

Full Length Research Paper

Occurrence of some pesticides in Bhoj wetland Bhopal and their effect on phytoplankton community: An ecological perspective

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Received 6 November 2014; Accepted 24 November, 2014

The present communication deal with the analysis of water samples from Bhoj wetland to detect three pesticides (chlorpyrifos, monocrotophos and endosulfan) and to assess their individual toxicity to the phytoplankton community. Higher concentration of most of the pesticides was found to be in the pre-monsoon (March to May) 0.9 µg/l (monocrotophos) and post-monsoon (September to December) 1 µg/l (monocrotophos and chlorpyrifos) period. Among the aquatic organisms, phytoplankton communities are the key targets for the pesticides because of their ecophysiological similarities with terrestrial plants. A standard 96 h static algal bioassay was followed to determine pesticide effects on the population growth rate of phytoplankton. At higher concentrations of all the pesticides elicited a significant effect on population growth rate by maximum inhibition of the cell division, but toxicity would not be expected at typical environmental concentrations. The population growth rate EC₅₀ average values determined for Chlorophyceae, Cyanophyceae and Bacillariophyceae varied in the range of 16.1 to 32.3 µg/l for chlorpyrifos, 8.6 to 14.3 µg/l for monocrotophos and 4.2 to 15 µg/l for endosulfan, respectively. Therefore, decrease in phytoplankton populations resulting from pesticide exposure could occur at higher concentrations in aquatic systems where pesticides are present in mixture. Detrimental effects on phytoplankton population growth rate could impact nutrient cycling rates and food availability to higher trophic levels. Characterizing the toxicity of chemical mixtures likely to be encountered in the environment may benefit the pesticide registration and regulation processes.

Key words: Pesticides, monsoon, phytoplankton, toxicity.

INTRODUCTION

Agricultural area has the potential to pollute the aquatic ecosystem via the popular use of pesticides. Several of

pesticides are being used in India both in agriculture and public health sectors. Although the uses of pesticides

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have resulted in increased food production and other benefits, it has raised concerns about potential adverse effects on environment and human health. Since the pesticides are lipid soluble in nature, cumulative accumulation of low concentrations of these in the body fat of mammals might pose potential hazards in the long run (Metcalf, 1997). Some of the pesticides like di-chloro di-phenyl tri-chloroethane (DDT) and di-chloro di-phenyl di-chloroethane (DDD) are also of significant concern because of their chiral structure (Ali et al., 2002). The greatest potential for unintended adverse effects of pesticides is through the contamination of the hydrologic systems, which supports aquatic life and related food chains and is used for drinking water, irrigation, recreation and many more purposes. The presence of pesticides in the environment even at nano/pico level can cause severe damage to human health (Ali et al., 2008). In aquatic bodies these pesticides affect many non target organisms like fish and birds due to biomagnifications through food chain (Kaushik et al., 2010). The persistence of these substances in aquatic ecosystem has special significance as they are picked up by aquatic organisms like plankton, and in the process pesticide residues enter in the food chain (Sarkar et al., 2003). These are bioaccumulative, relatively stable and carcinogenic (Jain and Imran, 1997), so needs close monitoring. Pesticides get introduced into natural aquatic system by various means incidentally during manufacturing and through surface water runoff from the agricultural land after their application. In addition, some pesticides are deliberately introduced into aquatic system to kill undesirable pests such as weeds, algae and vectors.

Plankton forms an important component of an aquatic ecosystem, reflects the condition in that environment and control the flow of energy besides production, as such their well being is of utmost importance viz-a-viz aquatic water body metabolism (Wetzel, 1975; Michael, 1985). When these chemical substances come in contact with the aquatic biota, the later changes their metabolic activity and thus affect the aquatic environment adversely (Nimmo and Mcwen, 1994). Algae are useful indicators of potential pollution as they respond by stimulation, inhibition or both to all toxicants affecting water quality (Rajendran and Venugopalan, 1983). The variability in their responses usually depends on the toxicant concentrations, duration of exposure and the test species subjected to study. Characterized by their differential sensitivity to chemical compounds, algae have been recommended as test species in detecting the occurrence of pesticides in aquatic environments (Hersh and Crumpton, 1987) and the current scientific understanding concerning the phytotoxic effects of several kinds of contaminants is based mostly on the studies of a few green algae. Algal assays are considered as a supplement to chemical analysis in the assessment of

pollution and have been proved to be more sensitive than *Daphnia* tests for monitoring toxicities in aquatic environments (Baun et al., 1998). Considering their importance in an aquatic ecosystem, some species of algae present in the water body were considered in the present investigation to assess the impact of pesticides viz., chlorpyrifos, monocrotophos and endosulfan commonly used in the agricultural catchment area. In spite of persistent nature and consequent interactions of pesticides with biosphere, relatively few studies have been conducted on the effect of pesticides on freshwaters.

