Iranian Journal of Fisheries Science 1(1), 47-58 (1999)

Herbicides and Insecticides Effects on Green Algae and Cyanobacteria Strain

M. Piri[1] and V. Ördog[2]

1) Gilan Fisheries Research Institue, P.O. Box 66, Bandar-e Anzali, Iran
2) Pannon Agric University, H-9200 - Mosonmagyaróvar Hungary - Kolbai - Karoly U.8

Abstract: The toxic effects of herbicides (Machete, Saturn) and pesticides (Diazinon and Malathion) on green algae, *Scenedesmus obtusiusculus*, and cyanobacteria, *Anabaena flos aquae*, were studied.
The results indicated that Machete and Saturn, in comparison to Diazinon and Malathion, were more toxic. On the other hand it was revealed that green algae was more sensitive to the pesticides than the cyanobacteria. When we added 3.199 mg/l Machete (EC_{50} of this toxicant for *Anabaena flos aquae*) to a mix algal culture, the growth rate of green algae after 6 days of treatment was reduced 0.95 times, while it was 4.5 for the cyanobacteria. Treating the culture with 10.53 mg/l of Saturn for the same period of time, revealed that the growth rate for green algae and the cyanobacteria increased 1.19 and 7 times, respectively. Hence, we can conclude that pesticides are capable of decreasing the diversity of the phytoplankton species in an aquatic ecosystem, that from the fisheries point of view could cause serious problems.
KEY WORDS: Herbicide, pesticide, Algae, toxicant, pollution

Introduction

Every year we use chemical substances extensively to protect the agricultural products. In order to reduce the hazard of pollution in the aquatic ecosystems, the correct application of these chemicals must be taken into consideration, because it has been documented that chemicals are accumulated in the water bodies, into which they are drained (Torstensson , 1989 ; Nilsen , 1989).

The declared concentration of such substances in the reports and studies are normally not high enough to cause acute environmental perturbation. The deleterious effects of many chemicals have been studied only on a limited number of species such as fish and invertebrates. However, the evidence of green algae and cyanobacteria sensitivity to chemicals are very clear. Studying the harmful effects of the chemicals on the aquatic organisms provide us with relevant information about the sensitivity of the aquatic ecosystems to a given chemical

(Sloff *et al.* , 1983). Although algae play a dominant role in the primary production of the aquatic ecosystems, their importance in detecting environmental hazards, due to chemical pollution, must be considered twice as important. Phytoplanktons species differ physiologically and genetically from each other, and therefore thier sensitivity to the exposed chemicals could vary in different species (Torsten and Romstad , 1994).

Uncontrolled disposal of agricultural wastewater into the rivers and irrigation canals, fish ponds and lagoons in the northern part of Iran, enter ultimately the Caspian sea and causing uncompensated damages to its ecosystem and adjacent waters. This problem not only induces mortality in the vertebrate and invertebrates, it demolish additionally the algae communities and sometimes alter the ecological equilibrium. The present study was carried out in order to investigate the effects of the above-mentioned chemicals on algae.

Materials and Methods

In the present study, herbicides Machete (60% pure Butachlor, solubility in water at 24°C, 23 mg/l) and Saturn (50% pure Bentiocarb, solubility in water at 20°C, 30 mg/l); pesticide Diazinon (60% effective matter, solubility in water at 25°C, 40 mg/l) and Malathion (57% effective matter, solubility 145 mg/l) were applied. After performing the initial tests, the experiment was carried out using three replicates. The alga *Scenedesmus obtusiusculus* and *Anabaena flos aquae* were treated with 1-10 mg/l and 2.5-6.7 mg/l of Machete, respectively. The applied concentration of Saturn, Malathion and Diazinon were 200-450 mg/l and 7-25 mg/l; 20-50 and 50-80 mg/l; and 54-86 and 110-200 mg/l, respectively. The algae used in the tests were isolated in the biotechnological laboratory and kept in Agar.

