The Effect of Methoxychlor on Periphyton Under Natural Conditions

Peter G. Meler
School of Public Health, The University of Michigan, Ann Arbor, Mich. 48109

Lawrence H. Hecker
School of Public Health The University of Michigan Ann Arbor, Mich. 48109

Donald D. Hultgren
Stearns-Roger Incorporated Environmental Consultants Denver, Colo. 80217

Methoxychlor has replaced DDT in many instances of pesticide application. The major advantages of methoxychlor, a DDT analogue, are its apparant low level of biomagnification in the food chain and its ability to be degraded rapidly in nature.

Although some knowledge has been gained in recent years concerning the behavior of methoxychlor in the environment (LEE et al. 1975; SIKKA et al., 1976), the amount of completed research has been very limited. This is particularly true in respect to the effects of methoxychlor on algal, protozoan, and bacterial communities.

A study was carried out to determine the effect of methoxychlor on the autotrophic periphyton community in seminatural streams. This community was chosen due to its relatively fast changes in species composition with alterations in the environment and also because it contains the primary producers in flowing streams.

Description of Study Area

This research was conducted at the Michigan Department of Natural Resources research station located near Saline, Michigan, U.S.A., during May and June of 1975. Koch-Warner Creek, which is spring fed, supplies water that is passed from a reservior to a duplicate series of three outdoor channels. Each section is 36.6 meters in length, 5.4 meters in width, and varies in depth from 0.3 to 1.1 meters.

Each channel was designed to have a flow rate of 7.1 liters/second. Two automoatic dosing mechanisms fed a methoxychlor-acetone mixture into the lower two sets of channels at rates to maintain concentrations of methoxychlor of 0.5 ppb and 2.0 ppb respectively, in the water. The control channels received no acetone addition, since earlier laboratory studies showed no effect on periphyton with similar concentrations (WEITZEL, 1973).

Materials and Methods

The methods for collection and examination of the periphytic community was done by means of the glass slide technique as described by WEBER (1973). In this investigation, eighteen 25mm x 76mm microscope slides were placed vertically in each one of thirty black plastic slide boxes. The backs of these boxes were removed so as to allow for a flow-through design when placed in water.

Bulletin of Environmental Contamination & Toxicology, Vol. 18, No. 3 © 1977 by Springer-Verlag New York Inc.

Five boxes containing slides were randomly positioned on the substrate in each channel at a depth of 0.3 to 0.45 meters. The boxes were held in place by means of an attached wooden clothespin which rested upon the substrate and positioned the boxes at a proper angle for optimal periphytic growth.

Four glass slides one from each channel were collected after 2,4,6,8,14,28,32, and 42 days of exposure for organic ash-free weight and chlorophyll and determination. After removal, each slide was placed in a small plastic bag and frozen so all samples could be analyzed simultaneously at the end of the experiment.

Another set of duplicate slides was removed from each channel section for enumeration and species identification of the periphyton. These slides were obtained after 2,4,6,8,16, and 32 days of exposure. For this portion of the study, the surfaces of each set of slides were scraped into plastic vials containing a known volume of water. Logol's solution was then added for preservation.

Ash-Free Weight

Each slide was removed from the plastic bag in which it had been frozen and the attached material was removed and the ash-free dry weight determined according to standard methods (APHA, 1971).

Chlorophyll *a*

Two slides from each collecting date were separately extracted with acetone and analyzed for chlorophyll *a* utilizing a Turner Model 110 fluorometer. The concentration of chlorophyll *a* was determined by employing a modification of the formula described by STRICKLAND and PARSONS (1968), and SLACK et al., (1973). From the ash free weight and chlorophyll *a* values the autotrophic index was calculated.

Identification and Enumeration

Samples for species composition and enumeration were in duplicate for each channel section on the various dates of exposure. Two counting techniques were employed. In one case, genus determination of all taxa was carried out on fresh material using a Palmer-Maloney counting cell under 400X. The other counting procedure involved the preparation of permanent diatom slides from cleaned diatom material which was mounted in Hyrax. Identification to species level was carried out under 1000X. Random lateral strips the width of the Whipple grid were examined until at least 250 frustules were tallied and identified. If very few diatoms were present, the analysis was limited to the number of cells found in 45 minutes of scanning. This procedure was also conducted in duplicate for each sample from each channel. Diatom diversity index was calculated from the permanent diatom slides utilizing the formula presented by WEBER (1973).

Water samples from the channels were also collected on days 14, 28, and 32 of the study for the purpose of nutrient analysis (NO_3^- and ortho PO_4^{-2}). These samples were collected in midchannel at a depth of approximately 0.3 meters below the surface.

