

## TOXICITY OF PESTICIDES ON PHOTOSYNTHESIS OF DIATOMS

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### Abstract

Pakistan being an agricultural country, a large amount of pesticides are used, including organophosphates and synthetic pyrethroids. These pesticides are released through rivers and other tributaries into the coastal environment, thus posing a continuous threat to marine organisms. In the present study two species of diatoms *Amphora* and *Navicula* were selected for the assessment of impact of organophosphate and pyrethroid toxicity on these primary producers. The study shows that rate of photosynthesis was inhibited in both *Amphora* and *Navicula* species exposed to pesticide. The acute toxicity of pesticide was determined by measuring IC<sub>50</sub> of the test organisms. IC<sub>50</sub> calculated for diatom species depicts that different pesticides had variable effects on the photosynthesis of microalgae. High sensitivity of marine organisms is alarming as it may have implications on the marine ecosystem and fisheries. The results are also useful in setting control limits for the release of these chemicals in nature.

### Introduction

Pollution in aquatic environment has been a major concern worldwide. Agrochemicals, such as pesticides pose a continuous threat to the marine ecosystem. Pakistan is an agricultural country and one of the major cotton producers in the world. Therefore, organophosphates and synthetic pyrethroids are largely used against cotton pests in Pakistan. Marine and freshwater microplankton species show a variable sensitivity to pesticides. Generally photosynthesis and growth of microplankton is negatively affected on exposure to pesticides. It is estimated that these microalgae may account for 40 to 45% of oceanic production and are considered as more productive than all the worlds' rainforests (Mann, 1999). Although diatoms are generally not employed in ecotoxicological studies due to their slow growth and difficulty in culture, but a number of reports show effects of pesticides on these organisms. Some previous studies showed reduction in photosynthesis and growth of diatoms exposed to 2, 4-D, atrazine, metobromuron, triazines (Andus 1970; Chai & Chung 1975; Brown & Lean 1995) and some organophosphates (Mohapatra & Schiewer, 1996; Mohapatra *et al.*, 1997). Considering ecological importance of diatoms and that these organisms may serve as affective indicators of pollution. They are sensitive to change in salinity, temperature, pH and pollution (John, 1983). Changes in diatom population structure would indicate changes in marine environment (John, 1983). Ecotoxicological effects of pesticides have not been observed on microplankton species inhabiting Pakistani waters, therefore, the present study was undertaken to assess the ecotoxicological effect of organophosphate and pyrethroid pesticides on photosynthesis of two diatom species.

### Materials and Methods

**Collection of samples, isolation & identification:** Diatoms were isolated and purified from water samples collected from Manora Channel using a phytoplankton net (55 µm mesh) towed for ten minutes in the surface water. Water samples were brought to the laboratory in a cool box for isolation of diatoms using spread plate and serial

dilution techniques (Rippka, 1988) employing f/2 medium (Guillard & Ryther 1962, Guillard 1975). The species of diatoms were identified on the basis of their morphological characters described previously (Kutzing, 1844; Heimdal, 1970; Tomas, 1997). Two organophosphates (methyl parathion and chlorpyrifos) and two synthetic pyrethroids (fenvalerate and fenprothrin) were used in this study.

**Experimental design:** Effect of pesticides on photosynthesis of two laboratory grown cultures of diatoms was assessed using 'Light and Dark bottle method' (Strickland & Parsons 1968). A set of triplicate Light and Dark bottles were used for control and for each pesticide concentration (0.02 ppm, 0.06 ppm and 1 ppm). A known volume (5 ml) of well homogenized culture was inoculated in all bottles. One set was incubated in light and the other set was secured in the dark at constant temperature (36±1°C) conditions for three hours. At the end of experiment, content of each of the light and dark bottles were fixed for the analysis of dissolved oxygen using Multimeter ion specific meter (Hanna C100). Gross photosynthesis was calculated as described by Strickland & Parsons (1968). The IC<sub>50</sub> values were determined by using Log log graph.

### Result

Effect of organophosphates and pyrethroids was observed on the photosynthesis of two species of diatoms, viz., *Amphora* sp. and *Navicula* sp. Results showed that the toxic effect of pesticides on both diatom species increases with increase in concentration of all pesticides tested (Tables 3 and 4). Both diatom species appeared to have variable sensitivity against different pesticides (Tables 1 & 2). For example, *Amphora* sp. is more resistant to fenprothrin (IC<sub>50</sub> = 52 ppm), whereas *Navicula* sp., showed high sensitivity to this pesticide (IC<sub>50</sub> = 0.03 ppm; Table 1). Table 2 depicts rank of each pesticide pertaining to the degree of its toxicity (IC<sub>50</sub> value) to each diatom species. The most toxic pesticide for *Amphora* sp., was fenvalerate, followed in decreasing order of toxicity by chlorpyrifos, methyl parathion and fenprothrin. On the other hand, fenprothrin had highest toxic effect on photosynthesis in *Navicula* sp.,

which was followed by chlorpyrifos, fenvalerate and methyl parathion in the decreasing order of toxicity. The minimum range of photosynthesis at 95% confidence limit was found to be 19.86-22.13% of fenprothrin for

*Navicula* sp., (Table 4) whereas the minimum range of photosynthesis at 95% confidence limit was found to be 26.00-28.53% of chlorpyrifos for *Amphora* sp., (Table 3).

