Uptake and Effects of Dichlobenil in a Small Pond[1]

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The herbicide 2, 6-dichlorobenzonitrile (dichlobenil, Casoron®) is used throughout the United States for control of submerged aquatic weeds. It is not effective against filamentous algae and has little effect upon plankton and fish except at high concentrations (1, 2, 3).

In this study, we treated a small pond with dichlobenil in order to measure uptake by organisms and effects of treatment upon oxygen production, plankton dynamics, and water chemistry.

Methods

Two biologically similar natural ponds, approximately 50 meters apart, were used. Analyses of yearly cycles of plankton and water chemistry were begun in January 1968 and were made at weekly intervals throughout the year. On 3 April, one pond was treated with a wettable powder formulation of dichlobenil at a concentration of one p. p. m. (parts per million); the other was maintained as an untreated "control."

Water for chemical analysis was taken at approximately 0800 and 1300 hours at the surface, mid-depth, and bottom of the water column of the basin of each pond on each sampling day. Oxygen

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content was measured by the Winkler method (4). The difference between afternoon and morning values for oxygen, after correction for diffusion across the surface (5), was considered to be the net amount of oxygen released to the water by vascular plants and phytoplankton. Net production of oxygen by phytoplankton was estimated by the light-and dark-bottle method (4). Light-and dark-bottle pairs were incubated at the surface, mid-depth, and bottom for five hours and all oxygen data are expressed as basin averages.

Diel studies were made in which water samples were taken at three-hour intervals from 0600 hours of one day to 0600 hours of the next. Gross production of oxygen was calculated by the diurnal curve method (6) and two sets of light-and dark-bottles were incubated in each pond. One set was incubated between 0600 and 1200 hours and the other between 1200 and 1800 hours. In each set, bottle pairs were incubated at surface, mid-depth, and bottom, and gross production of oxygen calculated (4).

At each sampling time, we also measured nitrate-nitrogen (7), phosphate-phosphorus (4), dissolved carbohydrate (8), total alkalinity and CO_2 (9), pH (Coleman Model 37A portable pH meter), and temperature, conductivity, and salinity (Beckman Model RS5-3 salinometer).

Plankton were collected each week by towing a net of No. 10 bolting cloth behind a boat for 100 meters. Species were identified and counted on the day of collection in a Sedgwick-Rafter chamber and filamentous algae were recorded as number of filaments per liter.

Dichlobenil was injected beneath the surface of the water from a Hudson sprayer and analysis of water samples three hours after application proved the concentration to be one p.p.m. The pond was then monitored at selected intervals for a period of 64 days.

The analytical procedure used to determine residues of dichlobenil was developed by Meulemans and Upton (10). Recoveries of the chemical from fortified samples were 98% for water, 90% for hydrosoil and aquatic plants, 82% for fish, and 80% for all others. All data were corrected for these values. All samples were also analysed for the presence of 2,6-dichlorobenzoic acid, a metabolite of dichlobenil (10).

Indentification and quantitation were made on a Varian Model 1200 gas chromatograph equipped with an electron capture detector. A 1.7 meter long glass column packed with 5% DC200 of Gas Chrom Q, 80/100 mesh, was used. Operating specifications were; injector-140° C, column - 110° C, detector - 170° C, N_2 flow-40 ml/min. Retention time was 4.1 minutes for dichlobenil and 5.3 minutes for 2,6 -dichlorobenzoic acid.

At each sampling time, 500 ml samples of surface and bottom water were collected randomly and combined to give composite samples of surface and bottom water. Sub-samples of one liter were taken from each, filtered through glass wool, and analyzed immediately upon return to the laboratory.

Samples of hydrosoil were also calculated on a dry-weight basis, whereas all other residues are reported as wet-weight.

