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FATE AND IMPACT OF 2,4-D IN A POND ECOSYSTEM

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Executive Summary

The recent invasion of Canadian waters by Eurasian watermilfoil has resulted in increased use of the herbicide 2,4-D and increased public concern with the safety of such chemical control measures. This study was an Environment Canada response to such concerns expressed in areas as diverse as the Trent-Rideau-Severn waterway and the Okanagan Lakes in British Columbia. Our study was designed to determine the fate and impact of the butoxyethyl ester and N,N-dimethylamine formulations of 2,4-D. The ester is the only approved formulation in Canada for aquatic use, and the amine was being considered by Agriculture Canada as a replacement. This report summarizes two previous reports and is intended as a synopsis of Environment Canada's research on 2,4-D in artificial pond ecosystems.

Both formulations were effective in milfoil control at nominal concentrations of 1 ppm. The half-life of 2.4-D in the water varied from 17 to 35 days in the two year study. The herbicide and its degradation product, 2,4-dichlorophenol, were detected in the water, sediment and various components of the biota throughout the study. Some direct effects of the herbicide were observed on fish fry and clams. Secondary effects were increased bacteria populations and snail populations, enhanced growth of clams and a shift in the benthic community after the milfoil collapse and decay.

The experimental pond ecosystems were found to maximize effects by containment of the chemical and to facilitate a study of the chemical's impact on components of the biota.

DEVENIR ET RÉPERCUSSIONS DU 2,4-D DANS UN ÉCOSYSTÈME D'ÉTANG

E. Nagy, D.S. Painter et B.F. Scott

RÉSUMÉ À L'INTENTION DE LA DIRECTION

Récemment, l'envahissement des eaux canadiennes par le myriophylle eurasien a provoqué une utilisation accrue de l'herbicide 2,4-D et a intensifié l'inquiétude manifestée par le public quant à la sécurité de telles mesures de limitation. La présente étude est la réponse d'Environnement Canada à l'inquiétude manifestée dans des régions aussi différentes que les voies navigables Rideau et Trent-Severn et les lacs de l'Okanagan en Colombie-Britannique. L'étude a été conçue de façon à déterminer le devenir et les répercussions des formulations à base d'ester butoxyéthylique et de sel de N,N-diméthylammonium du 2,4-D. L'ester est la seule formulation approuvée au Canada pour usage dans le milieu aquatique; Agriculture Canada a envisagé la possibilité de le remplacer par le sel d'ammonium.

À une concentration nominale de 1 ppm, les deux formulations limitent efficacement le myriophylle. Pendant l'étude d'une durée de 2 ans, le temps de demi-élimination du 2,4-D dans l'eau variait de 3 à 5 semaines. L'herbicide et son produit de dégradation, le 2,4-dichlorophénol, ont été décelés dans l'eau, les sédiments et diverses composantes du biote pendant toute la durée de l'étude. Certains effets directs de l'herbicide ont été observés sur le frai de poissons et sur les clams. Voici certains de ces effets secondaires : accroissement des populations de bactéries et

de gastropodes, croissance accrue des clams et modification de la communauté benthique après la destruction du myriophylle et sa décomposition.

L'utilisation de ce type d'écosystèmes expérimentaux a permis de rendre maximum les effets des produits en les confinant, ce qui a facilité l'étude de leurs répercussions sur les composantes du biote.

RÉSUMÉ

On s'est servi d'écosystèmes expérimentaux constitués d'un étang pour contrôler par une étude de 2 ans les effets et le devenir de deux formulations de 2,4-D. À un taux d'épandage donnant une concentration nominale de 1 ppm dans la phase aqueuse, ce produit chimique permettait de limiter efficacement un myriophylle. Les traitements n'ont eu aucun effet important sur les bactéries, les champignons, le phytoplancton et le zooplancton. On a observé, quelques jours après l'épandage, quelques cas de mortalité chez le frai de meunier noir au cours de la première année, mais aucun au cours de la seconde. Au cours des deux années, les poissons adultes n'ont pas été touchés. Après la destruction du myriophylle, on a observé une augmentation des populations de bactéries, une croissance accrue des clams et des gastropodes et un accroissement du nombre d'oligochètes dans les populations de zooplancton. Le 2,4-D persistait dans l'eau et les sédiments pendant le gros de l'été et de l'automne; au cous des deux années, le produit disparaissait de l'eau avec des temps de demi-élimination de 17 et de 35 jours. Certains des effets observés ont été attribués à la présence de 2,4-DCP dans le système.

