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Effect of Arabian Gulf Crude Oil on the Freshwater Fish, *Oreochromis niloticus:* Bioassay, Behavioural and Biochemical Responses

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ABSTRACT. Specimens of Oreochromis niloticus were exposed to lethal and sublethal concentrations of Saudi crude oil. The LC-50 value was computed as 13.18 ml/1. The fish exposed showed changes in behaviour. Hyperglycaemia was observed in the fish exposed to sublethal concentrations and it persisted for the whole period of investigation. Glycogen level in muscle and liver became depleted. The reduction was more in higher concentrations in the latter phase of treatment.

Various sources of petroleum hydrocarbons pollute the oceans, ocean floor and coastal regions. Estuarine and coastal organisms appear to be most endangered because of the concentration of production, shipping and refining activities near to coastline (Boesch *et al.* 1974) and because of the potential for confinement of a spill to a small coastal area (National Academy of Sciences 1975). Due to its acute toxic nature, the oil reduces the populations of aquatic animals and birds in the oiled estuaries, coastal areas and seas. Chronic low levels of crude oil which are not great enough to cause immediate death in an aquatic ecosystem could have subtle and potentially damaging effects on these organisms from chronic point of view. The physiological changes could result from either direct effect of oil on the organism or on the other elements of ecosystem (Yarbrough *et al.* 1976). The past studies demonstrated that crude oils adversely affect aquatic organisms (Rice *et al.* 1977, Less 1978, Linden 1978, Chamber *et al.* 1979, Thomas and Rice 1979, Kiceniuk *et al.* 1980, Fletcher *et al.* 1981, Moles *et al.* 1981, Moles and Rice 1983, Thomas *et al.* 1987, 89, and Panigrahi and Konan 1990). Crude oil exposure changes the level of plasma chloride and other essential elements (Payne *et al.* 1978 and Fletcher *et al.* 1979). It causes histopathological changes in fish (Sabo and Stageman 1977, Payne *et al.* 1978, Woodward *et al.* 1981 and Keke 1989). Fish exposed to crude oil show changes in the behaviour (Thomas *et al.* 1987, Panigrahi and Konan 1990 and Riebal and Percy 1990) and alterations in chemical composition of tissue (Sabo and Stageman 1977, Thomas *et al.* 1980 and Dey *et al.* 1983).

In the present study an attempt has been made to assess the toxicity of Arabian Gulf crude oil on a selected fish species by registering the mortality of fish, changes in behaviour and energy reserve (glycogen) by exposing them to acutely lethal and sublethal concentrations of crude oil.

Materials and Methods

Specimens of *Oreochromis niloticus* were obtained from a fish farm located at Deerab about 80 km south-west of Riyadh. The average length and weight of the fish were 14.53 cm and 44.56 gm., respectively. In the laboratory, the fish were kept in glass aquaria and left for four weeks to acclimatize to the laboratory conditions. During the period of acclimation the fish were fed a commercial fish food to satiety twice daily. The feeding was stopped during the period of oil exposure. The laboratory water was analysed for dissolved oxygen (7.5 ppm), hardness (273 ppm as $CaCO_3$), temperature (22°C) and pH (7.5). The acclimation period was Judged to be over when the fish showed normal feeding and swimming activity. Ten fishes were randomly selected and transferred to each of five aquaria. Known volumes of the crude oil were added to each of the test aquaria to obtain the required concentrations (5, 10, 15 and 20 ml/l). A control set was run at the same time with the same number of fish and the same volume of water but without crude oil. The water in the test aquaria was stirred mechanically for 30 minutes to mix the crude oil with water in order to dissolve the water soluble fractions of it. The mixing was repeated every hour until six hours after oil addition. All aquaria including the control were aerated with mechanical air pumps. The medium of experimental tanks and control was renewed daily. The exposure of crude oil extended up to 96 hours and the mortality of fish was registered separately for each concentration. LC-50 was computed from a graph prepared from $\log_{10}$ concentrations and probits of kill according to the method of Finney (1952).