Descriptions of the Ponds

The ponds were located near Pensacola, Florida, at approximately 87°9' west longitude and 30° 20' north latatude. Area, volume, and depth are given in Table 1. Each pond had a narrow band of emergent vegetation (Typha sp.) around the margin and

Table 1

Area, volume, and depth of ponds used in dichlobenil studies.

Pond	Area ha	Volume m^3	Average depth m
Treated	0.15	650	0.43
Untreated	0.91	9,660	1.06

was surrounded by nearly bare sand. Substrata were course sand intermingled with organic debris. Water color was light brown and a Secchi disc could be seen resting on the bottom at maximum depth in each pond. Thermal stratification was never observed. Chara sp. was the dominant hydrophyte, but Potamogeton pectinatus was also present.

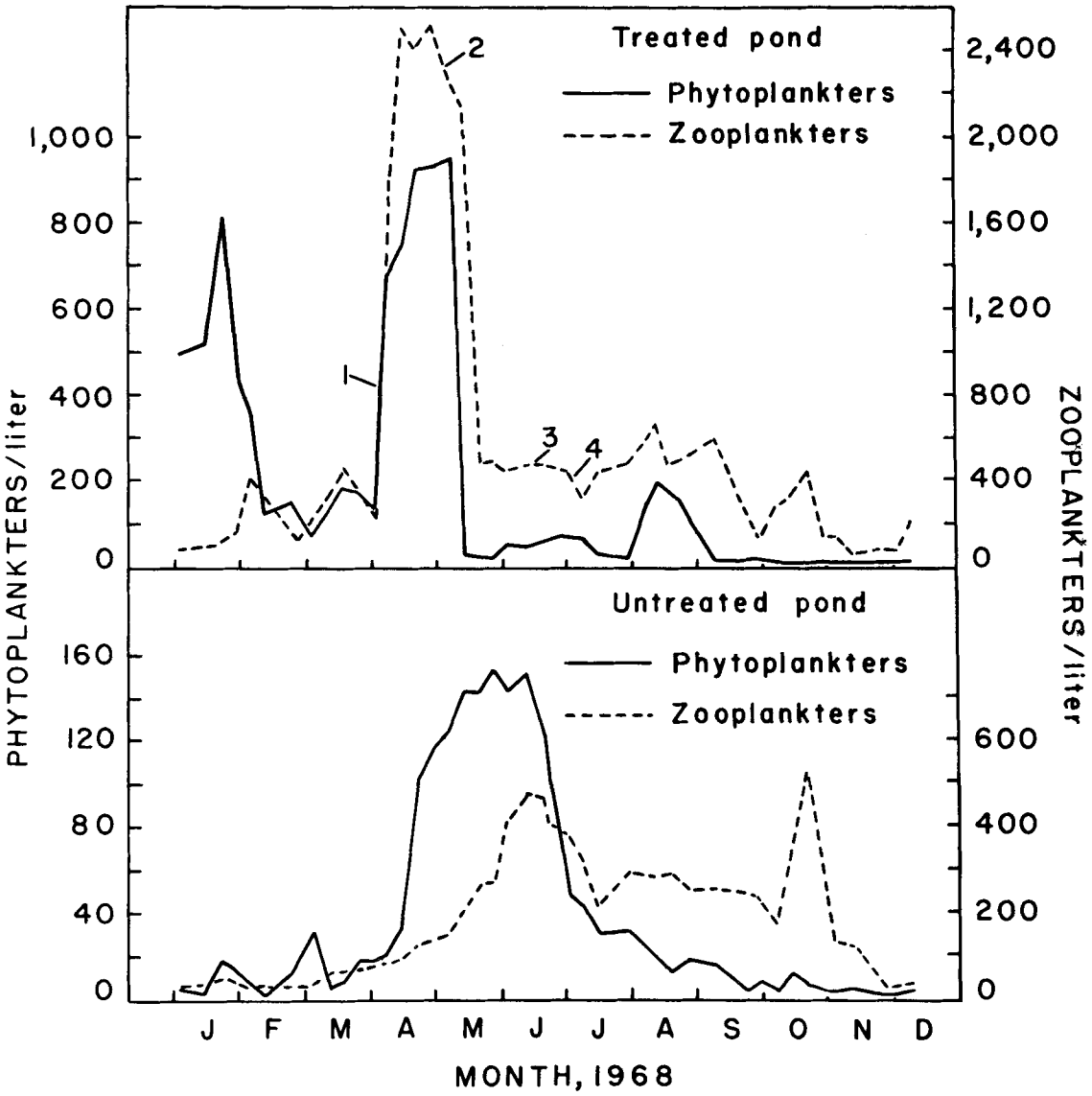

Figure 1. Annual variation in numbers of phytoplankters and zooplankters in two ponds, one of which was treated with one p.p.m. of dichlobenil. 1-date of treatment; 2-date of maximun kill of plants; 3-date of bottom covered by Chara; 4-pond at pretreatment state.

Effects Upon Water Chemistry and Biota

The sequence of treatment and response can be outlined as follows:

3 April....treatment
5 May.....maximum kill of aquatic plants
18 June.....bottom covered by Chara
5 July.....pond at pretreatment state

Except for death of plants, the greatest effect of treatment was upon relative rates of oxygen production by vascular plants and algae. Table 2 shows that in the untreated pond and the treated pond before treatment, phytoplankton contributed between 0 and 25% of the total daily increase in oxygen. In contrast, phytoplankton production in the