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Toxicity of aromatic hydrocarbons to several species of molluscs from Shatt Al-Arab river

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Abstract

The present study includes toxicity experiments carried out under laboratory conditions for 24- and 48- hours periods by using renewal toxicity test system to determine the comparative toxicities of three types of aromatic hydrocarbons{hydroxylated aromatic hydrocarbon (phenol and β–naphthol), azaarenes (quinoline and acridine) and polycyclic aromatic hydrocarbons (naphthalene and phenanthrene)} to several species of molluscs found in Shatt Al–Arab river. These species of molluscs are snails,Lymnaea auricularia,Theodoxus jordani,Physa acuta,Melanopsis nodosa,and Melanoides tuberculata and bivalves,Corbicula fluminea and Corbicula fluminalis. The toxicity experiments show that the more toxic aromatic compounds to species of molluscs is phenanthrene and the less toxic is quinoline. In each of these types of aromatics,the compound with the greater number of aromatic rings always exert a greater toxicity to species of molluscs.

The order of sensitivity of molluscs tested to aromatic hydrocarbons are as follows : L. auricularia> P. acuta > M. nodosa > T. jordani > M. tuberculata > C. fluminalis > C. fluminea . The overall acute effects of hydrocarbons on the species of molluscs tested are abnormal activities,narcosis and anesthesia,the loss of ability to react to the external cue,rapture the tissues and die.

1- Introduction

Each year, an estimated 4,000,000 t of petroleum enter the marine environment through sea and land based discharges, atmospheric fall–out and other events (API,2001). Aromatic petroleum hydrocarbons are tainting components of crude petroleum,and are potentially toxic because they are relatively soluble in water (Barron *et al.,* 1999). Depending on concentration, they are also carcinogenic (Birtwell and McAllister, 2000). Although there is general agreement that aromatic hydrocarbons are responsible for the toxic effects of crude oils and refined products,the relative importance of various aromatic hydrocarbons to toxicity is not clearly defined. Therefore,there has been an increasing interest in determining the degree contribution of individual aromatics to the toxicity of oil–water solutions (API,2001 ; Boelsterli ,2000).

Aromatic petroleum hydrocarbons contamination of Shatt Al–Arab river can occur from a variety of sources. A significant portion of the aromatic hydrocarbons present in the water of Shatt Al–Arab river may have been transported there through the atmosphere. However,chronic low level contamination of water of Shatt Al–Arab river originates primarily from the discharge of oil from ship operations and via urban and industrial sewage effluents. On a more intermittent basis, discharge of ballast waters by tankers and other vessels and accidental spills from offshore wells and shipping mishaps may heavily contaminate localized region of Shatt Al–Arab river (Al–Saad, 1995). Many laboratory studies elsewhere have been conducted with marine organisms, both vertebrates and invertebrates, to assess the effects of the aromatic petroleum hydrocarbons with respect to physiology, toxicity, and tainting (Ackman and Heras, 1992 ; Barron et al., 1999 ; Birtwell,and McAllister, 2000). While some studies have focused on the toxic effects of whole crude oil and its refinery products on the organisms of Shatt Al–Arab river (Al–Aabbawy, 1999 ; Farid, 1998 ; Farid,2002 ; Farid and Farid, 2002), little attention has been given to those compounds in crude oil thought most toxic to aquatic organisms, i.e. aromatic hydrocarbons. The objective of this research therefore investigates the comparative toxicities of three types of aromatic hydrocarbons {hydroxylated aromatic hydrocarbon (phenol and β–naphthol), azaarenes (quinoline and acridine) and polycyclic aromatic hydrocarbons (naphthalene and phenanthrene). All of these compounds have been identified as contaminants likely to originate from petroleum conversion processes (Landis, 2003 ; NRC,2003). The some properties of phenol, β–naphthol,

acridine, quinoline, naphthalene and phenanthrene compounds were represented in (Table 1). Several species of molluscs common in Shatt Al-Arab river are used as a toxicity test species. These are Corbicula fluminea, Corbicula fluminalis (bivalves), Lymnae aurticularia,Theodoxus jordani, Physa acuta, Melanopsis nodosa and Melanoides tuberculata (gastropods).