The Bhoj Wetland situated in the Western side of the city has a water stretch from west to east and basically bounded between the latitude 23° 13' to 23° 16' and longitude 77° 18' to 77° 24' was selected for the present study (Figure 1). The water body is a wetland of National importance and is one of the sixteen wetlands in the country that have been so far identified for conservation and management. It is the main source of potable water, besides, being a habitat for various migratory birds and a source of revenue by way of tourism. A lot of deterioration has been observed in its water quality with the passage of time. This is mainly because of various anthropogenic activities taking place in its widely spread catchment area. Keeping this in view, the present study was undertaken to assess residual pesticide concentration in water and study their toxicity on the plankton population.

MATERIALS AND METHODS

Water samples were collected from two different sites (30 cm below the surface) during the Premonsoon (March to May), Monsoon (June to August) and the Post monsoon (September to December) period in brown glass bottles of 1 liter capacity and were transported to the laboratory. For analysis of pesticides, 1 liter of the unfiltered water sample from each location was extracted by liquid-liquid extraction in a separatory funnel using n-hexane. The combined solvent extracts were demoinsturised using anhydrous granular sodium sulphate and concentrated in a rotary evaporator to a final volume of 2 ml. All the samples were analyzed for different pesticides viz. endosulfan, monocrotophos and chlorpyrifos on Antek-2000 gas chromatograph equipped with Ni⁶³ ECD. For all extraction, GC grade n-hexane (Spectrochem, India; 99%) was used. The pesticides standards of 99.9% purity were procured from Sigma-Aldrich, USA. All the analysis was carried out in duplicate and the recoveries of the individual pesticides were determined through spiked sample method. Recovery correction factor were applied to the final results.

Sample collection and culture

Fresh water phytoplankton samples were directly collected into one liter plastic bottles from Bhoj Wetland Bhopal. Environmental factors such as water temperature and pH of the sampling station was measured at the spots (date will be published elsewhere). Thereafter recommended nutrient media (Aquacentre Ltd. USA) was added to the bottles and were immediately transferred to plant



Figure 1. Satellite map of Bhoj wetland showing sampling stations.

growth chamber (Vision, VS-3D, Korea) with the temperature range of 20 to 25°C and then it was kept for three days. From the incubated samples, 1 ml of the sample was transferred to the Sedgwick rafter cell for the observation of the desirable abundance and community changes of phytoplankton under and inverted microscope.

Acute toxicity test

A standard 96 h static algal bioassay (ASTM, 1996) was followed to determine the potential toxicity of three pesticides (endosulfan, monocrotophos and chlorpyrifos) on phytoplankton. Known volume of multispecies algal cultures were exposed to a series of concentrations of test material and an untreated control was maintained to measure the normal growth of the algae incubated in plant growth chamber (Vision, VS-3D, Korea) with the temperature of 25°C, light intensity $180 \mu\text{E m}^{-2} \text{s}^{-1}$ and light: dark cycle of 12:12, respectively. Algal toxicity may be expressed in terms of a broad range of responses, encompassing those that are inhibitory (50% reduction in cell number at specified time interval) and cell death observed in Sedgwick rafter cell. The effects of test materials were evaluated by measuring the growth of phytoplankton in treated and untreated cultures. Cell density was assessed in control as well as treatment containers at an interval of 24 h up to 96 h using Sedgwick-rafter cell following the method given in American Public Health Association (APHA) (1995). Based on the cell count, data was analyzed for the determination of EC_{50} , 96 h and limiting percentage/percentage inhibition in the growth relative to control systems (Mary and Rubber, 1983).

Limiting percentage/percentage inhibition:

$$I = \mu_c - \mu_{tox} / \mu_c \times 100$$

Where I is the inhibition, μ_c is the average specific growth rate of control and μ_{tox} is the average specific growth rate of phytoplankton in the test series.

