



Original Research Article

## Toxic Effects of Dissolved and Dispersed Crude Oils on Eggs and Larvae of Some Fishes from Shatt Al-Arab River

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### ABSTRACT

Experiments had been made to study the effects of various Iraqi crude oils (Nahrn-Omar, Majnun and Rumella) on the eggs and young larvae of common carp (*C. Carpio*), carassin (*C. auratus*) and grass carp (*C. idella*). The eggs and larvae were exposed to different concentrations of dissolved and dispersed crude oils. The eggs mortality is directly proportional to the concentration of dissolved crude oils and the exposure duration. The eggs with developed embryos were less sensitive than young eggs. Nahrn-Omar was the most toxic crude oil to eggs. Majnun was the next toxic crude oil, while Rumella crude oil was almost non toxic. The eggs hatching during the test depended on the concentration of dissolved crude oil, the sensitivities of embryos, and various toxicities of crude oils. The crude oil had more obvious effects on young larvae than embryos. The Carassin larvae were less and grass carp larvae were more resistant to dissolved crude oils than common carp larvae of the same age. Dispersed crude oil was more toxic to the larvae than floating oil. The Corexit 9500 solution alone was non toxic in all concentrations used. The chemically dispersed crude oil, however, was more toxic than mechanically dispersed crude oil. A significant biological and behavioral effects of dissolved and dispersed oils on the larvae were demonstrated. The affected larvae were not able to avoid the dispersed crude oil due to destruction of the chemical receptors rapidly at the beginning of contact with the oil.

**Keyword:** Crude oil; toxicity; fish; Shatt Al-Arab river; dispersant

### INTRODUCTION

Oil spill affecting the aquatic life and their habitats in many ways [1]. The severity of the impact depends on the type and amount of oil

spilled, the season and weather, the type of shoreline, and the type of wave and tidal energy in the area of the spill [2].

Oils can range from very light to very heavy oils. Very light oils are highly volatile and evaporate quickly. They are one of the most acutely toxic oils to aquatic life. Medium oils are less likely to be mixed with water and can cause severe and long-term pollution in tidal areas. Heavy oil do not mix easily with water and be much less evaporation and dilution potential. They can cause severe pollution in the long term of the tide and sediment areas. Very heavy oils can mix, sink, or hang in the water. These oils become oil droplets and mixing in the water, or accumulate at the bottom, or mixing with sediment and then sink [3, 4].

Most of the oil has a lower density than water, so it floats. Oil tends to spread in a thin layer on the surface of the water [5]. Oil in the water is subject to weathering, a process that reduces the most toxic elements in petroleum products over the time, as exposure to air, sunlight, waves and tidal action, and certain microorganisms degrade and/or disperse oil. Weathering rates depend on factors such as the type of oil, weather, temperature, and type of the shoreline and bottom of that occur in the spill area [6]. Some oil components have the ability to persist in the environment for a long time after the spill event and have been detected in sediments 30 years after the spill [7].

Oil spill contaminated shores were treated with huge amounts of oil spill dispersants [8]. Adding dispersants to the oil does not reduce the total amount of oil entering the environment. They simply change the physical and chemical properties of the oil and allow it to enter the water column, making the water column and benthic animals submitted to the toxic effects of treated oil, and dispersants themselves [9]. Small amounts of oil dispersed naturally in the water column through the action of waves and other environmental processes [10]. Dispersant application to increase the amount of oil that mixes physically in the water column, and reduce the possibility that the contamination of surface slick and the shoreline habitats and

animals, or impact organisms that come into contact with the surface of the water. However, by promoting the dispersion of the oil in the water column, dispersants increase the potential exposure of the water column and benthic organisms to the spilled oil. Therefore, the application of dispersant represents a conscious decision to increase the load of hydrocarbons in the water column while reducing the load on the coastal wetland [11, 12]. This trade-off reflects the complex interplay of many variables, including the type of oil spilled, the volume of the spill, sea state and the weather, the depth of the water and the degree of mixing and dilution of oil, and the relative abundance and life stages of living organisms [13]. Field observations and the following tests have shown that most of these chemicals have been highly toxic to many aquatic organisms. According to [9] that Corexit dispersant, for example, can be bioaccumulate and persist in the sediment and water column (where it can impact larvae and others plankton) for decades, especially in deep water, where the low temperature inhibit biodegradation [14].

