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EFFECTS OF HYDROCARBONS ON GROWTH AND ^{14}C-UPTAKE BY *THALASSIOSIRA PSEUDONANA* (BACILLARIOPHYCEAE)

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ABSTRACT

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Exponentially growing cultures of *Thalassiosira pseudonana* were exposed to different concentrations of benzene, naphthalene and decane. The acute effects of algal growth were different for increasing concentrations of the three compounds. Benzene either did not affect cell division or it completely blocked it. Naphthalene reduced the growth rate gradually. Increasing concentrations of decane caused a prolonged lag phase. After the lag phase the growth rates were similar to those of the control.

The acute effects on ^{14}C-uptake followed a similar pattern for the three components. There was a rise in the uptake with low concentrations of hydrocarbons, followed by a sharp fall in the uptake with rising concentrations. The concentrations giving 50% reduction in ^{14}C-uptake were: 40 mg/l benzene, 2 mg/l naphthalene and 0.016 mg/l decane. There was a linear relationship between the concentrations of hydrocarbon giving a 50% reduction in ^{14}C-uptake and the solubility of the hydrocarbon.

INTRODUCTION

According to Hutchinson et al. (1979) the toxicity of hydrocarbons to algal photosynthesis is related to their solubility in water. They tested several different hydrocarbons on the freshwater algae *Chlorella vulgaris* and *Chlamydomonas angulosa*, and found the less soluble hydrocarbons to be the most toxic (per mole).

Different toxic agents are found to cause two major types of initial response on the growth of *Chlamydomonas reinhardti* and *Dunaliella bioculata* (Norland et al. 1982). Some substances cause immediate death to a fraction of the algal population, while the surviving fraction grow and develop similarly to the algae in the control culture. Other substances

reduce the mean growth rate of the algal population, but do not cause any significant mortality at low concentrations.

To investigate whether such phenomena exist for a species belonging to another taxonomic class, the growth and photosynthesis of the euryhaline diatom *Thalassiosira pseudonana* exposed to various concentrations of benzene, naphthalene and decane were studied.

METHODS

The experiments were performed on the alga *Thalassiosira pseudonana* clone W, isolated from Wümme (at Bremen, Federal Republic of Germany) by Prof. E. Paasche (Department of Marine Botany, University of Oslo) in 1973. The stock culture was axenic and was grown in enriched seawater (Eppley et al. 1967) at 17°C. The medium used in the experiment was sterilized by heating to 80°C. Light was supplied from white fluorescent tubes, at an intensity of 80 $\mu E\ m^{-2}s^{-1}$ with a 12:12 hour L:D cycle.

Stock solutions of benzene, naphthalene and decane were prepared by adding each respective hydrocarbon in excess to 2 l algal growth medium in a closed glass bottle. The details of the preparations are listed in Table 1. Analysis of the content of the hydrocarbon in the stock solutions were performed after extraction with dichloromethane. The extracts of naphthalene and decane were evaporated in a Rotavapor to 1 ml and further to 40 µl by gassing with nitrogen. The concentrates were injected by an automatic sampler on a Hewlett Packard 5880A gas chromatograph, equipped with flame ionization detector and electronic integrator. Nitrogen was used as carrier gas. The naphthalene and decane extracts were chromatographed on glass columns, 1/4" x 5' length, packed with 3% SP

Table 1

The preparation of stock solutions.

Hydrocarbon	Amount added to 2 l medium	Duration of stirring	Temp °C	Separation procedure	Conc. of stock sol. mg/l	% stock sol. used in the experiments
Benzene	20 ml	2 h	22	Separation 2 h after stirring	270	0, 2, 4, 10 20, 30, 50
Naphthalene	400 mg fine grinded	20 h	80	Filtration 20 h after stirring	20	0, 2, 4, 6, 8 10, 20, 50, 90
Decane	2 ml	20 h	80	Separation 20 h after stirring	0.029	0, 10, 20, 30 50, 70, 90

2100 80/100 Supelcoport. The benzene extracts were injected without evaporation and chromatographed on a 25 m Fused Silica SE 54 capilary column, I.D. 0.31 mm, split ratio 1:59. The recovering rate was higher than 90%. The quantitative calculations were based on external standards.

The concentration of diluted stock solutions of naphthalene was estimated on a Turner filter fluorometer (Model 111) using UV-light source (G4T4 1), a 254 nm interference filter for excitation and a 366 nm general purpose bandpass filter (7-60) for emission. The fluorescence was calibrated against the GC data. The fluorescence was a linear function of the dilution of the stock solution. The loss of naphthalene in the growth medium with 5% stock solution added was estimated by fluorescence in a separate bottle without algae. The concentration remained stable throughout the experimental period (Fig. 1). The two other components were not monitored at low concentrations. It is, however, reasonable to assume that major loss from the closed incubation bottles did not take place.

