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EFFECT OF DIELDRIN ON THE GROWTH OF TWO MARINE PHYTOPLANKTON SPECIES

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Abstract. Two marine phytoplankton species, *Dunaliella tertiolecta* and *Platymonas suecica*, were exposed to 10, 100, 500 and 1000 ppb of dieldrin for 8 days. The sensitivity of both species to dieldrin altered during experimental period. The inhibiting effect of dieldrin on the growth of two species decreased with increasing division rate or cell density.

I. INTRODUCTION

Certain chlorinated hydrocarbon pesticides, such as dieldrin, are known for their low biodegrability and high accumulation in living organisms [1,2].

Marine phytoplankton, as primary producers, occupy the first level of build-up in benthic and pelagic food chains. They have been found to accumulate the pollutants in significant amounts from water and transfer them to the herbivores [3,4].

Dieldrin is ubiquitously distributed [5] and is extremely persistent in the environment [6]. It was shown that this compound was taken up by unicellular algae [7] and transfered to the organisms which feed on them [8]. The sensitivity and response to chlorinated hydrocarbons may vary considerably in different species of marine planktonic algae [2,7,9,10]. Although the inhibiting effect of certain chlorinated hydrocarbons on the growth and photosynthesis of phytoplankton has been well documented [11,12,13,14,15,16], little is known about the accumulation of dieldrin by planktonic algae and its toxicity on these organisms. Therefore, it becomes important to study the interaction between algae and dieldrin.

In this study, the effect of dieldrin on the growth of two marine planktonic algae, *Dunaliella tertiolecta* Butcher and *Platymonas suecica* Kylin has been evaluated.

II. MATERIALS AND METHODS

Two species of marine planktonic algae, *Dunaliella tertiolecta* and *Platymonas suecica*, obtained from Laboratory Citadel Hill, Plymouth, U.K., were used in this study.

Seawater was collected from off-shore of our region. It was filtered as soon as possible after collection through a glass-fiber, Whatman GF/C, to remove particulate material and organisms. It was then passed through a column of precleaned Amberlite XAD-2 to remove any chlorinated hydrocarbons including dieldrin naturally present in the water. The filtrate was stored in glass containers. Before autoclaving, the salinity of seawater was adjusted to 34.5% with double distilled water. Autoclaved seawater, enriched with Provasoli nutrient medium [17], was inoculated with 1 ml of exponentially growing stock cultures to an initial cell density of ca. 10.000 cells/ml.

Experimental cultures were grown in 100 ml Erlenmayer flasks containing 50 ml enriched seawater medium. The flasks were plugged with cotton and were shaken by hand several times daily. Illumination was provided by cool-white 40-W fluorescent lamps with an intensity from above of 4000 lux. The temperature was maintained at 18 +1°C. Stock cultures were also maintained at the same light and temperature regime as described for experimental cultures.

As certain concentrations of dieldrin, used during this study, exceed its solubility in water [186 ppb; 18], stock and working solutions were prepared in acetone. Dieldrin was added from freshly prepared working solution to the culture medium providing final concentrations of 10, 100, 500 and 1000 ppb. Three Erlenmayer flasks were prepared for each concentration. Control cultures received equal volume of solvent (acetone) not containing dieldrin.

Every two days, samples of one ml were removed from each culture medium and fixed with formaldehyde solution (ca. 4 %). After fixing, cell counts were performed with Plankton Counting Plate (Hydro-Bios, Apparatebau, GmbH) under microscope (Nikon, Model S-Kt). Two cell counts were carried out for each of the triplicate flasks and cell number calculated according to the method described by Tedmondson [19]. Experiments were repeated two times. Responses of *D. tertiolecta* and *P. suecica* to dieldrin, added at differerent concentrations to the culture medium, were evaluated on the bases of specific growth rate (k) and on the changes in cell number.

Single Classification Analysis of Variance was used to find the degree of significance of the differences existing between the cell density of the control and of the exposed cultures.

III. RESULTS

Two marine phytiplankton species were tested for their responses to dieldrin. Cell densities of *Dunaliella tertiolecta* and *Platymonas suecica* exposed to 10, 100, 500 and 1000 ppb of dieldrin are plotted against time (Fig. 1).