In another set of experiments, 20 fishes (in a group of five fish) were exposed to two sublethal concentrations (2 ml/l, 3 ml/l) of crude oil for 8 days. Control set having the same volume of water and the same number of fish was run simultaneously for comparison. At an interval of 48 hours, five fishes from each test aquarium and control were sacrificed and their liver and muscle samples were taken for the estimation of glycogen. Blood samples were collected in heparinized vials by cutting the caudal peduncle. Samples of clotted blood were discarded. Blood samples were centrifuged at 6000 rpm for 15 minutes to separate plasma from blood cells. Glucose was estimated from the plasma by the method of Roe (1955). Glycogen of liver and muscles were estimated by the method of Montgomery (1957). Observations on mucus secretion and size of gall bladder are based on direct eye observation considering the colour of water and fullness of gall bladder, respectively. The behaviour of the fish monitored in the present study are described in Table 1. The group of fish selected for behavioural observation was sacrificed at the end of experiment. Observations were made every two days intervals for five fish separately for one hour. Time of observation and sequence of aquaria were rotated so that diurnal fluctuations in activity would not be confounded with oil effect. Rate of gill ventilation was registered for one minute for each fish. Data obtained in the present study were subjected to statistical analysis (student 't' test) for significance of results.

Table 1. Behaviour of *Oreochromis niloticus* monitored in the present study

Cough	Rapid, repeated opening and closing of mouth and opercular covering with partial extension of fins.
Yawn	Wide opening of mouth and hyperextension of fins
S-jerk	Movement of body sequentially from head to tail.
Partial-jerk	Movement of head or tail only.
Fin-Flickering	Repeated extension and contraction of dorsal fin
Chafe	Rubbing of the body against the inanimate object
Threat	Movement of the fish towards another fish
Nip	Bite

(After Henery and Atchison 1986)

Results

The results of the present investigations reveal that the crude oil ofArabian Gulf is moderately toxic to fish. The 96 hours LC-50 value computed from Fig. 1 was found to be 13.18 ml/l. Fish exposed to acutely lethal doses (5, 10, 15 and 20 ml/l) showed changes in behaviour. Swimming activity of oil exposed fish was reduced, the fish remained calm and motionless for most of the exposure time. The respiratory behaviour like cough and yawn and rate of gill ventilation were increased. The aggressive behaviour such as nip and nudge were changed and their frequency was slightly increased. In another set of experiments the fishes were exposed to two sublethal concentrations (2 ml/l and 3 ml/l) for 8 days. The behavioural observation indicate that fish were less active in both test solutions. Frequency of cough, yawn and rate of gill ventilation was increased (Table 3). The increase in the frequency of respiratory behaviour were high in the beginning of experiment and in high dose and became low at the latter period of exposure in all concentrations. Fish exposed to oil became more aggressive and secreted mucus in greater quantity from the mucus gland. The frequency of aggressive behaviour