Devenir et répercussions du 2,4-D dans un écosystème d'étang

E. Nagy, D.S. Painter et B.F. Scott

Fate and Impact of 2,4-D in a Pond Ecosystem

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Experimental pond ecosystems were used to monitor the fate and effect of two 2,4-D formulations in a two-year study. At an application rate that produced a 1 ppm nominal concentration in the water phase, the chemical was effective in milfoil control. The treatments did not produce significant effects on the bacteria, fungi, phytoplankton and zooplankton communities. White sucker fry exhibited some mortality during the first few days following treatment in the first year, but none in the second year. Adult fish were not affected in either year. After the collapse of the milfoil, increased bacteria populations were observed, clams and snails showed enhanced growth, and the zoobenthos shifted to oligochaete dominated populations. The 2,4-D persisted in the water and sediment for most of the summer and fall seasons, with its disappearance from the water showing half lives of 17 and 35 days in the two years. Some of the observed effects were attributed to the presence of 2,4-DCP in the system.

The recent invasion into Canadian waters of Eurasian watermilfoil, Myriophyllum spicatum, the chemical control of which relies on the use of 2,4-dichlorophenoxyacetic acid (2,4-D), has resulted in both an increased use of the chemical and increased public concern with the safety of its use. In Canada, hundreds of tonnes of 2,4-D are used annually for the control of terrestrial and aquatic weeds. In addition to water weed control, the chemical can enter the aquatic environment from terrestrial sources (1).

The chemical, 2,4-D, can be used in several forms such as esters, acid salts and amines. The only commercial formulation approved in Canada for aquatic weed control is the butoxyethyl ester in a slow release form (AQUA-KLEEN, by Union Carbide Agricultural Chemicals). In the late 1970s, Agriculture Canada had banned the terrestrial use of the butylester form because of drift problems, was reviewing the use of butoxyethyl ester, and was considering the N,N-dimethylamine formulation for specific aquatic uses. This study was designed to determine the fate and impact of the latter two forms in an aquatic ecosystem.

Previous studies have found that 2,4-D does not persist long in the aquatic environment (2). Although a laboratory study reported some effects on phytoplankton (3), field studies generally show no effect on phytoplankton, zooplankton, clams, or fish (4,5). For a perspective we may note that the 96-hour LD_{50} of the 2,4-D acid to bluegill is 350 ppm, whereas the recommended treatment concentration is 1 ppm (6). A laboratory study inferred that the decomposing vegetation after 2,4-D treatment could produce anoxic conditions, presenting a secondary hazard to fish (7).

Ecosystem studies on 2,4-D (4,8,9,10) have investigated several components of the food chain to determine uptake and persistence of the chemical in the biota, and possible toxic effects. Our approach utilized experimental ponds to study the impact of 2,4-D on the components of the ecosystem, and on community structures. The ponds, as closed systems, were considered well suited for the study of the fate and persistence of the chemical. A critique of using such ponds is given elsewhere (11).

This paper summarizes the results of a two-year study (1980/81) and is based on two internal reports which document all findings in detail (12,13).

Experimental

Site Preparation. Six ponds, about 10x20 m each with a depth of 1.5-1.8 m, were excavated in an isolated location near Winona, Ontario, a year before the 2,4-D experiments. The ponds were lined with four layers of 6 mil black polyethylene, with sediment placed over the bottom and the sides. They were filled with water from a nearby pond, in a way that insured the introduction of both planktonic and benthic organisms. Milfoil was planted in the level sediment area of each pond during the fall prior to the treatment year. Shortly thereafter, twenty common shiner (Notropis cornutus) were added to each pond.

Pisidiid clams (Sphaerium rhomboideum) were collected from a natural pond just prior to the experiment. Ten clams were placed in each of 72 containers with 12 placed in each pond.

Environmental Sample Collection and Analyses. Samples were collected from mobile bridges constructed for this study. A diving platform was attached to each bridge so that a Scuba diver lying on the platform could be moved about the pond for sediment sampling and plant growth measurements without agitating the sediment.

Composite water samples were subsampled for bacteria, water column fungi, phytoplankton, protozoa, water chemistry, and particulate material. Samples were generally collected fortnightly during periods of open water, monthly during the winter, but a more frequent sampling schedule was used immediately before and after the chemical treatments.

