MICROBIAL DEGRADATION OF THE LAMPREY LARVICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL IN SEDIMENT-WATER SYSTEMS

Great Lakes Fishery Commission

TECHNICAL REPORT No. 18

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MICROBIAL DEGRADATION OF THE LAMPREY LARVICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL IN SEDIMENT-WATER SYSTEMS

by

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ABSTRACT

The selective lampricide 3-trifluoromethyl-4-nitrophenol (TFM), maintained in the water at concentrations of 1 to 6 μg/ml for several hours, kills larval sea lampreys (Petromyzon *marinus)* in tributaries of the Great Lakes. Because the fate of TFM in the environment is a matter of concern, the interactions of this chemical with river and lake sediments were studied in laboratory experiments. In mixtures of TFM, water, and sediment held in aquariums, the TFM decreased progressively and nearly or completely disappeared in 1 to 4 weeks; concentrations of the fluoride ion increased; and the systems became nontoxic for sea lamprey larvae and goldfish *(Carassius auratus).* If the reduction in TFM ceased before ail of the chemical had disappeared, the process resumed when nutrient broth was added. Loss of TFM from the systems was prevented by the addition of an antiseptic (phenol) and by heat sterilization. Enrichment cultures of microorganisms isolated from stream and lake sediments degraded TFM in nutrient broths. I conclude that TFM is degraded by microorganisms that live in sediment-water systems.

INTRODUCTION

The sea lamprey *(Petromyzon marinus)* is controlled in the Great Lakes by the introduction of the sodium salt of 3-trifluoromethyl-4-nitrophenol (TFM), a quantitatively selective larvicide, into tributary streams (Applegate, Howell, and Smith 1958), usually at concentrations of 1 to 6 μg/ml (Baldwin 1968). Most of the TFM leaves the tributary within a few days but some probably is retained by bottom sediments. Its ultimate disposition in streams and the Great Lakes is unknown.

The present study, designed to gain insight into the fate of TFM in the environment, consisted primarily of two series of experiments. First, TFM concentrations were measured periodically in different mixtures of TFM, water, and bottom sediment held in laboratory aquariums under different conditions: (1) Concentrations of TFM were followed in the supernatant water after the chemical was introduced into sediment-water systems with different organic contents. (2) Attempts were made to recover TFM from the sediment in systems in which the chemical had disappeared from the water. (3) Lamprey larvae and goldfish *(Carassius* auratus) were exposed to systems from

1 Contribution 461, Great Lakes Fishery Laboratory, U.S. Fish and Wildlife Service, Ann Arbor, Michigan 48107.

which TFM could not be recovered. (4) Supernatant water in systems from which TFM was disappearing was analyzed for the presence of fluoride ions.

After these tests indicated that TFM was lost from the sediment-water systems, possible causes of the loss were investigated in the following experiments: (1) Sediment-water-TFM systems were held at different temperatures (6-85 C). (2) Systems were biologically inactivated with phenol or by heat sterilization. (3) Nutrients were added to systems in which the TFM concentration had stabilized after initial loss. (4) Culture media containing TFM were inoculated with enrichment cultures of bacteria that originated from river and lake sediments.

MATERIALS AND METHODS

TFM

Physical, chemical, and biological properties of TFM have been described (Applegate and King 1962; Smith, Applegate, and Johnson 1961) and methods for its detection and measurement have been recorded (Smith, Applegate, and Johnson 1960).

Pure TFM recrystallized from benzene was routinely employed for these experiments, although technical grade TFM (which is used in field applications) was occasionally used as a check on the results. Technical TFM (Maumee Chemical Company, Toledo, Ohio) was purified by first dissolving it in hot, reagent-grade benzene and cooling it to room temperature; the resulting crystals were then collected on a vacuum filter, washed with cold benzene, and dried in vacuum. The melting point of technical grade TFM was 70-72 C and that of the recrystallized material 74-76 C. Titration of the recrystallized TFM against a carbonate-free sodium hydroxide solution indicated the purity to be 96(±4)%. A standard 1,000 μg/ml solution of purified TFM in distilled water or 0.85% NaCl was used in routine experiments.

