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Toxicity of Crude Oil to the Metabolism of Freshwater Minor Carp, *Puntius sophore*

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Ecotoxicological effects of petroleum products and oil effluents have been studied extensively (Malins and Hodgins 1981; Woodward et al. 1981; Teal and Howarth 1984). Recently, the various toxicological aspects of oil pollution have been reviewed by Stephan et al. (1986). Stimulatory effects of petroleum fractions on the metabolic processes of marine fishes have been shown (Anderson et al. 1974; Neff et al. 1976) but Rice et al. (1977) observed a metabolic imbalance due to reduced respiration rate in the pink salmon fry by the petroleum hydrocarbons.

The effects of crude oil on the rate of metabolism in freshwater fishes have been little investigated. In the present investigation, the respiration rate in vitro and overall QO_2 in vivo of a freshwater minor carp Puntius sophore has been measured after exposing the fish to the lethal and sublethal doses of crude oil extracts for varying periods.

MATERIALS AND METHODS

Specimens of Puntius sophore were collected from local suppliers and were maintained in the cemented cisterns with continuous water flow. Water of the cistern contained 6.8 ± 0.2 ppm DO, 3.0 ± 0.1 ppm free CO_2, 7.8 ± 0.2 pH at 25°C temperature. Crude oil was obtained from Barauni (Bihar) oil refinery and its main fractions were analysed (Prasad and Kumari 1987).

Preparation of aqueous extracts. Sublethal (200, 500, 1000 ppm) and lethal (1500, 2000, 2500 ppm) extracts

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of crude oil were prepared in the laboratory with unchlorinated (bore-hole) water. Acetone was used as the solvent and emulsifier of the crude oil. 1 ml of crude oil was dissolved in 4 ml of acetone in a glass stoppered bottle and homogenized by shaking it for 10 minutes. The homogenate was kept as stock solution. 1 ml of stock solution after diluting with 1000 ml of water made an aqueous extract of 200 ppm strength.

Measurement of respiration rate (QO_2). 5 fishes of almost equal body size (length 5.50 $\pm$ 0.46 cm, weight 2.68 $\pm$ 0.31 g) were exposed to each concentration of crude oil for different periods (upto 168 h) and were accompanied by two controls: one with unpolluted water and the other mixed with a calculated amount of acetone which had been used for the emulsification of crude oil. Respiration rate of control and crude oil exposed fish was estimated according to the method described by Singh and Singh (1979). The results were analysed statistically employing the exponential equation: $QO_2 = aX^b$, where a = intercept, X = exposure period (h), b = slope of the regression coefficient. Level of significance was examined by Student's t-test.

Determination of QO_2 in excised tissues. 4 tissues (gill, kidney, liver and muscles) were selected for the determination of in vitro QO_2. Control and treated (1000, 1500 ppm) fish were killed by an abscission of the spinal cord, just posterior to the head, and promptly dissected for excising tissues. The excised tissues were washed in Cortland saline (Wolf 1963), chilled with ice to remove blood and contaminants and prepared for QO_2 determination by the method of Oikawa and Itazawa (1983). The value of QO_2 was determined at 25°C by a Warburg manometer shaking 120 times/min, using 20% KOH solution as CO_2 obserbent and ordinary air as the gas phase.

RESULTS AND DISCUSSION

The respiration rate in vivo (ml. h^{-1} and ml.h^{-1}.g body weight^{-1}) declined in almost all the six concentrations of crude oil. Regression analyses of data for QO_2 yielded negative b values for each concentration (Table 1). The percent decrease in O_2 uptake rate with respect to different concentrations of crude oil at different exposure periods has been plotted in figure 1. The overall metabolism decreased to 50% after 96 h of exposure in 200 ppm but a decrease of only 37.5% was recorded after 168 h at the same concentration.