Table (1): Some Properties of Phenol,β–Naphthol,Acridine,Quinoline,Naphthalene and Phenanthrene Compounds (Windholz et al.,1976).

Compound and its formula	Molecular weight	Melting point	Boiling Point	Flash point	Solubility in water
Phenol C_6H_6O	94.11	40.85ºC	182ºC	79ºC	Soluble
β–Naphthol $C_{10}H_8O$	144.16	121ºC- 123ºC	285ºC- 286ºC	161ºC	Soluble
Quinoline C_9H_7N	129.15	- 15ºC	bp_{76o} 237.7ºC bp_{100} 163.2ºC bp_{40} 136.7ºC bp_{20} 119.8ºC bp_{10} 103.8ºC bp_5 89.6ºC $bp_{1.0}$ 59.7ºC	---	Soluble
Acridine $C_{13}H_9N$	179.21	106ºC- 110ºC	bp_{76o} 346ºC	---	Slightly soluble in boiling water
Naphthalene $C_{10}H_8$	128.16	80.2ºC	bp_{76o} 217.9ºC bp_{400} 193.2ºC bp_{200} 167.7ºC bp_{100} 145.5ºC bp_{60} 130.2ºC bp_{40} 119.3ºC bp_{20} 101.7ºC bp_{10} 85.8ºC	Open cup 174ºF (79ºC) Closed cup 190ºF (88ºC)	Insoluble
Phenathrene $C_{14}H_{10}$	178.22	100ºC	340ºC	---	Insoluble

2- Materials and Methods

Collection of molluscs samples :

The specimens of molluscs, Lymnaea auricularia, Theodoxus jordani, Physa acuta, Melanopsis nodosa,Melanoides tuberculata (gastropods), Corbicula fluminea and Corbicula fluminalis (bivalves) were collected from Shatt Al–Arab river (along the region extended from Abu–Al-Khasib to Garmat-Ali) during 2004 and 2005 (Figure1). Each species consisted of at least 3500 adult and of uniform size individuals.