The colorimetric detection method used in the present studies is applicable when TFM concentration is 0.1 μg/ml or greater (Daniels et al. 1965). The chemical imparts an intense yellow color to water above pH 8.0; such solutions have a light absorption peak at 395 nm. When turbidity of the water interfered, a portion of the sample was first clarified in an angle-head clinical centrifuge. The pH of the supernatant fluid was raised slightly above 8 by the addition of a measured volume of NaOH, usually 5 or 6% (w/v), before samples were placed in a Beckman DU or Spectronic 20 spectrophotometer.

Degradation of TFM was defined by three criteria: (1) total loss of the yellow color of TFM from the supernatant water of mud-water systems during incubation; (2) loss of toxicity for sea lamprey larvae and goldfish; and (3) increase in F^- concentration in the water.

The stability of TFM at different temperatures and in boiling water or nutrient medium was demonstrated by the following observations and tests:

(1) Aqueous solutions of TFM were stable at room temperature. The concentration of recrystallized TFM did not change detectably in a 3.7 μg/ml solution stored for several weeks in a glass bottle on a laboratory shelf, or in a standard solution (1,000 μg/ml of recrystallized TFM in 0.85% NaCl) similarly

stored for more than a year; and no color was lost from the supernatant water in test aquariums unless bottom sediment was present. In the presence of bottom mud, some TFM always disappeared from the water almost immediately after initial mixing. This loss was believed to be due to the physical process of sorption by particles and colloids. The concentration of TFM in the water stabilized within one to several hours and remained constant indefinitely in sterile systems or in those in which the sediment consisted only of clean sand.

(2) TFM was also stable at elevated temperatures. When solutions containing 22.4 μg/ml TFM in distilled water were adjusted to pH 5.0, 7.0, and 10.0 by the addition of a concentrated NaOH solution, and 3-ml portions of these solutions in open test tubes were autoclaved in steam at 15 psi g pressure for 2 hours, the TFM concentration did not change. Thus no TFM was lost by either decomposition or volatilization under these severe conditions.

(3) TFM was stable in boiling water. When 100 ml of a solution of TFM in distilled water was adjusted to pH 8.0 with NaOH, diluted with distilled water to a concentration of 75 μg/ml, and boiled in an Erlenmeyer flask, and the water evaporated during boiling was replaced with distilled water, the measured concentrations of TFM after intervals of 10, 15, and 45 minutes were 75, 76, and 73 μg/ml TFM, respectively.

(4) TFM was also stable when boiled for 10 to 45 minutes in trypticase soy broth (TSB; Baltimore Biological Laboratories, Baltimore, Maryland) at pH 7.0.

Bottom sediments

Bottom sediments were collected by scraping (manually or with a Petersen dredge) the upper few centimeters of sediment from the bottom; they came principally from the Pine River, Iosco County, and the Pere Marquette River, Mason County, Michigan. Accessory tests (not discussed in detail here) were made with sediments from other Great Lakes tributaries and from various localities in the Great Lakes. The muds were placed in glass jars, covered loosely, and refrigerated until used. No preservatives or other chemicals were added. Where indicated, the organic contents of muds were determined by measuring the loss upon ignition in an electric muffle furnace at 600 C (American Public Health Association 1965).

Enrichment cultures

Mixed cultures of native microorganisms used in some of the present experiments were obtained from bottom sediments by transferring two loopfuls of mud into a sterile tube of TSB containing 75 μg/ml TFM, and then incubating the inoculated media at 22 C. Alternatively, 10 cc of mud were added to 120 ml of fluid thioglycollate medium (Difco Laboratories, Inc., Detroit, Michigan) containing 10 μg/ml TFM. When a culture developed, usually after 4 to 6 days of incubation, a loopful of the actively growing culture was aseptically transferred to another tube of sterile broth and incubated as before. The cultures were ready for use after four such transfers.

They were usually carried in the same medium from which they were isolated. Alternatively, the cultures were transferred to solid media of similar composition (except for addition of 2% agar), incubated until surface cultures developed, and then refrigerated in screw-cap culture tubes. These cultures remained stable for several weeks when stored at 4 C.

