TOXIC EFFECTS of WATER-SOLUBLE FRACTIONS (WSF) of BASRAH REGULAR CRUDE OIL to LARVAE of FRESH WATER SHRIMP (*CARIDINIA BABAULTI BASRENSIS* AL–ADHUB and HAMZA) and RIVER CRAB (*SEASARMA BOULENGERI* CALMAN) from SHATT AL-ARAB RIVER, IRAQ [+]

التأثيرات السامة للأجزاء الذائبة بالماء من نفط خام البصرة الاعتيادي اتجاه يرقة روبيان المياه العذبة *CARIDINIA BABAULTI BASRENSIS* (AL-ADHUB AND HAMZA) والسرطان النهري *SEASARMA BOULENGERI* (CALMAN) المتواجدان في نهر شط العرب، العراق

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Abstract:

In the present study, the toxicity experiments were conducted with intermolt and molting larvae (stage I) of freshwater shrimp *C. babaulti basrensis* and river crab *S. boulengeri* from Shatt Al–Arab river. The larvae of the two species were exposed to different concentrations (0.8, 1.6, 2.4, 3.2, 4.8 and 6.4ppm) of WSF of Basrah regular crude oil in the static toxicity system for 96 hours period. In the terms of median lethal concentrations (LC_{50}), The molting larvae of shrimp and crab were more sensitive than intermolt one to WSF of crude oil. The intermolt and molting larvae of shrimp were more sensitive than were intermolt and molting larvae of crab. Further toxicity experiments were conducted with the molting larvae of shrimp and crab. The larvae were exposed to different concentrations of WSF of crude oil, (0.3, 0.5, 1.0 and 1.9ppm) for shrimp and (0.6, 0.9, 1.8 and 2.8ppm) for crab, to different exposure periods (12, 24, 48, 72 and 96 hours) in the renewal toxicity system. After exposure periods were over, the larvae were transferred to clean river water for recovery and further observations. The total time for each test (exposure period+the period in clean river water) was 168 hours. The high concentrations of WSF of crude oil [1.9ppm (shrimp) and 2.8ppm (crab)] reduced molting success of shrimp and crab larvae by 80 and 91% respectively after 24 hours period of exposure and some deaths occurred. While these high concentrations declined the molting success of shrimp and crab larvae by 95 and 98% respectively after 96 hours period of exposure, and larvae usually died. The lowest concentrations of WSF of crude oil [0.3ppm (shrimp) and 0.6ppm (crab)] did not inhibit molting of shrimp and crab larvae at any period of exposure, but many larvae died after molting. The 168 hours LC_{50} values for molting larvae of shrimp and crab were lower than the 96 hours LC_{50} values of molting larvae of the both species.

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المستخلص:

