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Response of some freshwater micrometazoans to DDT

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Abstract. The response of a few members of freshwater micrometazoans to DDT have been ascertained by conducting laboratory and field bioassay experiments. The organisms tested in laboratory bioassays, have been found to respond at low concentrations with LC50 ranging between 10 and 49 ppb. *Cyclops* sp. and *Asplanchna brightwelli* respond to higher concentrations of DDT with LC50 as high as 515 ppb and 2218 ppb respectively. LC50 for the larvae of *Culex fatigans* has been found to be 180 ppb. In the field test of 70 ppb of DDT was applied and repeated after 23 days. The response of organisms is similar in both the treatments. The population of *C. cornuta* and *M. brachiata* reappeared after 22 days of the treatment whereas, *D. excisum* and *N. kamakhiae* failed to reappear. Analysis of the bottom soil of the treated tank revealed the presence of DDD, DDE and DDT. Some secondary effects of the DDT treatment have also been observed and discussed.

Keywords. Freshwater micrometazoans; bioassay; DDT; mortality; aquatic weeds.

1. Introduction

Insecticide pollution is a serious problem across the world especially in developing countries where it is used indiscriminately in crop protection programmes. During monsoon these toxic chemicals easily find their way along with water into ponds, lakes and rivers causing damage to biotic communities.

Literature perusal reveals that there have been excellent studies on toxicity of pesticides in fish but similar work on freshwater micrometazoans is limited (Anderson 1945; Ruber 1963, 1965; Sanders and Cope 1966, 1968; Patterson and Windeguth 1964; Ruber and Basker 1968; Hurlbert *et al* 1970, 1972; Khudairi and Ruber 1973; Hurlbert 1975; Ali and Mulla 1978). The present study aims at evaluating the toxicity of DDT on a few members of freshwater microfauna which are important in the aquatic food chain.

2. Material and methods

2.1 *Laboratory bioassay experiments*

Test organisms, *viz., Ceriodaphnia cornuta, Neodiaptomus kamakhiae, Moina brachiata, Diphanosoma excisum* and *Cyclops* sp. were collected from Jyotisar, a freshwater tank in Kurukshetra. Laboratory cultures of *Ceriodaphnia reticulata* and *Asplanchna brightwelli* raised by employing the methods of Deway and Parker (1964) and Singh and Dattagupta (1981), respectively, have been used in the bioassay. To obtain mosquito larvae, yeast contaminated tap water was left open in glass jars during the night. Egg rafts laid in these glass jars by wild *Culex fatigans* were collected next day. Each egg raft was kept in a separate container and allowed

to hatch in tap water. The newly hatched larvae were given yeast suspension as food till they reached their 4th instar stages.

The bioassay experiments were performed on the animals of uniform age in *A. brightwelli, C. cornuta, C. reticulata, D. excisum* and *M. brachiata* since these organisms reproduce young ones parthenogenetically. One day prior to testing, the brooded forms of the aforesaid animals were placed in the classified petri dishes, and the young ones which appeared on the following day were considered to be not more than one day old and were used in the bioassay. With regard to *Neodiaptomus kamakhiae* and *Cyclops* sp. the animals of uniform size, and for the larvae of *Culex fatigans* only 4th instar stages were put to laboratory bioassay treatments.

One percent stock solution of DDT was prepared in acetone. The insecticide was further diluted from this stock solution. The coarsely filtered water of the Jyotisar tank was used as dilution medium for experiments using the organisms collected from this tank. The rotifer media (Singh and Dattagupta 1981) were used for experiments using *A. brightwelli.* Aged tap water, kept for a week in a closed container, was taken as diluent for experiments using *C. reticulata* and the larvae of *Culex fatigans.*

The toxicity experiments on *N. kamakhiae, C. cornuta, C. reticulata, M. brachiata, D. excisum, A. brightwelli* and *Cyclops* sp. were conducted in cavity blocks of 15 ml capacity containing 10 ml of test concentration. The larvae of *Culex fatigans* were treated in glass beakers of 250 ml capacity containing 200 ml of test concentration. Ten micrometazoans were used in each assay treatment and 3 replications of each treatment were maintained. The experiments employing each species were repeated thrice. Controls were run for each of these experiments. Acetone was added to the control in an amount equal to the largest aliquot of stock solution used in the test concentration.