**Table 1. Effect of organophosphate and pyrethroid pesticides on photosynthesis of diatoms. Values are concentration of pesticides at which photosynthesis is reduced by 50% of control values.**

Diatoms	IC <sub>50</sub> ppm			
	Chlorpyrifos	Methyl Parathion	Fenvalerate	Fenprothrin
<i>Amphora</i>	0.18	0.34	0.15	52
<i>Navicula</i>	0.06	0.4	0.1	0.03

**Table 2. Ranking of toxicity of organophosphate and synthetic pyrethroid pesticides on some diatoms. Numbers 1-4 are assigned in decreasing order of toxicity.**

Diatoms	Chlorpyrifos	Methyl Parathion	Fenvalerate	Fenprothrin
<i>Amphora</i>	2	3	1	4
<i>Navicula</i>	2	4	3	1

**Table 3. Reduction in photosynthesis of *Amphora* sp. exposed to pesticides for three hours.**

Compound	ppm	Mean	S.D.	Range at 95% confidence limit
Chlorpyrifos	0.02	99.24 ±	0.295	98.90-99.57
	0.06	66.66 ±	0.33	66.29-67.04
	1	27.27 ±	1.11	26.00-28.53
Methyl parathion	0.02	89.77 ±	0.22	89.52-90.03
	0.06	74.99 ±	1	73.85-76.13
	1	34.09 ±	1.04	32.90-35.27
Fenvalerate	0.02	87.03 ±	1	85.89-88.16
	0.06	59.25 ±	1.25	57.83-60.66
	1	31.48 ±	2.5	28.65-34.30
Fenprothrin	0.02	97.07 ±	1.02	95.91-98.23
	0.06	73.03 ±	1	71.89-74.16
	1	71.91 ±	0.08	71.82-72.00

Values are percent as mean photosynthesis of control value

**Table 4. Reduction in photosynthesis of *Navicula* sp. exposed to pesticides for three hours.**

Compound	ppm	Mean	S.D.	Range at 95% confidence limit
Chlorpyrifos	0.02	75.62 ±	2	73.35-77.88
	0.06	50.41 ±	2.5	47.57-53.24
	1	45.37 ±	1.59	43.56-47.71
Methyl parathion	0.02	62.18 ±	1.46	45.39-48.70
	0.06	57.14 ±	1.05	55.95-58.32
	1	47.05 ±	1	61.04-63.31
Fenvalerate	0.02	62.74 ±	1.25	61.32-64.15
	0.06	53.92 ±	1.02	52.76-55.07
	1	38.23 ±	1.23	36.83-39.62
Fenprothrin	0.02	62.18 ±	0.84	61.22-63.13
	0.06	33.61 ±	0.38	33.18-34.04
	1	21 ±	1	19.86-22.13

Values are percent as mean photosynthesis of control value.

## Discussion

The general inhibition of photosynthesis in both *Amphora* and *Navicula* species, tested in the present study, is in good agreement with some previous studies showing inhibitory effect of pesticides on algal species (Andus, 1970; Chai & Chung, 1975; Subramanian, Lingaraja & Venugopalan, 1979; Rajendran *et al.*, 1983; Lorenzo *et al.*, 2000; Mohapatra *et al.*, 2003).

The data on the effect of different pesticides on two indigenous diatom species inhabiting coastal waters of

Pakistan is being reported for the first time and these two species have not been examined before elsewhere. Our results showed that a diatom species has variable sensitivity against a given pesticides and that different pesticides have variation in the toxicity to particular species. This inter-species variation has been shown earlier (Kallqvist & Romstad 1994) which would lead to selective success of more resistant species to grow in natural waters. The consequential ecological imbalance may cause population explosions of one or more resistant species (Korringa, 1968) thereby altering the species

composition of a natural phytoplankton community (Wurster, 1968a, b). Implication of absorption and uptake of pesticide by diatoms and other primary producers in fishery industry may be evident from the fact that the absorbed pesticides are efficiently transferred to organisms of higher trophic levels (Barron 1990) including fish and shrimp on which the fishery industry thrives in Pakistan. Our results (IC50) demonstrate the effective concentration of pesticides and are useful to set control limit for the release of such chemicals in the natural waters.