treated pond constituted 94.5% of the total production in May, the time of maximal effect of herbicide, when all P. pectinatus and about 80% of the Chara were eliminated. A phytoplankton bloom was in progress at that time. Dominant genera were Lyngbya (533 filaments/liter), Oedogonium (327 filaments/liter), and Oscillatoria (36 filaments/liter); smaller numbers of Anabena, Spirogyra, and Desmidium were also present. Concentration of chlorophyll in the phytoplankton was 25.1 mg/liter. Presumably, this phytoplankton bloom was supported by nutrients released from dead and dying plants.

Table 2

Gross amounts of oxygen given to the water in the ponds during this study.

Date	Total g O_2/m^2/day		Phytoplakton g O_2/m^2/day		% Contributed by Phytoplankton	
	untreated	treated	untreated	treated	untreated	treated
12/7 - 12/8/67	2.2	6.8	0.4	0	18.2	0
3/28 - 3/29/68	4.0	10.2	1.0	1.6	25.0	15.7
5/2 - 5/3/68	6.5	9.1	1.1	8.6	16.9	94.5

We found no effect of treatment upon temperature, conductivity, salinity, nitrate-nitrogen, phosphate-phosphorus, dissolved carbohydrate, total alkalinity, total CO_2, and pH.

On an annual basis, numbers of zooplankton and phytoplankton increased greatly shortly after application of dichlobenil (Figure 1). The dominant zooplankter was Gonyaulax sp. which attained a desity of 1,950 individuals/liter on 29 April. Other zooplankters were Keratella cochlearis (426/liter), K. quadrata (5/liter), Diaptomus dorsalis (126/liter), Lecane luna (11/liter), ostracods (44/liter), and cladocerans (7/liter).

Table 3

Residues of dichlobenil, in parts per million, in water, hydrosoil, and organisms of a small pond, 4 April to 6 June 1968.