Results and Discussion

Ash-Free Weight

Data collected from this study indicates that the average standing crop of organic weight increased quite rapidly through days 6-8 in all channels (TABLE I). Subsequently the rate of biomass accrual declined. The rate of periphytic accrual was independent of methoxychlor concentration below at the levels investigated.

Observed variation in standing crop was attributed to sloughing and recolonization. This natural phenomenon occurred in an unpredictable manner in these channels, even though, the periphyton substrates were exposed to nearly the same physical factors of temperature, light level, and water velocity.

Chlorophyll *a*

The accumulation of chlorophyll *a* measured in terms of $mg/m^2/d$ was the highest after 6-8 days in the control and low level dosed channels, but occurred after 16 days in high concentration channel (TABLE I). The trend in the accrual of chlorophyll *a* was similar to that displayed by organic ash-free weight, except that the increase proceeded at a much slower rate in the channel dosed with 2.0 ppb methoxychlor.

These findings were somewhat similar to those reported by WEITZEL (1973) who carried out laboratory work with the same water supply in flow-through aquaria. The trend of organic weight was the same in his study except that the standing crop was nearly an order of magnitude higher and the exposure period was much shorter (14-16 days). Differences in biomass between WEITZEL'S study and this one are attributed to more controlled light conditions and a slower velocity in the aquaria which would enhance periphyton growth and minimize sloughing.

Autotrophic Index

The autotrophic index was calculated for each collection date on each channel and all values exceeded 100 (indicative of water quality degradation, WEBER, 1969). It is interesting to note that in the control channel a heterotrophic community becomes first established and

TABLE I

PERIPHYTON ACCRUAL PER DAY AFTER RESPECTIVE DAYS OF INCUBATION

Days of Accrual	Ash-free Weight in g/m^2			Chlorophyll *a* Concentration mg/m^2		
	Control*	Low**	High***	Control*	Low**	High***
2	.07	.22	.08	.02	.18	.09
4	.17	.35	.08	.07	.16	.06
6	.38	.87	.15	.12	.60	.06
8	.14	.24	.47	.50	.24	.12
14	.15	.24	.16	.12	.09	.15
16	.12	.1	.11	.14	.45	.37
28	.05	.05	.12	.09	.1	.07
32	.03	.04	.1	.13	.11	.14
42	.13	.08	.09	.28	.05	.07

*Control - no Methoxychlor **Low - 0.5 ppb Methoxychlor ***High - 2.0 ppb Methoxychlor

NOTE: Each value represents the mean of two observations

with an exposure period of 42 days becomes autotrophic. This phenomena can be explained on the basis that the water is drawn from the bottom of the reservior and is piped underground to the control channels. It is logical to assume that the major plankton component in this supply water is heterotrophic and therefore is the first community to establish itself on the glass substrates. Over time, however, this population which contains autotrophs, is exposed to nutrients and light which enhances the growth and results in a less heterotrophic community.

Since the low and high level channels were downstream from the control, their indigenous population that initially colonized these substrates were autotrophs. The fluctuations of the autotrophic index displayed by the periphyton population of the dosed channels are the result of its species composition. Data in TABLE II support this hypotheses that an increase in the percentage of the green alga, Spirogyra, decreases the autotrophic index. This phenomenon is shown in both treated channels and is continued through day 16 after which similar autotrophic conditions were observed in all channels.

The increase in heterotrophy, on the other hand, could not be related to any algal species that was identified; although, it has been documented that some algae can utilize organic compounds (STEWART, 1974). No attempt was made to identify and enumerate the nonalgal components of the periphytic community.

Identification and Enumeration

Data collected from the Palmer-Maloney cell counts revealed that the number of periphyton followed a similar pattern of fluctuations as those displayed by organic weight and chlorophyll a. The channel receiving 0.5 ppb methoxychlor had a slightly higher average of total organisms per unit area, which seems to agree fairly well with the slightly higher mean organic weight (TABLE III). Also, those channels that were dosed with methoxychlor appeared to contain a higher average percentage of filamentous green and blue green algae than the control.

Microscopic examination of permanent mounts of the diatom population showed that the periphyton community was dominated by Achnanthes spp., Gomphonema olivaceum, and Navicula spp., especially N. cryptocephala and N. canalis. Fragilaria spp., Nitzschia acicularis and N. palea. and Synedra spp. were also quite common.