In the aquatic environment, the oil spills may cause shifts in population structure and abundance of species and diversity, and distribution. Loss of habitat and loss of prey elements also have the potential to affect living populations [15, 16]. Oil causes harm to aquatic life through physical contact, ingestion, inhalation and absorption. Floating oil can contaminate plankton, which includes algae, fish eggs and larvae of various invertebrates. Aquatic organisms that feed on these objects can subsequently become contaminated. Large animals in the food chain, including the largest fish, birds and terrestrial mammals, and even humans may then consume contaminated organisms [2, 17].

The fishes exposed to spilled oil in different ways. They may come into direct contact and contaminate their gills. Water may contain toxic and volatile components of oil that can be absorbed by their eggs, larvae, and juvenile

stages and they may eat contaminated food [18, 19]. Fish exposed to oil may suffer from changes in heart and respiratory rate, enlarged livers, reduced growth, and fin erosion, a variety of biochemical and cellular changes and reproductive and behavioral responses. Oil has the capacity to influence the success of spawning eggs and larvae of many fish species [20, 21]. Chronic exposure to some chemicals found in oil may cause genetic abnormalities or cancer in sensitive species of fish. If chemicals such as dispersants are used to respond to a spill, there may be an increased potential for tainting of fish by increasing the concentration of oil in the water column. This can affect humans in areas that have commercial and recreational fisheries [22, 23].

In recent study, the toxic effects of dissolved and dispersed forms of different origin and character of Iraqi crude oils (Rumella, Nahran-Omar and Majnun) to eggs and young larvae of some commercially species of Shatt Al-Arab River fishes (common carp *Cyprinus carpio*, carassin *Carassius auratus*, and grass carp *Ctenopharyngodon idell*) have been investigated.

## MATERIALS AND METHODS

The different stage eggs of common carp *C. carpio* (Linnaeus, 1758) and young larvae of carassin *C. auratus* (Linnaeus, 1758), common carp and grass carp *C. idella* (Valenciennes in Cuvier and Valenciennes, 1844) [Phylum:

Chordata, Class: Actinoptergii, Order: Cypriniformes, Family: Cyprinidae] were subjected to oil contamination in the present experiments. The fishes were obtained from Shatt Al-Arab River. They had been kept and reared at laboratory in glass containers that contained river water. The containers were vigorously aerated to maintain dissolved oxygen levels at 90 to 100%. Laboratory temperature was maintained at  $20 \pm 2$  °C. Photo regime was used to provide a constant 12 hours light and 12 hours dark photoperiod at the water surface of containers. The animals were fed aquatic plants, detritus and live nauplii.

The tests were achieved with different origin and character of Iraqi crude oils, Rumella (heavy-API gravity < 28), Nahran-Omar (light-API gravity > 34), and Majnun (medium-API gravity 24 - 40) as dissolved and dispersed forms. The crude oils were obtained from Iraqi South Oil Company with properties shown in Table 1 according of Ali et al [24] The crude oils were transferred to the laboratory by dark glass bottles closed tightly and stored in laboratory temperature ( $20 \pm 2$  °C) in dark place prior to use. The two liter of each crude oil was stirred for 24 hours by a magnetic stirrer at laboratory temperature to allow evaporation. This was done to minimize the changes in toxicity that would have taken place during the experimental period due to evaporation of the more volatile fractions and to simulate the condition of an actual oil spill at water.