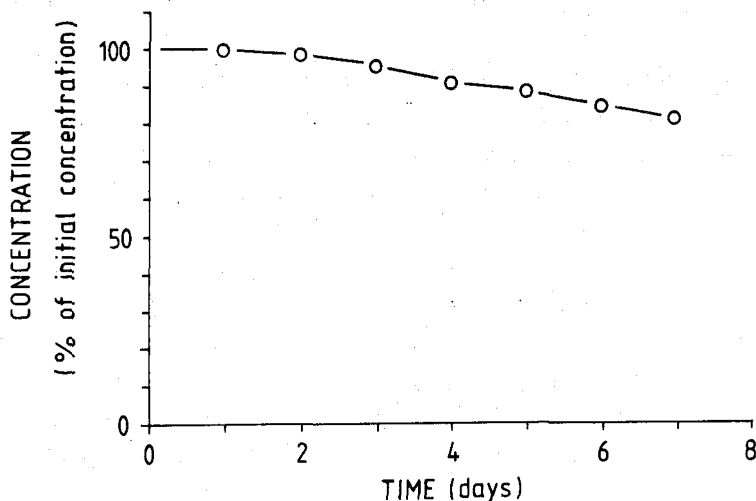

Fig. 1. The loss of naphthalene against time from a bottle with 5% stock solution. The bottle was treated in the same way as the ones used for cultures in the experiment. The values are given as percentages of initial value.

The experiments were carried out by exposing exponentially growing algae to different concentrations of diluted hydrocarbon stock solutions (Table 1). The growth chambers were 50 ml Sovirel bottles with screw-caps sealed with teflon packing. Temperature and light conditions were as for the stock cultures. All experiments were performed in triplicate in

separate bottles. The growth was measured by cell counting in a light microscope, and the fraction of abnormal cells was noted. The ^{14}C-uptake (Steemann Nielsen 1952) was measured during 4 hours incubation, 24 hours after first exposure to the hydrocarbons. The filters were acid-fumed before counting in a Packard Tri-Carb Liquid Scintillation Spectrometer Model 2450.

RESULTS

The effects on algal growth by the three compounds, benzene, naphthalene and decane, were quite different (Fig. 2). Benzene initially had no effect, but concentrations above a certain level caused total inhibition. Naphthalene caused a gradual reduction of the growth rate with increasing concentration. Increasing concentrations of decane in the growth medium resulted in a prolonged lag phase. After the lag phase the growth rate was similar to that of the control culture. There was no growth in the 20, 50 and 90% additions of naphthalene and the 20, 30 and 50% additions of benzene, but the growth in all these bottles resumed when the screw-caps were removed and the hydrocarbons evaporated.

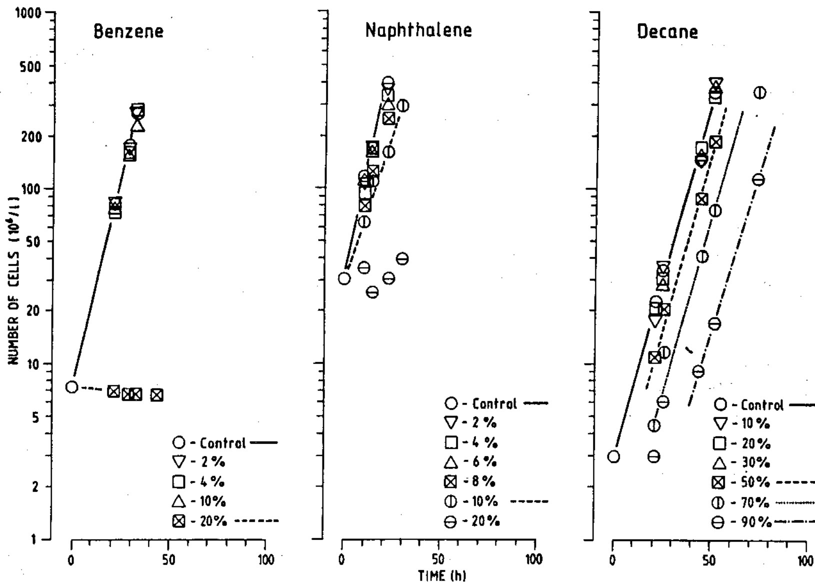

Fig. 2. Growth of *T. pseudonana* at different concentrations of hydrocarbons. The concentrations are given as percentages of the stock solution.