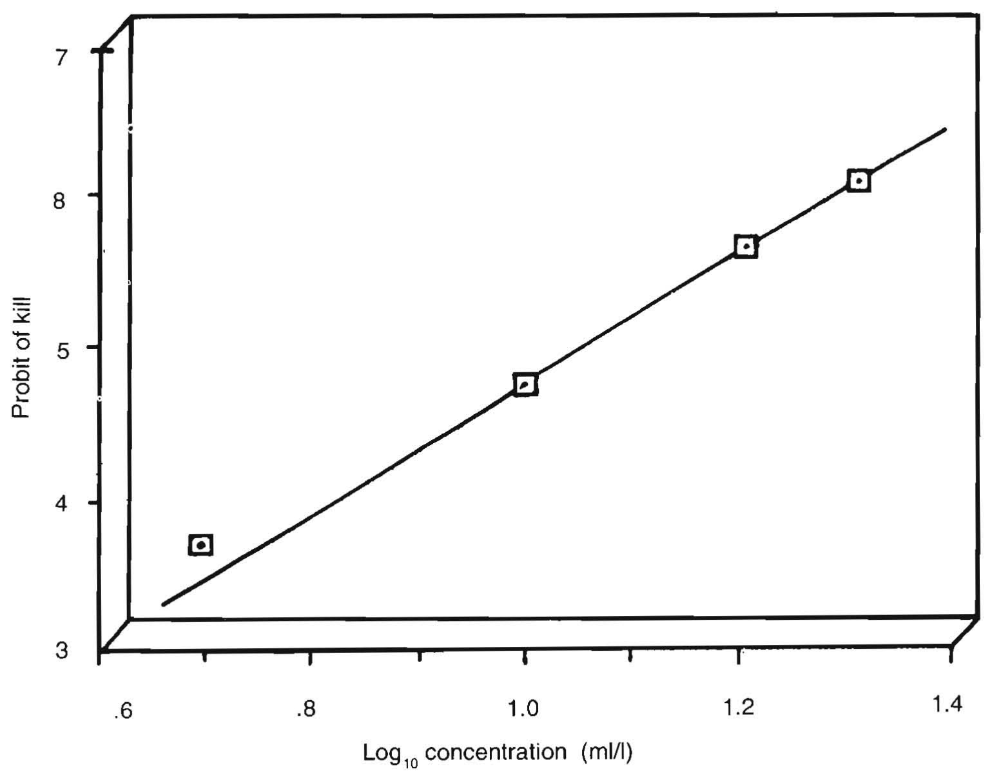

Fig. 1. Relationship between probit of kill and Log $_{10}$ concentration of crude oil (ml/l)

Table 2. Changes in glycogen content (liver and muscle) and plasma glucose of *Oreochromis niloticus* exposed to crude oil. Values are mean ± standard error

Time (Days)	Liver glycogen (μg/g)			Muscle glycogen (μg/g)			Plasma glucose (mg/100ml)		
	Control	2ml/l	3ml/l	Control	2ml/l	3ml/l	Control	2ml/l	3ml/l
2	11691 ± 152.87	11205±* 76.93	10746±** 174.26	4530 ± 58.67	4143±* 88.28	4053±** 72.93	73.07 ±2.25	96.41** ±1.20	96.10** ±1.33
4	10093 ± 143.66	9192±* 205.26	8888±** 162.21	4230 ± 25.98	3861±** 51.87	3813±** 43.06	81.02 ±2.67	99.32** ±0.82	100.26** ±0.92
6	9198± 134.53	8220±* 208.55	8163±** 154.48	4050 ± 26.83	3762±* 75.81	3729±** 59.55	81.32 ±3.57	103.5** ±0.99	101.81** ±0.74
8	8856 ± 202.01	7737±** 161.65	7692±** 112.59	3951± 36.62	3696±* 67.18	3687±** 57.59	86.73 ±0.91	102.65** ±0.36	108.37** ±1.01

Significant difference between control and treatment means. * = (p < .005), ** = (P < .001)

Table 3. Frequency of occurrence of different behaviour of *Oreochromis niloticus* at different times (2, 4, 6 and 8 days) in relation to crude oil. Values are mean of frequencies of five fish registered for one hour (No./fish/h) ± values are standard errors.

Behaviour	Control				2 ml/l				3 ml/l			
	2	4	6	8	2	4	6	8	2	4	6	8
Cough	3.2 ±0.583	3.4± 0.748	4.0± 0.707	3.8± 0.735	4.8± 0.583	4.2± 0.585	3.6± 0.678	4.0± 0.548	5.2± 0.663	5.0± 0.707	3.8± 0.663	4.0± 0.707
Yawn	1.4± 0.509	2.4± 0.510	2.0± 0.707	1.6± 0.510	3.6± 0.509	3.0± 0.707	2.8± 0.374	2.4± 0.510	3.8± 0.663	4.0± 0.894	2.8± 0.374	2.4± 0.510
Gill ventilation (beats/m)	78.61± 1.09	79.45 ±1.25	75.92± 1.45	77.63± 1.99	105.25± 1.89	105.45± 2.01	103.45± 2.11	98.45± 2.15	115.25 ±2.43	118.23 2.16	113.46± 1.99	110.45± 2.11
Fin-Flickering	24.0 ±2.168	23.6± 1.749	23.2± 2.871	22.0± 1.613	29.8± 1.356	28.8± 1.772	22.4± 3.043	20.0± 2.470	33.0± 1.483	32.2± 1.685	22.8± 2.396	20.0± 1.703
S-jerk	2.6± 0.678	3.0± 0.894	2.2± 0.735	2.0± 0.707	2.0± 0.548	2.6± 0.509	1.8± 0.374	1.6± 0.510	2.4± 0.510	3.2± 0.583	2.6± 0.678	3.0± 0.448
P-jerk	3.6± 0.509	3.6± 0.678	4.6± 0.510	2.8± 0.374	2.6 ±0.509	2.2± 0.583	2.0± 0.316	1.2± 0.374	2.6± 0.748	2.6± 0.748	2.2± 0.374	3.2± 0.735
Chafe	3.8± 0.663	3.4± 0.510	3.2± 0.860	4.4± 0.812	3.6± 0.927	4.4± 0.509	3.6± 0.678	3.4± 0.740	4.2± 0.735	3.8± 0.860	4.4± 0.927	3.0± 0.447
Nip	19.8± 1.772	20.0± 1.703	18.8± 1.463	17.4± 1.503	23.0± 1.517	22.8± 2.417	21.0± 2.236	19.8± 1.655	26.0± 1.231	23.0± 1.871	19.6± 1.806	18.2± 2.107
Threat	4.2± 0.583	3.6± 0.812	3.8± 0.671	4.4± 0.510	5.4± 0.678	5.0± 0.707	4.4± 0.509	4.0± 0.548	5.8± 0.800	5.0± 0.894	4.4± 0.510	4.0± 0.707