After performing the tests, the algae were transferred into the culture medium of Z-8 based on the procedure proposed in Miller *et al.*, (1978). Each test phase lasted for 6 days and the amount of the applied algae in each tube was 1 mg dry matter per litre. According to the practice, which has recently been elaborated by Piri (1996), the algae were exposed to different concentration of herbicides and pesticides. The toxic effect on the algae was determined by measuring the turbidity by spectrophotometer (wavelength 750 nm). The algae were treated with EC_{10}, EC_{50} and EC_{90} of the toxic chemicals and the results were measured using the Probit analysis (Finney , 1971). After finishing this phase, we continued the experiment with studying the mixculture. In addition to the inoculation, the algae were dissolved in nutrient; 10-ml samples were taken and fixed with formaline, and then 3.199 mg/l Machete (EC_{50} for *Anabaena flos aquae*) was added to the samples. After 6 days 10 ml of the samples was taken and fixed. The algae were counted using an inverted microscope. The same test was performed with 10-53 mg/l Saturn. At the end, the growth rate of both the sample with toxic content and the control were calculated.

Results

Effective concentrations of the four tested toxic substances appeared to be different. Based on NOEC (Non Observe Effective Concentration, EC10), the Machete and Saturn were extremely toxic for the *Scenedesmus obtiusiusculus* and *Anabaena flos aquae*.

Measuring the turbidity revealed that this approach was more appropriated for determining the harmful toxic effect of chemicals on the algae. Compared to measuring dry weight, it was easier and cheaper. It seemed that there was a negative correlation between solubility of the herbicides in water and their toxicity, because Machete and Saturn, which are very toxic, had a low solubility in the water. EC_{10} and EC_{50} of Machete and Saturn for green algae were less than 5.8 and 21.55 mg/l, while these concentrations for cyanobacteria were less than 0.05 and 0.38 mg/l, respectively.

Despite of low solubility in water, Machete and Saturn, were moderately toxic for the these two alga species. The results also showed that EC_{10} and EC_{50} of both chemicals for *Scenedesmus obtiusiusculus* were less than 82.26 and 46.95 mg/l and for *Anabaena flos aquae* less than 180.23 and 71.59 mg/l, respectively (Table 1 and Figs. 1-8).

In the mixed culture of *Scenedesmus obtiusiusculus* and *Anabaena flos aquae* we observed that, after six days, the growth rate of the green algae and cyanobacteria increased 41.5 and 39.8 times, respectively. By adding further 3.199 mg/l of Machete, the total concentration of Machete reached to the EC_{50} for cyanobacteria causing the growth rate of green algae and cyanobacteria to be decreased 0.95 and 4.5 times, respectively. Applying 10.53 mg/l of Saturn, EC_{50} for cyanobacteria, caused the growth rate of green algae and cyanobacteria to reach 1.19 and 7, respectively (Table 2).

Table 1 : The effect of chemical toxicant on the growth rate of *Scenedesmus obtusiusculus* and *Anabaena flos aquae* .

Chemical Toxicant	*Scenedesmus obtusiusculus* EC_{10}	EC_{50}	EC_{90}	$EC_{50/10}$	*Anabaena flos aquae* EC_{10}	EC_{50}	EC_{90}	$EC_{50/10}$
Machete	0.0019	0.0108	0.0605	0.0011	2.5800	3.1990	5.8000	0.3190
Saturn	0.1220	0.2150	0.3800	0.0215	5.1400	10.5300	21.5500	1.0530
Diazinon	11.0500	22.7800	46.9500	2.2780	43.7500	55.9600	71.5900	5.5960
Malathion	37.3200	55.3800	82.1600	5.5380	92.9400	129.0800	180.2300	12.9080

Table 2 : The effect of Machete and Saturn on mixed culture of *Scenedesmus obtusiusculus* and *Anabaena flos aquae*

Toxic Concentration	*Scenedesmus obtusiusculus* No. alge ml, test initial	No. alge ml, test initial	*Gr. Ratio changes	*Anabaena flos aquae* No. alge ml, test initial	No. alge ml, test initial	*GF. Ratio changes
Control	983449	4088180	41.50	76478	3046927	39.80
Machete (3.19 mg/l	63177	60522	0.95	108670	490231	4.50
Saturn (10.53 mg/l)	48670	58272	1.19	78670	554565	7.00