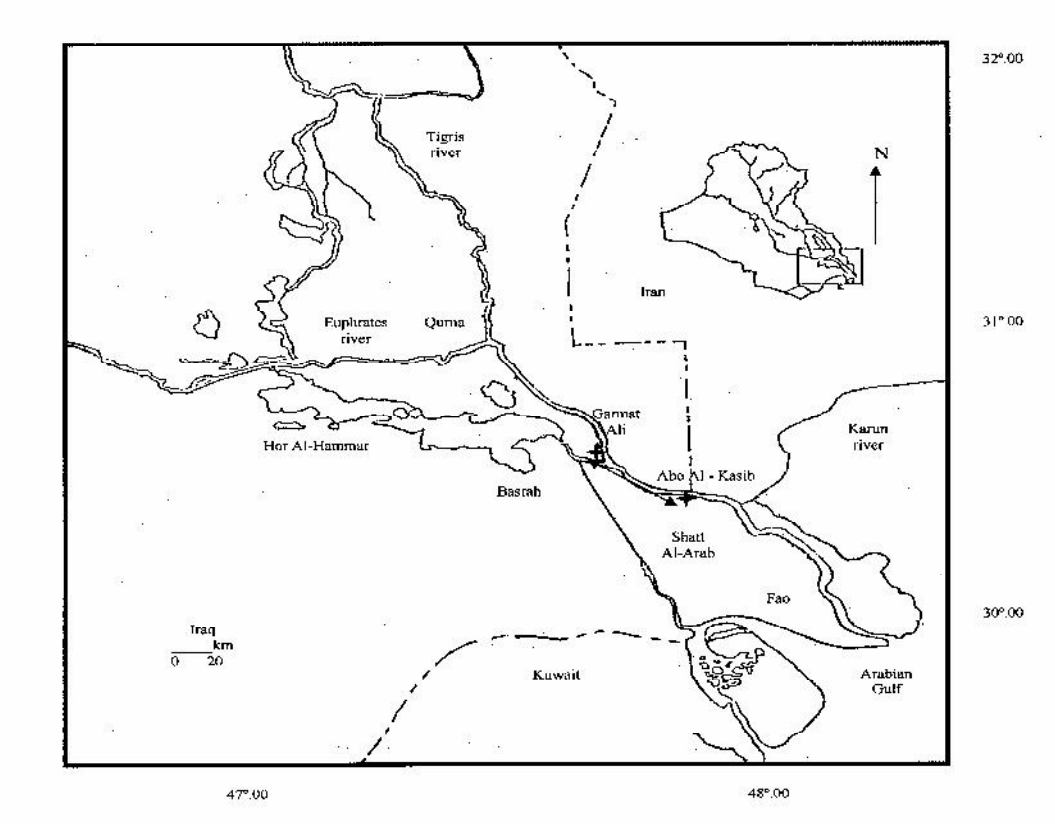

Figure (1) : Map of Sampling Location

Acclimation of the test species (molluscs):

The species of molluscs were transferred to an aquarium for acclimation period of ten days prior to the toxicity experiments,under laboratory temperature of 20 ± 2 °C with light / dark cycle (12 : 12) under aerated conditions.

Toxicity experiments **:**

Toxicity tests were conducted with three types of aromatic hydrocarbons,I ncluding hydroxylated aromatic hydrocarbons (phenol and β–naphthol), azaarenes (quinoline and acridine) and polycyclic aromatic hydrocarbons (naphthalene and phenanthrene).

The experimental procedure adopted for toxicity determination was based on the method established by Pace *et al*. (1995). Batches of 50 animals of each species were exposed to each type of aromatic hydrocarbons for a period of 24-and 48-hours in glass aquarium (40 × 22 × 15 cm^3 in size),followed by the 5–days recovery period in clean aerated river water. The aquariums were covered with glass lids to reduce evaporation of hydrocarbons. The test solution concentration of each type of aromatic compounds consists of manually mixing 10 mg of aromatic compound with each liter of the river water. The animals were left without food during the exposure period. Mortalities in species of gastropods were taken as the number of animals still immobile and remaining in the water after 5–days, in the case of bivalves,mortalities were taken as the number of individuals showed no signs of life and with permanently gaping shells. These tests were set up in three replicates together with three control (untreated). All experiments were carried out in the renewal toxicity system for test in November and December,2004

Test solutions :

The tests solutions of all experiments were monitored for temperature (simple thermometer with range from 0 to 100 °C graduated at 0.2 °C made by Hanna company), dissolved oxygen (dissolved oxygen meter type YSI 556 MPS-USA),pH (pH–meter type HI 8915 made by Hanna company) and salinity (digital salinometer E–202 type Tsurumi Seiki-Japan) at regular intervals. The temperature was at 20 ± 2°C. The dissolved oxygen ranged was 8.5 to 10.1 mg / l. As to the other characteristics of tests solutions,pH was 7.1 to 7.8 and salinity was 1.6 to 1.8 ppt.

3- Results and Discussion

Tables (2 and 3) represent the percentage mortalities of the tested species after exposure to different types of aromatic hydrocarbons,followed by the 5–days recovery period in the river water. Although

the order of toxicity of aromatic hydrocarbons varied somewhat for the different species of molluscs. It is clear,that the more toxic compound to species of molluscs is phenanthrene and the less toxic is quinoline. A striking structure–toxicity relationship is observed for the three types of aromatic compounds,including hydroxylated aromatic hydrocarbon (phenol and β–naphthol), azaarenes (quinoline and acridine) and polycyclic aromatic hydrocarbons (naphthalene and phenanthrene). In each of these types, the compound with the greater number of aromatic rings always exerted the greater toxicity to species of molluscs.

