Indian J. Fish., 48(1) : 49-53, Jan. - Mar., 2001

Uptake of an organochlorine insecticide by a microalga *Tetraselmis gracilis*

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ABSTRACT

Estuarine microalga *Tetraselmis gracilis* was studied to find out the bioaccumulation of an organochlorine insecticide. The gas chromatographic technique was employed to find out the insecticide uptake by *T. gracilis*. The microalga had the ability to concentrate the organochlorine insecticide from the medium in which they are growing. Algae grown in 0.5 ppm of BHC in the medium accumulated 0.106 ppm i.e. about 21.2% of what was present in the medium. But at 4 ppm concentration, about 29.43% was accumulated after 10 days of exposure.

Introduction

The problem of environmental contamination by persistent chlorinated pesticides is of major concern due to the presence of their residue in the environment and in the human tissues. In developing countries like India, organochlorines are extensively being used in agriculture and vector control programmes. The cumulative effects of these chlorinated pesticides on the coastal environment can be expected to be considerable.

Some algal species show a marked capacity to concentrate DDT from the surrounding medium, although the degree of accumulation varies with DDT concentration and algal species (Sodergren, 1968; Vance Drummond, 1969; Keil and Priester, 1969; Cox, 1970; Rice and Sikka, 1973). In a comparative study of the uptake of the organochlorine - DDT and the organophosphate Fenvalerate by algae, *Daphnia*, snails and fish in a model ecosystem (Ohkawa *et al.*, 1980) showed that algae had the highest bioaccumulation ratio for DDT. The interaction of DDT with two species of fresh water algae was studied by Goulding and Ellis (1981). Accumulation, degradation and biological effects of lindane on *Scenedesmus obliques* was reported by Lin-yi-xing and Scen-Bo-Zen (1987). Dhanraj *et al.* (1989) reported on the bioconcentration and metabolism of aldrin and phorate by the blue green algae, *Anabaena* sp. and *Aulosira fertilissima*.

Eventhough there are reports about the bioaccumulation of different organochlorine and other pesticides, most of them were of short-term experiments. So in order to find out long term effects, particulary the bioaccumulation of organochlorine some experiments were carried out on *Tetraselmis gracilis* for 20 days and the organochlorine insecticide used was Benzene Hexa Chloride (BHC).

Materials and methods

Tetraselmis gracilis, an estuarine form of microalga was used for the present study. This species is of mariculture importance in rearing the molluscan larvae in hatcheries. For the experiment purpose, the alga was taken from the culture collection of CMFRI algology laboratory and the culture was maintained in the laboratory condition. The Walne's medium (Walne, 1974) was used for the culture of this alga. The organcochlorine pesticide used for present study was technical grade Benzene Hexa Chloride gamma isomer or lindane - 50% WDP.

Four different concentration of the pesicides was introduced into the culture during exponential phase of growth. Control culture received no pesticide. Because BHC has low solubility in water, stock solution were made by dissolving the pesticide in a minimum of acetone and then made up the desired concentration of toxicants. One control was maintained in acetone also.

The experiment was of 20 days duration. After 10 and 20 days of the experiment, the algal cells were harvested by centifugation and lyophilized to ensure uniformity among samples. The method followed by Schauberger and Wildman (1977) was adopted to find out the bioaccumulation of organochlorine pesticide.

Extraction of the pesticides from the algal cells was done three times with 2.5 ml acetonitrile. An equal volume of 2% aqueous Na_2SO_4 was added to the collected supernatant. The mixture was subjected to extraction three times with 2 ml hexane. The extracts were evaporated to 1 ml under nitrogen.

Clean-up procedure : A glass column of size 200 mm x 14mm was packed with florisils and anhydrous Na_2SO_4 (1:1). After elution with 200 ml 6% ethyl ether/petroleum ether (v/v), and then 200 ml 15% ethyl ether/petroleum ether (v/v) the eluate was evaporated to 1 ml on a steam bath.

Chromatographic analysis was performed on a Chemito - 8510 gas chromatograph equipped with 1 bar stainless steel column packed with 5 % SE 30 as Ni 63 electron capture detector and nitrogen as carrier gas. Operating temperatures for various components of the instrument were, oven temperature 200°C, injector temperature 230°C and detector temperature 250°C.

The bioaccumulation of the organochlorine pesticide was found out using the formula (AOAC, 1984).

$$\text{Each residue ppm} = \text{Conc. of std } (\mu g/ml) \times \frac{\text{Peak size of sample}}{\text{Peak size of std}} \times \frac{\mu L \text{ std.}}{\mu L \text{ sample}} \times \frac{\text{Dilution volume}}{\text{Wt. of sample}}$$

Results

As seen from Table 1, the particular algal species concentrated BHC from the aqueous solution. The control and acetone samples with no pesticides in the meduim did not accumulate BHC. Alga grown in 0.5 ppm of BHC in the medium accumulated 0.106 ppm i.e., about 21.2% of what was present in the medium. But when it reached 4 ppm concentration, it was observed that about 29.43% was accumulated after 10 days of exposure.