The mortality was recorded after 24 hr treatment in all experiments except those performed on *A. brightwelli.* The mortality of *A. brightwelli* was observed after 12 hr treatment because a longer exposure period would cover over 20% of the average life span of this species. The toxicity of the insecticide was calculated as LC50 (concentration lethal to 50% of the test organisms) using the standard methods (Busvine 1971).

2.2 *Field bioassay experiment*

Two small tanks (5 × 5 × 2 ft) were constructed to study the DDT effect on the population of different micrometazoans in their natural habitat. The natural population of different micrometazoans we obtained from their resting eggs present in each experimental tank. The study began in August 1979 and terminated in November 1979 and during this period one of the experimental tanks was given two DDT treatments at the same concentration of 70 ppb on 11 September and 4 October, respectively. The other tank was left untreated to run as a control. Microfauna samples were collected from each experimental tank. The first sample was taken on the third day after the treatment; thereafter the samples were drawn every 10 days. For a sample, water (20 litres) taken from different spots of the tank was filtered through Hand plankton net. The concentrated mass of microfauna thus obtained was estimated for the different organisms. The total organisms present in one litre were estimated for each sample. Temperature, pH, dissolved O_2 of water were also measured at the time of each collection. After the experiment, the soil

samples taken from the top 3 cm bottom sediments of each experimental tank were examined for various residues of DDT using gas liquid chromatograph, Packard A7400 equipped with electron capture detector with tritium foil.

3. Results and discussion

The concentration tested and the percentage of mortality were converted to logs and probits respectively and calculated to get regression equation by the least square method for each test species (figures 1 and 2). Table 1 represents the LC50 value of the different organisms tested in the laboratory. It is evident from these values that *N. kamakhiae* is more sensitive to DDT than the other organisms tested. Further, the susceptibility of the other micrometazoans is in the order as *C. cornuta, D. excisum, C. reticulata, M. brachiata,* larvae of *Culex fatigans, Cyclops* sp. and *A. brightwelli;* the latter being least susceptible. These test organisms could be grouped into two broad categories based on their tolerance to DDT concentrations. *N. kamakhiae, C. cornuta, D. excisum, C. reticulata, M. brachiata* respond to low concentrations with LC50 ranging between 10 and 49 ppb. Compared with these microcrustaceans, *Cyclops* sp. and *A. brightwelli* have been found to respond to much higher concentration with LC50 as high as 515 ppb and 2218 ppb, respectively.