Concerning the hydroxylated aromatic hydrocarbons,the percentage mortalities in present study indicated that β–naphthol was more toxic than phenol to all molluscs species. This was in agreement with results of acute tests reported by other investigators. Millemann *et al.* (1983) tested the toxicity of phenol and β–naphthol for seven aquatic plant and animal species . Comparisons of the acute LC_{50}'s and EC_{50}'s for the two compounds revealed that β–naphthol was about 7 to 44 times more toxic than phenol. NRC (2003) reported that the toxicity of β–naphthol compound was approximately twice as toxic as phenol compound with fish species.

Based on the percentage mortalities of the present study for the azaarenes,acridine was more toxic to species of molluscs than quinoline. Barron *et al*. (1999) performed acute studies on these compound (acridine and quinoline) with crabs species and found that acridine was about 5 to 30 times more toxic than quinoline. NRC (2003) reported that acridine was 7 to 34 times more toxic to fish species than was quinoline.

In molluscs species tests of the present study on the polycyclic aromatic hydrocarbons,phenanthrene was more toxic to molluscs than naphthalene. As noted for the hydroxylated aromatic hydrocarbons and azaarenes,toxicity within this type of compounds increased with the number of aromatic rings,as illustrated in (Figure 2). In acute tests with five species of algae and arthropods,Naes and Knutzen (1998) found phenanthene to be about three to nine times more toxic than naphthalene. NRC (2003) reported that the phenanthrene was nearly three times more toxic to fish species than naphthalene.

It is clear that within each type of compounds discussed above,the chemical with the greater number of rings produced the greater toxicity to species of molluscs tested. Other investigators also reported that within a given type of aromatic hydrocarbons,toxicity to aquatic organisms generally increased with increasing ring number in tests with Selnastrum capricornutum, Meador *et al*. (1995) found

that one–ring compounds always were less toxic than related two–ring compounds. A similar toxicity relation was observed by Barron *et al.* (1999) for three azaarenes administered to embryo–larval stages of the frog.

It should also be noted that within each chemical type a close correlation was observed between toxicity and n–octanol : water partition coefficients ($P_{o\,w}$). The latter is expressed as the equilibrium concentration ratio of an organic chemical partitioned between an organic liquid (i.e., n–octanol) and water (NRC,2003). As given by Sanemasa *et al.* (1994),the log $P_{o\,w}$ values for the compounds considered in this investigation were 1.46 and 2.84 for phenol and β–naphthol, 2.03 and 3.40 for quinoline and acridine, and 3.37 and 4.46 for naphthalene, and phenanthrene. Thus,for each of three types of compounds,both toxicity and the potential for bioconcentration increased with increasing ring number.

The relationship between the n–octanol : water partition coefficient and a compound's potential for biological uptake,lipophilic storage,biomagnification,and toxicity has been shown by numerous investigators. Southworth *et al.* (1978) administered three azaarene compounds (isoquinoline, acridine,and benzo (a) acridine) to Daphnia pulex and observed that bioaccumulation and acute toxicity increased with the n–octanol : water partition coefficient and the number of aromatic rings. As discussed above,the relationship between biological activity and chemical structure were restricted to compounds within a given chemical type,and such relationship were less consisted when comparisons were made among the three types. Taking data for the six compounds evaluated in the present study,phenanthrene with the highest log $P_{o\,w}$ was always the most toxic in all mollusks and acute tests,whereas phenol and quinoline with the lowest log $P_{o\,w}$ values generally were the least toxic. However,as noted by other investigators (Sterling *et al*.,2003),this relationship does not always hold when certain other compounds in different types are compared.

It is apparent that structure–toxicity relationship provides a reliable basis for estimating bioconcentration factors for aquatic toxicants and that the relative order of acute toxicity can be predicted with reasonable accuracy for at least certain types of compound. It is possible that the use of appropriate chemical descriptors in computer models (McCay,2001) may increase the precision of such predictions,but reliable estimates of toxicity values may prove difficult to achieve. Nevertheless,structure toxicity correlation

should prove useful in initial assessments on new or untested compounds,providing a basis for identifying and prioritizing the testing of those likely to possess significant biological activity.