تضمنت الدراسة الحالية تجارب للسمية اتجاه يرقة (المرحلة الأولى) روبيان المياه العذبة*C. babaulti basrensis* والسرطان النهري *S. boulengeri* المتواجدان في نهر شط العرب قبل الانسلاخ وخلال الانسلاخ. إذ عرضت يرقة كلا النوعين إلى تراكيز مختلفة من الأجزاء الذائبة بالماء من نفط خام البصرة الاعتيادي(0.8 و1.6 و2.4 و3.2 و4.8 و6.4 جزء بالمليون) في نظام مستقر للاختبار لمدة 96 ساعة. بمصطلحات متوسط التركيز المميت ، كانت يرقة الروبيان والسرطان خلال الانسلاخ أكثر حساسية اتجاه الأجزاء الذائبة بالماء من النفط الخام من اليرقة قبل الانسلاخ. وكانت يرقة الروبيان خلال الانسلاخ وقبل الانسلاخ أكثر حساسية من يرقة السرطان خلال الانسلاخ وقبل الانسلاخ. كما تضمنت الدراسة الحالية تجارب أضافية للسمية اتجاه يرقة الروبيان والسرطان خلال الانسلاخ، إذ عرضت اليرقة إلى تراكيز مختلفة من الأجزاء الذائبة بالماء من النفط الخام (0.3 و0.5 و1.0 و1.9 جزء بالمليون) ليرقة الروبيان و(0.6 و0.9 و1.8 و2.8 جزء بالمليون) ليرقة السرطان، لفترات زمنية مختلفة (12 و24 و48 و72 و96 ساعة) في نظام متجدد للاختبار. بعد انتهاء كل فترة تعرض، تم نقل اليرقات إلى ماء نضيف من نهر شط العرب لغرض الاسترجاع والملاحظة، بحيث كان الزمن الكلي للاختبار (فترة التعرض+الفترة في ماء النهر النظيف) تساوي 168 ساعة. سببت التراكيز العالية من الأجزاء الذائبة بالماء من النفط الخام [1.9 جزء بالمليون (الروبيان) و2.8 جزء بالمليون (السرطان)] اختزال في نجاح انسلاخ يرقة الروبيان والسرطان بنسبة 80% و90% على التوالي بعد 24 ساعة من التعرض إلى الأجزاء الذائبة بالماء من النفط الخام، مع ظهور بعض الوفيات بين اليرقات، بينما سببت تلك التراكيز اختزال في نجاح انسلاخ يرقة الروبيان والسرطان بنسبة 95 % و98 % على التوالي بعد 96 ساعة من التعرض إلى الأجزاء الذائبة بالماء من النفط الخام، مع موت اليرقات. في حين لم تسبب التراكيز الواطئة من الأجزاء الذائبة بالماء من النفط الخام [0.3 جزء بالمليون (الروبيان) و0.6 جزء بالمليون (السرطان)] اختزال في نجاح انسلاخ يرقة الروبيان والسرطان في أي فترة من فترات التعرض إلى الأجزاء الذائبة بالماء من النفط الخام، ولكن العديد من اليرقات تموت بعد الانسلاخ. كانت قيم متوسط التركيز المميت ليرقة الروبيان والسرطان خلال الانسلاخ بعد 168 ساعة من التعرض إلى الأجزاء الذائبة بالماء من النفط الخام اقل من قيم متوسط التركيز المميت ليرقة الروبيان والسرطان خلال الانسلاخ بعد 96 ساعة من التعرض إلى الأجزاء الذائبة بالماء من النفط الخام.

Introduction:

The Shatt Al–Arab river is the most important river and the only source of fresh–water in the arid surroundings of southern of Iraq. It is the prime freshwater source and pours about $5x10^6 m^3$ nutrient rich water into the Arabian Gulf each year [1]. Shatt Al–Arab river waters are liable to contain petroleum origin wastes [2]. It is reported that the petroleum hydrocarbons in the Shatt Al–Arab river are likely originated from oil refineries, rural runoff, electricity generating stations, sewage discharges and river transportation activities [3]. It is estimated that this river transports about 48 tones of oil residues to the Arabian Gulf annually [1]. Pollution by petroleum hydrocarbons in the Shatt Al–Arab river would be particularly damaging to aquatic organisms. Stress in aquatic animals exposed to even low levels of oil pollutants has been repeatedly demonstrated [4]. The breeding and larval stages of aquatic animals are considered to be the most sensitive to natural environmental stresses [5]. In crustacean larvae this sensitivity is compounded during molting. Factors contributing to a high natural mortality in molting crustaceans include increased permeability and altered ionic

regulation, the mechanical process of casting off the old exoskeleton, and increased predation while the cuticle is still soft and locomotion is slowed down [6]. Studies on the toxic effects of oil pollutants on the crustacean larvae from Shatt Al-Arab river are very few. Oil is more toxic to molting larvae than intermolt larvae in the tests performed with different crustacean [7].
In this study, the larvae (stage I) of two crustaceans species, the freshwater shrimp, *Caridinia babaulti basrensis* Al–Adhub and Hamza and the river crab, *Seasarma boulengeri* Calman are chosen for exposed to various concentrations of WSF of Basrah regular crude oil. These two crustaceans are important members of the aquatic community of Shatt Al–Arab river. Several toxicity tests were conducted on the larvae of the two species during intermolt and during the molting. These tests aimed to determine the sensitivity differences between the intermolt and molt periods and the toxic effects of concentrations and exposure periods of WSF of crude oil on molting success and survival.