such as threat and nip was high in the beginning of oil exposure and in high concentration but when the exposure time prolonged *i.e.* in the last period of exposure the frequency of aggressive behaviour lowered in all concentrations of crude oil. Biochemical analysis indicated that the level of glycogen in liver and muscle was depleted in oil exposed fish (Table 2). The depletion of glycogen was more in the higher concentration than the lower concentration. The reduction of glycogen was low at the beginning of the treatment (2nd and 4th days) and gradually increased in the latter phase (6th and 8th day). Hyperglycemic condition was noted in fish exposed to oil and it remained for the whole experimental period. It was also noted that the gall bladder of oil exposed fish was completely filled with bile.

Discussion

Crude oils are acutely toxic to fish which is evident from the reduction of fish populations in oiled areas. The extent of toxic effects of crude oils varies because of quantitative and qualitative differences of hydrocarbons present in the oils. The LC-50 value (13.18 ml/l) registered in the present study is higher than the value reported for Persian oil (0.79 ml/l) and lower than the value recorded for Senai oil (14.5 ml/l) for the fish *Siganus rivulatus* (Ronald and Kissil, 1976). Ronald (1975) has investigated the LC-50 value of the same oils (Persians and Senai) for the fishes and invertebrates. In 1975 Ronald reported that the fish were most sensitive among all the animals tested and LC-50 values were between 0.74 to 30 ml/l for Persian oil and 14.5 to 30 ml/l for Senai oil for different animals. This differential toxicity exhibited by the crude oils may be related to compositional differences among the oils. LC-50 values for the water soluble fractions (WSDs) of crude oils and refined oils investigated in the past were different for different oils and the authors have given the possible explanations for these variations as the qualitative and quantitative differences of hydrocarbons present in the oils and experimental conditions (Rice *et al.* 1976, Anderson *et al.* 1974 and Neff *et al.* 1976). It has been noted that oil exposed fish exhibited less activity and most of the time remained calm, motionless and settled at the bottom. This is in agreement with the findings of Berge *et al.* (1983) and Thomas *et al.* (1987). They reported that the fish exposed to water soluble fractions of oil showed less activity than the control. This reduction in the activity of fish can be related to anaesthetic effect of hydrocarbon present in the oil.

Oil exposed fish experience stress caused by the hydrocarbons which is apparent from the fish behaviour and decline in the energy reserve (glycogen) of fish. It is not beyond to the expectations that diffusion of gases across the gills in oil exposed fish would be obstructed, partly because it damages the respiratory organs (Blanton and Robinson 1973) and partly because of deposition of oil droplets and mocus on the gills. Secretion of mucus in large quantity under stress condition is reported by Mustafa and Murad (1984), Al-Akel *et al.* (1988) and Alkahem *et al.* (1990). Hence, to improve the gaseous diffusion across the gills, the fish perform more coughs, yawns and increases the rate of gill ventilation because the former two are known to have clearing effect on gills (Henery and Atchison 1986 and Alkahem 1989) and latter is responsible to increase the water flow through the gills (Alkahem *et al.* 1990). Changes in aggressive behaviour like nudge and nip observed in oil exposed fish may be due to physiological disturbances caused by oil. Changes in behaviour of oil exposed fish, especially swimming and gill ventilation is reported by Berge *et al.* (1983) and Thomas and Rice (1979).

It is well known that metabolites of hydrocarbons present in liver are excreted through the biliary excretion (Varanasi *et al.* 1979 and Varanasi and Gmur 1981). Hence the apparent increase in the size of the gall bladder of oil exposed fish appears to be due to increased secretion of bile from the liver for the excretion of metabolites of petroleum hydrocarbons. Similar to the present observation Kiceniuk *et al.* (1982) and Khan *et al.* (1981) in cunner (*Tautogolabrus adspersus*) and cod (*Gadus morhua*) observed an increase in size of gall bladder after the exposure of petroleum hydrocarbons.