After 20 days of exposure of the microalga to the insecticide BHC, it was observed that nearly 86% of the toxicant present in the medium was concentrated by the alga at 4 ppm concentration. While 50, 36.5 and 35.4% were accumulated at 2, 1 and 0.5 ppm concentration respectively.

From the results, it was observed that

TABLE 1. *Concentration of organochlorine insecticide extracted from* microalga Tetraselmis gracilis.

Initial concentration of BHC in the medium ppm	B.H.C. extracted from alga - ppm			
	After 10 days exposure		After 20 days exposure	
	Conc. of BHC accumulated	Percentage accumulated	Conc. of BHC accumulated	Percentage accumulated
0.5	0.106	21.2	0.177	35.4
1.0	0.253	25.3	0.365	36.5
2.0	0.586	29.3	1.0	50.0
4.0	1.176	29.43	3.423	85.58

as the concentration of BHC in the culture medium increased, the amount of BHC accumulated also increased. Further long term exposure of the algal cells to the organochlorine insecticide increased the chance for the accumulation of the BHC.

Discussion

In the present study the accumulation of organochlorine insecticide enhanced with increased duration of contact to the insecticide. A similar observation has been reported by Lin-yi-xing and Sun-bo-zen (1987). They tested the accumulation of y-BHC after 1, 3 and 5 days and found high accumulation after 5 days of exposure.

Rice and Sikka (1973) reported the uptake of dieldrin, another organochlorine by *Tetraselmis chuii*. As observed in the present study, they also reported that uptake of dieldrin increased linearly with an increase in its concentration. But the accumulation of toxicant reported was many times greater than the original concentration in the culture medium. This is in contradiction to the present finding. However, it may be due to the nature of pesticide used.

The same insecticide y-BHC was tested in *Chlorella pyrenoidosa* also by Sodergen (1971). The insecticide which contained at least 99% y-BHC was less readily accumulated by the species from the aqueous medium containing 0.18 ppb lindane than DDT, which again has much lower affinity for water.

Another organochlorine insecticide, DDT was found to accumulate in marine diatom to about 265 times to that present in the culture medium, as reported by Keil and Priester (1969). Contrary to the present investigation, diatoms accumulate DDT several times greater than what is actually present in the medium. This may be because the diatoms store food as oil and leucosin. But the species which was investigated during the present study stores food as starch. The oil and leucosin may serve as storage or `pick up' substances for oil soluble insecticides (Keil and Priester, 1969).

The present investigation shows that alga has the capacity to accumulate certain amount of organochlorine insecticide from the medium in which they are grown. The uptake of organochlorine by *Tetraselmis gracilis* could be considered as adsorption into the cell followed by absorption as reported by various authors (Sodergren, 1968, Rice and Sikka, 1973).

Tetraselmis gracilis being very small in size has a larger surface area to absorb the insecticide. If this species is kept in the same medium for a long time, there is a tendency to absorb more and more toxicant into the cell. That is why the rate of accumulation is high after 20 days of exposure when compared to 10 days exposure. The rate of magnitude of bioconcentration of chemicals is governed by different chemicals and physical processes which vary among different taxa of algae (Sodergren, 1968, 1971; Rice and Sikka, 1973)

The uptake of BHC from the medium by algal cells may be the result of several processes. The insecticide may be metabolically active and may act as an essential nutrient or mineral and may be transported across the cell membrane and thus enter into biochemical processes (Boyle, 1984).

Glooshcenko *et al.* (1970) suggest that bioaccumulation in algae is not affected by the rate of cell growth or metabolism. But contrary to this report Lakshminarayana and Bourque (1980) suggested that the degree of absorption of organochlorines by plankton depends on various environmental conditions, particularly their population number, seasonal variation, bloom formation, growth rate etc.

Eventhough the present investigation shows the capabilities of algal species to accumulate the insecticide, tests on single species of algae are of limited applicability in assessing the effects of pollutants on algal communities. The reason is that the algal communities are composed of an array of species with different sensitivities.

Further research is needed to understand the magnitude at which the algae are able to bioconcentrate or biodegrade pesticides. It is important to understand how the pesticides move through food chain and also how alga provide some sort of protection to other organisms in the aquatic ecosystem. Further research in this area should also focus on the identification of kinds of algae that take up different pollutants into their bodies or how the pollutants are degraded or are passed into consumer organisms.

Acknowledgements

The authors are grateful to Dr. P.S.B.R. James, former Director of Central Marine Fisheries Research Institute for providing necessary facilities and encouragement to carry out the work. The first author thank ICAR for the award of a senior fellowship during the programme. This work formed part of Ph.D. thesis submitted to Cochin University of Science and Technology, by the first author.

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