Materials and Methods:

Crude oils (Basrah regular-medium-API gravity between 28–34 and Nahran–Omar– light-API gravity>34) were supplied by Iraqi South Oil Company, Basrah, Iraq. They were transferred to laboratory by dark glass bottle closed tightly and kept in a cold and dark place prior to use. Carbon tetrachloride (CCl_4), n-hexane and methylene chloride were supplied from Burdick and Jackson laboratories, Inc. Sodium sulphate was supplied by Supelco SA. It was extracted with methylene chloride for 36 hours in a soxhlet. Following clean up by extraction, It was dried in an oven at 130˚C for about 24 hours and deactivated with deionized water at the recommended percentage prior to use.

The freshwater shrimp *C. babaulti basrensis* and river crab *S. boulengeri* were collected during the low tide from Shatt Al-Arab river in 2009 (Figure 1). The animals were held in the laboratory into aquaria contained the water from Shatt Al–Arab river. The females of the both species were isolated in containers before hatching and release of larvae. When the larvae were released the females were removed from containers. The river water in the containers was changed daily. The salinity of the water was 1.6 to 1.8‰ and the pH was 7.1 to 7.8. The larvae were maintained under aerated conditions with light/dark cycle (12:12). The laboratory temperature was 20±2°C. The larvae were fed daily on detritus and *Artemia*.

The stock solution of water–soluble fractions of Basrah regular crude oil (WSF) were prepared freshly according to the procedure of Singer *etal.* [8]. A known volume of the crude oil (20ml) was added to volumetric flask containing about 150ml of river water (1.6‰ salinity) which was filtered and boiled then cooled before use. The resulting mixture was then mixed for about three hours on magnetic stirrer at a temperature of 20± 2°C. After stirring, the volume was brought up to one liter of a filtered and boiled river water. The solution was to be poured into one liter separating funnel. It was allowed to separate for two hours at temperature 20±2°C. Following separation, the lower 950ml of solution was removed to one liter flask and mixed for 30 minutes while the insoluble fraction was discarded.

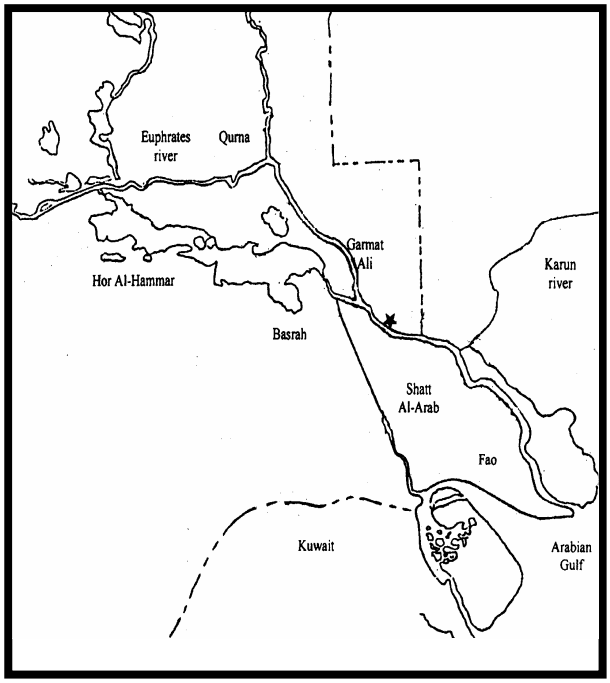


Figure (1): Map of Sampling Location.

To determine the concentrations of WSF of crude oil, the procedure of UNEP [9] was used. 100ml of nanograde carbon tetrachloride (CCl_4) was used in two successive 50ml extractions and the extracts were combined. The mixture was vigorously shaken to disperse the CCl_4 thoroughly throughout the water sample. The shaking is repeated several times before decanting the CCl_4. To these extracts a small amount of anhydrous sodium sulphate was added to remove excess water. The CCl_4 extracts were reduced in volume to less than 5ml by using a rotary evaporator. The reduced extract was carefully pipetted into a precleaned 10ml volumetric flask, making sure any residual particles of sodium sulphate were excluded and evaporated to dryness by a stream of pure nitrogen. The flask was then rinsed with fresh hexane and the rinsing used to make the sample volume up to exactly 5ml prior to UVF analysis (spectrofluorometer).