The hyperglycemia and depletion in the glycogen level of liver and muscle observed in the present study indicate that oil exposed fish produces more glucose probably by the glycogenolysis. Thomas *et al.* (1980) reported a hyperglycemic response of fish exposed to oil. Similar to above findings, hyperglycemia was also registered in fishes after the exposure of oil and hydrocarbons by Wardle (1972), Perrier *et al.* (1977) and Dimichele and Taylor (1978).

In response to the various physical and chemical stresses, ahyperglycemic condition was registered in brook trout (Houston *et al.* 1971), in rainbow trout (Schrek *et al.* 1976 and Nakano and Tomlinson 1967), in flounder (Fletcher 1975) and in killifish (Pickford *et al.* 1971).

Hyperglycemia and reduction in glycogen level in liver and muscle of the oil exposed fish appear to be due to glycogenolysis. Nakano and Tomlinson (1967) found that hyperglycemia produced in *Salmo gairdneri* was accompanied by a decline in liver glycogen level. Similarly Mcleay and Brown (1975) observed that the depletion in the glycogen reserves coincided with the hyperglycemia in coho salmon exposed to pulpmill effluent. Many investigators, such as, Sabo *et al.* (1974), Hawkes (1977), Sabo and Stageman (1977), Thomas *et al.* (1980) and Dey *et al.* (1983) have reported that the fish exposed to the crude oils and water soluble fractions of crude oils and refined oils mobilizes the energy reserves like glycogen and lipids and utilize them to meet the energy demand of fish.

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استجابات البلطي النيلي *Oreochromis niloticus* البيوكيمائية والسلوكية لتأثير النفط الخام من الخليج العربي

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يعتبر النفط ومشتقاته من أكثر الملوثات تأثيراً على الكائنات الحية في المسطحات المائية المختلفة وخاصة المسطحات المائية المالحة والتي تعبرها ناقلات النفط التجارية بين الفينة والأخرى .

ولايقتصر تأثير النفط على الطيور المائية بل يشمل الكائنات الحية الأخرى وخاصة عناصر الثروة السمكية .

ولقد اختيرت في هذه الدراسة أسماك البلطي النيلي والمنتشرة في المياة العذبة في المملكة العربية السعودية لمعرفة مدى تأثير النفط الخام على سلوك هذا النوع وكذلك ما تحتويه عضلاته وكبده من نشاء حيواني ، ناهيك عن تأثيرة على سكر الدم ، فلعل مثل هذه الملاحظات التجريبية تنير السبيل على مدى التأثير النفطي الخام على الأسماك في المياه الملوثة .

ولقد تم في هذه الدراسة غمر عدة عينات من سمك البلطي النيلي في تراكيز مختلفة هي على النحوالتالي :

٥ مل / لتر ، ١٠ مل / لتر ، ١٥مل / لتر ، ٢٠ مل / لتر . وتتبع في هذه التجربة استجابة الأسماك لكل تركيز من التراكيز السابقة وكذلك حسب التركيز النفطي الحرج LC50 ، واستخدمت طريقة مونتوقمري في عام ١٩٥٧م تقدير كمية النشاء الحيواني في عضلات وأكباد الاسماك والتي غمرت في ٢مل / لتر ، و٣ مل / لتر لمدة ثمانية أيام ، حيث أن مثل هذه الطريقة ناجعه حتى الآن في تقدير النشاء الحيواني . وكذلك قدرت كمية سكر الدم في البلازما حسب طريقة روي عام ١٩٥٥م وقورنت العينات المعالجة مع عينات الكنترول .

وتبين من هذه الدراسة أن قيمة التركيز النفطي الحرج LC50 تساوي ١٣,١٨ مل / لتر .

ويمكن حصر التأثيرات النفطية المختلفة والتي تعرضت لها هذه الأسماك بتغيرات في السلوك كزيادة السلوك العدواني بين الأسماك وزيادة حركة الغطاء الخيشومي ، وكذلك إرتفاع نسبة سكر الدم ولوحظ النقص المطرد مع زيادة التركيز في كمية النشاء الحيواني في عضلات وأكباد الاسماك المفحوصة .

ويبين المنحى العلاقة التي توضح التركيز الحرج للنفط وكذلك تبين الجداول كمية النشاء الحيواني وسكر الدم ، ناهيك عن الاستجابات السكلوكية المختلفة للاسماك .