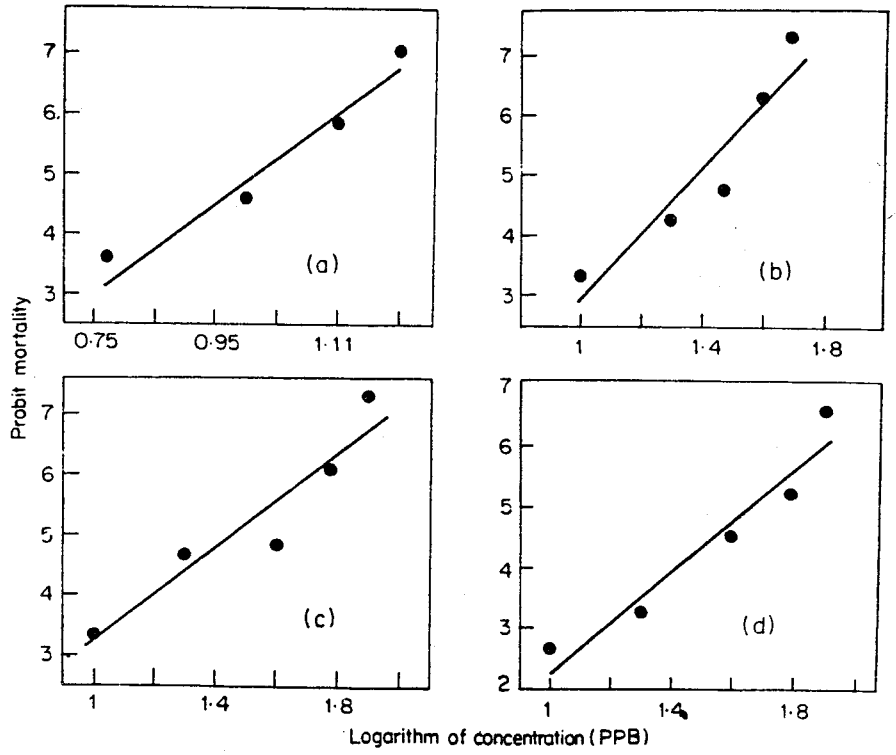

Figure 1. Response of some freshwater micrometazoans to DDT. **a** = *Neodiaptomus kamakhiae*, **b** = *Ceriodaphnia cornuta*, **c** = *Diphanosoma excisum*, **d** = *Ceriodaphnia reticulata*.

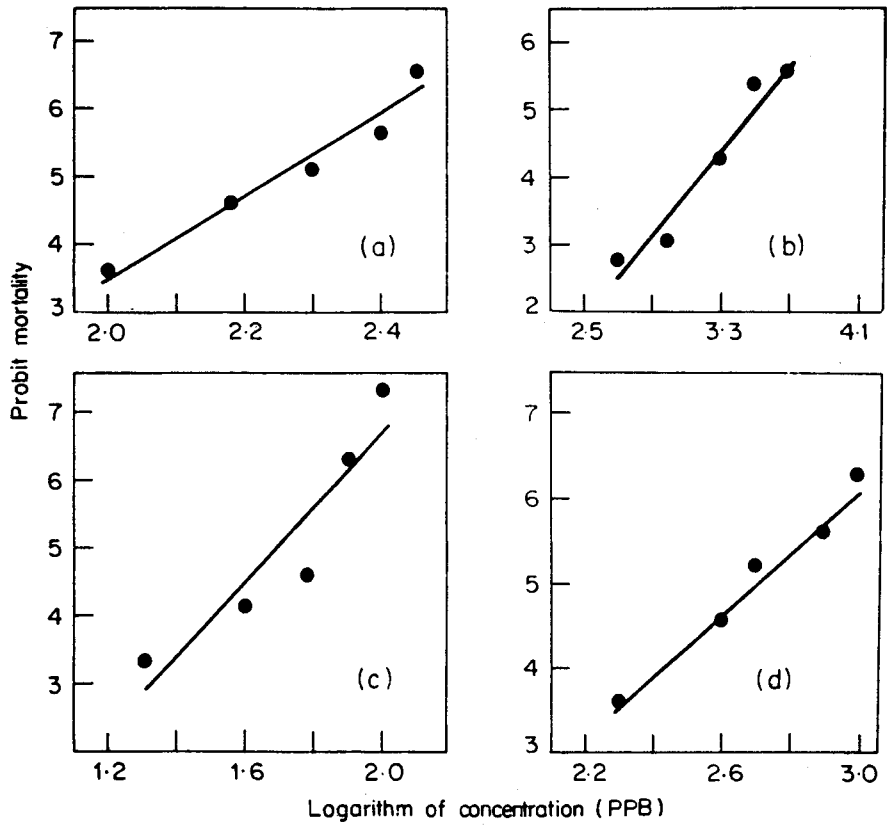

Figure 2. Response of some freshwater micrometazoans to DDT. **a** = larvae of *Culex fatigans*, **b** = *Asplanchna brightwelli*, **c** = *Moina brachiata*, **d** = *Cyclops* sp.

Table 1. LC50 values of different organisms.

Organisms	LC50 (ppb)
Neodiaptomus kamakhiae	10
Ceriodaphnia cornuta	23
Diphanosoma excisum	28
Ceriodaphnia reticulata	44
Moina brachiata	49
Larvae of *Culex fatigans*	180
Cyclops sp.	515
**Asplanchna brightwelli*	2218