Static and renewal toxicity tests were conducted with the Intermolt and molt larvae of shrimp and crab. The tests were carried out in the 500ml glass jars. The jars were sealed to reduce evaporation of hydrocarbons. 10–20 larvae were placed in each jar. Three replicates for each treatment were used. Control treatment were established. Intermolt larvae of shrimp and crab were tested within 24 hours of release from the females. The tests on molting larvae were begun before the larvae started to molt. The intermolt and molting larvae of shrimp and crab were exposed to static 96 hours toxicity tests to the crude oil WSF concentrations of 0.8, 1.6, 2.4, 3.2, 4.8 and 6.4ppm.

Renewal toxicity tests were also conducted on the molting larvae of shrimp and crab. The larvae were exposed to crude oil WSF concentrations of 0.3, 0.5, 1.0 and 1.9ppm (for shrimp) and 0.6, 0.9, 1.8 and 2.8ppm (for crab), for exposure periods of 12, 24, 48, 72 and 96 hours. The test solutions were changed every 24 hours. In the end of each exposure period, the larvae of shrimp and crab were transferred to clean river water for recovery and further observations. The total time for each test (exposure period+the period in clean river water) was 168 hours. Observation of death and molting success of larvae were made without removing the larvae from the test jars. The larvae were considered to be dead when there was no visible motion. The oxygen concentration in the test solutions was about 75–85% saturation at the end of exposure periods. The larvae were not fed during the exposure.

The Shimadzu RF–540 spectrofluorometer equipped with a DR–3 data recorder was used to determine the concentration of WSF of crude oil. The basis quantitative measurements were made by measuring emission intensity at 360nm with excitation set at 310nm and monochrometer slits of 10nm. The reference oil used for calibration was Nahran-Umar crude oil obtained from South Oil Company.

To compute the median lethal concentration (LC_{50}) (i.e. the concentration of WSF causing death in 50% of exposed larvae in a given amount of time) and their upper and lower 95% confidence limits, the method described by UNEP [9] was used.

Results and Discussion:

The results of the present study showed that the molting larvae of shrimp and crab were more sensitive to crude oil WSF than intermolt one (Figure 2) and (Table 1). This was in agreement with the previous studies on the toxic effects of oils to the crustacean larvae [10]. The study also showed that the intermolt and molting larvae of shrimp were more sensitive than were intermolt and molting larvae of crab. This suggested the differences in the sensitivity between crustacean species in the molt and intermolt periods to crude oil WSF , as had been reported in the previous studies [11]. These differences in the sensitivity to oils also occur interaspecifically in the crustacean at different life stages [10].

In this study, the molting success in shrimp and crab larvae exposed to WSF of crude oil at different exposure periods followed the same pattern. As the concentrations and exposure periods of WSF of crude oil increased, the individuals of shrimp and crab larvae that molting were decreased. In the lower concentrations of WSF of crude oil, (0.3ppm) for shrimp and (0.6ppm) for crab, no effect on the molting success was found, but many larvae died after molting. The concentrations of WSF of crude oil, (0.5 and 1.0ppm) for shrimp and (0.9 and 1.8ppm) for crab did not affect molting success when the larvae were exposed to 12 and 24 hours for shrimp, and 12 hours for crab, but in the exposures of 48, 72 and 96 hours for shrimp and 24, 48, 72, 96 hours for crab the molting inhibited. At higher concentrations of WSF of crude oil, (1.9ppm) for shrimp and (2.8ppm) for crab, the molting success inhibited in the exposures of 24 hours or longer for shrimp and 12 hours or longer for crab and deaths of larvae usually occurred. Control shrimp and crab larvae were completed molting (Tables 2 and 3) and (Figures 3 and 4). This pattern has also reported for larvae of the tanner crab (*Chionoecetes bairdi*) when exposed to different concentrations of hydrocarbons [12].