*GF: Growth Factor

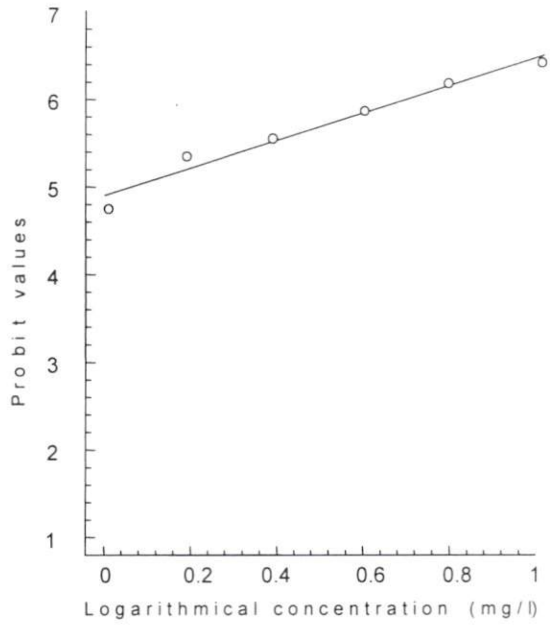

Fig. 1 : Effect of herbicide Machete on growth rate of green alge *scenedesmus obtusiusoulus*

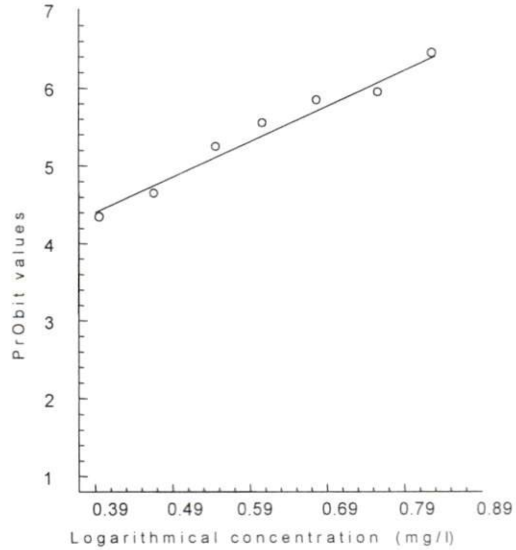

Fig. 2 : Effect of herbicide Machete on growth rate of Cyanobactria *Anabaena flos aquae*

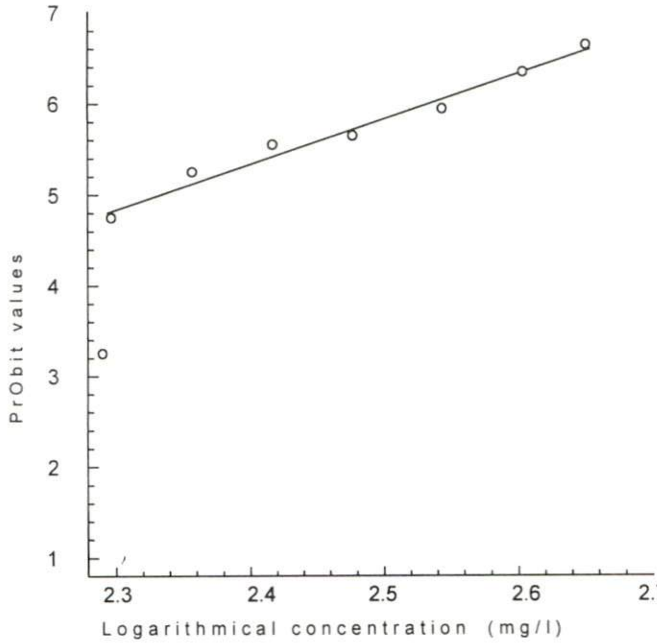

Fig. 3 : Effect of herbicide Saturn on growth rate of green alge *scenedesmus obtusiusoulus*