*Exposed for 12 hr, others for 24 hr

Although it is not possible to attribute any reason to the tolerance, both *A. brightwelli* and *Cyclops* sp. are raptorial feeders (Hutchinson 1967). Compared with the latter organisms *N. kamakhiae, C. cornuta, C. reticulata, D. excisum, M. brachiata* are readily susceptible to DDT and interestingly enough these organisms are filter feeders (Hutchinson 1967). Filter feeding zooplankters have been known to ingest more pesticide than other forms of freshwater micrometazoans (Hurlbert 1975). Ruber (1963) reported that the rotifer, *Philodina* sp. was unaffected at a concentration of 500 ppb of DDT exposed for 193 hr. Further, Hurlbert *et al* (1972) reported an increase in population and increase in sexual forms of *A. brightwelli* in ponds treated with Dursban, an organophosphate. The LC50 of 180 ppb for the larvae of *Culex fatigans* as has been found in the present study is considerably higher than what has been reported by WHO (1968) for larvae of *Culex fatigans.* The larvae for the present experiment have been obtained from eggs laid by wild *Culex fatigans.* Since, the university campus is regularly sprayed with chlorinated hydrocarbons as a measure of malaria control, it is possible that tolerance level of these mosquito larvae have reached a higher level consequent upon their exposure to insecticide residues. Table 2 shows the toxicity of DDT to a variety of freshwater micrometazoans reported by several authors. But these authors worked on species of aquatic animals which are different from the present ones. Also there could be a difference in response owing to the intrinsic heterogeneity of the test organisms.

Table 2. Showing the toxicity of DDT to a variety of freshwater micrometazoans.

Organism	Toxicity measurements (in ppb)			References
	Acute Range (0 to 100% mortality) (24 hr)	LC50 (24 hr)	EC50** (48 hr)	
Daphnia pulex	–	–	0.36	Sanders and Cope (1966)
Simocephalus serrulatus	–	–	2.50	–do–
Daphnia magna	–	–	1.40	Boyd (1957)
Microcyclops bicolor	30-250	–	–	Ruber (1963)
Ceriodaphnia quadrangula	6-120	–	–	–do–
Cypridopsis vidua	250-1000	–	–	-do–
Cyclops apocyclops	10-500	–	–	Ruber and Basker (1968)
Diaptomus sp.	1-100*	–	–	–do–
Larvae of *Culex fatigans*	–	70	–	WHO (1968)
Larvae of *Anophelese albimanus*	–	10	–	Georghiou (1972)
Stonefly naid (*Pteronarcys californica*)	–	41	–	Sanders and Cope (1968)

*Acute range for 40 to 100% mortality

**50% immobolization of the organisms

In field bioassay experiment, *C. cornuta* and *M. brachiata* were found completely absent within 2 days of the treatment. However, these organisms reappeared in the samples which were collected after 22 days of the treatment (figure 3). These reappeared forms apparently were hatched from their respective dormant eggs. The treatment of the same concentration was repeated and the response of the organisms has been similar to that after the first treatment. Therefore, the young ones emerging from the dormant eggs seem to have the same level of tolerance as that of their previous generations. Shiff and Bridget (1961), Hurlbert *et al* (1970, 1972) Didia *et al* (1975) and Ali and Mulla (1978) found similar micrometazoans reappear at an interval of 1 to 3 weeks after they were eliminated partially or totally by insecticide treatments in the field.

N. kamakhiae and *D. excisum* were completely absent after 2 days of the first DDT treatment (figure 4). However, they were present throughout the study period in the control tank. Miura and Takahashi (1976) also reported similar failure of some cladocerans to reappear after the water was treated with 0.03 ppm of SD 47775. In a similar situation, *Diaptomus pallidus* did not develop in the experimental