The mortalities of molting larvae of shrimp and crab often occurred later in the present experiments (after 96 hours of exposure). The 96hoursLC_{50} values of molting larvae of shrimp and crab were approximately similar in all exposure periods. The more toxic effects on

the molting larvae in the present study usually occurred after 96 hours, when the larvae were transferred in clean river water, so they were not as clear from the 96hoursLC_{50} as they were from the 168hoursLC_{50} (Figures 5-8) and (Table 4). Delayed mortalities of crustacean larvae after exposure to toxic pollutants has also been reported in other studies [13].
The higher sensitivity in the crustacean to environmental stresses during the molting periods than intermolt ones was a general phenomenon. This phenomenon reflected in the tests on crustacean at any life stages and with pollutants other than oil [14]. The sensitivity to oil during molting was probably related to physiological changes associated with the molting process [12]. Lewis and Haefner [15] observed depression in the metabolism of adult blue crab (*Callinectes sapidus*) exposed to oil and impairment the ability of its molting larvae to metabolize and excrete the oil accumulated in the tissues. Because molting had influence on the sensitivity of crustaceans to toxic pollutants. Anderson [13] reported that the comparisons of the sensitivities between life stages and species will be valid if based on animals tested in the same stage of the molt cycle, such as intermolt. Testing the sensitivity of intermolt larvae to pollutants may be difficult for many crustacean species because the intermolt periods were often as short as 2 or 3 days. Thus, larvae would begin to molt during the standard 96 hours bioassay.

Conclusions and Recommendations:

From this study, we concluded that the molting larvae of freshwater shrimp *C. babaulti basrensis* and river crab *S. boulengeri* were more sensitive than intermolt one to WSF of Basrah regular crude oil. The intermolt and molting larvae of shrimp were more sensitive than were intermolt and molting larvae of crab. As the concentrations and exposure periods of WSF of Basrah regular crude oil increased, molting success in shrimp and crab larvae inhibited and deaths increased. This pattern was clear in the 168hoursLC_{50} than 96hoursLC_{50}.
Further studies to test the toxicity of oils, oils products, oil fractions, oil components, and oil emulsions in the Shatt Al-Arab river were recommended. This would help to predict the effects of oil pollution on living organisms and ecology of the river, to determine permissible effluent discharge rates into the aquatic environment of the river, and to monitor levels of oil pollution in stream with respect to water quality standards.