**Table 1: Properties of Iraqi crude oils**

Index	Value		
	Rumaila	Nahran-Omar	Majnun
Sulfur content, % mass in crude oil	1.30	0.73	1.30
fraction (i.b.t.-180 °C )	0.144	0.029	0.17
fraction (180-360 °C)	1.12	0.64	1.67
Content of fractions boiling up to 350 °C, % mass	50.02	59,87	44.27
Content of water, % mass.	Ab	Ab	Ab
Content of mechanical admixtures % mass	Ab	Ab	Ab
Concentration of chlorine salts, mg/dm <sup>3</sup>	14.06	23.01	19.22
Content of paraffin in crude oil, % mass	3.4	3.0	1.4
Freezing point of kerosene fraction	-54°C	-59°C	-73°C
Pour point of diesel fraction	-11°C	-12°C	-12°C

Ab= Absence

The dissolved crude oils were prepared by the extraction of oils in 20-liter plastic containers to avoid the direct application of oils films in the test containers. Different amounts of crude oils (100, 1000 and 10000 ppm) were poured on the water and left for two days. A calm water circulation in containers were maintained by means of small pumps to ensure maximum saturation of soluble compounds. The clear oils extracts were transferred into the test containers and renewed every two days [25]. The mount of crude oils used to prepare the extracts, however, no criterion for the actual amount and type of dissolved hydrocarbons. The initial chemical analysis shows that under the described test conditions the amount of hydrocarbons dissolved from 10000 ppm of crude oils are in the range of 10 ppm.

The dispersed crude oils were obtained by mechanical mean or by additional aid of chemicals. In mechanical dispersion, 1000 ppm of the crude oils were stirring for 1 minute at 10000 revolution/minute. Dilutions were then

prepared at once. For chemical dispersion, the dispersant Corexit 9500 was used for this purpose. 10 and 100 ppm of Corexit were added when the crude oil was mixed with the water, representing 1 and 10 % of the crude oil dispersed. The dilutions were then prepared. The experiments were started 1 and 50 hours later.

The Corexit 9500 (Nalco Energy Services Company, USA) is contains propylene glycol 1-5%, w/w; distillates, petroleum, hydrogenated light 10-30%, w/w; organic sulfonic acid salt (proprietary) 10-30 %, w/w. This dispersant is harmful by inhalation on repeated or prolonged exposure and may cause irritation of respiratory tract eyes and skin. If swallowed, it may cause nausea and vomiting and central nervous system (CNS) depression. The organic portion of this preparation is expected to be biodegradable and component substances have a potential to bioconcentrate. The physical and chemical properties of Corexit 9500 was shown in Table 2 [26, 27].

**Table 2: The properties of Corexit 9500**

Index	Value
Physical state	liquid
Appearance	clear hazy amber
Odor	hydrocarbon
Specific gravity	0.95 at 60°F/15.6°C
Density	7.91 lb/gal
Solubility in water	miscible
pH	(100%) 6.2
Viscosity	177 cst at 32°F/0°C, 70 cst at 60°F/15.6°C, 22.5 cst at 104°F/40°C
Pour point	< -71°F/< -57°C
Boiling point	296 °F/147°C
Vapor pressure	15.5 mm Hg at 100°F/37.8°C

The natural river water obtained from Shatt Al-Arab River which was filtered through 0.45 µm Whatman sterile membrane filter and boiled was used for the kept and reared the fishes, achievement tests, and preparation dilutions in bioassays. The water quality measurements of river water used in the experiments are dissolved oxygen (6.4-7.6 mg/l), pH (7.5-7.9), temperature (28.7-32.8 °C), salinity (4.5-7.3 ‰), conductivity (1155-1187 µ mhos/cm), Hardness (192-207 mg/l) and alkalinity (196-219 mg/l).