RESULTS AND DISCUSSION

The present study revealed the occurrence of different pesticides viz. chlorpyrifos, monocrotophos and endosulfan in the surface water of the Bhoj Wetland, Bhopal in different seasons, summarized in the Table 1. The average water temperature and pH in the system varied in the range between 20 to 25°C and 7 to 8, respectively (Data communicated elsewhere). Higher concentration of most of the pesticides was found during the pre-monsoon and post-monsoon period. In the present study, the concentration of endosulfan in the surface water collected from Site-A (under the influence of agriculture) detected the lowest concentration (0.01 $\mu\text{g/l}$) during post-monsoon whereas; highest concentration (0.12 $\mu\text{g/l}$) was detected during monsoon. Minimum concentration (0.7 $\mu\text{g/l}$) of monocrotophos was detected during monsoon and the maximum concentration (1 $\mu\text{g/l}$) was detected during post-monsoon at Site-A. Concentration of chlorpyrifos detected during the present investigation at the same site ranged between 0.5 $\mu\text{g/l}$ (monsoon) to 1 $\mu\text{g/l}$ (post-monsoon), whereas, the concentration of these pesticides were found to be below detection limit at Site-B during the study period. The contamination of water by pesticides at Site-A may be due to the magnitude of the

Table 1. Residual pesticide concentration in surface water of Bhoj Wetland, Bhopal.

<i>Period of sampling</i>	<i>Site A</i>			<i>Site B</i>
	<i>Endosulfan</i> ($\mu\text{g/l}$)	<i>Monocrotophos</i> ($\mu\text{g/l}$)	<i>Chlorpyrifos</i> ($\mu\text{g/l}$)	
<i>Pre monsoon</i>	0.073	0.9	0.79	*Nil
<i>Monsoon</i>	0.12	0.7	0.5	*Nil
<i>Post monsoon</i>	0.01	1	1	*Nil
<i>Average</i>	0.07	0.87	0.76	*Nil
<i>EPA (1994) criteria for aquatic organisms</i>	0.22	1	20	-

*Pesticide residual concentration was below detection limit

agriculture fields associated with the pesticide use whereas, absence of pesticides or the below detection limit of pesticides at Site-B may be due to the dilution factor and the non agricultural catchment. The presence of pesticides in the water body can be attributed to the maximum usage of these chemical substances in the agricultural catchment which finds their way to the waterbody during rainy period and during the period of their applications also.

While comparing the concentrations of endosulfan, monocrotophos and chlorpyrifos with the Environmental Protection Agency (EPA) (1994) water quality criteria for fresh water aquatic organisms, it appears that all the values were found to be within the prescribed limit. Among other investigators who contributed in the same field are Lalah et al. (2003) who reported low concentration of endosulfan and other pesticides in Tana and Sabaki Rivers in Kenya. Bakre et al. (1990) found maximum pesticides in water from Mahala reservoir with the onset of rains which was attributed to the sub soil movement of water. The levels of pesticides in the surface water systems in India as well as outside India depends on many factors like drainage basin, flow rate, particulate matter content, depth of water body etc. Several studies were conducted on pesticide contamination in lakes and reservoirs from other parts of the world (Kucklick et al., 1994; Tanabe et al., 1983). However, some investigators have assessed organochlorine residues in water reservoirs in India, that is, Mahala water reservoir, Jamuna water (Agarwal et al., 1956), Ganga water (Nayak et al., 1995; Sinha, 1991) and rural pond water (Dua et al., 1996).

Hosokawa et al. (1995) reported the presence of pesticide residues in rivers receiving discharge from agricultural activities. Jensen et al. (1969) reported ultra trace levels of pesticides at places like North Pole snow which was far from the site of their application. Nayak et al. (1995) reported many water samples from Ganga river contaminated with pesticides which exceeded the safe limit. Dua et al. (1996) assessed the organochlorine

insecticide residue in water from five lakes of Nanital (UP). Various other workers who also contributed in the field of environmental research include the works of Ahad et al. (2006), Singh et al. (2006), Moore et al. (2007), Singh et al. (2005), Sarkar et al. (2003) and Abbassy et al. (2003). Pesticides not only do their intended job, but may also adversely affect non targeted beneficial species. Contamination of surface waters by pesticides has been reported to have direct toxic effects on populations of phytoplankton. Pesticides can affect the structure and function of aquatic communities by changing the species composition of an algal community. The toxicity of some pesticides on algae is higher than toxicity reported by several authors on organisms like zooplankton, filter-feeding invertebrates and fishes (Kreutzweiser et al., 1998; Kreutzweiser and Faber, 1999). This is an important fact because in the freshwater ecosystem, algae are important primary producers in the food chain, with phytoplankton providing food for a diverse community of invertebrates and fishes. Depending on pesticide toxicity, aquatic contamination could result in a die-off of many algal species, thus decreasing the food source (Gomez et al., 2004). During the present 96 h acute toxicity tests of different pesticide chemicals to phytoplankton population, the later were affected in a different manner, indicating significant decline in population growth at higher concentrations. Concentration of chlorpyrifos tested in the range of 4.3 to 129 $\mu\text{g/l}$ recorded a considerable inhibitory effect in growth of Chlorophyceae, Cyanophyceae and Bacillariophyceae groups by 87.1, 85.6 and 91.3%, respectively at concentration of 129 $\mu\text{g/l}$ as compared to the control sample (Figure 2). Monocrotophos at the concentrations in the range of 0.19 to 28.5 $\mu\text{g/l}$ recorded inhibition in growth by 79.8, 80.2 and 69.3% at concentration of 28.5 $\mu\text{g/l}$ in the Chlorophyceae, Cyanophyceae and Bacillariophyceae. groups, respectively in comparison to the untreated control sample (Figure 3).

