Bull. Environ. Contam. Toxicol. (1986) 36:150–158

Bulletin of Environmental Contamination and Toxicology

Crude Dubai Oil Toxicity on Some Fresh-Water Invertebrates

Mario Cotta Ramusino and Daniele Zanzottera

Dipartimento di Biologia—Sezione di Ecologia, Via Celoria, 26–20133 Milano, Italy

Six hundred and sixty-three tons of crude oil were accidentally spilled a bursting pipeline into the Po River in April,1980.

Toxicity tests (Gray et al.1981;Cotta Ramusino et al.1984) were carried out to determine if beavioural changes occurred in fish exposed to the oil.

The purpose of the present study is to evaluate the effects of the oil on a common invertebrate species in the Po River, so as to obtain a more comprehensive picture of the possible damage caused to the river ecosystem.

MATERIALS AND METHODS

Asellus aquaticus L. is a common detritivore in the Po River and is readily collected during all season (it reproduces in spring and autumn and withstands the winter cold). In addition, it can be easily cultured in the laboratory.

For all these reasons this isopod seems to be the ideal organism for our toxicity tests, both with crude Dubai oil "as it is" and with its "water-soluble fraction" (SFW), obtained by mixing 20 liters of normal tap water and 500 grams of crude Dubai, for 24 hours at room temperature, at a velocity of 230 revolutions per minute.

At the end of mixing it is left to settle for 2 hours, so that the solution stabilizes into two distinct strata: a more or less large deposit on the surface and below it the SFW, which is taken from the bottom.

Furthermore, in order to obtain more data on the toxicity of the latter, the behaviour of various organisms has been observed at equal SFW concentrations.

This approach has been prompted by the major methodological difficulties encountered in trying to follow the traditional procedure (progressive restriction on the range of test

concentrations) for calculating LC_{50}s, which turned out to be impossible to apply in this case (Mc Auliffe 1969; Percy and Mullin 1975; Rice et al.1976; Cotta Ramusino et al. 1984), since dilutions of the stock solution were used and the actual concentration was determined later, after analysis by infrared spectrophotometry.

A second reason for following this experimental procedure is connected with the difficulty in finding and culturing in our laboratory the species treated in the second part of the study, that is to say: Phisa acuta (Draparnaud), Echinogammarus stammeri (S. Karaman), Limnephilus (Leach) sp., Hydropsyche (Pictet) sp., Nemoura (Pictet) sp., Baëtis rhodani (Pictet), Ecdyonurus helveticus (Eaton).

All tests have been conducted under "static conditions", i.e. without changing the Dubai crude-contaning solution and have lasted no longer than 48 hours.

Plastic basins have been used for this type of test (each one with 2 liters of tap-water) and left in a thermostat-controlled bath (16 C +/- 1 C) throughout the test.

The measuring of dissolved oxygen has been performed according to the Winkler method, as modified by Alsterberg (APHA 1971) and results have been expressed in mg/l.

Before introducing the animals, the water was aerated for 20 minutes. Then the amount of dissolved oxygen was measured , first, before adding the crude oil and then periodically, until test completion.

The data collected on Asellus aquaticus have subsequenty been processed statistically on the basis of the probit method, underlying principle being that by turning toxicity curves (mortality % /concentration) into regression straight lines, (probit mortality rate /concentration log.), it is possible to estimate the average lethal concentration or LC_{50}. Statistical processing has been based upon program n 45, taken from "Computer Programming in Quantitative Biology" (Davis 1971).

The results of the other tests performed on Invertebrates are described in frequency histograms.

RESULT AND DISCUSSION

Following the treatment with crude Dubai oil "as it is", Asellus aquaticus has a 36h LC_{50} = 71.78 ml/l and a 48h LC_{50} = 14.44 ml/l (Fig. 1 and Fig 2).

Treatment with greater concentrations was deemed unnecessary so as to obtain clear effects at 24h, considering that a 100 ml/l concentration is, per se, extremely unlikely to be found in nature.

Table 1. Dissolved oxygen concentrations reported during the tests carried out on Asellus aquaticus with crude Dubai oil "as it is".

Dubai conc.	Initial time	12 hours	24 hours	36 hours	48 hours
ml/l	mg/l O_2	Δ mg/l O_2	Δ mg/l O_2	Δ mg/l O_2	Δ mg/l O_2
2.5	12	1.0	2.0	3.0	4.5
5.0	11	1.0	2.0	2.5	3.0
10.0	12	1.0	3.0	5.0	5.0
12.5	9	0.5	1.0	1.5	2.0
25.0	10	0.7	1.2	2.0	2.2
50.0	12	-	1.5	-	2.5
100.0	10	0.2	1.0	1.7	2.2
control	10	0.5	1.0	1.5	1.7

In fact, if rectangular-shaped basins are used, having a surface area of 75 cm^2, this concentration produces a surface layer about 3 cm thick.
In the light of this, we have thought it wise to determine to what extent oxygen exchanges are prevented between the solutions, thus formed,and the overlying amount of air, namely to see whether this phenomenon could be related to the thickness of the surface crude oil layer.