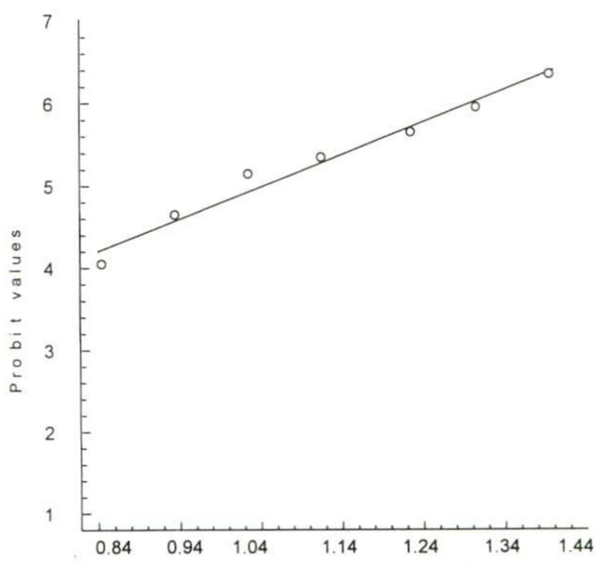

Fig. 4 : Effect of herbicide Saturn on growth rate of Cyanobactria *Anabaena flos aquae*

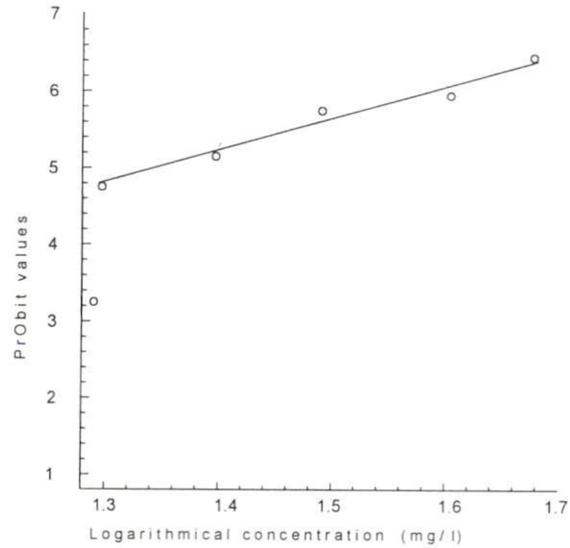

Fig. 5 : Effect of pesticide Diazinon on growth rate of green alge *scenedesmus obtusiusoulus*

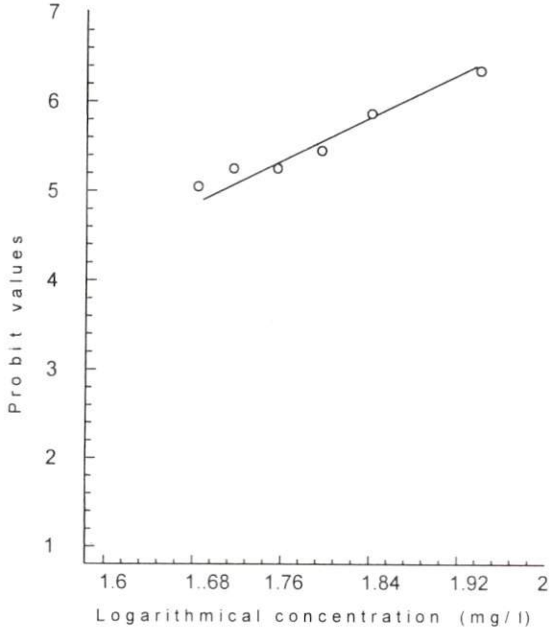

Fig. 6 : Effect of pesticide Diazinon on growth rate of Cyanobactria *Anabaena flos aquae*

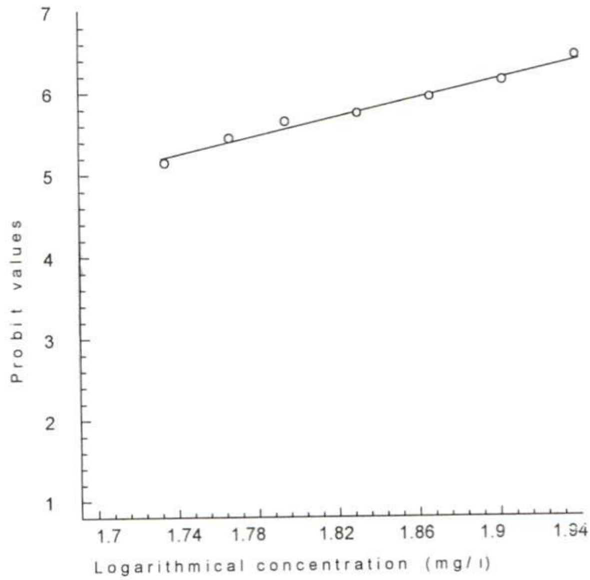

Fig. 7: Effect of pesticide Malathion on growth rate of green alge *scenedesmus obtusiusoulus*