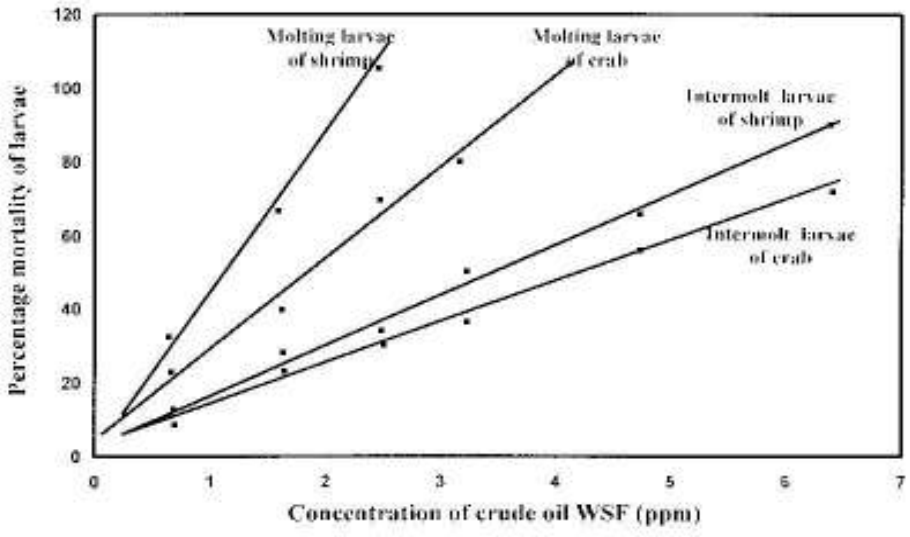


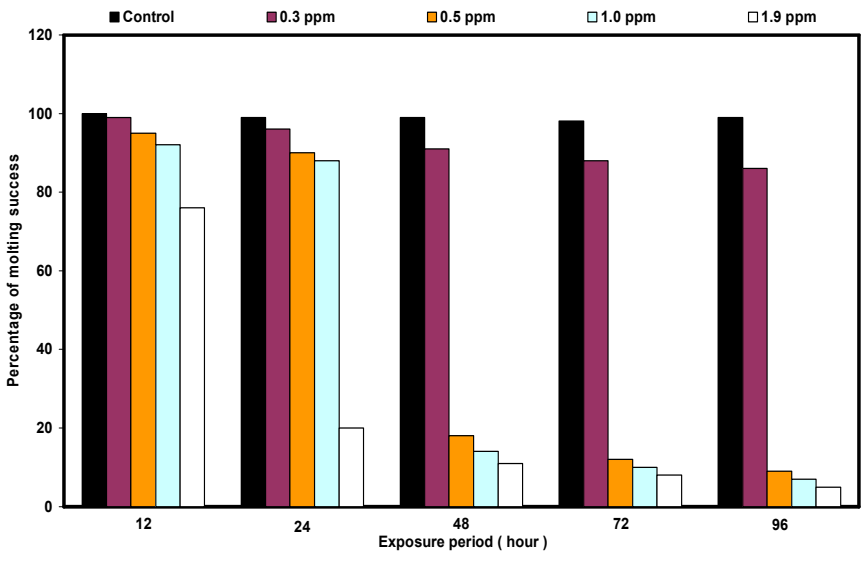
Figure (2): The response of larvae of freshwater shrimp *C. babaulti basrensis* and river crab *S. boulengeri* to exposure to WSF of crude oil for 96 hours at varying concentrations.

Table (1): The LC_{50} values and their upper and lower 95% confidence limits of freshwater shrimp *C. babaulti basrensis* and river crab *S. boulengeri* larvae exposed to WSF of crude oil for the period of 96 hours.

Species	LC_{50} (ppm)		
	96hoursLC_{50}	Upper 95 % confidence limit of 96hoursLC_{50}	Lower 95 % confidence limit of 96hoursLC_{50}
Intermolt larvae of shrimp, *C. babaulti basrensis*	3.6	3.9	3.2
Molting larvae of shrimp, *C. babaulti basrensis*	1.1	1.6	0.7
Intermolt larvae of crab, *S. boulengeri*	4.2	4.6	3.9
Molting larvae of crab, *S. boulengeri*	1.9	2.5	1.3

Table (2): The percentage of molting success of freshwater shrimp, *C. babaulti basrensis*, larvae exposed to WSF of crude oil for different exposure periods.

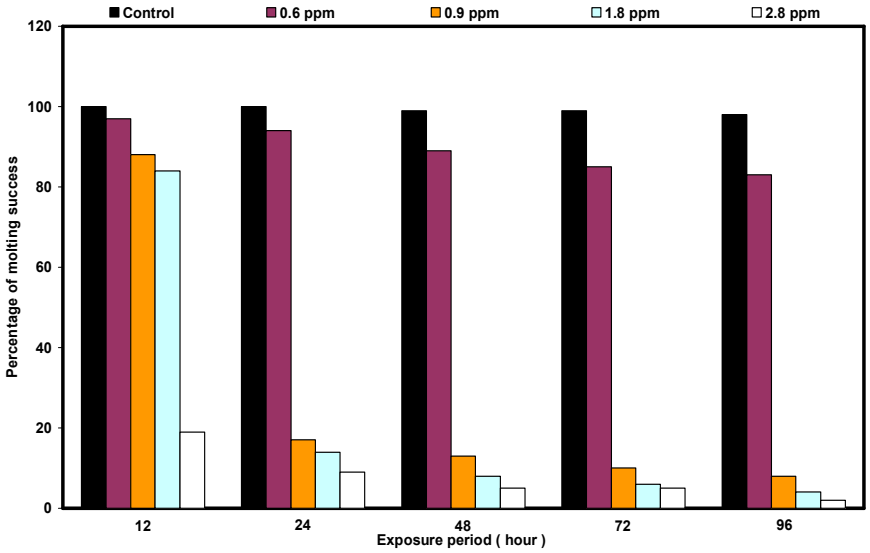
Exposure period (hour)	Concentration (ppm)				
	Control	0.3	0.5	1.0	1.9
	Percentage of molting success of larvae				
12	100	99	95	92	76
24	99	96	90	88	20
48	99	91	18	14	11
72	98	88	12	10	8
96	99	86	9	7	5



Figures (3): The molting success of freshwater shrimp, *C. babaulti basrensis*, larvae exposed to WSF of crude oil for different exposure periods.