When assessing the potential impact of spill oil,it is crucial to know if some species or life stages are more sensitive than other to oil toxicity (Zakaria et al.,2000 ; Vernberg and Vernberg,2001).

In the present study,the order of sensitivity of species of molluscs tested are not exactly the same for all aromatics toxicants,but some trends are clearly evident that the sensitivity of animals are as the following trend ; L. auricularia > P. acuta > M. nodosa > T. jordani > M. tuberculata > C. fluminalis > C. fluminea (Tables 1 and 2) .

The difference in sensitivity to petroleum toxicants among the species of molluscs may be due to the difference in the membrane structure of their bodies,their ability on the metabolism,excretion and storage of the toxicants and/or the difference in the transportation of the toxicants into the site of action.

The difference in sensitivity to varying environmental conditions between different species is well known. Zakaria et al. (2000) reported that the tolerance of marine fauna exposed to oil pollutants increases in the series from fish,to higher vertebrates then to lower invertebrates. Tolerance also increases in the marine habitat series from pelagic animals to subtidal benthic animals and intertidal animals. Rice et al. (1976) tested 27 species of marine fish and invertebrates,and permitted the best comparisons of species sensitivities since methods,temperature,ect.,were all similar. Fish and shrimp were usually among the more sensitive species tested,while intertidal animals were generally more tolerant. Intertidal animals are probably more tolerant to static exposure because they can temporarily insulate themselves from the exposures,at least until the concentration in the static exposure declined to sublethal levels. The intertidal limpets and chitons were sensitive than the other intertidal animals,but this might be due to damage occurring when they were collected. Vernberg and Vernberg (2001) reported that the sensitivities of cold–water species appear greater than sensitivities of similar species from warmer climates. Talley (2000) speculated that the cold–water species may appear more sensitive because lower temperatures increase the persistence of toxic aromatic hydrocarbons,even though there are differences in oils and species between the studies. Jaweir and Habash (1987) in their experiments on the toxicity of water – soluble hydrocarbons (WSF) of kerosene to two species of polychaetes from Shatt Al–

Arab river (Namalycastic indica and Dendronereides heteropoda),found that D. heteropoda was slightly more sensitive to WSF of kerosene than N. indica. The 96-hours LC_{50} values recorded were 1.5 ml / l for D. heteropoda and 4 ml / l for N. indica. Laboratory studies on the snails (M. nodosa,T. jordani,L. aurcularia and M. tuberculata) and the bivalves (C. fluminalis and C. fluminea),a common inhabitant in Shatt Al–Arab river estuary area,were made with crude oils refined products and oil fractions (Farid,1998 ; Al–Aabbawy 1999 ; Farid,2002 ; Farid and Farid,2002 ; Farid et al.,2002 ; Farid,2003). These studies showed that the sensitivity of molluscs to oil pollutants were very different as following trends ; L. aurcularia > M. nodosa > T. jordani > M. tuberculata > C. fluminalis > C. fluminea .

In the present study,some acute effects of oil hydrocarbons on the species of molluscs were observed by monitoring the animals closely during exposure periods. These effects are ; restrict their normal activities, prevent their adhering ability to test vessels (including the snails only),bring narcosis and anesthesia,loss their ability to reach to the external cue,rapture their tissues,lead them to leave their shells and finally lead them to die. Such effects are due to physical and / or chemical toxic effects of hydrocarbons which produce the abnormal activities in molluscs and die.

Boelsterli (2000) reported the toxicity significance of the various fractions of hydrocarbons,the low boiling saturated hydrocarbons have been showed to produce anesthesia and narcosis at low concentrations,and at higher concentrations cell damage to a wide variety of animals. Higher–boiling saturated hydrocarbons may not be directly toxic,although it is suggested that they may interfere with nutrition. The aromatic hydrocarbons are the most dangerous fractions. The low–boiling aromatics represent the acute toxic hazard,while the higher molecular weight polynuclear species may well be of significance in their long–term effects. Similar toxic effects of hydrocarbons on other species of aquatic organisms were also reported by (Dicks,1998 ; Singer et al.,2001 ; Birtwell and McAllister,2000 ; Farid, 2005).

