتراكم الحيوي للتولوين وتأثيراته النسيجية المرضية على الغلاصم. عرضت الحيوانات لثلاثة تراكيز تحت قاتلة للتولوين (2.5 و5.5 و10.5 جزء بالألف) لمدة 14 يوم وتحت درجة حرارة 28?م. النتائج بينت وجود ارتباط موجب بين معدل استهلاك الأوكسجين وتركيز التولوين، حيث وصل معدل استهلاك الأوكسجين إلى 0.223 ملغم أوكسجين/غم/ساعة في تركيز 10.5 ملغم/لتر تولوين. كذلك سبب التعرض للتولوين زيادة في المحتوى المائي للجسم حيث وصل تركيز الماء في الجسم إلى 71.7% في تركيز 10.5 ملغم/لتر تولوين. تأثر التنظيم الأيوني بشدة نتيجة التعرض للتولوين حيث لوحظ حصول انخفاض معنوي في مستوى الصوديوم (245.5 ملي مول/لتر) في اللمف في اليوم العاشر من التعرض لتركيز 10.5 ملغم/لتر تولوين. بينما ارتفع مستوى البوتاسيوم في اللمف الى 12.4 ملي مول /لتر في اليوم الرابع عشر من التعرض إلى 10.5 ملغم/لتر تولوين. التراكم الحيوي للتولوين في غلاصم السرطان النهري المعرض لتركيز 5.5 ملغم/لتر وصل إلى 2.98 ملغم/غم وزن جاف خلال اليوم الثالث من التعرض بينما انخفض مستواه إلى 1.34 ملغم/غم وزن جاف بعد مرور 11 يوم من التعرض.التأثيرات النسيجية المرضية درست أيضا وسجلت حصول انتفاخ وفرط تنسج وتورم في الخيوط الغلصمية. نتائج عملية الاسترجاع (الاستشفاء) بعد مرور أربعة أيام من النقل للماء النظيف بينت إن القياسات الفسلجية المدروسة لم ترجع إلى مستوياتها الطبيعية ما قبل التعرض.