The effects on photosynthesis 24 hours after the addition of the hydrocarbons (Fig. 3) were similar for all three components. There was an enhancement of the ^{14}C-uptake with low additions of hydrocarbons, followed by a sharp drop in the uptake with increasing concentrations. The enhancement was most pronounced in the exposure to decane. The drop in the ^{14}C-uptake rate was particularly steep in the benzene treat-

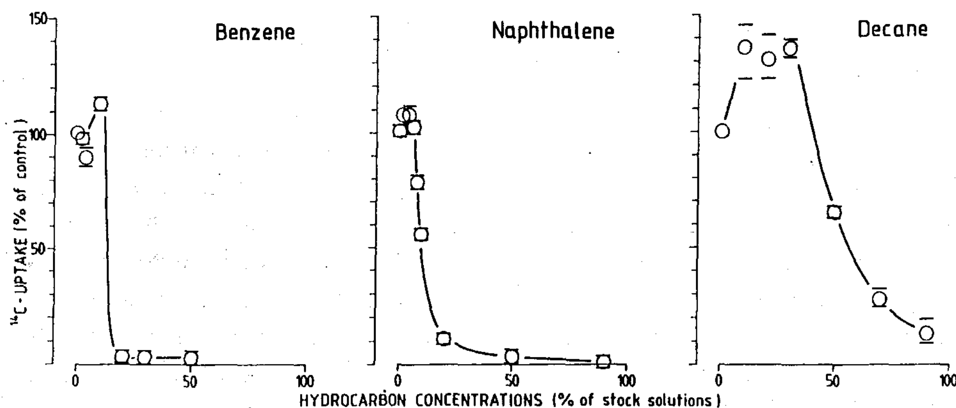

Fig. 3. ^{14}C-uptake of *T. pseudonana* after exposure for one day to different concentrations of hydrocarbons. The concentrations are given as percentages of the stock solution.

ment. The concentrations for each of the three hydrocarbons that would give a 50% reduction of photosynthesis were calculated and are listed in Table 2. The relationship between the concentration of hydrocarbon that

Table 2

Initial hydrocarbon concentration corresponding to a 50% reduction of ^{14}C-uptake.

Hydrocarbon	mg/l
Benzene	40
Naphthalene	2
Decane	0.016

would cause a 50% reduction in photosynthesis and the solubility of the hydrocarbon in the growth medium was linear (Fig. 4).

When the growth of *T. pseudonana* was inhibited by exposure to benzene or naphthalene, the frequency of abnormal cells with prolonged

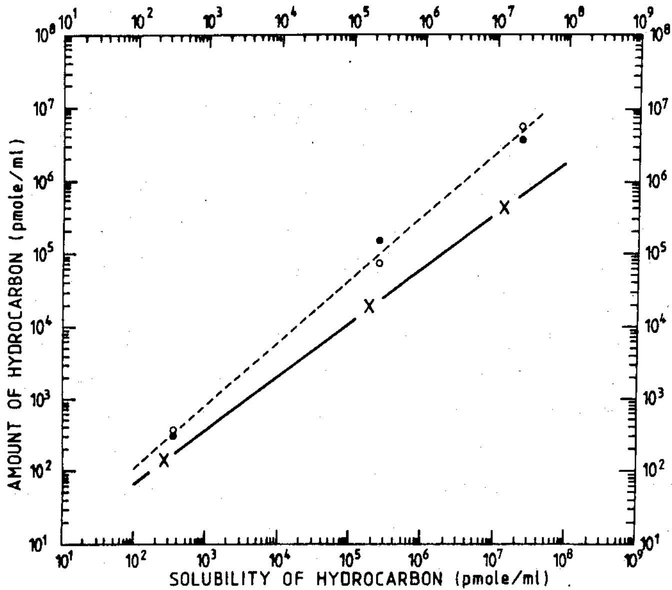

Fig. 4. The molar hydrocarbon concentrations[1] that caused a 50% reduction in photosynthesis against solubility of the hydrocarbons[2]. The open and closed circles and the dotted line represent values from Hutchinson et al. (1979). 1. Based on the concentrations of the stock solutions (Table 1). 2. Based on the concentrations of the stock solutions concerning naphtalene and decane and on solubility in seawater (Table 3) concerning benzene.

Table 3

Hydrocarbon solubility values previously published.