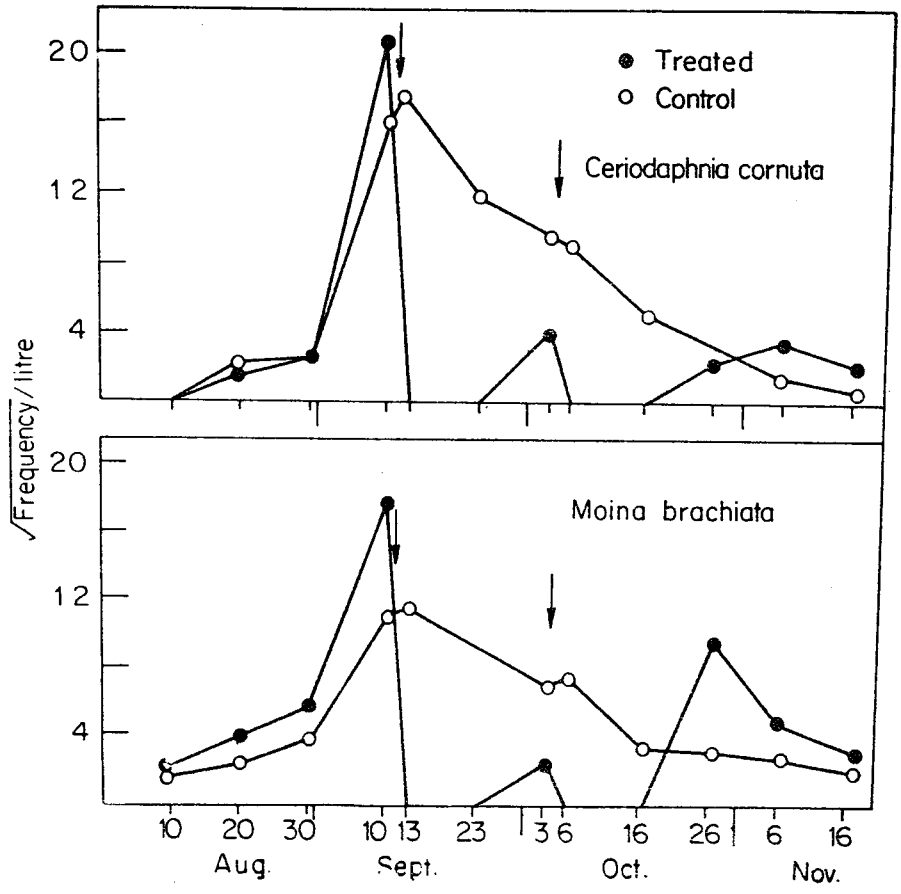

Figure 3. Effect of DDT treatment on the natural population of *Ceriodaphnia cornuta* and *Moina brachiata*. The arrows indicate the treatment day.

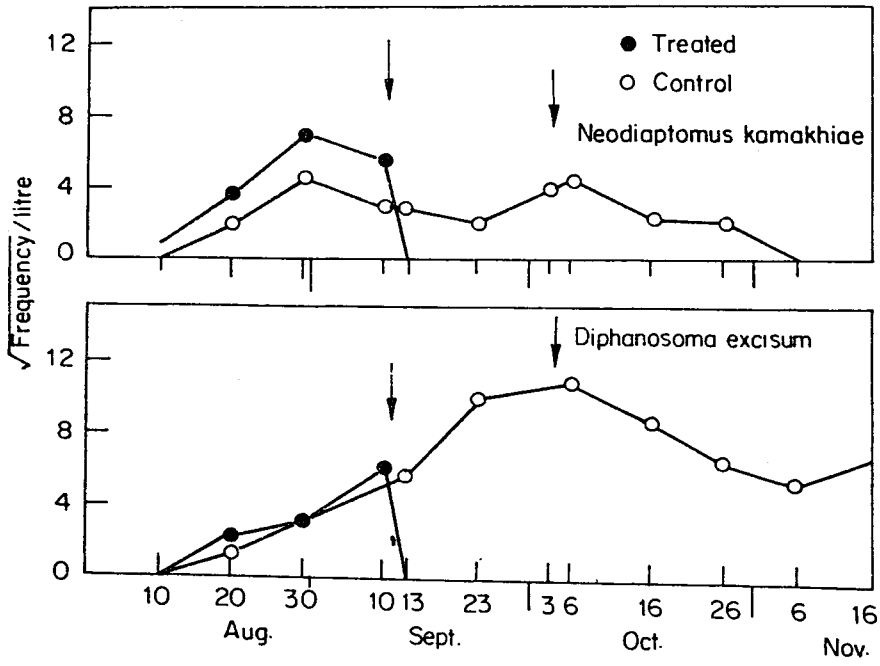

Figure 4. Effect of DDT treatment on the natural population of *Neodiaptomus kamakhiae* and *Diphanosoma excisum*. The arrows indicate the treatment day.

tank which received 1.0 lb/acre of Dursban treatment (Hurlbert *et al* 1970). One reason for such a failure on the part of certain plankters may be that the organism died owing to the insecticides treatment before they could produce any dormant eggs. It is also possible that the dormant eggs of *N. kamakhiae* and *D. excisum* are susceptible to DDT residues in the tank bed as in the present study (*vide infra*).