All muds tested by this enrichment process yielded bacterial cultures. When plated out on trypticase soy agar and grown under aerobic conditions, the cultures were all of one colonial type. Cultural, staining, and sugar reactions identified the colonies as *Pseudomonas* sp.

Aquarium experiments

Experiments were carried out in laboratory aquariums, which usually consisted of 500-cc Erlenmeyer flasks. The flasks were covered with glass plates or loosely fitting foil overlaps that reduced evaporation and excluded debris but permitted free interchange of gases.

In a typical experiment, 117 ml of distilled water, 3 ml of a 1,000 μg/ml TFM solution, and 50 cc of bottom sediment were mixed in a flask. Controls, consisting of mixtures of mud and distilled water, were used in all experiments; this precaution allowed for correction of the slight color that often developed at 395 nm in mud-water systems after prolonged incubation. The flasks were shaken and the mixtures were then incubated at rest, at 22 C (unless otherwise indicated). Initial TFM readings were taken in the supernatant water in about 1 hour, after the solids had settled. Most of the mixtures provided an initial TFM concentration of about 25 μg/ml in the supernatant water. The implied assumption that the mud was inert and contained little free water followed from the observation that the mud compacted to a gelatinous paste when stored in a refrigerator. The small amount of water that sometimes accumulated on the surface was drained before the mud was used. The status of the remaining water was debatable; for example, it could have been adsorbed to solids or present as a component of gels. In the present experiments such water was regarded as part of the mud rather than as an independent phase. The pH of the water in the aquariums was normally 6.8 ± 0.2. Distilled water was used routinely both for setting up the experiments and for replacing small evaporative losses (less than 1% per week). Substitution of water from Lake Erie for distilled water had no apparent effect.

EVIDENCE OF LOSS OF TFM IN SEDIMENT-WATER SYSTEMS

Effect of bottom sediments on TFM

In a typical experiment all TFM disappeared from the water in about 24 days in a flask containing Pine River mud, whereas the TFM concentration stabilized at 1 to 2 μg/ml in a flask containing mud from the Pere Marquette River and at 23 μg/ml in a flask containing clean sand (Fig. 1). Muds from the Pine and Pere Marquette Rivers contained 11.7 and 5.2% organic matter, respectively; the sand contained none. These results suggest a correlation