	Days after treatment									
	1	2	3	7	14	21	28	35	50	64
Water	0.836	0.641	0.403	0.221	0.046	0.017	0.012	0.006	0.002	0.001
Hydrosoil	0.526	0.431	0.334	0.212	0.162	0.051	0.022	0.015	0.009	0.004
Potamogeton sp.	0.953	1.333	0.553	0.634	0.266	(-)	(-)	(-)	(-)	(-)
Chara sp.	1.16	0.771	0.690	0.670	0.191	0.123	0.080	0.050	Neg.	Neg.
Poecilia latipinna	4.903	4.216	3.407	2.795	0.820	N. S.	0.271	0.071	Neg.	Neg.
Gambusia affinis	10.957	6.628	4.491	3.061	0.824	0.437	0.343	0.084	0.015	Neg.
Hyallela azteca	0.210	0.552	0.691	0.223	0.060	0.021	Neg.	Neg.	Neg.	Neg.
Orthemis sp.	1.36	1.58	0.43	0.53	0.05	Tr.	Neg.	Neg.	Neg.	Neg.
Leuchorrhinia sp.	0.260	0.146	0.067	0.146	0.078	0.035	0.014	Tr.	Neg.	Neg.
Plankton	7.231	2.917	1.625	1.250	0.882	0.733	0.312	0.192	0.050	Neg.

Explanation of terms: N. S. = no sample; (-) = no material available because plants were eliminated by treatment; Tr. = less than 0.05 p.p.m.; Neg. = herbicide not detected.