The levels of hydrocarbons were monitored during the tests by Shimadzu RF-540 spectrofluorometer equipped with a DR-data recorder following the procedure of Wattayakorn and Rungsupa [28] with some modification. At least two sets of samples were collected from each concentration. Samples were then extracted with 100 ml of nanograde carbon tetrachloride (CCl<sub>4</sub>) in two successive 50 ml extractions, and the extracts were combined. The mixture was vigorously shaken to disperse the CCl<sub>4</sub> thoroughly throughout the water sample. The shaking was repeated several times before decanting the CCl<sub>4</sub>. A small amount of anhydrous sodium sulphate was added to these extracts to remove excess water. The CCl<sub>4</sub> extracts were reduced to volume less than 5 ml by using a rotary vacuum evaporator. The

reduced extracts were carefully pipette into a precleaned 10 ml volumetric flask, making sure that any residual particles of sodium sulphate were excluded and evaporated to dryness by a stream of pure nitrogen. The flasks were then rinsed with a fresh hexane. The rinsing was used to make the sample volume up to exactly 5 ml. The basis quantitative measurements were made by measuring the emission intensity at 360 nm, with excitation set at 310 nm and monochromatic slits of 10 nm.

The experimental procedure adopted for toxicity determination was based on the method established by USEPA [29] with some modification. The eggs and larvae were subjected to crude oils into each 20 liter glass container. The containers were kept without cover and aerated. Controls were achieved without the addition of the oils. The eggs and larvae were monitored and the mortalities were measured at different time intervals. Recovery was determined by transferred the organisms to clean river water after exposure. Dissolved oxygen and pH were measured at the beginning, middle, and end of each test. Alkalinity and hardness were measured at the beginning and end of each test.

All the data were statistically analyzed. The one way analysis of variance (ANOVA) were used to

test for significant difference ( $p > 0.01$ ) of obtained values.

## RESULTS AND DISCUSSION

The addition of oils to test water caused a lowering of pH and dissolved oxygen concentration, but had no effect on hardness or alkalinity. This was in agreement with conclusion of Puglisi and Hedtke [30] in their experiments on the toxicity of five oils on four freshwater species. The pH in all tests was between 7.0 and 7.1. The dissolved oxygen concentration was between 5.7 and 5.9 mg/l Table 3.

The eggs mortality in the controls was ranging from 2 to 15 %. The extract of 10000 ppm of Nahran-Omar crude oil had caused a high mortality of 58 % (0.5 day age), 52 % (1 day age)

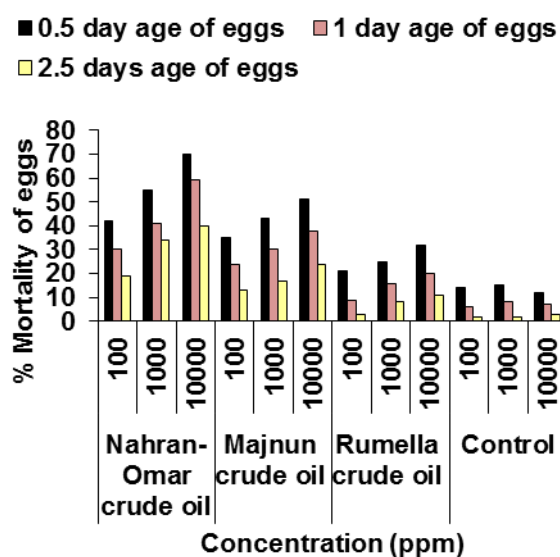
and 37 % (2.5 days age) than in the control. Majnun crude oil extract caused a mortality of about 39 % (0.5 day age), 31 % (1 day age) and 21% (2.5 days age) above the control. While, the extract from Rumella crude oil had caused the 20 % (0.5 day age), 13% (1 day age) and 8 % (2.5 days age) higher mortality than in the control. The extract from 100 ppm crude oils still caused 28 % (0.5 day age-Nahran Omar) to 1 % (2.5 days age-Rumella) higher mortality compared with the controls Table 4 and Figure 1. Rumella crude oil was almost non-toxic, perhaps because of the smaller content of toxic fractions. The toxicities vary between the crude oils, which is to be expected because the concentration and composition of individual hydrocarbons within the oils vary.