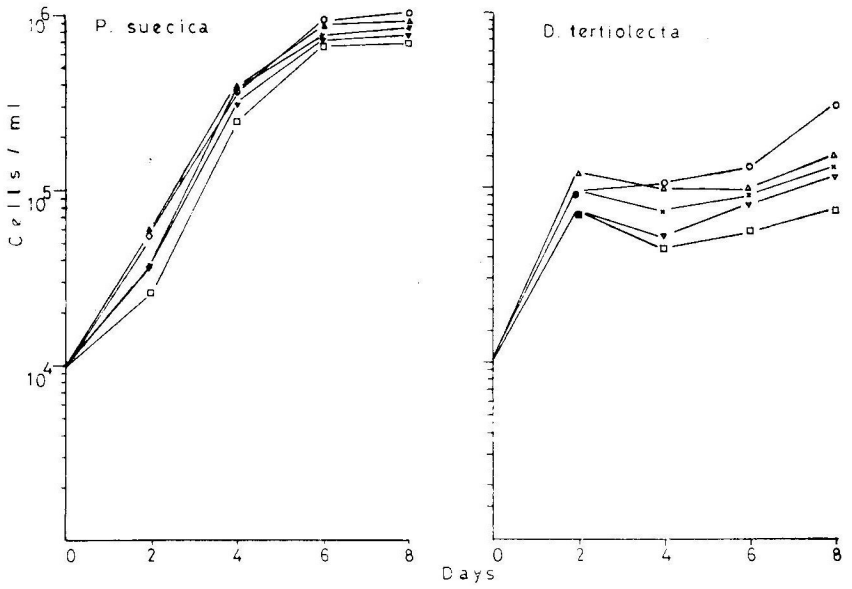

Figure 1. Effect of dieldrin on two phytoplankton species, *Dunaliella tertiolecta* and *Platymonas suecica* growing at (○-○) = 0 ppb; (△-△) = 10 ppb; (x-x) = 100 ppb; (▽-▽) = 500 ppb and (□-□) = 1000 ppb and as a function of time.

The Fs values were calculated for every two days for each test concentration to be able to evaluate the effects of different dieldrin concentrations on the exposed cultures versus control as a function of time and also to establish the safe concentration of dieldrin for these two phytoplanktonic organisms.

The growth of *D. tertiolecta* was almost unaffected by dieldrin for the first 2 days of experiment, while that of *P. suecica* was inhibited at 100, 500 and 1000 ppb of dieldrin. The results of Single Classification Analysis of Variance [20] showed that this inhibition was significant at these concentrations (Table 1).

Table 1. Single Classification Analysis of Variance of the growth of treated *Dunaliella tertiolecta* and *Platymonas suecica* showing the effect of dieldrin. The analysis were based on the cell densities.

Days	Treatement (ppb)	Fs			
		D. tertiolecta		*P. suecica*	
2	10	3.51	n.s.	0.439	n.s.
	100	0.00004	n.s.	13.453	$P < 0.005$
	500	0.793	n.s.	10.453	$P < 0.01$
	1000	1.181	n.s.	41.973	$P < 0.001$
4	10	0.934	n.s.	0.918	n.s.
	100	6.66	$P < 0.025$	0.233	n.s.
	500	37.981	$P < 0.001$	0.946	n.s.
	1000	51.668	$P < 0.001$	14.317	$P < 0.005$
6	10	4.543	n.s.	0.053	n.s.
	100	9.933	$P < 0.01$	4.94	$P < 0.05$
	500	24.809	$P < 0.001$	6.775	$P < 0.025$
	1000	37.813	$P < 0.001$	14.317	$P < 0.005$
8	10	15.53	$P < 0.005$	2.971	n.s.
	100	17.308	$P < 0.001$	24.436	$P < 0.001$
	500	22.779	$P < 0.001$	23.707	$P < 0.001$
	1000	34.055	$P < 0.001$	55.997	$P < 0.001$

n.s.: not significant.