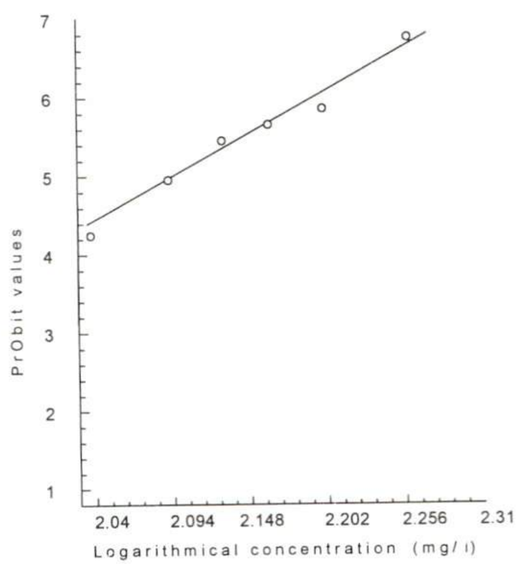

Fig. 8 : Effect of pesticide Malathion on growth rate of Cyanobactria *Anabaena flos aquae*

Discussion

Despite of low solubility of Malathion and Diazinon in water, the existing algae in the aquatic ecosystem exhibited different sensitivities to them and the obtained *in vitro* results could not be directly employed. However, Machete and Saturn could always be extremely toxic for green algae. Study on toxicity of these two herbicides for *Scenedesmus obtiusiusculus* indicated that EC_{10} of these herbicides for green algae were lower than 10 mg/l.

Diazinon have normally little effect on the photosynthetic cells and the biomass of *Scenedesmus obtiusiusculus* (Stadnyk *et al.*, 1971), while blue-green algae could tolerate over 400 mg/l (Singh, 1973). In contrast, the growth of some freshwater algae would be terminated at the concentration of 5–25 mg/l (Butlor, 1977). Murray and Guthrie (1980) have reported that 5 mg/l chlorpyrifos could reduce natural phytoplankton colonies by 45%. Also 0.25–2.0 mg/l of chlorpyrifos could reduce the diversity of the diatoms, while 0.07 mg/l of this substance would contribute to the blooming.

It has been reported by Brown *et al.*,(1976) that 0.24 mg/l of chlorpyrifos could affect the freshwater phytoplanktons differently. Concentration between 1-100 mg/l could stimulate the growth of *Scenedesmus obtiusiusculus* and *Anabaena flos aquae* (Birmingham and Coiman, 1977), but in our experiment the EC_{10}, EC_{50} and EC_{90} tested *Anabaena flos aquae* were less than 100 mg/l. It is clear that if this amount of Diaxinon enters the ecosystem of ponds and logoon, it could trigger the phytoplankton bloom.

These toxic chemicals contribute undoubtedly to the planktonic bloom during spring and summer in the fish culture ponds. Ördog and Kuivasniemi, (1989) have reported that chemicals have no effect on the reduction of phytoplankton biomass in natural ecosystems, but could play an important role in eliminating sensitive species and altering the diversity of species. Various researchers (Mula and Main, 1981; Moore and Dorward, 1968) have studied Malathion effects on non-target species. These researchers have shown that 1 mg/l of Malathion could reduce the metabolism activity of *Gonium pectrorale* by 56%. Also 0.3 – 7.2 mg/l of this pesticide could reduce the growth of *Euglina gracilis* by 4% – 49%. (Murray and Guthrei, 1980). Other reports showed that this algae could tolerate 100 mg/l of Malathion (Poorman, 1973). It has also been demonstrated that 5 mg/l of this pesticides inhibits the natural growth of phytoplankton by 70% (Moore, 1970), while 25 –50 mg/l of Malathion hinders the growth of algae species (Butler, 1977).

Based on Wasserscandstoff-katalog, (1975) Machete and Staturn are extremely toxic for green algae, and medium toxic for blue-green algae. The maximum allowable concentration (MAC) of toxic matters in the natural ecosystems could be calculated using EC_{10} test. Other researchers (Ördog and Kuivasniemi, 1989) have demonstrated measuring the turbidity as a suitable evaluation method for toxic effects of the algae.