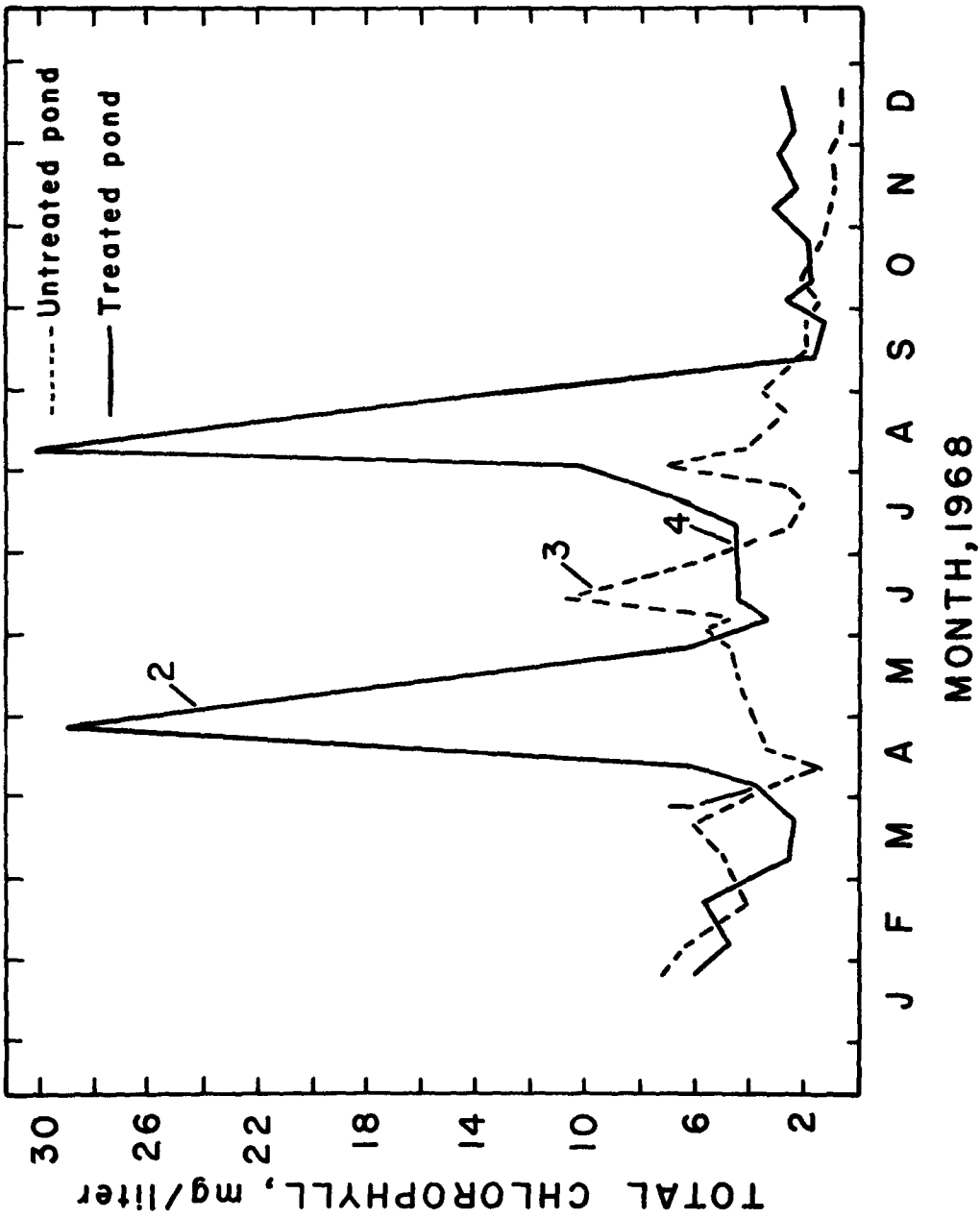

Figure 2. Annual variation of phytoplankton chlorophyll in two ponds one of which was treated with one p.p.m. of dichlobenil. 1-date of treatment; 2-date of maximum kill of plants; 3-date bottom covered by Chara; 4-pond at pretreatment state.