Specific growth rate (division per day) of *D. tertiolecta* growing for two days in a medium containing 10 ppb of dieldrin was higher (1.82) than that of control (1.62). The cultures containing 100 ppb of dieldrin had also a value of 1.63 division per day, which is equal to that of control (Table 2). The specific growth rate of *P. suecica* exposed to 10 ppb of dieldrin was 1.26, which is also found to be slightly higher than the 1.20 value of control (Table 2). (Specific growth rate was calculated according to the equation described by Guillard [21]: $k = \log 2 (Ni/No)/(ti\text{-}to)$, where Ni and No are cell densities at times ti and to respectively).

Apart from control, all the cultures of *D. tertiolecta* exposed to dieldrin showed a decrease in cell number at the 4 th day of the experiment. But this decrease has not been persistent and was followed by an increase during the following 2 days of the experiment (Fig. 1.) During the later period (from Day 4 to Day 6), the growth of cultures containing 500 ppb of dieldrin, were faster than the others. Thus the division rate of *D. tertiolecta* was highest (0.29) at 500 ppb, but lowest (0.04) at 10 ppb (Table 2).

Table 2. Specific growth rate (division per day) of the treated and cultures of *Dunaliella tertiolecta* and *Platymonas suecica* at different experimental days.

Days	*Dunaliella tertiolecta*					*Platymonas suecica*				
	Concentration (ppb)									
	0	10	100	500	1000	0	10	100	500	1000
2	1.62	1.82	1.63	1.49	1.47	1.20	1.26	0.92	0.92	0.66
4	0.10	−0.14	−0.15	−0.29	−0.37	1.37	1.38	1.68	1.57	1.64
6	0.14	0.04	0.13	0.29	0.16	0.70	0.62	0.55	0.61	0.72
8	0.62	0.26	0.29	0.30	0.20	0.08	0.02	0.06	0.08	0.05

The cell density of *P. suecica* continued to increase exponentially from Day 2 up to Day 6. But, because the division rates of all cultures, including control, were diminished at 6 th day, the growth slowed at this time (Table 2).

By the end of 8-days experimental period, the growth was inhibited in all treated cultures of *D. tertiolecta*. This species was affected by dieldrin as low as 10 ppb, and cell density reduced to 50% of the control. So, inhibition was significant even at 10 ppb (Table 1). The growth of *P. suecica* was also significantly affected at the concentrations of 100, 500 and 1000 ppb, but it remained unaffected at 10 ppb (Table 1). The division rate decreased in all cultures, including control, and so, a steady-state level was reached during this period (Fig. 1).

No morphological differences between the treated and the control culture cells of both species were observed under the light microscope throughout the experiments.

IV. DISCUSSION

The results obtained at the first 2 days of the experiment, show that concentrations of dieldrin as high as 1000 ppb did not inhibit the growth of *Dunaliella tertiolecta*. But this compound stimulated the growth of of the cultures growing at 10 ppb. Menzel et al. [7] showed

that C uptake by phytoplankton increased gradually from 10 to 1000 ppb of dieldrin over 24 hours. The stimulating effect of dieldrin at low concentrations was also observed on the growth of *Exuviella baltica* by Powers et al. [22]. However, the response of *Platymonas suecica* to dieldrin, for the same experimental period, was different from that of *D. tertiolecta*. The growth of the former species was affected significantly by dieldrin in the cultures containing 100, 500 and 1000 ppb of this compound (Table 1). Rice and Sikka [2] found that various species differed significantly in their ability to remove dieldrin from the medium. Menzel et al. [7] also studied the sensitivity and response of four marine phytoplankton species to DDT, dieldrin and endrin. They observed that the effect of dieldrin was considerably different among the species. We therefore suggest that dieldrin was taken up in different amounts from the medium by the two algae, and its effect has been different on them.