Table (3): The percentage of molting success of river crab *S. boulengeri*, larvae exposed to WSF of crude oil for different exposure periods.

Exposure period (hour)	Concentration (ppm)				
	Control	0.6	0.9	1.8	2.8
	Percentage of molting success of larvae				
12	100	97	88	84	19
24	100	94	17	14	9
48	99	89	13	8	5
72	99	85	10	6	5
96	98	83	8	4	2



Figures (4): The molting success of river crab, *S. boulengeri*, larvae exposed to WSF of crude oil for different exposure periods.

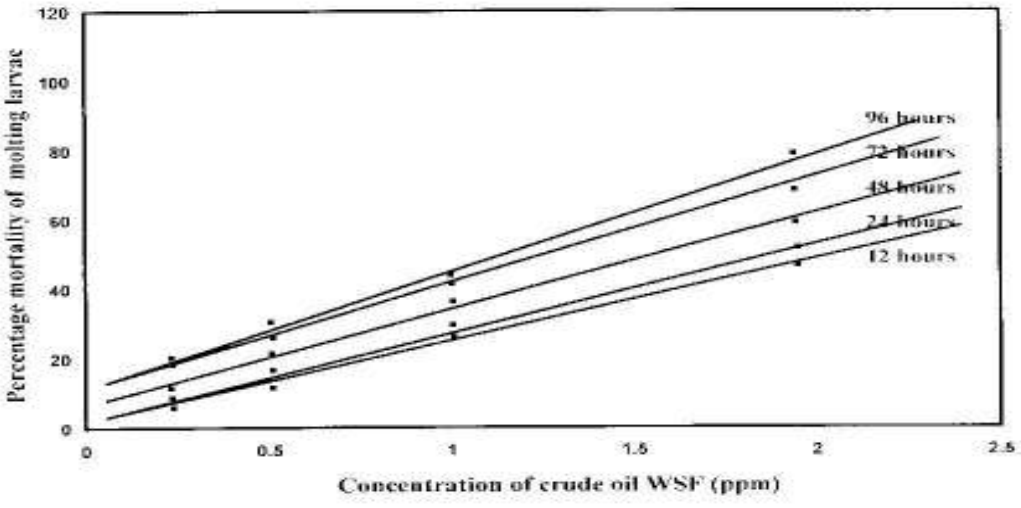


Figure (5): The response of molting larvae of freshwater shrimp *C. babaulti basrensis* to exposure to WSF of crude oil for 96 hours at varying concentrations.

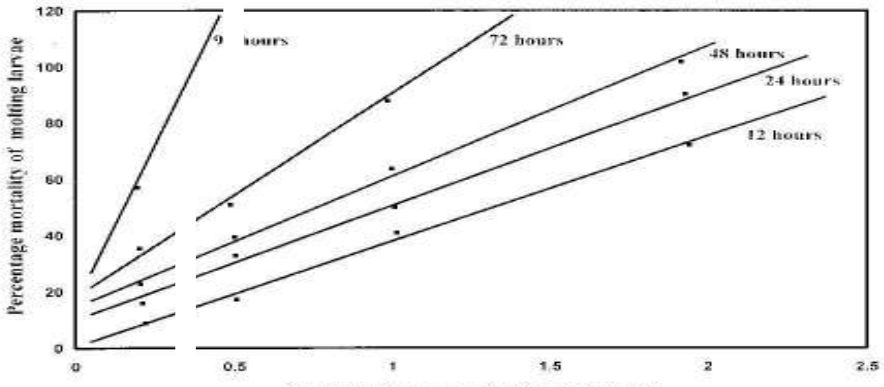


Figure (6): The r ponse of molting larvae of freshwater shrimp *C. babaulti basrensis* to exposur o WSF of crude oil for 168 hours at varying concentrations.