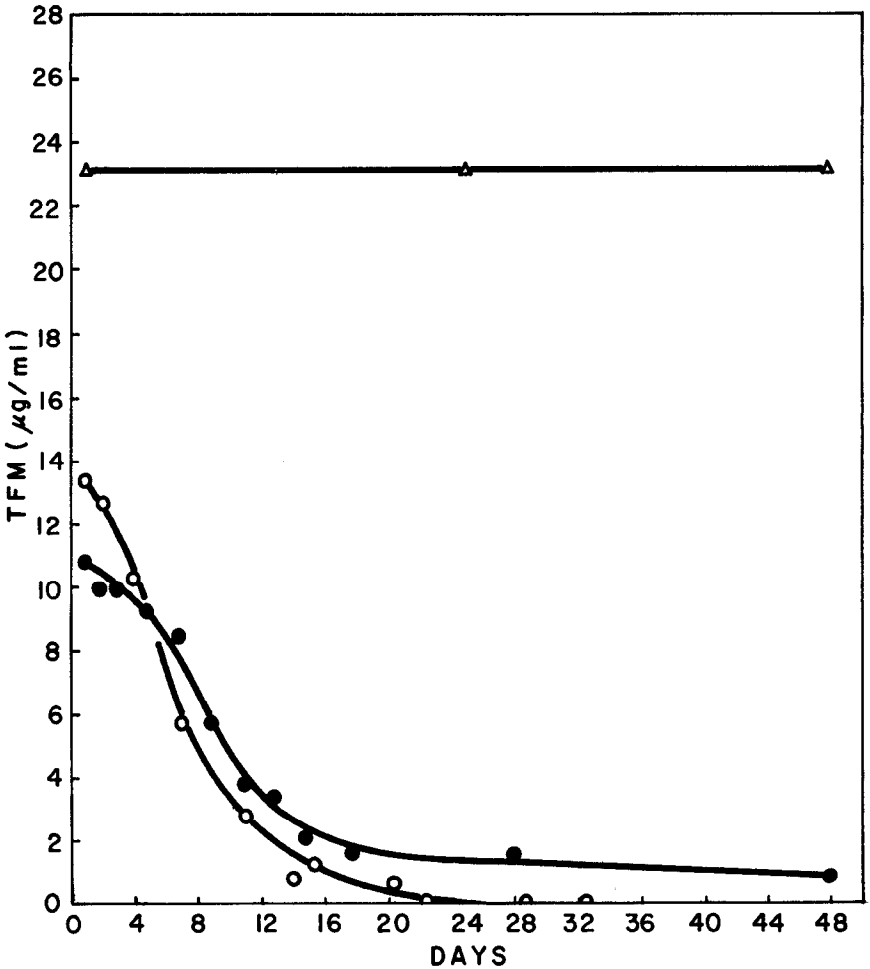

Figure 1. Changes in concentration of TFM in water incubated with sand (triangles), mud from the Pere Marquette River (solid circles), and mud from the Pine River (open circles). Each flask contained 50 cc of mud or sand, 117 ml of distilled water, and 3 ml of a 1,000 μg/ml TFM solution. Initial concentration of TFM, about 25 μg/ml; incubation temperature, 22 C. Curves drawn by inspection.

between disappearance of TFM from the supernatant water and the organic content of bottom sediments.

In experiments on the recovery of TFM from sediments, muds from experiments similar to that shown in Figure 1 were first examined for the presence of TFM that could have possibly been retained in particles by sorption, base exchange, or capillary entrapment. For this purpose, 4 ml of 1,000 μg/ml TFM solution, 30 g of Pere Marquette River sediment, and 46 ml of distilled water were added to each of nine flasks. Nine control flasks contained 30 g of sediment and 50 ml of distilled water. After the successive