**Table 3: Dissolved oxygen, pH, alkalinity and hardness of test solutions measured in toxicity experiments**

Character	Value
Dissolved oxygen (mg/l)	5.7-5.9
pH	7.0-7.1
Alkalinity (mg/l)	202-208
Hardness (mg/l)	199-205

**Table 4: The mortality percentage of common carp eggs in dissolved crude oils and control at different ages**

Crude oil and control	Concentration (ppm)	Age of eggs (day)		
		0.5	1	2.5
Nahran-Omar	100	42	30	19
	1000	55	41	34
	10000	70	59	40
Majnun	100	35	24	13
	1000	43	30	17
	10000	51	38	24
Rumella	100	21	9	3
	1000	25	16	8
	10000	32	20	11
Control	100	14	15	12
	1000	6	8	7
	10000	2	2	3



**Fig. 1: The mortality percentage of different ages eggs of common carp at different concentrations of dissolved crude oils**

The light crude oils are generally considered to be more toxic than heavy crude oils [31]. The increased toxicity of light crude oils is primarily caused by two factors: (1) light crude oils often have concentrations of aromatic hydrocarbons, and (2) light crude oils are usually less viscous than heavy one thus requiring less mixing energy for toxic concentrations to be mixed into the water [32, 33]. Neff et al [34] had shown that the toxicity of heavy oils to be of physical or mechanical nature and chemical toxicity due to light oils. The light oils are rich in aromatic hydrocarbons. These are known to be readily soluble and toxic.

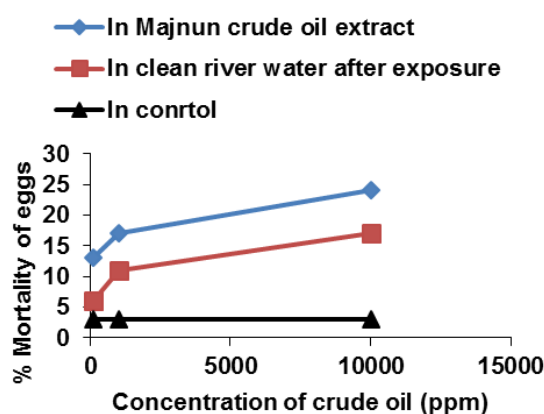
The young eggs that have been subjected to crude oils extract after fertilization appeared to be the most sensitive. Whereas, the 2.5 days eggs age with developed embryos were less sensitive to all three concentrations of crude oils extract and did not appear larger mortality than the controls. This agrees with work of Venesjarvi and Karjalainen [35] who found that the newly

fertilized eggs (from fertilization to eyed stage) were more sensitive to water soluble fraction (WAF) than eggs in the late embryonic stage (from eyed stage to hatching). The sensitivities to oils are different for each life stage of a species and for the same life stages of different species [31]. According to Rice [36] the tolerance of eggs to the effects of oil increases as their development proceeds. Components of oil can penetrate the plasma membrane of eggs and this penetration ability is highest after fertilization [37].

In the case of transfer a series of eggs from Majnun crude oil extract to clean river water after exposure, the mortality of eggs was obviously lower Table 5 and Figure 2. Whereas, the eggs with developed embryos contaminated with Nahran-Omar extract did not recover when they were transferred to clean river water. It seems that the harmful impact of Nahran-Omar crude oil to be manifested much more rapidly.

**Table 5: The mortality percentage of common carp eggs in Majnun crude oil extract, clean river water and control**

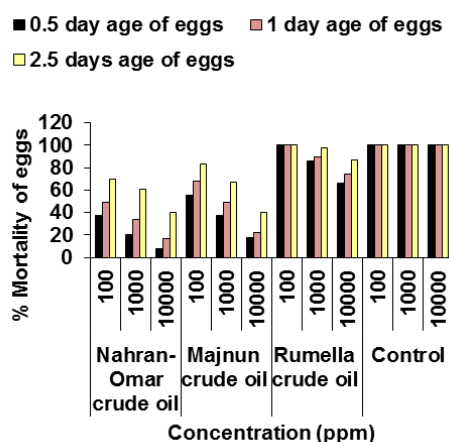
Concentration (ppm)	In crude oil extract	In clean river water
100	13	6
1000	17	11
10000	24	17
Control	3	3



**Fig. 2: The relationship between mortality percentage of common carp eggs and concentration of Majnun crude oil extract**

In all crude oils extract the mortality of eggs based on the duration of the exposure and concentration Table 4 and Figure 1. This was in agreement with the previous studies on the toxic effects of oils to a fish eggs [38, 39]. Generally, Kazlauskiene and Tadjanskis [40] reported that the toxic effect of oil on marine life depends on the duration of exposure and oil concentration in the environment.