The relevant results are shown in Table 1., in which the figures in the first column are the concentration of crude oil, the figures in the second column are initial concentrations of oxygen and the figures in the subsequent columns are the reduced values with respect to column 2.

Comparing the 2.5 mg/l, 5 mg/l and 10 mg/l concentrations with the control group (at 48h they all have a similar survival rate), the following observations have been made.

In the control group the reduction of the oxygen value from the initial value is only 1.75 mg/l, whereas in the case of the previously mentioned concentrations the reduced values are 4.5, 3 and 5 mg/l respectively.

With greater crude oil concentrations and after 48 hours, lower values than Δ O_2 were reported, due to a previous mortality that reduces precisely the overall oxygen consumption.

Our findings clearly indicate the impossibility of oxygen exchange between the solutions and the overlying air and that this phenomenon does not depend on the thickness of the surface crude oil.

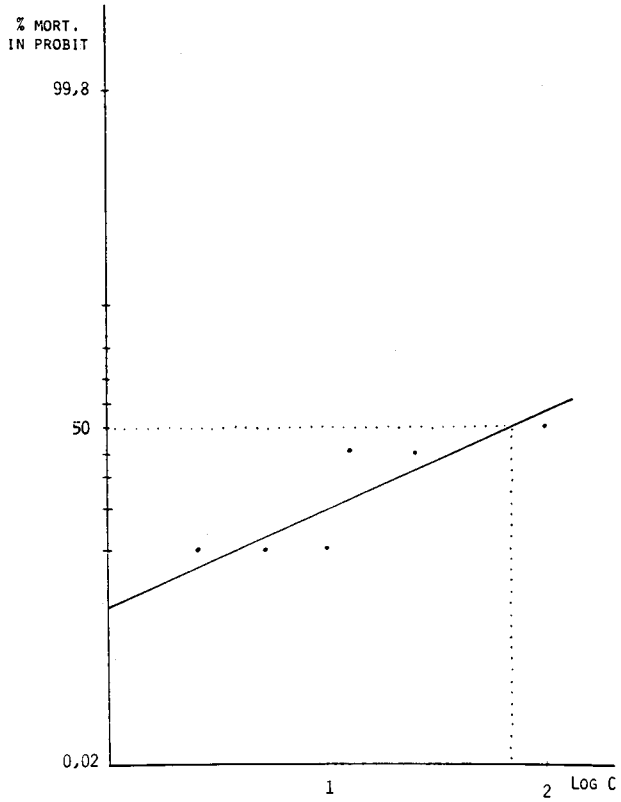

Figure 1. Asellus aquaticus treated with crude Dubai oil "as it is ".Situation at 36h. C= ml/l of crude oil.

With regard to crude Dubai oil SFW toxicity, Asellus aquaticus has a 36h LC_{50} = 12.55 mg/l and a 48h LC_{50} = 11.58 mg/l (Fig.3 and Fig.4).
Already after a few hours, severe toxicity effects can be observed in the basins with concentrations higher than 9.8 mg/l.
In fact, after one hour the surviving organism in these basins have very limitated mobility and are mainly turned upside down.
On the other hand,in the second hour of the test,mortality rates already range between 70% and 80% in the basins with 16.3 mg/l and 19.9 mg/l concentrations respectively.

Once they overcome this initial phase, the surviving animals recover "normal" behaviour, which is maintained until end of test,thus confirming the extreme volatility and toxicity of the SFW (Cotta Ramusino et al. 1984).