The tests of the this study were performed under laboratory conditions and therefore inevitably did not provide a complete description of the undoubtedly more complex processes which took place in the natural environment. The results indicate,however,a number of toxic effects on an ecologically important species and that these may occur at comparatively high

levels of oil pollution in the waters of Shatt Al–Arab river.

Table (2) : Percentage mortalities of several species of intertidal molluscs from the Shatt Al–Arab river after 24-hours exposure to different types of aromatic hydrocarbons, followed by the 5–days recovery period in the river water .

Species	Number	Exposure period (hour)	Hydroxylated aromatic hydrocarbons		Azaarenes		Polycyclic aromatic hydrocarbons	
			Phenol	β - Naphthol	Quinline	Acridine	Naphthalene	Phenanthrene
L. aurcularia	50	24	66	85	49	74	78	90
T. jordani	50	24	13	29	10	17	20	36
P. acuta	50	24	60	67	35	70	74	71
M. nodosa	50	24	39	53	24	43	50	57
M. tuberculata	50	24	9	17	10	10	12	21
C. fluminea	50	24	5	9	4	7	9	10
C. fluminalis	50	24	7	11	5	7	8	13

Table (3): Percentage mortalities of several species of intertidal molluscs from the Shatt Al–Arab river after 48-hours exposure to different types of aromatic hydrocarbons, followed by the 5–days recovery period in the river water .

Species	Number	Exposure period (hour)	Hydroxylated aromatic hydrocarbons		Azaarenes		Polycyclic aromatic hydrocarbons	
			Phenol	β - Naphthol	Quinline	Acridine	Naphthalene	Phenanthrene
L. aurcularia	50	48	73	91	61	81	86	95
T. jordani	50	48	18	34	14	22	30	40
P. acuta	50	48	71	79	40	76	81	74
M. nodosa	50	48	42	64	30	46	56	60
M. tuberculata	50	48	13	22	11	12	16	26
C. fluminea	50	48	8	10	6	9	11	12
C. fluminalis	50	48	10	13	9	11	11	17