When the eggs have been kept under test conditions until hatching, the spectrum of the hatching success occurred that shown in Table 6 and Figure 3, which show more clearly the graduated correlation between crude oil concentration and sensitivities of embryos and the various toxicities of the crude oils.



**Fig. 3: The hatching percentage of different ages eggs of common carp at different concentrations of dissolved crude oils**



**Table 6: The hatching percentage of common carp eggs in dissolved crude oils and control at different ages**

Crude oil and control	Concentration (ppm)	Age of eggs (day)		
		0.5	1	2.5
Nahran-Omar	100	37	49	70
	1000	20	34	61
	10000	8	17	40
Majnun	100	55	68	83
	1000	37	49	67
	10000	18	22	40
Rumella	100	10	10	100
		0	0	
	1000	86	90	98
Control	10000	66	74	87
	100	10	10	100
		0	0	
	1000	10	10	100
		0	0	
	10000	10	10	100
		0	0	

The most biological effects on the larvae that hatched had deformed bodies or abnormal flexures of the tail so that they could not swim normally and died and sometime a delay of development was observed. In some cases hatching was delayed or did not happen. These observations corroborated with previous studies on the effect of crude oils on fish larvae [18, 39]. Shen et al [21] who mention that the hatched larvae of fish are especially vulnerable to the toxic effects of crude oil and its refined products. Carls et al [41] reported that many of fish larvae, after hatching, was found to be deformed and incapable of swimming. Karam et al [39] noted that many oil exposed eggs of orange spotted grouper hamoor that had appeared to develop normally ultimately failed to hatch. Whereas, Venesjarvi and Karjalainen [35] found that the exposure to WAF caused high mortality among the hatched larvae of baltic herring and the newly hatched larvae were the stage most sensitive to crude oil exposure in all oil WAF levels.

In crude oils extract, the larvae showed typical behavioral symptoms include: increased activity

especially in high concentration followed by a low swimming activity, which finally stopped with the exception of sporadic spasms. Then, the larvae showed signs of the beginning narcosis, which slowly deepened until the critical point when no responses of larvae were obtained even by touching or prodding. Such symptoms had also been reported by Kazlauskienė et al [18] who showed that the typical fish larval responses to toxic concentrations of petroleum include a brief increase in activity, followed by reduced activity, sporadic twitching, narcosis, and ultimately death.

After the critical point had been reached no recovery from narcosis was observed when the larvae were placed in clean river water. This point was regarded as the most important for the judgement of the toxic effect of the oil tested [25]. The mean critical time (i.e. the mean time until reaching the critical point) was plotted against concentration of oil.

It was noted that the young larvae were much less resistant to the dissolved crude oils than embryos. The differences among the tow early life stages may be due to the different body

structures and behaviors. The early life history stages (embryos and larvae) of fish can be especially sensitive to environmental contaminants due to the rapid proliferation, differentiation, and growth of tissues [41]. Similarly to the present study Karam et al [39] had reported that embryos of orange spotted grouper hamoor fish were more tolerant to short-term exposures of WSF of crude oil than young larvae. The author showed that the resistance decreased with the advancing resorption of the yolk sac. The same conclusion was arrived by Venesjarvi and Karjalainen [35] for the larvae of baltic herring exposed to WSF of crude oil. According to Rice [36], fish in the early stages of development are particularly sensitive to the impacts of oil because they do not yet

have the necessary structures to process detrimental substances. In larval stage organs are still undeveloped and the accumulation routes of the substances differ from those in adult fish [42]. Levels of oil that can be resisted by embryos protected by a chorion can often be lethal to the larvae, since fish larvae accumulate harmful substances intensively through their skin [37]. Common carp larvae had a mean critical time of 5.3 days in the highest concentration of Majnun crude oil at the age of 1 day and 0.4 day at 12 days of age. In less concentration, the values were 15.1 days and 3.7 days, respectively Table 7. In control the larvae reach almost the same values because they were not toxic.