The present investigation report is in complete agreement with the findings of Megharaj et al. (1986) who also

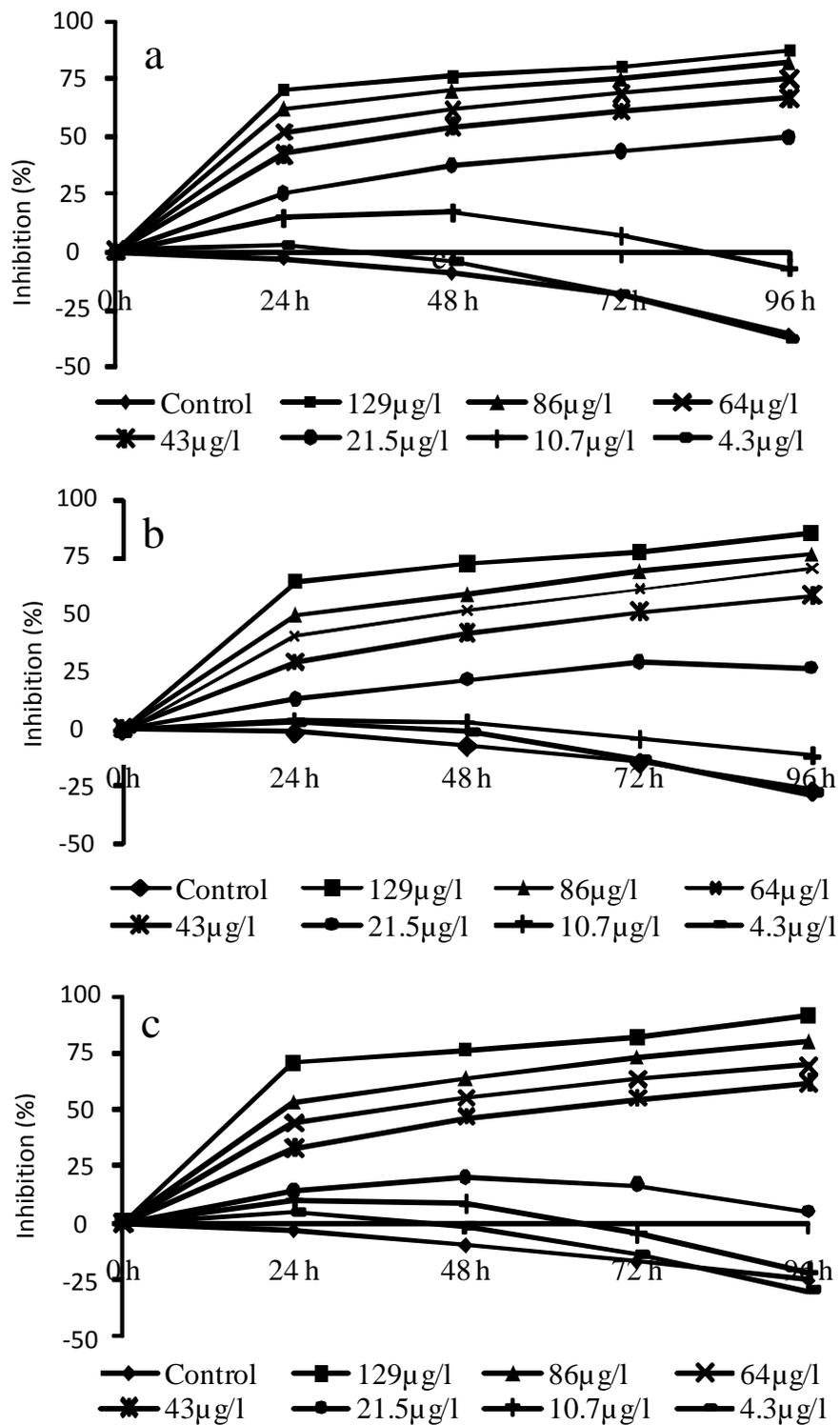


Figure 2. Growth inhibitory effect of chlorpyrifos on (a) Chlorophyceae, (b) Cyanophyceae and (c) Bacillariophyceae.