On June 25, 1980, two ponds were treated with the N,N-dimethylamine formulation of 2,4-D, and two with the butoxyethyl ester (AQUA-KLEEN). The additions were calculated to produce nominal concentrations of about 1 ppm, the recommended dosage for milfoil control. The two remaining ponds were used as controls. On July 5, 1981, the two former control ponds were treated with the amine and the ester, respectively.

The water chemistry parameters of nitrate, nitrite, ammonia, TKN, particulate nitrogen, filtered, unfiltered and reactive phosphorus, particulate and dissolved organic carbon, alkalinity, calcium, magnesium, chloride and sulphate ions were determined according to the methods of IWD, Environment Canada (14).

Water samples for 2,4-D analysis were collected in 1 L amber bottles containing 2 g each of XAD-2 and XAD-7 ion exchange resin. The samples were acidified with 4 mL concentrated sulphuric acid and stored at 4°C in the dark. The 2,4-D was desorbed from the resin with ethyl ether. The extract was dried, reduced in volume, diazotized, and analyzed on a gas chromatograph with an electron capture detector (12,13,15).

Sediment samples were first extracted with 50 mL of 0.1M Na_3PO_4, then the centrifuged and filtered extract was acidified and extracted with ethyl ether. The extract was then treated as those from the water samples and analyzed by GC.

Biological Sample Collection and Analyses. Clam and fish samples were digested in concentrated HCl, then extracted with benzene. The extract was treated as above. Milfoil samples were Soxhlet extracted with a 60:40 benzene-methanol mixture. The extracts was washed with 0.5M NaOH and the aqueous phase was extracted, after acidification, with ethyl ether. The extract was handled in the same way as the water extracts.

Acute toxicity tests were conducted on clams and white sucker fry. Young clams from a permanent pond were used in 120-hr laboratory test to determine the acute toxicity curves for 2,4-D acid at pH 7.9 and 2,4-DCP at pH's of 6.8 and 8.8. Acute toxicity to white sucker fry was determined by placing several mesh-covered containers of the fry in the experimental ponds daily, for one week, after the 2,4-D additions, and recording the mortalities for each 24-hour period.

Bacteria and fungi were quantified in the 1980 study season, using standard sampling and plating techniques. Phytoplankton samples were collected in both years to determine both populations and community structures. Equally intensive efforts were made to analyze zooplankton (protozoans and mesozooplankton) and zoobenthos (including clams and snails).

Milfoil growth was monitored by measurements of total stem length and carbon dioxide uptake. General observations were made about the makeup of the whole macrophyte communities before and after the milfoil treatments.

Results and Discussion

Both herbicide formulations killed the milfoil in 2 to 3 weeks at the nominal concentration of 1 ppm in the water. Actual concentrations in the water column were significantly below 1 ppm during most of the study period. Attempts were made to recolonize the treated ponds with fresh milfoil 55 and 86 days after the treatments. Residual 2,4-D concentrations above 0.1 ppm killed the new milfoil, confirming reported laboratory results (16). Concentrations just below 0.1 ppm caused excessive sublethal effects (fused leaves). The nominal 1 ppm 2,4-D concentration recommended for milfoil control thus provides a tenfold margin to allow for drift and dilution in natural water bodies.

The main difference between the two formulations was the immediate availability of 2,4-D in the water column from the amine, compared to its slow release from the ester pellets. The spraying of the amine (in pond 2) resulted in aerial drift of the chemical, causing sublethal effects on the milfoil in an adjacent control pond one week later. Analysis of the water showed no 2,4-D in the affected pond, but 0.075 ppm 2,4-DCP was detected for about 1 hour after the drift event. Applying the amine under the water surface (in pond 5) eliminated the drift and resulted in a more immediate distribution of the chemical in the water column.

The 2,4-D concentrations in the water are shown, for the two years of the study, in Figures 1 and 2. The short term data show that 2,4-D was almost immediately available from the amine, but was more slowly released from the ester-containing pellets. After this initial variation, the disappearance of the 2,4-D from the water followed similar patterns.

The 2,4-D remained detectable in the water phase for about four months after application, with the concentrations remaining above the recommended drinking water quality standards of 0.1 ppm for most of this period. The disappearance of 2,4-D from the water column followed first order kinetics, with the calculated half lives of 17 and 35 days in the two years.

Dichlorophenol (2,4-DCP) was present in the system both as an impurity in the formulations and as a degradation product of 2,4-D. Its presence was observed in the water column for most of the study period in 1980. In the following year, probably due to the higher water pH, it was present at lower concentrations and for a shorter period. The 2,4-DCP concentrations were generally about ten times lower than that of the parent acid, and were more variable during the study.