**Table 7: Mean critical time of common carp larvae in dissolved Majnun crude oil**

Larvae age (days)	Crude oil concentration (ppm)		
	10 <sup>2</sup>	10 <sup>3</sup>	10 <sup>4</sup>
1	15.1	9.4	5.3
3	13.6	8.7	4.3
5	6.6	4.5	2.6
12	3.7	1.3	0.4

The difference in toxicity between the crude oils was also observed on the larvae. Every type of crude oil had a different density, pour point, boiling point and different concentration of hydrocarbons. Therefore, there were different biological accumulative and persistent effects to fish larvae stressed by oil, which led to the different biological toxicity levels of the different types of oils. The crude oils had a more pronounced effect on the larvae than embryos. Carassin larvae were less and grass carp larvae were more resistant than common carp larvae of the same age. The difference in sensitivity to crude oils among the fish larvae may be due to the difference in the structure of their bodies, their ability on the metabolism, excretion and storage of the oils and/or the difference in the transportation of the oils into the site of action. This speculation is compatible with other studies [23, 43]. The difference in sensitivity to varying

environmental conditions between the different species is well known and had been reported by many investigators [23, 43].

Dispersants are commonly used for crude oil spill clean-up because they promote the formation of small crude oil droplets, enhancing their rate of natural dispersion, and reducing the risk of oil slicks arriving to coastal areas and physical contamination [44]. However, the use of dispersants often counteracted with their toxicity [45].

Table 8 and Figure 3, 4 and 5 show the mean critical time of 1-day old carassin larvae in dispersions Majnun crude oil with and without Corexit 9500 and in dissolved crude oil and pure Corexit 9500. The pure Corexit 9500 solution of the mentioned concentrations were non-toxic to the larvae. This result was in concomitant with previous reports [46]. Modern oil spill dispersants have been assessed by standard

toxicity testing techniques (LC50 testing for 24, 48, 72 or 96 hours) and most have been found to be of relatively low toxicity, or at least less acutely toxic than the spilled oils that they are used to disperse [10, 47]. This is in direct

contrast to the first generation dispersants, or more accurately, detergents used in an attempt to clean up the oil from the Torrey Canyon in 1967; these were much more acutely toxic than the spilled crude oil [46].

**Table 8: Mean critical time of 1-day old carassin larvae in dispersions Majnun crude oil with and without Corexit 9500 and in dissolved crude oil and pure solution of Corexit 9500**

Oil pollutant	After 1 hour of oil pollutant preparation				After 50 hours of oil pollutant preparation			
	10 ppm	100	1000	10000	10 ppm	100	1000	10000
A	100	7 0	8	7	10 0	70	3 0	2 0
B	100	6 0	6	5	98	50	7	6
C	100	2 0	5. 9	4	80	10	4	2
D	---	---	---	---	---	10 0	6 0	5 0
E	---	---	---	---	---	10 0	2 0	8 0

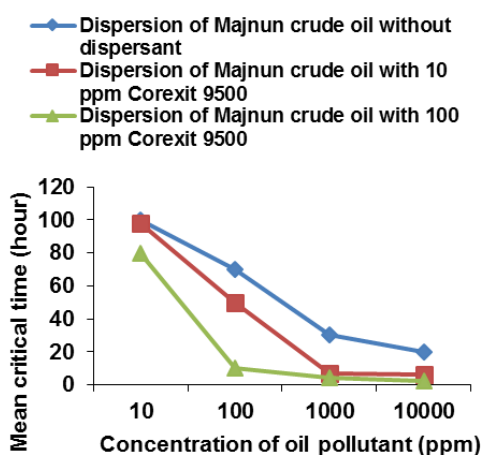
A= Dispersion of Majnun crude oil without dispersant