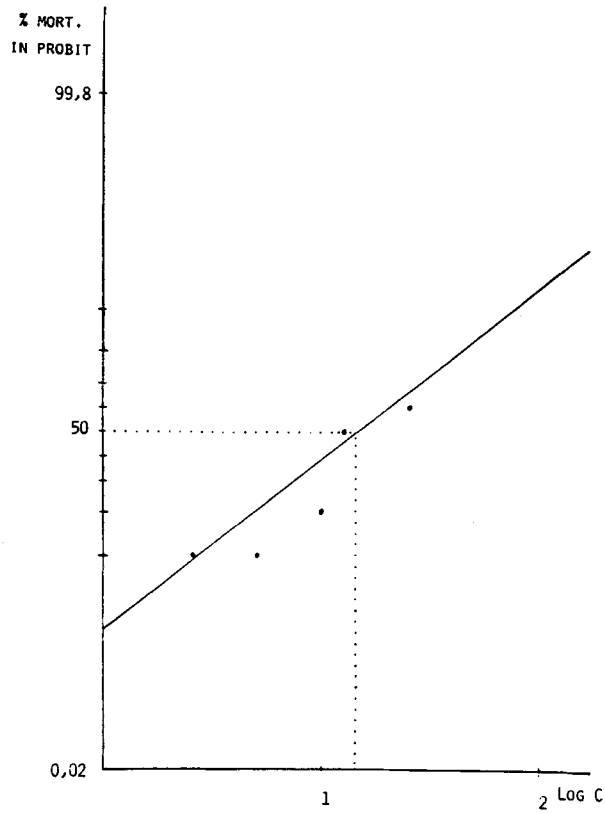

Figure 2. Asellus aquaticus treated with Crude Dubai Oil "as it is". Situation at 48h. C = ml/l of crude oil.

Controlling on Plecoptera and Tricoptera larvae the experimental trend in the course of time, (fig. 5), one can clearly note that the behaviour of animals treated with 35.8 mg/l and 17.9 mg/l concentrations of SFW is not very different from the behaviour which emerged during the test conducted on Asellus aquaticus.

Both Tricoptera species have a 100% mortality rate under the above-mentioned conditions and after only two hours.
On the other hand, Plecoptera have a 100% survival rate at 48h although they remain almost completly still throughout the test.
The 8.9 mg/l concentration has no severe toxic effect on any of the three species.

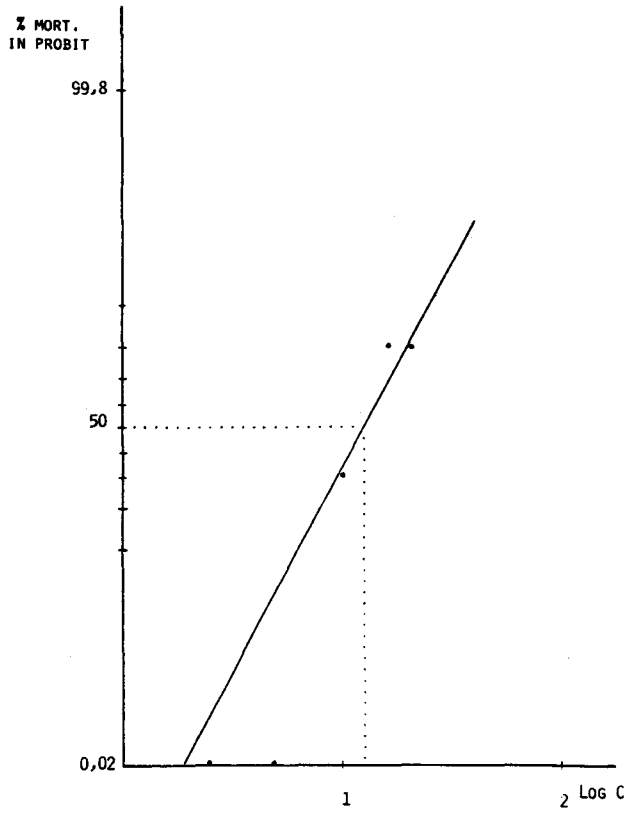

Figure 3. Asellus aquaticus treated with SFW of Crude Dubai Oil.Situation at 36h. C = mg/l of SFW.

Fig.6 shows that no death has been reported in the basins containing Phisa acuta (Gasteropod). As they are put into the solution, these animals react to the toxic substance by withdrawing into their shell and settling on the basing botton.

Later on, once a considerable part of the soluble fraction has evaporated, the Molluscs behave very much like the animals in the control basin.

In the case of Anphipodous Echinogammarus stammeri, just like Asellus aquaticus (Isopod) - as previously reported - these animals have extremely limited mobility and are mainly turned upside down. After 24 hours, the death rate is 100% in the basin with the 20 mg/l concentration solution.