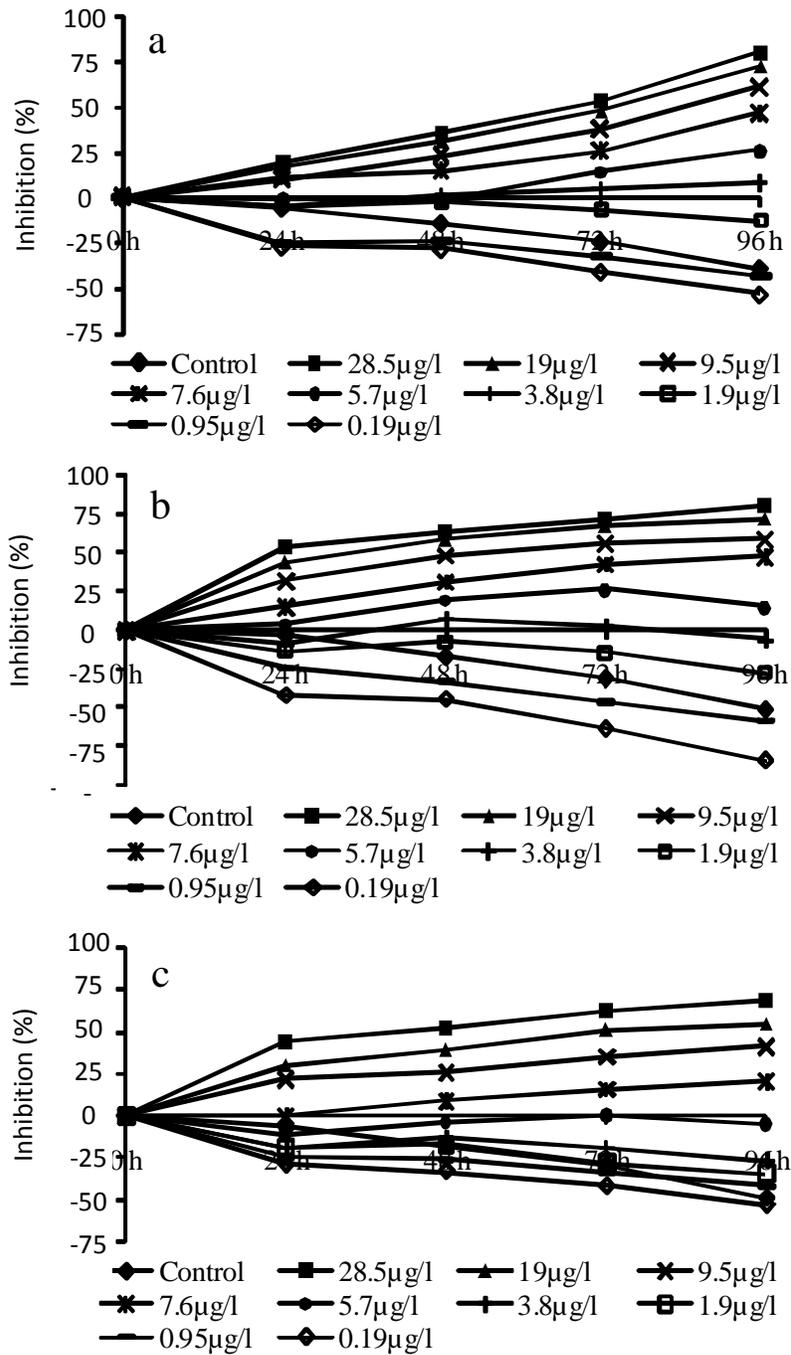


Figure 3. Growth inhibitory effect of monocrotophos on (a) Chlorophyceae, (b) Cyanophyceae and (c) Bacillariophyceae.

recorded higher concentration of monocrotophos inhibiting the growth of green algae and at lower concentration recorded an increase in growth rate of

algae. Similarly, concentrations of endosulfan in the range of 0.06 to 18 µg/l recorded growth inhibition by 70.7, 72.7 and 56.8% at higher concentration (18 µg/l) in