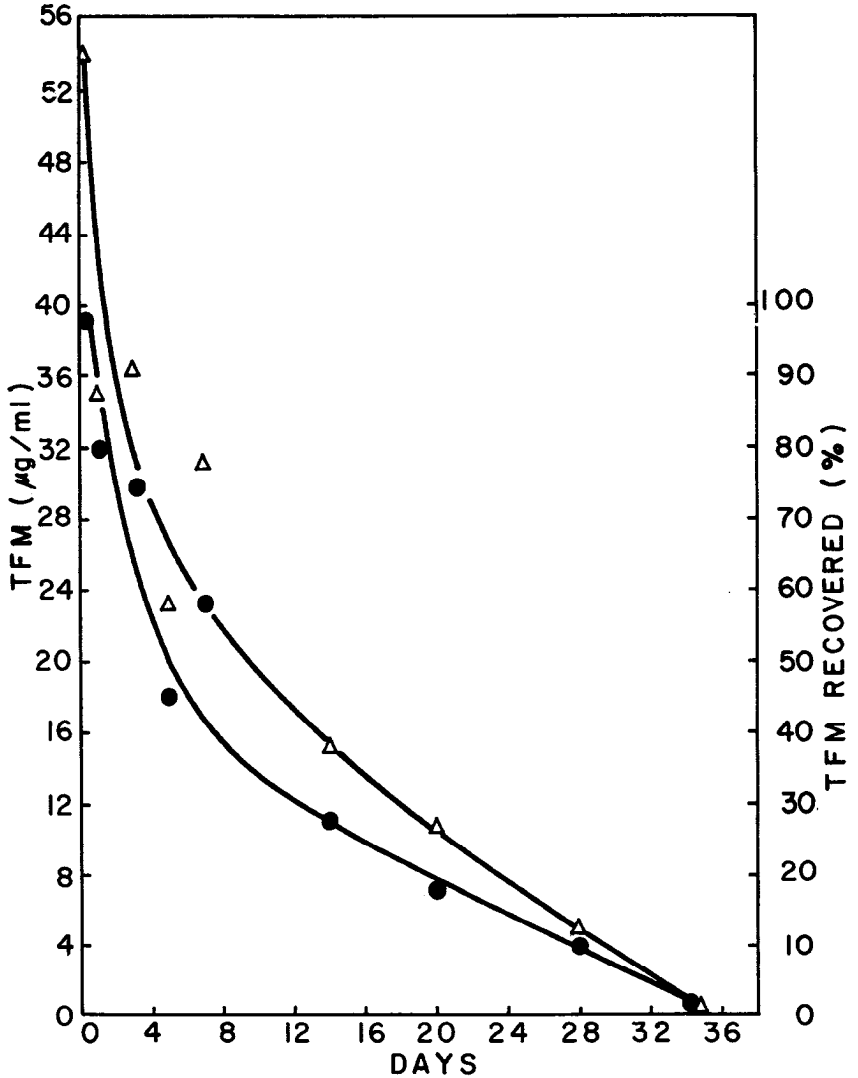

Figure 2. Percentage of total TFM recovered (solid circles, scale at right) after different intervals, from a mixture of distilled water and Pere Marquette River mud in flasks containing a TFM solution in which the concentration (triangles, scale at left) progressively decreased during incubation. Initial concentration of TFM in the water, about 80 µg/ml; incubation temperature, 22C. Curves drawn by inspection.

periods of incubation shown in Figure 2 (1 hour to 34 days), determinations were made of the amount of TFM remaining in the supernatant water (by the method previously described) and in the bottom sediment. The TFM content of the sediment was determined by filtering the mud on a Büchner funnel, washing the residue with distilled water, and then combining all of the washings with the supernatant fluid. Approximately 99% of the TFM initially

added could be consistently recovered within the first hour by this procedure. As incubation progressed, however, less and less TFM could be recovered from the system, until after 34 days (in the experiment illustrated by Figure 2) none could be recovered by this technique.

Recovery of TFM from muds was next attempted by extracting the mud with acid (for release of substances attached to calcium carbonate and other acid-soluble components and for conversion of TFM into a less ionized condition) and then with benzene (because unionized TFM should be more soluble in a nonpolar solvent than in water). In this procedure, 120 ml of a 25 μg/ml solution of TFM in distilled water and 50 cc of Pine River mud were placed in each of four flasks and incubated at 22 C. After 32 days no TFM could be detected in the water. Then, 2.5 ml of 2N H_2SO_4 were added to each flask, the mixture was shaken and allowed to stand for about 1 hour at room temperature, and the mud was filtered out on a Büchner funnel. The pH of the filtrates, which reached 1 to 3 during this treatment, was then raised to slightly above 8 by the addition of 5% NaOH so that the TFM level could be measured. No TFM was found in any of the filtrates.

The acid-extracted muds were next dried in air, pulverized with mortar and pestle, extracted with benzene, and suction filtered. The benzene filtrates were extracted with 5% NaOH and the water phase was then tested for TFM. Again, no TFM was found.

Toxicity of mud-water-TFM systems to sea lampreys and goldfish

The degradation products of TFM that remained in the mud-water systems after incubation for sufficient time to render TFM colorimetrically undetectable in the supernatant water were harmless to sea lamprey larvae and to goldfish. This lack of toxicity was demonstrated in an experiment in which Pine River mud and 25 μg/ml technical grade TFM solution were incubated at room temperature until the TFM concentration in the water fell to 0.2 μg/ml; three lamprey larvae subsequently held for 2 months in the mud and three goldfish held for 3 days in the water showed no ill effects.

The toxicity of the original TFM to goldfish was demonstrated by placing five goldfish in a 25 μg/ml solution of this chemical. All of the fish jumped violently within 5 minutes, turned on their sides within 10 minutes, and died within 30 minutes.

Release of fluoride ion

The next experiment concerned the possible presence of F^- in the supernatant liquid of mud-water systems in which TFM (i.e., the trifluoromethane group) was degraded: In one flask 250 cc of Pine River mud were mixed with 1,170 ml of distilled water and 30 ml of a 1,000pg/ml TFM solution; a control flask had the same contents except that no TFM was added. The mixture in the test flask initially contained less than 0.1 μg/ml F^- as measured with an ion-selective electrode (Rechnitz 1967).