#### References

- Andus, L.J. 1970. The action of herbicides on the microflora of the soil. *Proc. 10<sup>th</sup> Brit. Weed Control Conf.*, 3: 1036-1051.
- Barron, M.G. 1990. Bioconcentration: will water-borne organic chemicals accumulate in aquatic animals. *Environ. Sci. Technol.*, 24: 1612-1618.
- Berard, A., C. Leboulanger and T. Pelte. 1999. Tolerance of *Oscillatoria limnetica* Lemmermann to atrazine in natural phytoplankton populations and in pure culture: influence of season and temperature. *Archives Environ. Contam. Toxicol.*, 37(4): 472-479.
- Brown, L.S. and D.R.S. Lean. 1995. Toxicity of selected pesticides to lake phytoplankton measured using photosynthetic inhibition compared to maximal uptake rates of phosphate and ammonium: *Environ. Toxicol. Chem.*, 14(1): 93-98.
- Chai, I.K. and Y.S. Chung. 1975. Physiological effects of 2,4-dichlorophenoxyacetic acid (2,4-D) on *Chlorella ellipsoidea*. *Misaengmul Hakhoe Chi.*, 13: 101-108.
- DeLorenzo, M.E., G. I. Scott and P.E. Ross. 2000. Effects of the agricultural pesticides atrazine, deethylatrazine, endosulfan, and chlorpyrifos on an estuarine microbial food web. *J. Appl. Phycol.*, 12: 461-467.
- El-Bestawy, E.A., A. Z.A. El-Salam and A.H. Mansy. 2007. Potential use of environmental cyanobacterial species in bioremediation of lindane-contaminated effluents. *Science Direct. International Biodeterioration and Biodegradation*, 59(3): 180-192.
- Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26-60. In: *Culture of Marine Invertebrate Animals*, (Eds.): W.L. Smith and M.H. Chanley. Plenum Press, New York, USA.
- Guillard, R.R.L. and J.H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. *Can. J. Microbiol.*, 8: 229-239.
- Heimdal, B.R. 1970. Morphology and distribution of two *Navicula* species in Norwegian coastal waters. *Nytt Magasin for Botanikk*, 17: 65-75.
- John, J. 1983. The diatom flora of the Swan River Estuary, Western, Australia, *Bibliotheca Phycologia.*, 64: 1-359, J. Cramer, Veduz: 360 pp.
- Kallqvist, T. and R. Romstad. 1994. Effects of agricultural pesticides on planktonic algae and cyanobacteria-examples of interspecies sensitivity variations. *Norweg. J. Agricult. Sci. Suppl.*, 13: 117-131.
- Korringa, P. 1968. Biological consequences of marine pollution with special reference to the North Sea fisheries. *Helgolander wiss. Meeresunters.*, 17(1-4): 126-40.
- Kutzing, F.T. 1844. Die kieselschaligen Bacillarien oder Diatomeen, pp. 152. *Ferd. Forstmann, Nordhausen*, 1865, Zweiter Abdruck.
- Mann, D.G. 1999. The species concept in diatoms. *Phycologia*, 38(6): 437-495.
- Mohapatra, P.K. and U. Schiewer. 1996. Influence of dimethoate on structure and function of the natural phytoplankton assemblage of the Darss-Zingst bodden chain reared in a laboratory. *Pol. J. Environ. Stud.*, 5: 31-36.
- Mohapatra, P.K., H. Schubert and U. Schiewer. 1997. Effect of dimethoate on photosynthesis and pigment fluorescence of *Synechocystis* sp. PCC 6803. *Ecotoxicol. Environ. Saf.*, 36: 231-237.
- Mohapatra, P.K., S. Patra, P.K. Samantaray and R.C. Mohanty. 2003. Effect of the pyrethroid insecticide cypermethrin on photosynthetic pigments of the cyanobacterium *Anabaena doliolum* Bhar. *Pol. J. Environ. Stud.*, 12(2): 207-212.
- Rajendran, N. and V.K. Venugopalan. 1983. Effect of pesticides on phytoplankton production. *Mahasagar Bulletin of the National Institute of Oceanography*, 16(2): 193-197.
- Rippka, R. 1988. Isolation and purification of cyanobacteria. In: *Methods in Enzymology*, (Eds): A.N. Glazer and L.Packer L. Academic press, San Diego, California, 167: 1-7.
- Strickland, J.D.H. and T.R. Parsons. 1968. A manual of seawater analysis. *Bulletin of Fisheries Research Board.*, Ottawa, Canada.
- Subramanian, B.R., T. Lingaraja and V.K. Venugopalan. 1979. Effects of low concentrations of DDT on the growth and production of marine diatom *Skeletonema costatum* (Grev), *Current Science.*, 48(5): 226-228.
- Tomas, C.R. 1997. *Identifying marine phytoplankton*. Florida Department of Environmental Protection. Florida Marine research Institute. Florida. pp. 240-281.
- Wurster, C.F. Jr. 1968a. DDT threatens ocean life, chemical balance of atmosphere. UST. pp.2.
- Wurster, C.F. Jr. 1968b. DDT reduces photosynthesis by marine phytoplankton. *Science.*, N.Y., 159 (3822), 1474-1475.

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