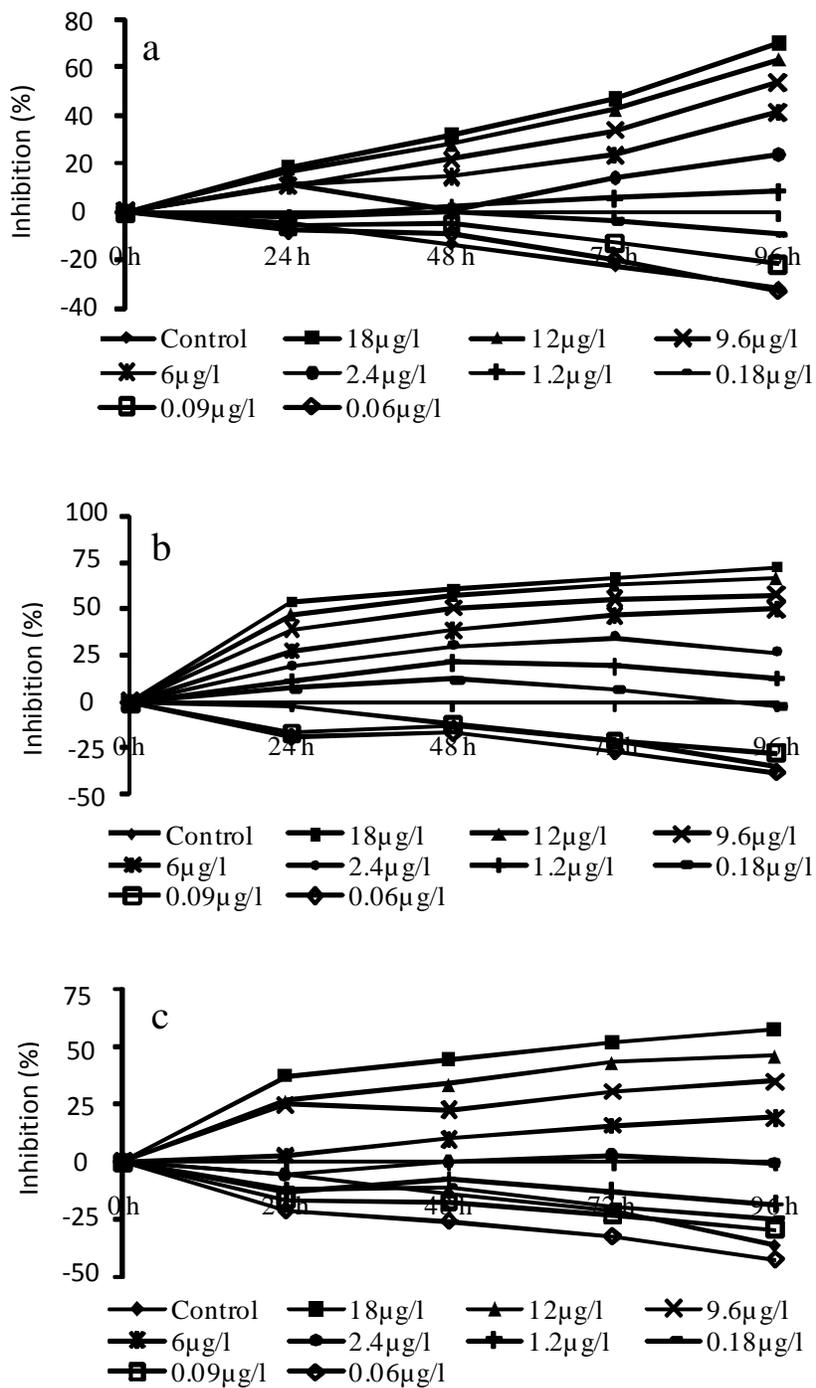


Figure 4. Growth inhibitory effect of Endosulfan on (a) Chlorophyceae, (b) Cyanophyceae and (c) Bacillariophyceae.

the group, Chlorophyceae, Cyanophyceae and Bacillariophyceae, respectively (Figure 4). The tested pesticides recorded stimulatory growth effect at lower concentrations while increasing the concentration and

time of exposure maximum inhibitory effect in the growth of algal groups was observed at higher concentrations during the 96 h acute toxicity testing procedure.