The population of cyclops, ostracods and larvae of *Anophelese* sp. was not affected by DDT treatment (figure 5). Didia *et al* (1975) and Ali and Mulla (1978) also found a number of insecticides extremely toxic to certain species of cladocerans but little or no toxicity to ostracods and copepods. The present results of the laboratory bioassay also indicate tolerance of *Cyclops* sp. to DDT (*vide supra*).

The variation of temperature and pH was almost similar in both the tanks (figure 6). However, a distinct maxima of dissolved O_2 content were observed in the sample of 23 September 1979 and 16 October 1979 respectively. These collections, which exhibited the maxima of dissolved O_2, were made after 12 days of each DDT treatment, and were probably due to increase in the population of phytoplankton and decrease in respiratory activity of microfauna. There are several reports which indicate the increase of phytoplankton population following the treatment of insecticides (Hurlbert 1975).

A bloom of aquatic weeds, *viz.*, *Wolfia* sp. and *Lamna* sp. was observed in the treated tank. These aquatic weeds proliferated during the period from 26 October to 6 November 1979. The water surface of the treated tank was thickly covered by these plants around 16 November when the present experiment was terminated. On the other hand these aquatic weeds were not observed in the control tank throughout

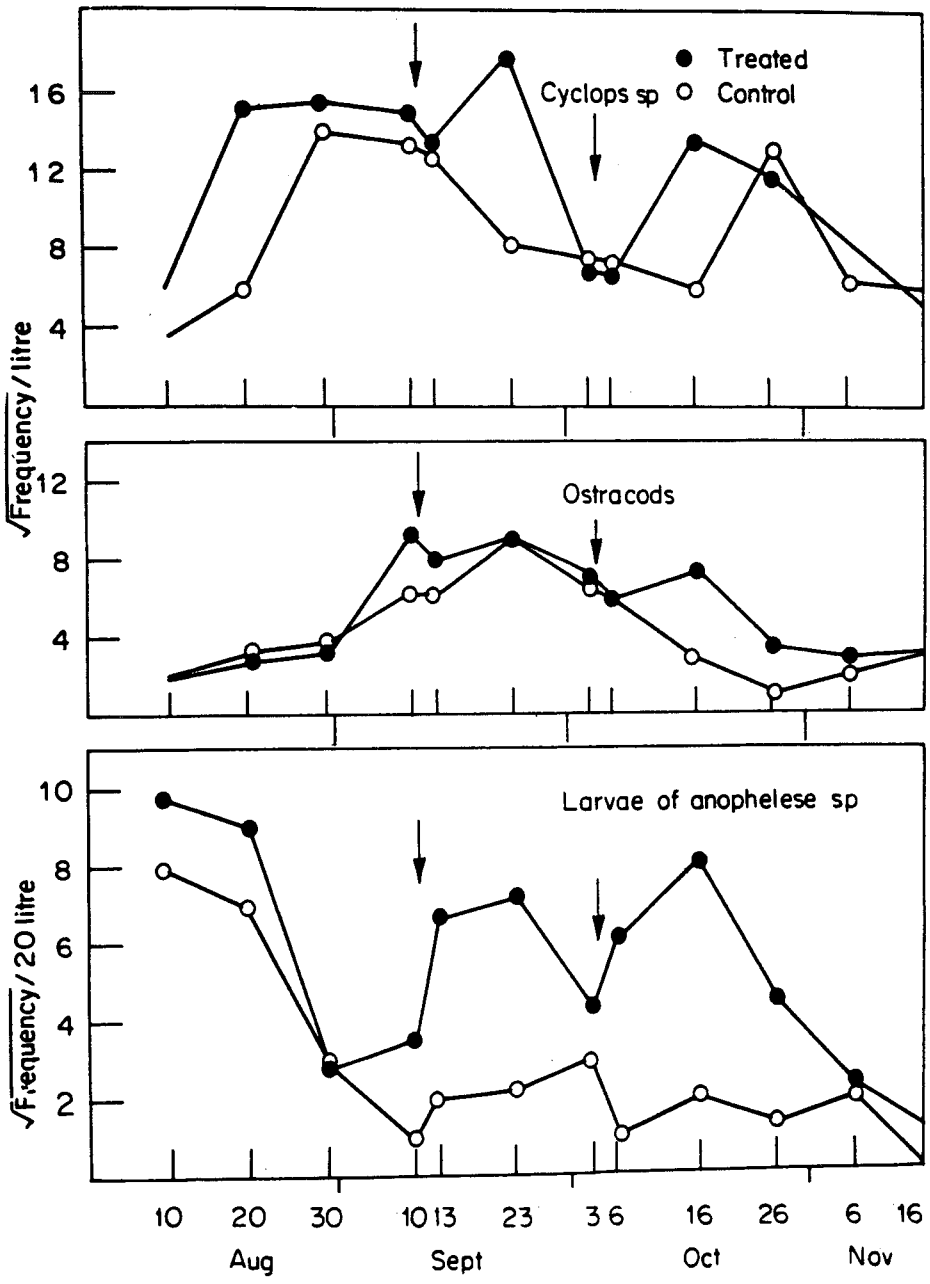

Figure 5. Effect of DDT treatment on the natural population of *Cyclops* sp., ostracods and larvae of *Anophelese* sp. The arrows indicate the treatment day.

the period of study. Similar blooms of aquatic macrophytes, *viz.*, *Chara* and *Nitella* have been reported by other workers in their water bodies following herbicide or insecticide treatments (Walsh *et al* 1971; Brook and Edwards 1973).

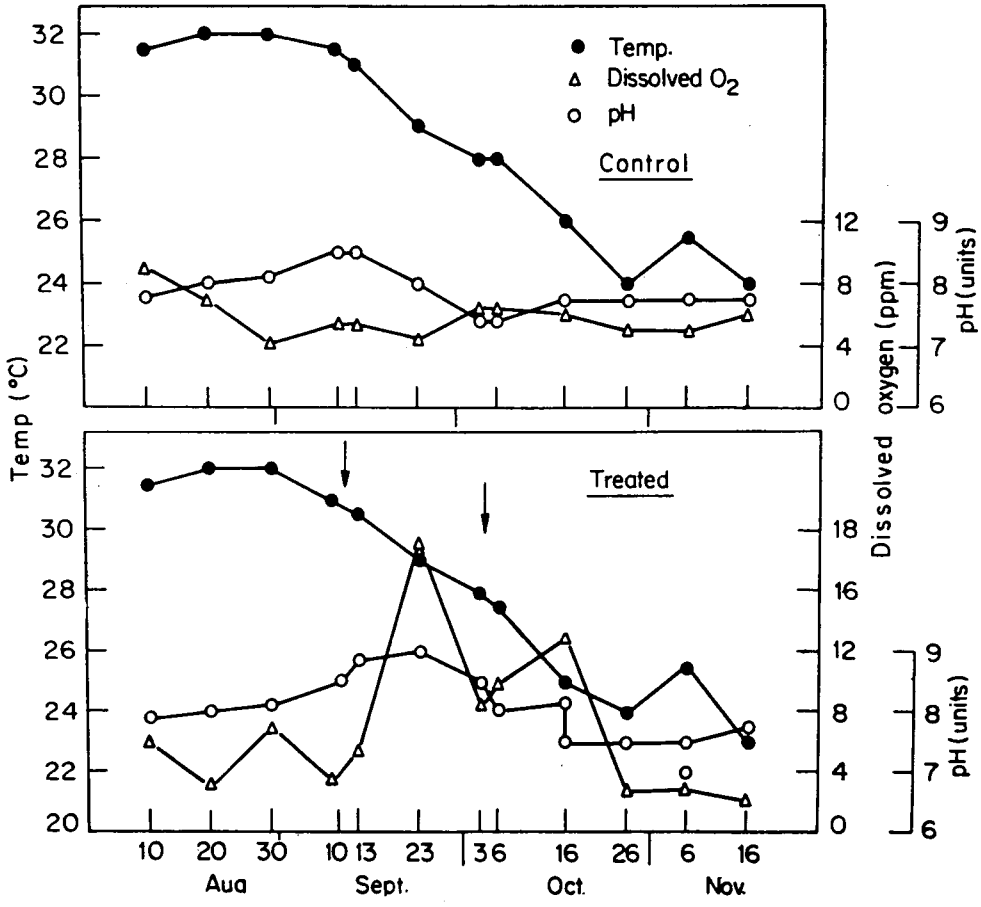

Figure 6. Physical and chemical characteristics of water in control and treated experimental tank.