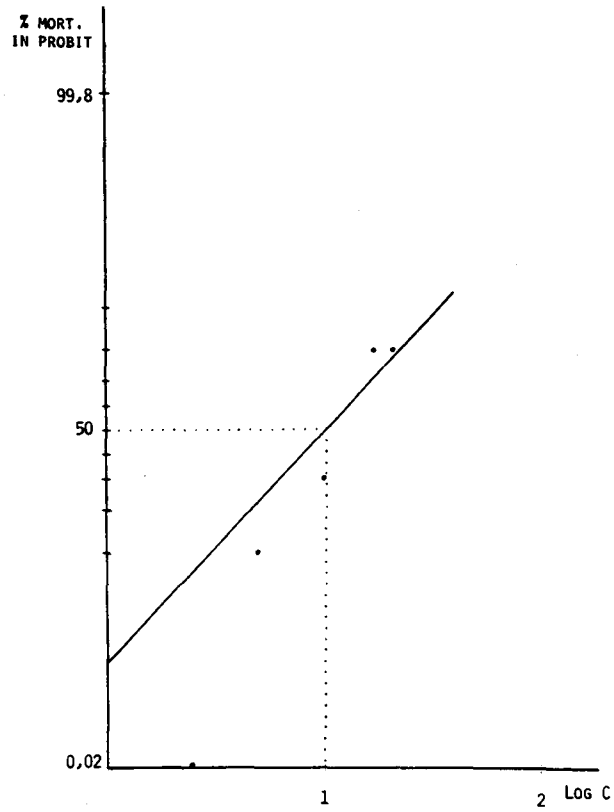

Figure 4. Asellus aquaticus treated with SFW of Crude Dubai Oil Situation at 48h. C = mg/l of SFW.

Ephemeroptera larvae turned out to be the most sensitive animals among those treated with these concentrations.

After 16h-treatment, they had a 100% mortality rate at a concentration of 10 mg/l.

It would be interesting to conduct further studies in this respect in order to assess the danage caused to animals by the SFW initial phase of extreme toxicity in terms of a long-term survival rate, species reproduction and viability, physiological, histological, biochemical and immunological alterations.

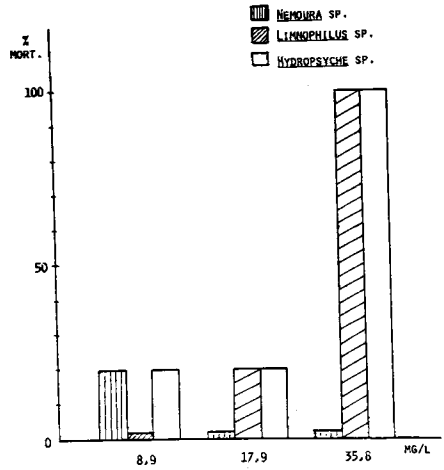

Figure 5. Comparison between the results obtained using equal concentrations of SFW on three different Invertebrate species. The situation remains the same at 24h as well as at 48h. Survival percentages are expressed in ordinates.

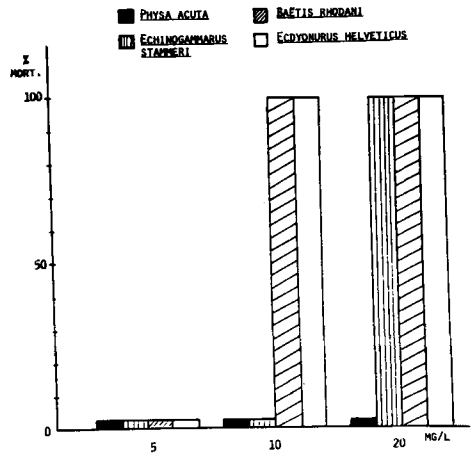

Figure 6. Comparison of the results obtained by treating four different species with equal concentrations of SFW. The situation is exactly the same at 24h and 48h. Survival percentages are expressed in ordinates.

REFERENCES

APHA, AWWA, WPCF, (1971) Standard Methods for the examination of water and waste water (New York, APHA)

Cotta Ramusino M, Dellavedova P , Zanzottera D (1974) Effects of crude Dubai oil on Salmo gairdneri Rich. and Carassius auratus L. Bull Environ Contam Toxicol 32:368-376

Davis RG (1971) Computer programming in quantitative biology. Acad Press, London and New York

Gray RH, Page TL, Saroglia MG, Tibaldi E, Pacchetti G,(1981) Behavioural responses of carp (Cyprinus carpio L.) to Dubai crude oil. Inserm. 106:553:562

Mc Auliffe C (1969) Determination of dissolved hydrocarbons in subsurface brines. Chem Geol: 225-233

Percy JA, Mullin TC (1975) Effects of crude oils on artic marine invertebrates Canadian Fisheries and Marine Service Dept of the Environment, Beaufort Sea Tech rep 11, 167 pp

Rice SD, Short JW, Karinen JF (1976) Toxicity of Cook Inlet crude oil and no 2 fuel oil to several Alaskan marine fishes and invertebrates. In: Sources, effects and sinks of hydrocarbons in the aquatic enviroment. AIBS, Washington DC,pp 394-406

Received June 4, 1984; accepted July 12, 1984.