Rajendran and Venugopalan (1983) are of the opinion

that the highly toxic nature of organochlorine insecticides on algae might be due to their uptake metabolism involving surface adsorption followed by very rapid absorption across the cell wall. Higher concentration of endosulfan also suppressed growth in *Chlorella ellipsoidea* (Asma and Mathew, 1995). Goebel et al. (1982) noticed the impact of endosulfan in higher concentrations on algae. Kasai and Hanazato (1995) investigated that Volvocales and Cryptophyceae were clearly reduced by herbicides application. Khalil and Mostafa (1986) recorded inhibition in the growth of green alga *Phormidium* sp. at higher concentration of insecticides. Maule and Wright (1984) also reported the inhibitory effect of some pesticides to green algae. A similar growth pattern had been reported in *Chlorella protothecoides* by Saroja and Bose (1982). Tandon et al. (1988) also observed the inhibitory effect of endosulfan on *Aulosira* sp. Further, Kumar et al. (2008) observed reduction in growth of *Aulosira fertilissima* and *Nostoc muscorum* at higher concentration of endosulfan. Kapoor and Arora (2000) while working on blue green algae reported the level of tolerance of BHC (an insecticide) found to be very low. Tuba et al. (1981) however, reported increase in growth inhibition in *Lyngbya* sp. as herbicides concentration increased. A good number of investigations point out significant growth reduction of unicellular algae when treated at different concentrations of organochlorine insecticides like DDT, heptachlor and lindane (Menzel et al., 1970; Powers et al., 1975; Subramaniam et al., 1979; Ramachandran et al., 1980; Sahu et al., 1992; Penalva and Fernandez, 1992; Fliedner and Klein, 1996; Krishnaswamy, 1997).

As discussed earlier, the present study indicates that lower concentrations of pesticides have a stimulatory effect on algal growth. This may probably be attributed to the fact that the pesticides selected in the present investigation are mostly phosphate based and may have acted as growth enhancers at lower concentrations. Menzel et al. (1970) also reported the enhanced growth and cell division at lower concentrations of DDT and heptachlor. Peterson and Batley (1993) have also emphasized the possibility of a nutrient effect at lower concentrations of endosulfan where it may act as a carbon source for plankton growth. Further, lower concentrations of endosulfan recorded in an increase in the cell density of Bacillariophyceae when exposed to furadan (Kar and Singh, 1978).

EC₅₀ values determined by certain investigators with respect to different algae prove to be advantageous for evaluating the toxicity of various organophosphates (Walsh and Alexander, 1980; Ibrahim, 1983; Kent and Currie, 1995; Chen et al., 1997; Zou et al., 1998). In the present investigation, effective concentrations (concentration responsible for 50% inhibition in the cell density of the phytoplankton) were recorded based on the

sensitivity of different classes to different pesticides. Sensitivity not only varies among toxicants but also among taxonomic groups and species within taxa. No species is always most sensitive or always the least sensitive (Wang and Freemark, 1995).

Chlorophyceae, Cyanophyceae and Bacillariophyceae in the present study recorded average EC₅₀ value for chlorpyrifos in the range of 16.1 to 32.3 µg/l, monocrotophos in the range of 8.6 to 14.3 µg/l and endosulfan in the range of 4.2 to 15 µg/l, respectively (Figure 5). Chlorophyceae was found to be more sensitive to chlorpyrifos than Cyanophyceae. Tang et al. (1997) also recorded some Chlorophycean species to be more susceptible to atrazine than diatoms. Based on the toxicity of these pesticides to different phytoplankton communities, endosulfan was found to be comparatively highly toxic whereas, chlorpyrifos was found to be least toxic amongst the tested pesticides.

Conclusion

It has been observed that higher concentration of various pesticides used in the experimental work affected phytoplankton community as a whole. The tests of acute toxicity with pesticides confirmed the high toxicity of the chemical to selected species of aquatic community. The results proved Chlorophyceae to be the most sensitive test group among the phytoplankton. It is necessary to protect natural surface waters from accidental escape of pesticides from treated areas. Assuming that the application of pesticides is done in an appropriate way, the pesticide should degrade quickly and never be present in surface waters at lethal concentrations. Toxicity is a biological response and this needs to be taken into account in formulating realistic guidelines on the acceptable upper limits on pesticide contamination of the environment.

Toxicity testing has a clear role in safeguarding environmental quality but a considered selection of the testing methods is essential for obtaining relevant results. Toxicity of pesticides is highly dependent on the duration, frequency, intensity of exposure, and the susceptibility of the target organism which is influenced by age, sex, fitness and genetic variation. Though a new generation of bioassays has been developed and is used in ecotoxicological practice, "standard" toxicity tests on living organisms are still essential and more relevant in the evaluation of the toxicity of compounds for higher animals, including humans.