After 4 days, the growth of *D. tertiolecta* was significantly inhibited in the cultures exposed to 100, 500 and 1000 ppb of dieldrin, while no significant inhibition was observed at 10 ppb. On the other hand, the growth of *P. suecica* was affected significantly only at 1000 ppb. The difference in the sensitivity of two species to dieldrin after certain period of time presumably resulted from their different division rates, since the increase in cell numbers at 2 and 4 days were different for the two species. Thus, the inhibiting effect of dieldrin on the growth of these species decreases with increasing division rate or cell concentration. We suggest that the decreased effect of dieldrin with increasing cell concentration is caused by a decrease in the amount of this compound per cell. Since the concentration of dieldrin in cells remained probably below the threshold concentration, and thus it could not exert its inhibiting effect on the growth of phytoplankton. A similar suggestion was also made by Petrocelli et al. [8]. They suggested that after 24 h, dieldrin concentration in *Dunaliella peircei* decreased presumably due to the production of new cells. Likewise, Wurster [16] who studied the effect of DDT on photosynthesis by marine phytoplankton, found an inverse relationship between number of cells and DDT concentrations in cells. He expected that the effect of DDT increased with decreasing cell concentration, itself due to an increased amount of DDT per cell.

At Day 6 of the experiment, the effect of dieldrin was similar on both species: with the exception of the cultures of *D. tertiolecta* and

P. suecica exposed to 10 ppb of dieldrin, the growth was significantly inhibited in all treated cultures (Table 1). Although dieldrin concentration as high as 500 ppb had no inhibiting effect on *P. suecica* at the 4 th day, it became toxic at the 6 th day in spite of its increased cell density at this time. The possible explanation for this inhibition is that, dieldrin was first adsorbed on the cells and then exerted its inhibiting effect on the growth of this algae. Our explanation was supported by that of Petrocelli et al. [8]. They showed that after 24 hours *Dunaliella peircei* took up 45 % of the initial concentration of dieldrin added to the culture and they suggested that the majority of the uptake observed was due to adsorption of dieldrin residues by algal cells.

By the end of the 8 th day of the experimental period, all the treated cultures of *D. tertiolecta* were affected significantly by dieldrin. The growth of *P. suecica* was also affected in the cultures growing at 100, 500 and 1000 ppb, but remained unaffected at 10 ppb (Table 1). Our results for *D. tertiolecta* were different from those found by Menzel et al. [7]. They observed that, *D. tertiolecta* was insensitive to DDT, endrin and dieldrin up to 1000 ppb over a 7-day period. We assume that, this difference is probably due to different culture conditions. Since the response of phytoplankton to pollutants differs depending on the environmental conditions. They become more susceptible to stress when the environment is not optimal for growth [9].

Our findings show that the sensitivity of marine phytoplankton species to dieldrin differed in two species tested. High cell densities, resulting from high division rate, affected considerably the toxicity of dieldrin; i.e. the greater the number of cell, the lower the effect of dieldrin. As the increase or decrease in cell densities depends mainly on the environmental conditions under which the phytoplankton grow, then these conditions should play a major role in the toxicity of pollutants, including chlorinated hydrocarbons, in these organisms. It is therefore inevitable to take into consideration the environmental or culture conditions in the evaluation of the effect of pollutants on phytoplankton species.

In natural environments, the conditions required for the growth of phytoplankton vary depending on the time of the year, on the area and on some other factors. The species living under optimal conditions would grow better and then would have more cell density than those living under stressful conditions. They would therefore be affected less

by chlorinated hydrocarbons than the species having less cell density. Thus, a species-differantiation would arise in the effect of chlorinated hydrocarbons and this would probably after the species composition of a natural phytoplankton community. Alteration of the species composition could also affect other trophic levels, because the consumers of phytoplankton take and digest selectively their foods [23,24,25]. So it is worth concluding that, alterations in the species composition of phytoplankton communities, caused by chlorinated hydrocarbons, could result in deleterious effects on marine ecosystems, and these effects appeared mostly as qualitative, rather than quantitative, changes in the food supply of herbivores.

ÖZET

DİELDRİNİN DENİZEL İKİ FİTOPLANKTON TÜRÜNÜN GELİŞİMİ ÜZERİNE ETKİSİ

İki denizel fitoplankton türü olan, *Duneliella tertiolecta* ve *Platymonas suecica*'nın 10, 100, 500 ve 1000 ppb'lik dieldrin konsantrasyonlarında 8 gün süreyle gelişmesi incelenmiştir. Her iki türün dieldrine karşı olan duyarlılığı deney süresince değişiklik göstermiştir. Hücre yoğunluğu ya da bölünme oranının artmasıyla, dieldrinin büyümeyi engelleyici etkisinin azaldığı gözlenmiştir.

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