TABLE II
PERCENTAGE COMPOSITION OF DOMINANT ALGAE

Organisms	Days of Accrual	2			4			6			8			16			32		
	Treatment	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H	C	L	H
Bacillariaceae (Total)		81	52	60	86	50	70	62	20	62	43	20	64	98	68	59	81	80	97
Achnanthes		-	1	1	-	3	3	2	7	6	4	6	13	33	25	28	34	30	50
Fragilaria		1	2	3	2	4	4	9	1	2	1	1	4	4	1	-	2	2	1
Gomphonema		1	1	1	-	1	2	1	-	2	-	2	-	36	26	14	25	30	19
Navicula		32	24	32	38	19	31	13	7	41	6	6	28	24	16	13	16	15	23
Nitzschia		35	13	3	36	15	13	30	2	5	25	2	6	1	-	1	-	-	-
Synedra		6	7	19	8	6	9	6	2	4	6	2	8	-	1	2	1	2	1
Chlorophyta (Total)		18	48	40	14	50	30	38	80	3	57	80	9	2	32	39	19	20	3
Cladophora		2	-	-	-	-	-	2	1	-	-	-	-	2	5	-	4	-	-
Spirogyra		12	45	40	10	50	30	33	78	3	49	78	9	-	24	33	10	19	2
Ulothrix		3	-	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Zygnema		-	1	-	-	-	-	-	2	-	-	2	-	-	-	-	2	1	-
Cyanophyta (Total)		-	-	-	-	-	-	-	-	35	-	-	27	-	-	2	-	-	-

Control - no Methoxychlor Low - 0.5 ppb Methoxychlor High - 2.0 ppb Methoxychlor

TABLE III

CONCENTRATION OF ALGAL CELLS FOR RESPECTIVE DAYS OF ACCRUAL (NUMBER/mm^2 x 10^3 OF SLIDE SURFACE)

Days of Accrual	Control	Low	High
2	.915	1.12	1.16
4	3.70	3.84	1.87
6	4.92	11.3	5.42
8	5.57	4.21	8.00
16	1.74	14.8	6.15
32	2.75	10.1	7.20
average	3.27	7.56	4.97

Diatom diversity did not seem to point out any specific trend as shown in TABLE IV. Little overall difference in diversity was observed between the control and those channels dosed with methoxychlor.

The greatest average diversity of diatoms in the control channels was observed after 4 days of exposure, while the least diversity was found after 32 days. This phenomenon is the opposite of what one would expect. A gradual decline from day 4 through day 32 of diversity was observed in all channels.

Nutrients

The effects of methoxychlor on periphytic communities could be associated with nitrate nitrogen changes that occurred during this study (TABLE V). Although the phosphorous concentration remained constant as the water passed through the channels, the nitrate level decreased markedly with increasing concentrations of methoxychlor. This nitrate reduction may be attributed to the characteristic filamentous growth in the 0.5 ppb dosed channel which exposed a larger algal surface area to the surrounding water. This lower nitrogen concentration although not limiting, that entered the 2.0 ppb dosed channel could have affected the production of photosynthetic organisms. Hence further investigations with the same levels of nitrogen in all channels are warranted to isolate the potential effect of methoxychlor on the indigenous periphytic communities.

TABLE IV

PERIPHYTON DIVERSITY FOR RESPECTIVE DAYS

Days of Accrual	Control	Low	High
2	2.77	3.50	3.23
4	3.51	3.02	3.67
6	3.18	2.14	2.89
8	2.18	2.33	2.50
16	1.43	0.78	1.03
32	1.21	1.30	0.56

TABLE V

NITRATE AND ORTHO PHOSPHATE CONCENTRATION (mg/l)

	NITRATE			PHOSPHATE		
Day	Control	Low	High	Control	Low	High
14	.428	.181	.099	.021	.023	.026
28	.967	.455	.180	.033	.029	.023
32	.395	.233	.199	.021	.027	.024

References

APHA. 1971. Standard Methods for the Examination of Water and Wastewater, 13th. Ed. Amer. Public Health Assoc., Washington, D.C.

LEE, J.H., J.R. SYLVESTER, and C.E. NASH. Bull. of Environmental Contamination & Toxicology 14, 180 (1975).

MERNA, J.W. Personal Communication (1975).

SIKKA, H.C., G.L. BUTLER, and C.P. RICE. U.S. EPA REPORT, 600/3-76-048 (1976).

SLACK, K.V., R.C. AVERETT, P.E. GREESON, and P.G. LIPSCOMB. U.S. Geological Survey Publication, (1973).

STRICKLAND, J.D.H. and T.R. PARSONS. Fisheries Research Board of Canada, Bulletin No. 167 (1968).

WEBER, C.I. and B.H. McFARLAND. Paper presented at the 17th Annual Meeting, Midwest Benthological Society, Gilbertsville, Ky. 1969.

WEBER, C.I. Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents. U.S. EPA. (1973).

WEITZEL, R.L. OWRR Report, Project A-0530Mich. (1973).