Hydrocarbon	Solubility mg/l	Solute	Reference
Benzene	1780	DW	McAuliffe (1966)
	1740	DW	Price (1976)
	1390	SW	Price (2976)
Naphthalene	22	SW	Eganhouse & Calder (1966)
n-Decane	0.052	DW	McAuliffe (1969)

DW = distilled water
SW = seawater

pervalvar axis increased from less than 10% to more than 50%. Exposure to decane resulted in only a few abnormal cells. The prolonged pervalvar axis is due to inhibition of mitosis (Badour 1968).

DISCUSSION

Table 3 shows some reported solubility values of hydrocarbons. Sutton and Calder (1974) compared solubilities of n-parafins in distilled water and seawater, and found, due to "salting out" effects, the highest solubilities in distilled water. In view of this, our concentrations of dissolved naphthalene and decane (Table 1) are reasonable. In the case of benzene, the concentration of 270 mg/l is low in comparison with the results of McAuliffe (1966) and Price (1976), even when taking into account the "salting out" effect. Our low concentration is probably mainly due to the short time used for preparing the stock solution (Table 1), although benzene was the most soluble hydrocarbon in the experiments.

The concentrations of hydrocarbons which caused acute effects on algae in the present study, are far above concentrations normally found in the sea, also after heavy oil spills of petroleum, e.g. Berge et al. (1977). The results should therefore be used with caution.

The different patterns of growth response of *T. pseudonana* caused by napththalene and decane in this experiment were similar to the two types found by Norland et al. (1982): 1) Increasing exposure to naphthalene caused a gradual decrease in the growth rate. 2) Increasing exposure to decane caused mortality of an increasing fraction of the cells while the survivors continued to grow with rates similar to the control. Thus, the lag phase observed may have been an apparent one and not a real one where the cells were adapting to a new medium. It was, however, not possible to directly verify mortality among cells when exposed to decane, but Soto et al. (1975) showed that cell mortality caused an apparent lag phase when *Chlamydomonas angulosa* was exposed to naphthalene. The lower frequency of cells with prolonged pervalvar axis when *T. pseudonana* was exposed to decane may also indicate a different toxic mechanism in this case.

The response to benzene, both of growth and photosynthesis of *T. pseudonana*, resembled a switch reaction at a certain threshold value. This might be an extreme response in the gradual growth reduction response. Kusk (1978) observed a more gradual reduction of photosynthesis, as O_2-evolution, when a pennate diatom, *Nitzschia palea*, was exposed to benzene. He also demonstrated that photosynthesis decreased with prolonged exposure time, which complicates the comparison of results from different authors.

The recovery of *T. pseudonana*, inhibited by high concentrations of benzene and naphthalene, when the exposure ended, is consistent with observations made on other species of algae (Kusk 1978, Soto et al. 1975).

Subinhibitory levels of toxic compounds often stimulate growth of the exposed species, an effect called hormesis (see review by Stebbing 1981, 1982). In the present experiments hormesis was detected only in the ^{14}C-uptake rates, and not in the growth rates of the algae. Similar observations were made by Karydis (1982) with crude oil extracts in cultures of *Cyclotella cryptica* and *Amphidinium carterae.* Parsons et al. (1976) observed stimulation of photosynthesis in natural blooms and in culture of *Nitzchia* sp. and *Skeletonema costatum* when exposed to low levels of hydrocarbons. Both Prouse et al. (1976) with crude oil extracts and Dunstan et al. (1975) with low molecular weight hydrocarbons, observed stimulated growth rates only in some of the species they tested. In view of these and the present results, the degree of enhanced ^{14}C-uptake and growth rate appear to vary with both the species and the hydrocarbons.

The linear relationship between the concentrations of hydrocarbons that caused a 50% reduction in photosynthesis and the solubility of the same hydrocarbons in the growth medium (Fig. 4) support the findings of Hutchinson et al. (1979): a strong inverse correlation between the solubility of hydrocarbons and their toxicity to algae. The less soluble hydrocarbons are the most toxic, on a per mole basis. However, from an ecological point of view, the more soluble hydrocarbons represent a larger problem because they may occur in much higher concentrations in the water.

T. pseudonana showed a higher sensititivy to hydrocarbons than the species tested by Hutchinson et al. (1979). In the case of benzene, the difference between the two lines is one order of magnitude (Fig. 4), which shows that the validity of the absolute values of the relationship might be limited. Different species are known to be sensitive in varying degree to oil extracts. *T. pseudonana* is rated as a sensitive organism (Pulich et al. 1974).

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