B= Dispersion of Majnun crude oil with 10 ppm Corexit 9500

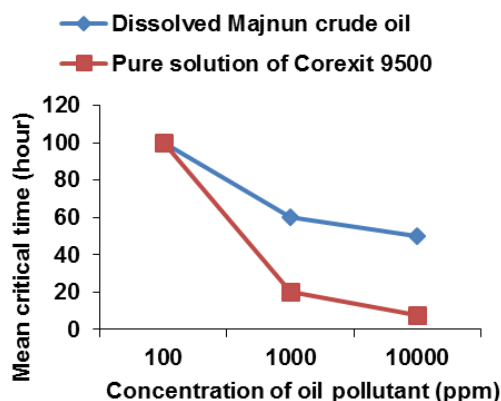
C= Dispersion of Majnun crude oil with 100 ppm Corexit 9500

D= Dissolved Majnun crude oil

E= Pure solution of Corexit 9500



**Figure 4: The relationship between mean critical time of 1-day old carassin larvae and concentration of dispersions Majnun crude oil with Corexit 9500**



**Figure 5: The relationship between mean critical time of 1-day old carassin larvae and concentration of dissolved crude oil and pure solution of Corexit 9500**

Crude oil dispersions with and without Corexit 9500 had caused the means critical times of 2 to 4 hours and 7 to 20 hours in 10000 ppm of Majnun crude oil respectively and 80 to 100 hours and 100 hours respectively at 10 ppm. This suggest that the chemically dispersed crude oil is more toxic than natural crude oil dispersions for larval fish. After 2 days the pure crude oil water dispersion had lost a lot of its toxicity, while the Corexit 9500 crude oil dispersion contains 100 ppm of dispersant had kept or even slightly increased its toxicity at all dilutions tested. This was in agreement with the previous studies on toxicity testing of dispersed oils. According to Committee [46] 75 % of researchers of the recent toxicity studies found that chemically dispersed oil was more toxic than physically dispersed oil. Most of them found that the cause for this was the increased polycyclic aromatic hydrocarbons–PAHs (typically about 5 to 10 times) in the water column. Others noted the increased amount of total oil in the water column and little researchers noted the damage to fish gills caused by the increased amount of droplets. Among the numerous examples in literature related to toxicity testing of dispersed oil are the researches conducted by Fuller et al [48], Koyama and Kakuno [49], Couillard et al [23], Khan and Payne [50], and Farid [46]. However, Less than 25 % of researchers noted

that chemically dispersed oil was roughly equivalent to physically dispersed oil such as the works of Long and Holdway [51] and Fuller et al [49].

The larval integument was damaged especially in higher concentrations. Typical rows of blisters formed on the primordial fin and the end tail looked gnawed. Surprisingly, the larvae did not avoid well defined milky clouds of even highly concentrated oil dispersions. The larvae entered and crossed the clouds though they suffered typical tissue damage. It seems that the chemical receptors to be blocked very quickly in the first contact with the oil components. Similar and other toxic effects of dispersed oils were also reported by many researchers, such as Couillard et al [23], Vosyliene et al [52], and Wise and Wise [53].

## CONCLUSION

Since these tests did not simulate the natural conditions in the river, the results cannot be directly applied to aquatic pollution. Although, there are many additional factors that may increase or reduce the toxic limits. It can be shown that the toxic compounds are extracted from crude oils, injuring larvae and young stages of eggs, and even if the concentration of dissolved compounds is sublethal to eggs, has been infected embryos and the hatched larvae.

It is important to note that the observed effects can vary greatly depending on the type of crude oil. Therefore it must be tested more types of crude oil, and countermeasures against the oil spill should be relevant to the possible biological effect that crude oil may be involved.

Natural oil dispersions obtained without chemicals gradually lose their toxicity with the oil film is being resumed on the surface of the water. Dispersions oil with dispersants to keep or increase their adverse effect for several days. The larvae do not seem able to avoid oil contaminated water, especially oil dispersions, as chemical receptors may have prevented or destroyed fairly quickly in the first contact with the oil compounds. If the larvae remain in the oil dispersions they have little chance of survival.

#### CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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