The 2,4-D concentrations in the sediment are shown in Figures 3 and 4. In 1980, the chemical appeared in the sediment of the ester ponds immediately after treatment, but was found in the amine pond sediments only after the milfoil's collapse. The release of 2,4-D from decomposing milfoil appeared to be a significant source. The chemical persisted in the sediment for about as long as it was detected in the water column. The 2,4-DCP was also observed in the sediments, at concentrations about an order of magnitude lower than that of the parent acid, as long as the 2,4-D was detected.

Fish and clam tissues contained some 2,4-D and 2,4-DCP in all treated and control ponds, but no significant trends were observed. Milfoil from the treated ponds contained measurable amounts of 2,4-D, but no 2,4-DCP was detected.

Laboratory toxicity tests showed that 2,4-D was not toxic to clams at a concentration of 250 ppm. The 2,4-DCP, on the other hand, was toxic with a 96 hr LC_{50} of 23.4 and 65.9 ppm at pH 6.8 and pH 8.8, respectively, indicating that the phenol was more toxic than the phenolate ion (17). White sucker fry exhibited limited mortality during the first six days in the treated ponds in the first treatment year. No mortality was observed after six days. On the first day only, the amine ponds produced a higher mortality than the ester ponds (18). The lower water pH in the first year shifted the phenol-phenolate equilibrium for DCP in favour of the more toxic phenol form, resulting in the observed mortalities, in agreement with the laboratory clam studies. Adult common shiners in the ponds were not affected.

The various biological components of the pond ecosystems showed minimal or no response to the treatments. The bacteria populations were unaffected following the treatment, but showed some increases in the treated ponds after the collapse of the milfoil beds. Phytoplankton and zooplankton biomasses and diversity were generally unaffected, although the 1981 samples indicated possible marginal effects on a few of the phytoplankton species (19). The zoobenthos were not directly affected by the treatment. After the collapse of the milfoil, a community shift occurred from chironomid to tubificid dominated communities as a secondary effect of the treatment. The number of snails was observed to increase as a secondary effect, i.e. after the milfoil collapse. The growth of the clams was inhibited in the treated ponds during the first one to three weeks after the applications. Their subsequent growth, on the other hand, was enhanced by the increased food supply. No effects were observed on the clams' reproductive capacity.

The macrophytes predominant in the control ponds (Elodea, Potamogeton and Typha) were inhibited in the treated ponds, in which Chara dominated after the milfoil treatments.

The collapse of the milfoil produced anoxic conditions in the sediment, but did not result in significant oxygen depletion or nutrient enrichment in the water column. Calcium concentrations and alkalinity were observed to increase in 1981, once milfoil decomposition began, probably due to dissolution of calcium carbonate from the milfoil surfaces. Young plants of cattails (Typha) did not colonize the shoreline of the treated ponds until the 2,4-D concentrations had declined.

The pond ecosystems used in this study showed remarkably little variation from pond to pond with respect to water quality parameters and most of the biological components. Phytoplankton populations, on the other hand, were extremely variable, masking possible subtle effects on community structures. The year allowed for the stabilization of the ponds appeared to be sufficient for the establishment of indigenous ecosystems.

Summary

Both 2,4-D formulations were shown to be effective in milfoil control at the recommended 1 ppm nominal concentration.

The 2,4-D concentrations in the water were halved in 17 to 35 days, probably dependent on water pH. Concentrations decreased to non-detectable levels in both water and sediment by late fall.

The 2,4-DCP was present both as an impurity in the formulations and a degradation product of 2,4-D. Although its concentrations were generally an order of magnitude lower than that of the parent acid, it was probably responsible for an initial fish fry mortality, an initial inhibition of clam growth, and some sublethal effects on milfoil in an aerial drift event.

The six individual ponds were similar and were "in phase" in the variations of several water quality parameters. Significant water quality changes included increased pH in all ponds during the second year due to increased macrophyte growth, and increased alkalinity and calcium ion concentrations in the treated ponds in the second year arising from the dissolution of calcium carbonate from the decaying milfoil.

The ponds were also similar in terms of most biological variables, but were often "out of phase" with respect to abundances of individual species of phytoplankton.