The bottom soil of the treated tank revealed the presence of 36 ppb of DDD , 26 ppb of DDE and 3 ppb of DDT , whereas the soil of the untreated tank showed the presence of only traces of DDE, *i.e.*, 2 ppb (figure 7). Absorbed DDT in the soil from an aqueous dispersion has been reported as high as 80% within a period of 24 hr (Bowman and Schmidt 1961). It is interesting to note that although DDT was applied to the tank, it is the least abundant isomer found in the bottom mud. This indicates that DDT is probably degraded in water or in the bottom mud. The biodegradation of DDT to DDD and DDE by micro-organism present in water or soil has earlier been reported (Keil and Priester 1969; Johnson *et al* 1971).

4. Conclusion

The present paper provides basic information on DDT toxicity to some members of freshwater micrometazoans. *Neodiaptomus kamakhiae,* the most susceptible form

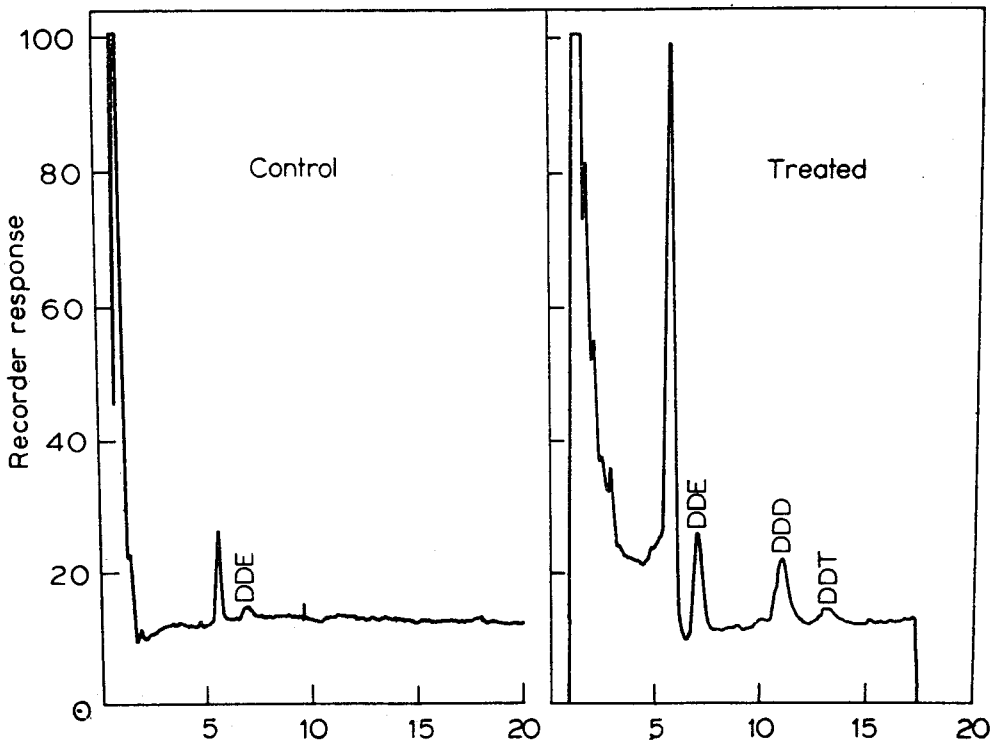

Figure 7. Gas chromatograms of the soil of the control and the treated experimental tank.

amongst the organisms tested during the present investigation could possibly be used to detect insecticide pollution in freshwater bodies. Apparently, the presence or absence of a particular type of zooplankton or the prolific growth of weeds now observed could indicate the nature and degree of pollution in the water.

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