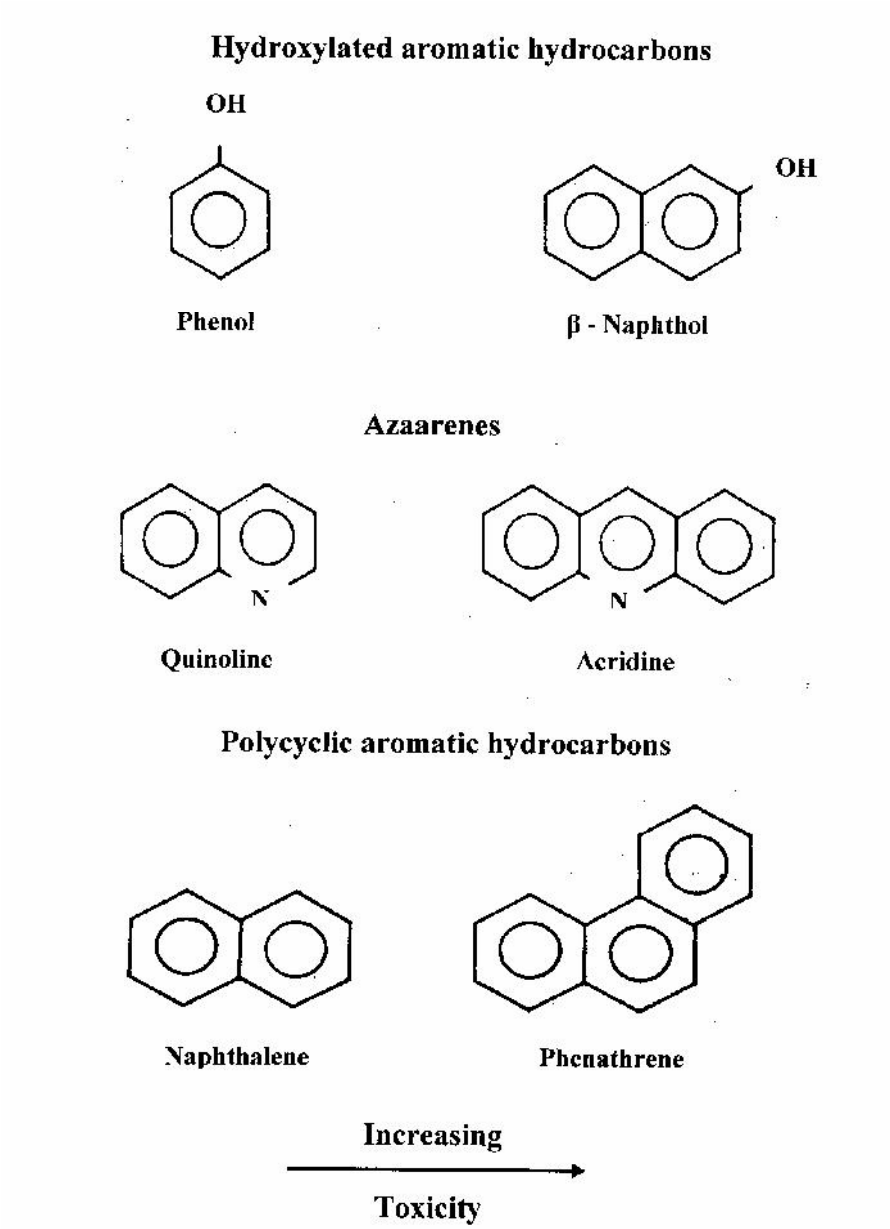

Figure (2) : The toxicity of aromatic hydrocarbons to molluscs species of Shatt Al – Arab river consistently increased with ring number .

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الخلاصة

تضمنت الدراسة الحالية تجارب للسمية أجريت تحت الظروف المختبرية في نظام متجدد للاختبار لمدة 24 و 48 ساعة لمقارنة سميات ثلاثة انواع من الهايدروكاربونات الاروماتية (مركبات الهيدروزايليتد أروماتك هيدروكاربون – الفينول و البيتا نفثول و مركبات الازارنس – الكوينولين و ألاكردين والهيدروكاربونات الاروماتية متعددة الحلقات – النفثالين و الفينانثرين) اتجاه عدة أنواع من النواعم (كائنات اختبار) المتواجدة في نهر شط العرب وهي خمسة أنواع من القواقع (*Lymnaea auricularia* و *Theodoxus jordani* و *Physa acuta* و *Melanopsis nodosa* و *Melanoide tuberculata*) و نوعين من المحار (*Corbicula fluminea* و *Corbicula fluminalis*) . لقد بينت تجارب السمية مايلي : كان الفينانثرين اكثر المركبات الاروماتية سمية اتجاه كائنات الاختبار بينما كان مركب الكوينولين اقلها سمية . خلال كل نوع من انواع المركبات الاروماتية كان المركب الذي يحتوي اكثر عدد من الحلقات الاروماتية اكثر سمية اتجاه كائنات الاختبار من المركب الذي يحتوي على اقل عدد من الحلقات الاروماتية .

كان ترتيب حساسية كائنات الاختبار المدروسة اتجاه جميع السموم النفطية كما يلي : *C . fluminea* < *C . flumineali* < *M . tuberculata* < *T. jordani* < *M . nodosa* < *P . acuta* < *L . auricularia* . كانت مجمل التأثيرات السامة الحادة للهيدروكاربونات النفطية اتجاه كائنات الاختبار المدروسة (النواعم) هي عرقلة الفعالية الطبيعية وأصابتها بالخدر و الخمول و فقدانها القدرة على الاستجابة للحوافز الخارجية وتمزيق أجزائها الجسمية (أنسجتها) واخيرا موتها .