This present study elaborated that toxic chemicals in the aquatic ecosystems contribute to the phytoplankton bloom affecting other living animals, both vertebrates and invertebrates in the fishponds and lagoons. The same conclusion has also been drawn by Huribert *et al.* (1972).

Acknowledgment:

We would like to express our gratitude to the assistance provided by the staff of Algae Biotechnology Lab, Mr. Sefaie, Mr. Khodaparast, Mrs. Falahy, Mr. Karimpour, Mr. Hosseinpour and Mr. Salvatian.

References :

Brown, J.R ; Chow, L.Y. and Deng, C.B. , 1976.The effect of Dursban upon freshwater phytoplankton, Bull. Environ. Contam. Toxicol. 437.

Butler,G.L. , 1977. Algae and pesticides, Residue Rev.19 p.

Felfoldy, L. ; Szabo, E. and Toth, L. , 1964. Apparatus for algal cultivation with a volume of two cubic meters in Tihany. Annal. Biol. Tihany.185-222.

Finney, D. , 1971. Probit analysis. Cambridge, Cambridge Univ. Press.1-333.

Hurlbert, S.H. ; Mulla., M.S. and Willson, H.R. , 1972. Effects of an organo-phosphorous insecticide on the phytoplankton, zooplankton, and insect populations of freshwater ponds, Ecol.Monogr. 269-299.

Miller,W.E. ; Greene, J.C. ; Mervim, E.A. and Shiroyamal, T. , 1978. Algal bioassay techniques for pollution evaluation. 9-16.

Moore, R.B. , 1970. Effect of Pesticides on growth and Survival of *Euglena gracilis*. Z., Bull. Environ.Contam.Toxicol. 226.

Moore, R.B. and Dorward, D.A. ,. 1968. Accumulation and metabolism of pesticides by algae, J.Phycol.7.

Mulla, M.S. and Main, L.S. , .1981. Biological and enviromental impacts of the insecticides Malathion and Parathion on nontarget biota in aquatic ecosystems , Residue. Rev. 101.

Murray, H.E. and Guthrie, R.K. , 1980. Effects of carbaryl, Diazinon and Malathion on native aquatic populations of micro-organisms. Bull. Environ. Contam. Toxicol. 535.

Nilsen, H.G. , 1989. Investigations concerning the occurrence of pesticides in drainage water ground water and surface water. Present Knowledge and planned projects in Norway. Nordic Plant Protection Conference, Helsingor. 417-422.

Ördog,V. and Kuivasniemi, K. , 1989. Studies on the effect of cell division inhibiting herbecides on unialgal and mixed algal cultures .Int . Revue. Hydrobiol. 221-226.

Piri, M. , 1996. The effect of herbicides on *Selenastrum capricornutum* and *Daphnia magna* .Gilan Fisheries Research Institute, Bandar-e Anzali,(in Persian). PP. 1-10.

Poorman, A.E. , 1973. Effects of pesticides on *Euglena gracilis* .I.Growth Studies, Bull. Environ. Contam. Toxicol. 10-25.

Singh, P.K. , 1973. Effect of pesticides on blue-green algae, Arch. Microbiol. 317.

Sloff, W. ; Canton, J.H. and Hermens, J.I.M. , .1983. Comparison of the susceptibility of 22 freshwater species to 15. chemical componds. I.(Semi) Acute toxicity tests. Aquatic Toxicology. 113-128.

Stadnyk, L. ; Campbell, R.S. and Johnson., B.T , 1971. Pesticide effect on growth and 14C assimilation in a freshwater alga ,Bull. Environ. Cntam. Toxicol. 1.

Torsten, K. and Romstad., R. , 1994. Effects of agricultural pesticides on planktonic algae and cyanobacteria examples of interspecies sensitivity variations. 118-131.

Torstensson, L. , 1989. Pesticides in surface and ground water in Sweden. Nordic Plant Protection Conference, Elsinor. 422-431. (in Swedish)

Wasserschadstoff-Katalog. , 1973. Instiute Wasser Wirtschaft. Berlin.