The treatment showed no measurable effect on bacteria, fungi, phytoplankton and zooplankton, with possible secondary effects on bacteria and phytoplankton. Some fish fry mortality was observed in the first days of the 1980 treatment, probably due to the presence of 2,4-DCP and to low water pH. An initial inhibition of clam growth was also attributed to the presence of 2,4-DCP. The growth of other water weeds was also inhibited and Chara became the dominant macrophyte in the treated ponds.

Secondary effects of the treatment included increased bacteria populations, enhanced growth of clams and snails after the collapse of the milfoil beds, due to an increased food supply, and a shift in the zoobenthic community from chironomid to oligochaete populations.

The pond study allowed a thorough survey of chemical and biological variables in a closed ecosystem. The absence of drift and dilution processes produced a "worst-case" scenario. The observed primary and secondary effects of 2,4-D application would probably be reduced or undetected in more open ecosystems.

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M. Dickman (Brock U.) - phytoplankton;
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One of the authors, B.F. Scott, was responsible for the pond design and overall coordination of the study.

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Figure 1. 2,4-D concentrations in the water (1980).

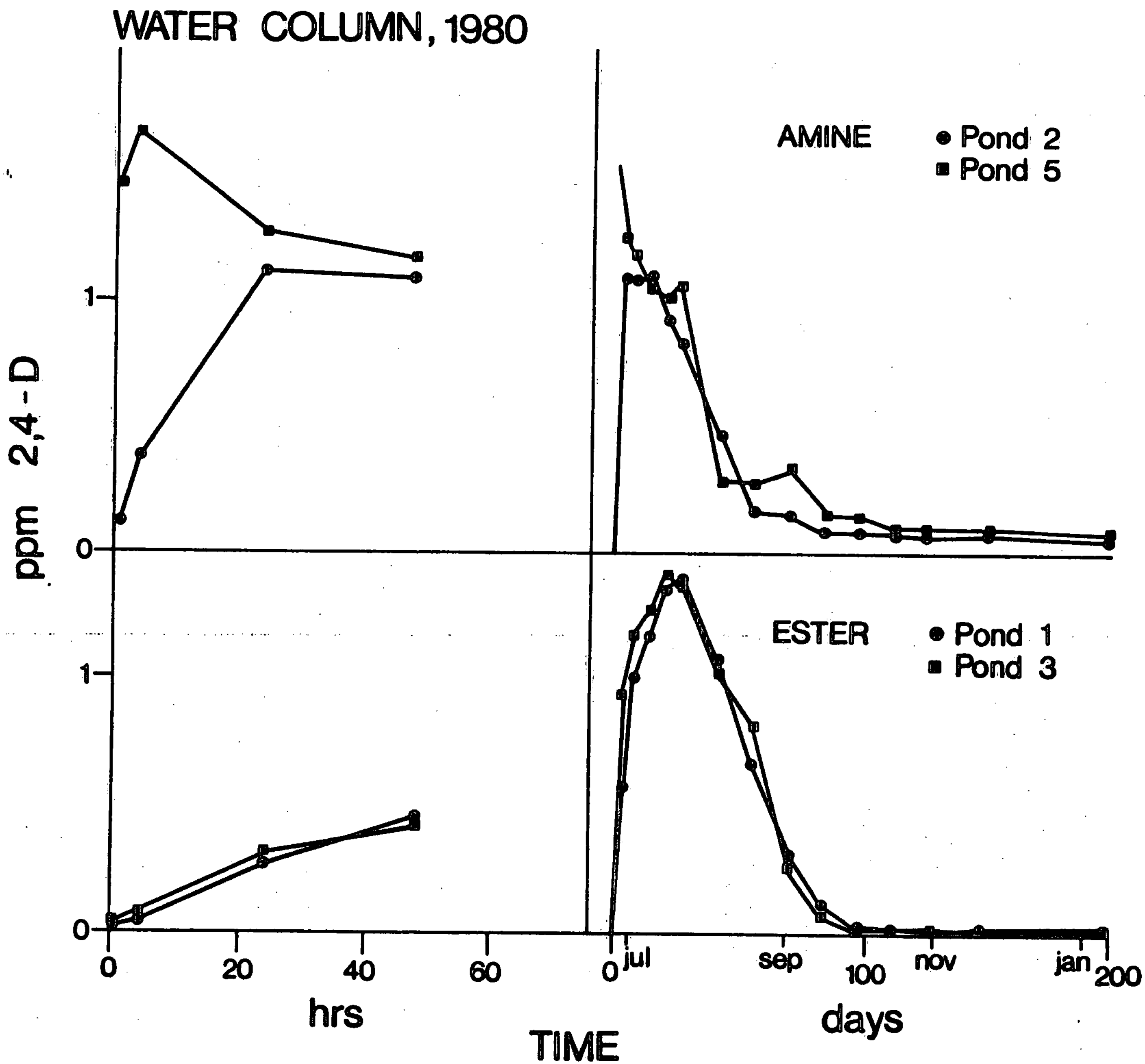

WATER COLUMN, 1980
ppm 2,4-D
AMINE
Pond 2
Pond 5
ESTER
Pond 1
Pond 3
0
1
0
1
0
20
40
60
hrs
0
jul
sep
100
nov
jan
200
days
TIME

Figure 2. 2,4-D concentrations in the water (1981).

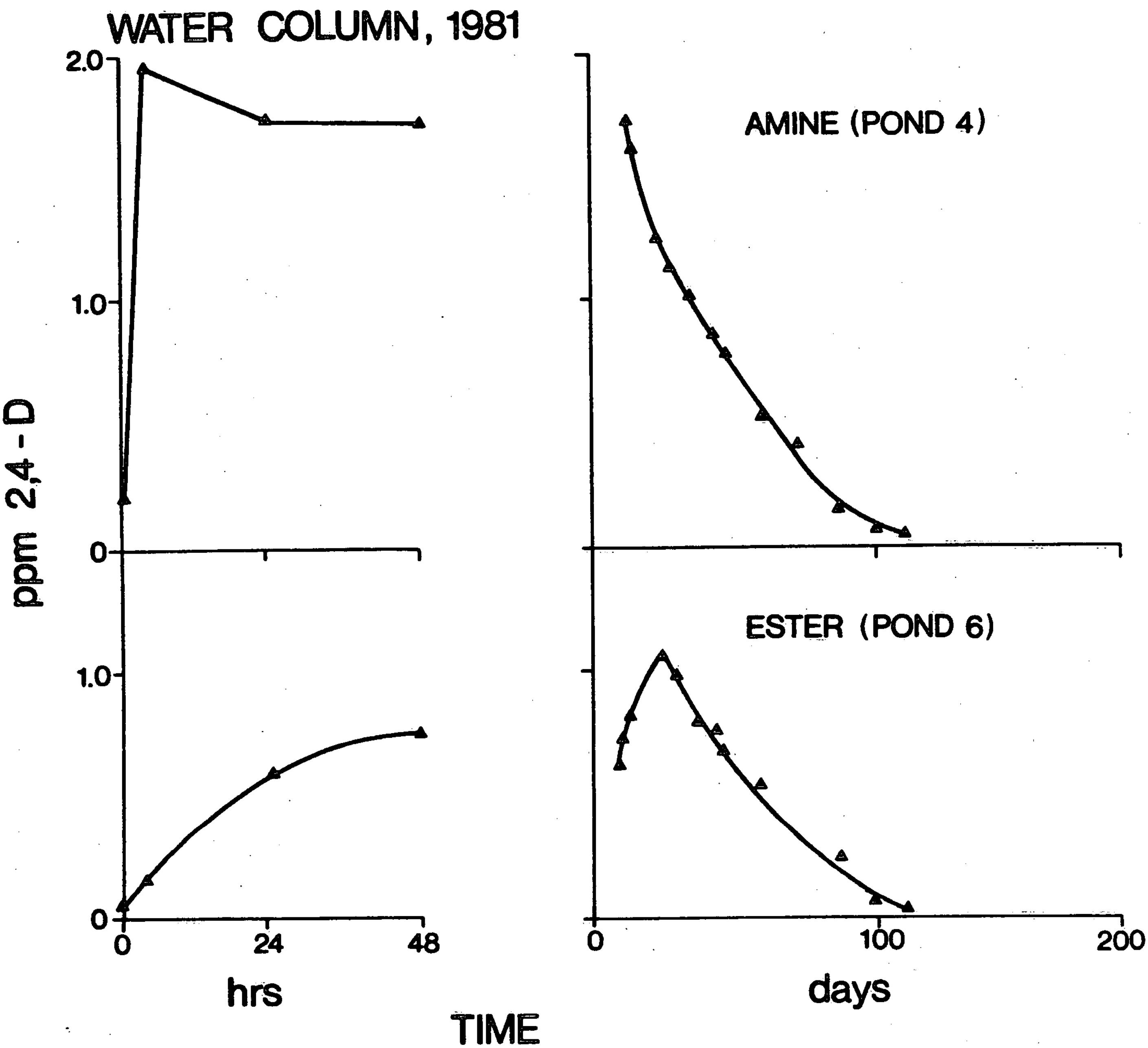

WATER COLUMN, 1981
AMINE (POND 4)
ESTER (POND 6)
ppm 2,4-D
2.0
1.0
0
1.0
0
0
24
48
hrs
0
100
200
days
TIME