In the test samples, slightly more than one-fifth of the fluorine added as TFM was released into the supernatant fluid during incubation, as a degradation product of TFM (Fig. 3). Concentrations of F^- in the control varied

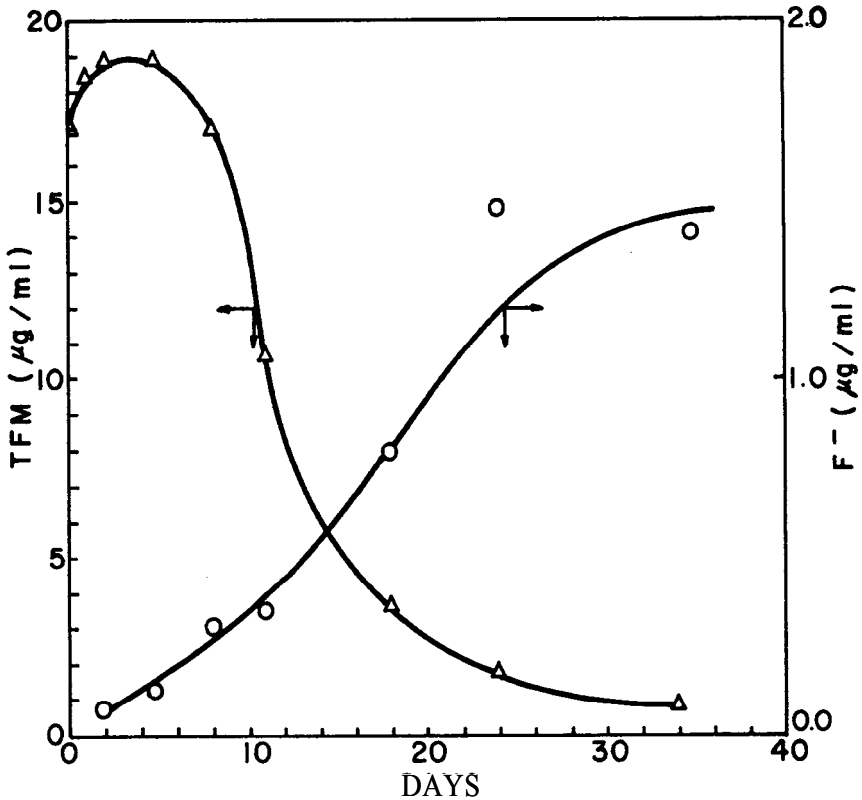

Figure 3. Release of F^- µg/ml, open circles, scale at right) to the supernatant fluid of a system containing 1,170 ml distilled water, 250 cc of Pine River mud, and 30 ml of a 1,000 µg/ml TFM solution (concentration in µg/ml, triangles, scale at left). Incubation temperature, 22 C. Curves drawn by inspection.

between 0.05 and 0.25 µg/ml and were usually less than 0.10 µg/ml (these values were subtracted from the concentrations reported in the test samples). The use of mud from Lake Erie and the SPADNS analytical technique (American Public Health Association 1965) yielded identical results.

It is not known whether the remaining fluorine in the TFM remains unchanged or is also released as F^- and then complexed into the mud.

EFFECTS OF TEMPERATURE, STERILIZATION, AND NUTRIENTS ON LOSS OF TFM IN SEDIMENT-WATER SYSTEMS

Disappearance of TFM from supernatant water in mud-water systems might have a number of causes. These could include physical sorption, strictly chemical reactions, or the action of enzymes present in dead cells or functioning in the metabolism of living microorganisms. Any of these mechanisms could operate alone or in unison; probably all are involved but to different degrees under various conditions of such factors as time, temperature, and pH.

Effects of temperature

In general, the mechanisms that may cause degradation of TFM should operate most rapidly at the higher temperatures in the physiological temperature range of normal organisms. In the present study it was not necessary to be overly concerned with temperatures above 60 C, since they do not occur in waters of the Great Lakes or their tributaries.