The measurement of respiration rate in vitro revealed that crude oil inhibited the QO_2 of all the experimental tissues (Table 2). The inhibition of respiration rate

Table 1. Regression analyses based on equation, $QO_2 = aX^b$ showing relationship between overall respiration rate in vivo and exposure periods (4, 8, 24, 48, 72, 96, 168 h) in different concentrations of crude oil (N = 5).

Concentration (ppm)	QO_2 (ml. h^{-1}) a	b $\bar{X}$ ± SE	Correlation coefficient (r)
0.0	0.2556	-0.013 ± 0.002	-0.982_{***}
200	0.2262	-0.100 ± 0.018	-0.990_{*}
500	0.2382	-0.099 ± 0.034	-0.856_{**}
1000	0.2397	-0.163 ± 0.023	-0.973_{**}
1500	0.2235	-0.106 ± 0.027	-0.941_{***}
2000	0.3698	-0.347 ± 0.082	-0.998_{**}
2500	0.3375	-0.585 ± 0.095	-0.882
	QO_2 (ml. h^{-1}. g body wt^{-1})		
0.0	0.1164	-0.012 ± 0.004	-0.893_{**}
200	0.0990	-0.104 ± 0.037	-0.965_{***}
500	0.1058	-0.485 ± 0.092	-0.952_{*}
1000	0.1180	-0.195 ± 0.028	-0.858_{*}
1500	0.1257	-0.218 ± 0.065	-0.986_{**}
2000	0.1725	-0.334 ± 0.051	-0.977_{***}
2500	0.1371	-0.485 ± 0.072	-0.995

0.0 = Control, Significance level from control:
*P<0.05, **P< 0.005, ***P< 0.001

within the tissues was in the following order:
Gill >Liver>Kidney >Muscle.

During hypoxia, the breathing rate is known to increase in order to compensate for the decreased PO_2 level of the blood. Puntius sophore, which succumbs to lethal doses of crude oil, has its breathing rate increased. The increased breathing rate implies more amount of toxicants to be brought in contact with the secondary lamellae of the gill, thereby causing a greater damage to the respiratory epithelium including the gill rakers. Damage to the secondary lamellae by the polluted environment has been recognized by many workers (Anderson et al. 1974; Woodward et al. 1981; Solangi and Overstreet 1982; Prasad and Kumari 1987). They emphasized that separation of the surface epithelium and the edematous condition of the gills follow due to exposure of the fish to a relatively high concentration of crude oil.

Decrease in oxygen uptake and increase in opercular frequency in crude oil appear mainly due to the fusion