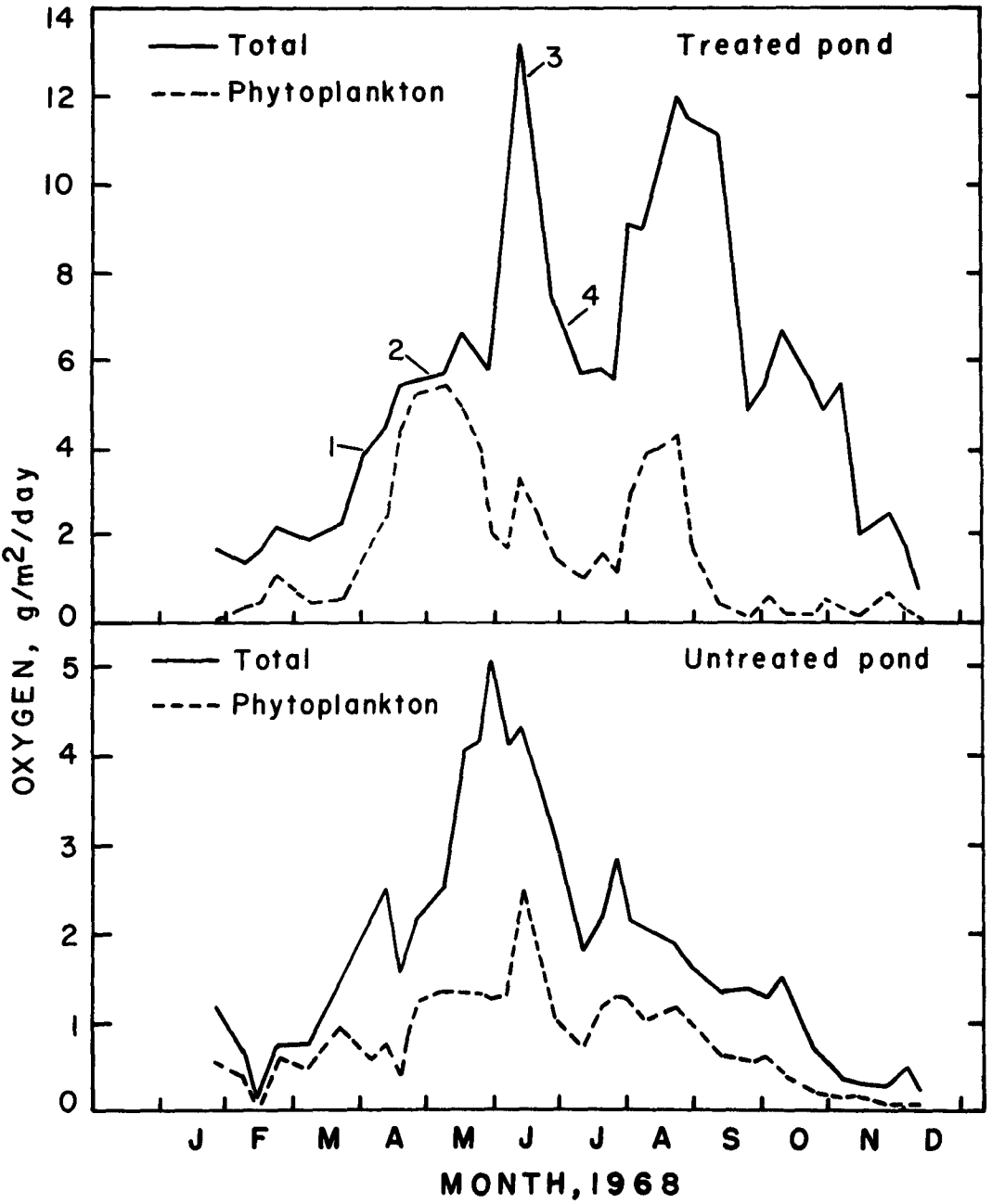

Figure 3. Annual variation in oxygen production in two ponds, one of which was treated with one p.p.m. of dichlobenil. 1-date of treatment; 2-date of maximum kill of plants; 3-date bottom covered by Chara; 4-pond at pre-treatment state.

Both zooplankton and phytoplankton blooms were sustained through April, but numbers fell precipitously between 8 and 13 May, when Chara began a period of rapid growth. Although the treated pond was more productive than the untreated one throughout the year, there was no difference in relative numbers of each genus in the two ponds. In the untreated pond, maximum density of plankters was attained at a slower rate and numbers fell less precipitously than in the treated pond.

In the treated pond, phytoplankton played a major role in the maintainence of normal conditions during the period of maximal herbicide effect upon vascular plants. Concentration of phytoplankton chlorophyll rose sharply to 29.3 mg/liter after application of herbicide and then fell rapidly during the period of recovery (Figure 2). The large numbers of phytoplankters produced over 90% of the oxygen released to the water at the time of maximal herbicidal effect upon benthic plants (Figure 3) but after the bloom, phytoplankton resumed its secondary role in oxygen production. Even in August, when vascular plants had returned to pretreatment numbers and concentration of phytoplankton chlorophyll reached 30.2 mg/liter due to a bloom of Lyngbya, net release of oxygen by phytoplankton was only 38.3% of the total.

Residues

Residues of dichlobenil found in water, hydrosoil, and organisms are given in Table 3. After 14 days, hydrosoil contained larger amounts than did water, and the herbicide was present in water and hydrosoil 64 days after treatment. None, however, was detected in organisms at 64 days and the metabolite 2, 6-dichlorobenzoic acid was never detected.

Relatively large amounts were found in mosquitofish (Gambusia affinis) and the sailfin molly (Poecilia latipinna) one day after treatment. Plankton also concentrated the chemical, but no deleterious effect was seen. Uptake by the dragonfly nymphs Orthemis sp. and Leuchorrhinia sp. differed greatly. One day after treatment, Orthemis contained over five times more dichlobenil than did Leuchorrhinia. The amphipod Hyallela azteca responded differently than all other organisms. Residues in H. azteca were greatest on the third day, whereas the others contained greatest residues on the first day after treatment.

Summary

Dichlobenil, when applied as a wettable powder at a concentration of one p.p.m., eliminated all P. pectinatus and about 80% of the Chara from a small pond. As benthic plants died, blooms of phytoplankton and zooplankton occurred, presumably because nutrients were released from dead and dying plants. No change was observed in water chemistry at any time during the period of herbicide effect. The normal oxygen regime was maintained by phytoplankton in the bloom condition. As benthic plants returned, numbers of plankters dropped sharply, and the pond returned to the pretreatment state approximately three months after treatment.

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