ACKNOWLEDGEMENTS

The authors are thankful to the Director ITRC Lucknow

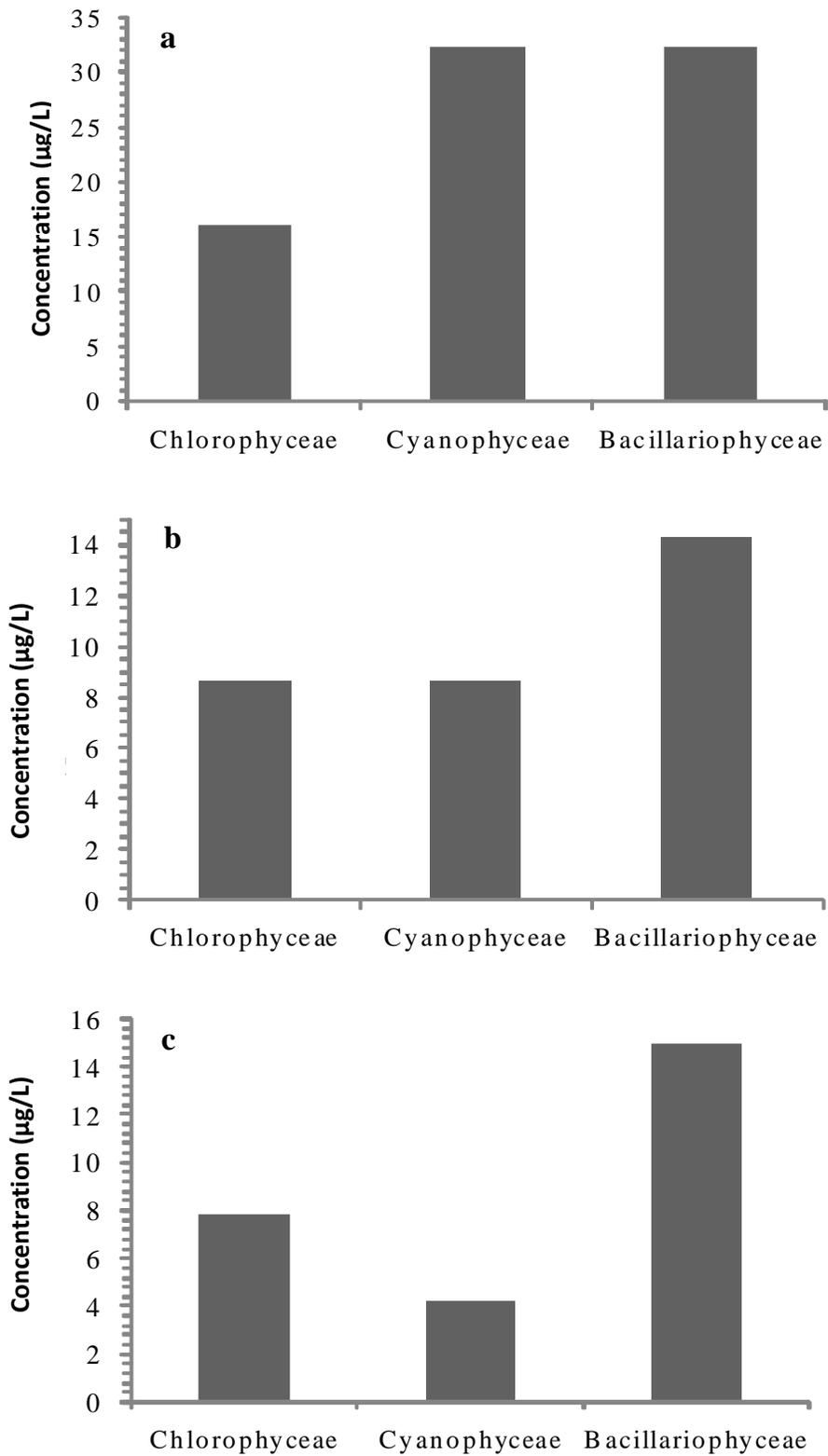


Figure 5. Population growth rate effective concentration (EC-50) of (a) Chlorpyrifos, (b) Monocrotophos and (c) Endosulfan for phytoplankton

for providing support for the analysis of the samples. The Vice Chancellor of Barkatullah University Bhopal is greatly acknowledged for providing the Laboratory facilities and authors are also grateful to the reviewers and editor for structuring the manuscript.

Conflicts of interest

No competing interests exist.

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