Figure 3. 2,4-D in the sediment (1980).
(per 10 cm² of bottom)

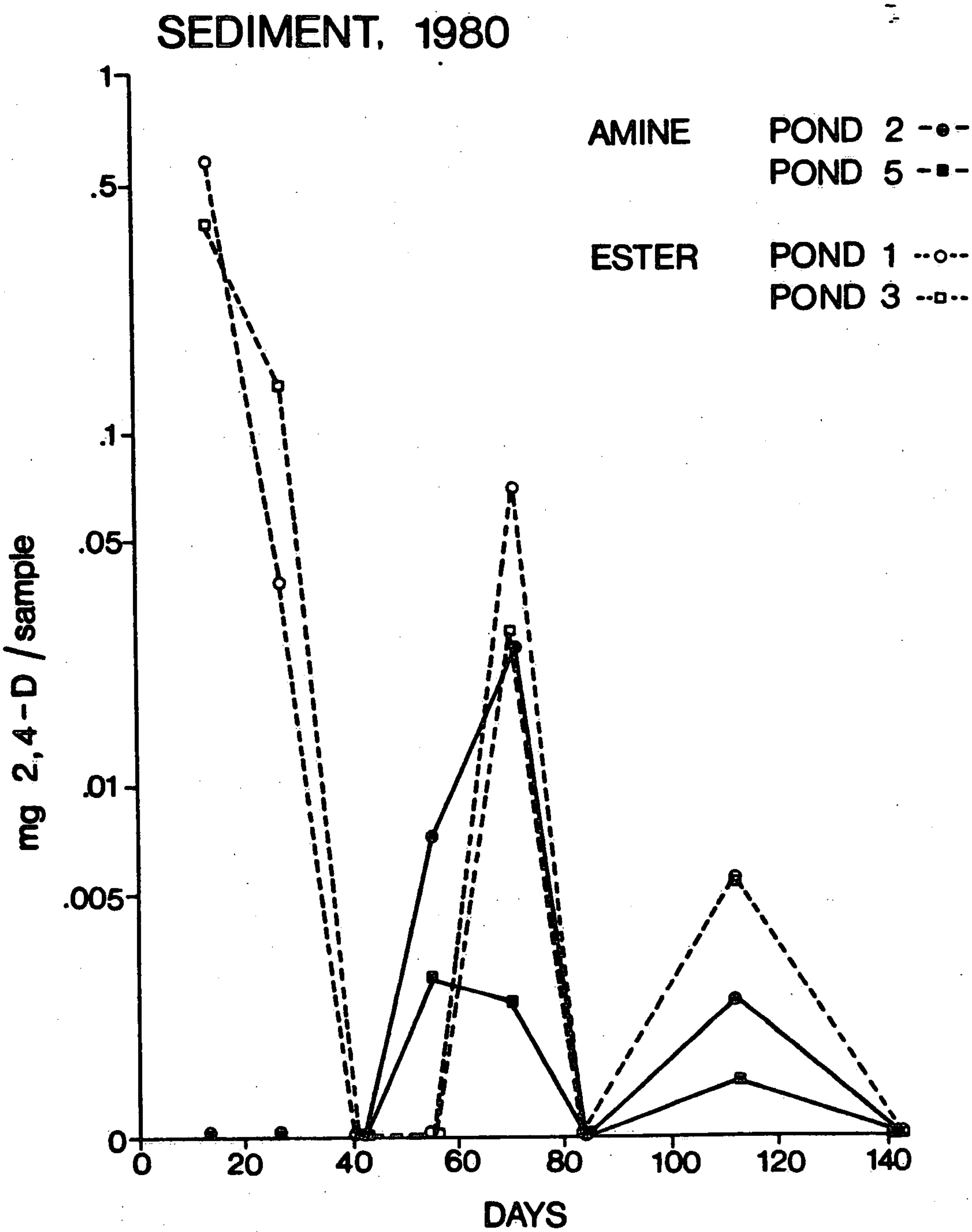
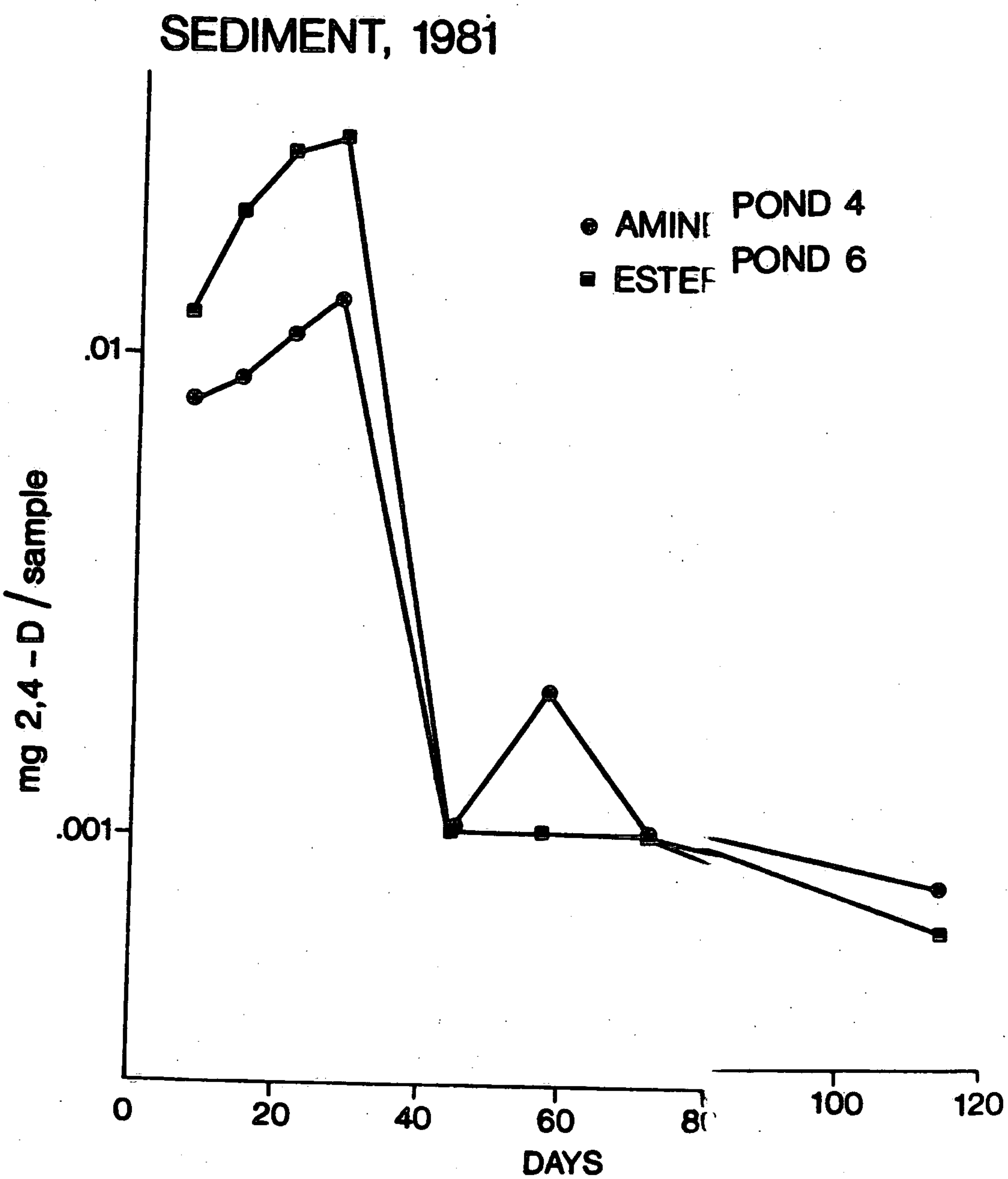

SEDIMENT, 1980
AMINE
POND 2
POND 5
ESTER
POND 1
POND 3
mg 2,4-D /sample
1
.5
.1
.05
.01
.005
0
0
20
40
60
80
100
120
140
DAYS

Figure 4. 2,4-D in the sediment (1981).
(per 10 cm^2 of bottom)



SEDIMENT, 1981
● AMINE POND 4
■ ESTER POND 6
.01
.001
mg 2,4 - D / sample
0
20
40
60
80
100
120
DAYS