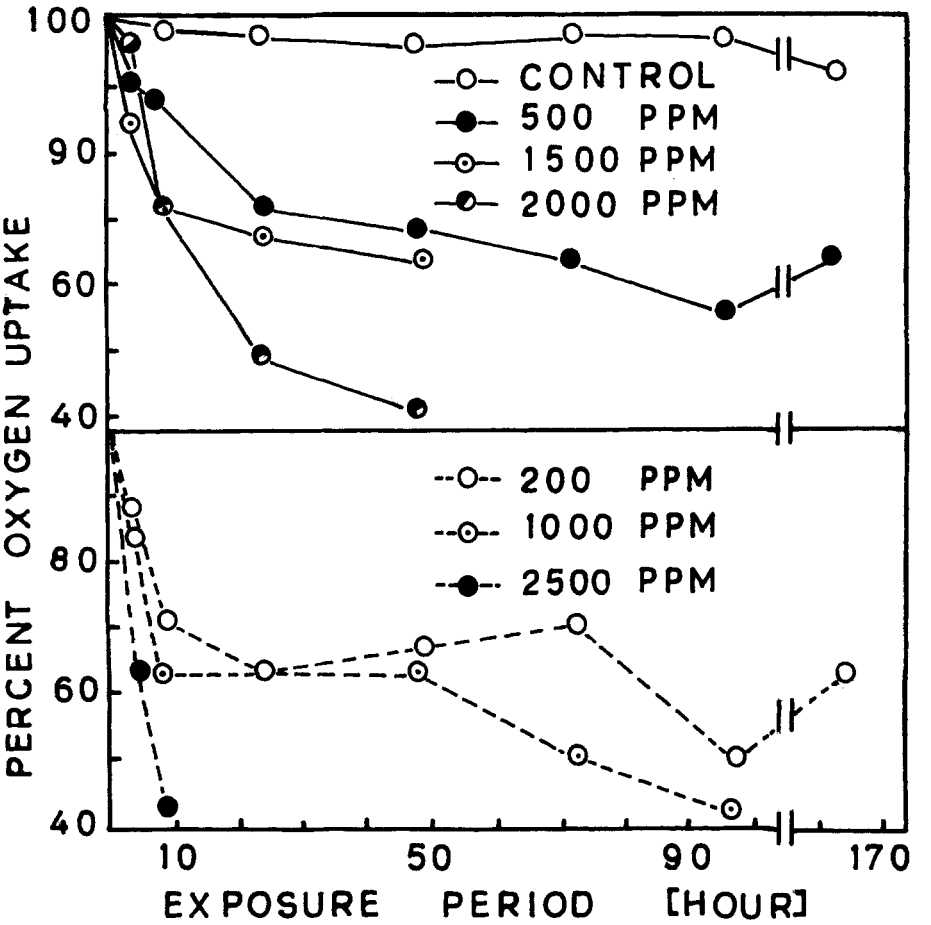

Figure 1. Effect of crude oil on the overall metabolism (% QO_2) in Puntius sophore.

of the secondary lamellae. Lesions in the respiratory epithelium and enlargement of water-blood diffusion barrier are other factors leading the fish towards respiratory distress. Solangi and Overstreet (1982) observed the hyperplastic and hypertrophic respiratory epithelium and enlargement of the water-blood barrier at the gills in different concentrations of crude oil and its water-soluble fractions. On the basis of anatomical studies, Prasad and Kumari (1987) inferred that formation of coagulated mucous film over the gills and body surface by the action of crude oil caused mortality to the fish by inhibiting oxygen transfer.

Among the experimental tissues, maximum inhibition in the respiratory rate occurs at the gills. Sensitivity of the gills towards crude oil toxicity may be related to the mode of uptake of petroleum fractions to the fish.

Table 2. Effect of crude oil on tissue respiration rate (QO_2) in vitro of Puntius sophore (Exposure time 24 h)

Tissue	Concentration (ppm)	N	QO_2 (ml. h^{-1}) $\bar{X} \pm$ SD	% decrease in QO_2
Gill	0.0	6	0.18 ± 0.024	-
	1000	6	0.15 ± 0.018	16.7*
	1500	6	0.11 ± 0.021	38.9*
Liver	0.0	7	0.09 ± 0.03	-
	1000	7	0.08 ± 0.01	11.2**
	1500	7	0.06 ± 0.02	33.3***
Kidney	0.0	5	0.13 ± 0.05	-
	1000	5	0.12 ± 0.02	7.7***
	1500	5	0.10 ± 0.03	23.0*
Muscle	0.0	6	0.020 ± 0.007	-
	1000	6	0.018 ± 0.005	10.0[a]
	1500	6	0.016 ± 0.002	20.0**

0.0 = Control, Significance level from control:
*P< 0.05, **P< 0.005, ***P<0.001,
a Not significant (P >0.001).

Petroleum fractions enter the fish tissues by positive transfer via the gills (Lee et al. 1972) causing a series of pathological effects. Furthermore, on the basis of in vitro measurement of QO_2 Oikawa and Itazawa (1984) established that the gills were metabolically the most active among the tissues experimented upon here.

Oxygen consumption is known to increase in fish exposed to petroleum hydrocarbons (Anderson et al. 1974; Neff et al. 1976). The results obtained in this study differ from the results of petroleum hydrocarbons possibly due to the fact that crude oil consists of many other components besides the hydrocarbons (Prasad and Kumari 1987) which may exhibit individual effects.

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