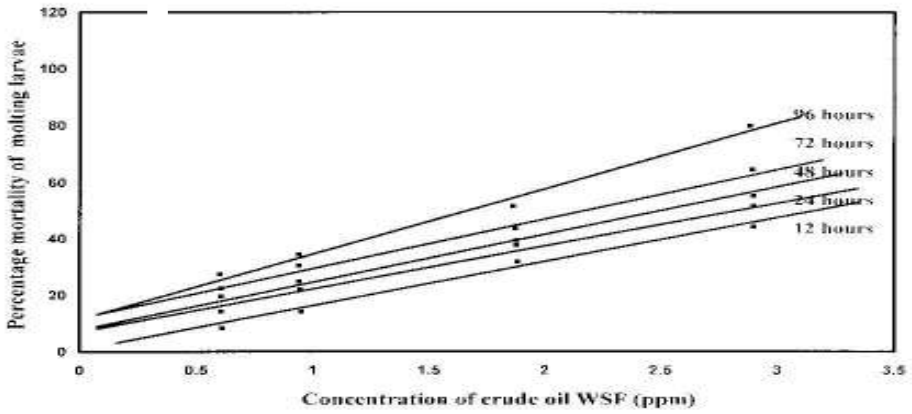


Figure (7): The response of molting larvae of river crab *S. boulengeri* to exposure to WSF of crude oil for 96 hours at varying concentrations.

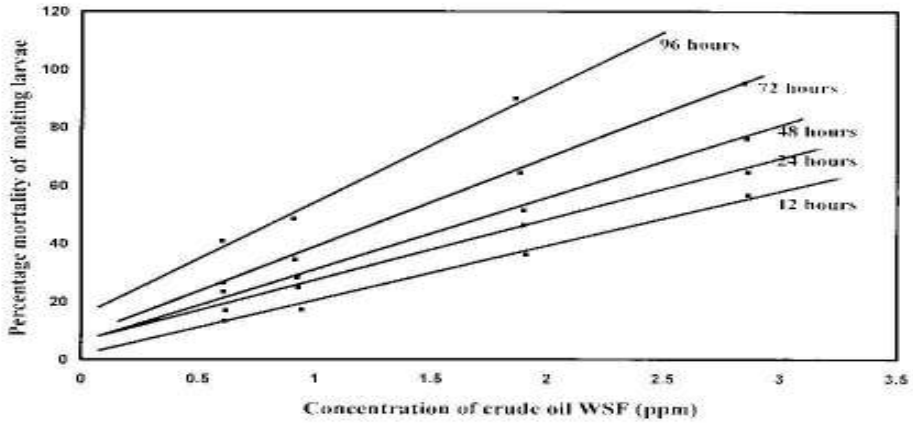


Figure (8): The response of molting larvae of river crab *S. boulengeri* to exposure to WSF of crude oil for 168 hours at varying concentrations.

Table (4): The LC_{50} values and their upper and lower 95% confidence limits of freshwater shrimp *C. babaulti basrensis* and river crab *S. boulengeri* larvae exposed to WSF of crude oil during the molting for the periods of 96 and 168 hours.

Species	LC_{50} (ppm)	Exposure period (hour)				
		12	24	48	27	96
Molting larvae of shrimp, *C. babaulti basrensis*	96hoursLC_{50}	2.1	1.9	1.6	1.3	1.2
	Upper 95% confidence limit of 96hoursLC_{50}	2.3	2.4	2.0	1.7	1.5
	Lower 95% confidence limit of 96hoursLC_{50}	1.5	1.6	1.3	0.7	0.5
	168hoursLC_{50}	1.4	1.1	0.8	0.5	0.2
	Upper 95% confidence limit of 168hoursLC_{50}	1.9	1.3	1.0	1.2	0.6
	Lower 95% confidence limit of 168hoursLC_{50}	1.1	0.5	0.5	0.7	0.2
Molting larvae of crab, *S. boulengeri*	96hoursLC_{50}	3.2	2.9	2.5	2.2	1.7
	Upper 95% confidence limit of 96hoursLC_{50}	3.7	3.3	3.0	2.8	2.1
	Lower 95% confidence limit of 96hoursLC_{50}	2.7	2.5	2.1	1.8	2.0
	168hoursLC_{50}	2.6	2.2	1.8	1.4	0.9
	Upper 95% confidence limit of 168hoursLC_{50}	2.9	2.7	2.2	1.7	1.3
	Lower 95% confidence limit of 168hoursLC_{50}	2.4	1.5	1.6	0.9	0.7

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