Since the temperature quotient (Q_{10}) for reaction rates at temperatures differing by 10 C is approximately 2 for chemical or biochemical reactions (Buchanan and Fulmer 1930) the rate of degradation of TFM would be expected to double for each 10 C temperature rise if the reaction were strictly chemical. If the reaction were microbiological, however, the Q_{10} would be

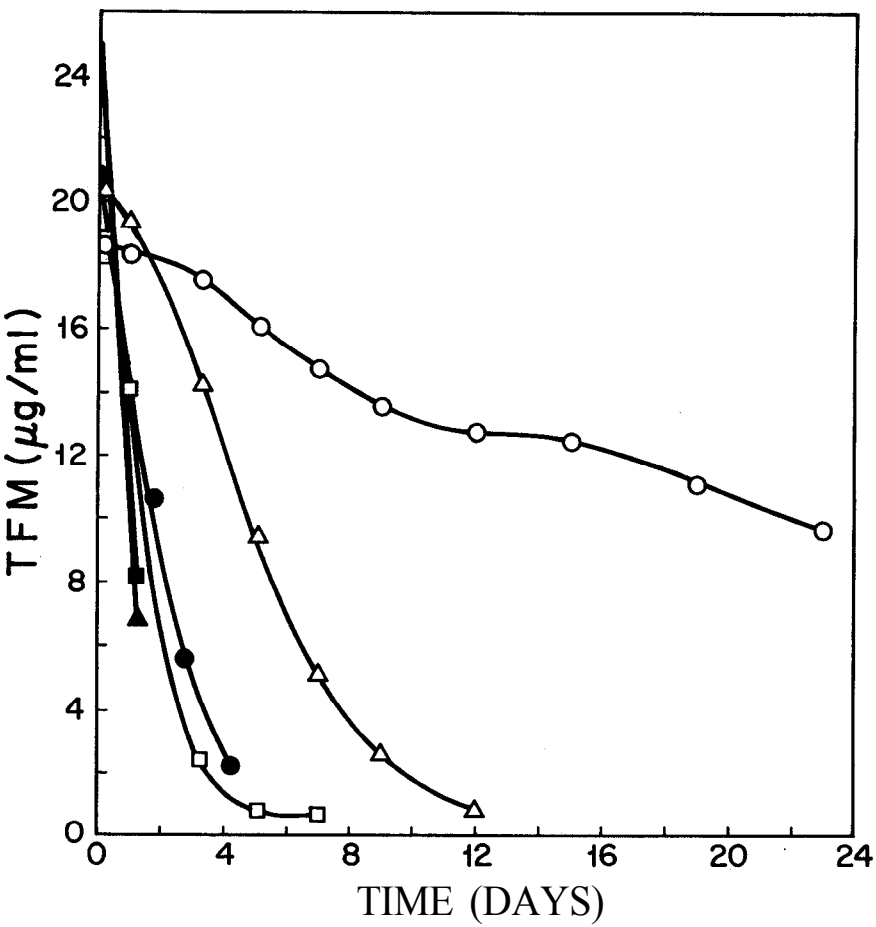

Figure 4. Loss of TFM from mud-water-TFM systems at different incubation temperatures: 6 C (open circles), 22 C (open triangles), 36 C (open squares), 50 C (solid squares), 60 C (solid triangles), and 85 C (solid circles). The flasks originally contained 117 ml of distilled water, 25 cc of Pine River mud, and 3 ml of a 1,000 µg/ml TFM solution. Curves drawn by inspection.

expected to remain near 2 only until an optimum temperature was reached, and then to drop sharply as temperature increased beyond that optimum.

The rates at which TFM disappeared from water incubated with Pine River mud increased with temperature in the range of 6-60 C; however, the rate was slower at 85 C than at 36, 50, or 60 C (Fig. 4).

Effects of an antiseptic

In the tests of the effects of phenol (the antiseptic chosen because it is chemically similar to TFM), 50 cc of mud from the Pere Marquette River and

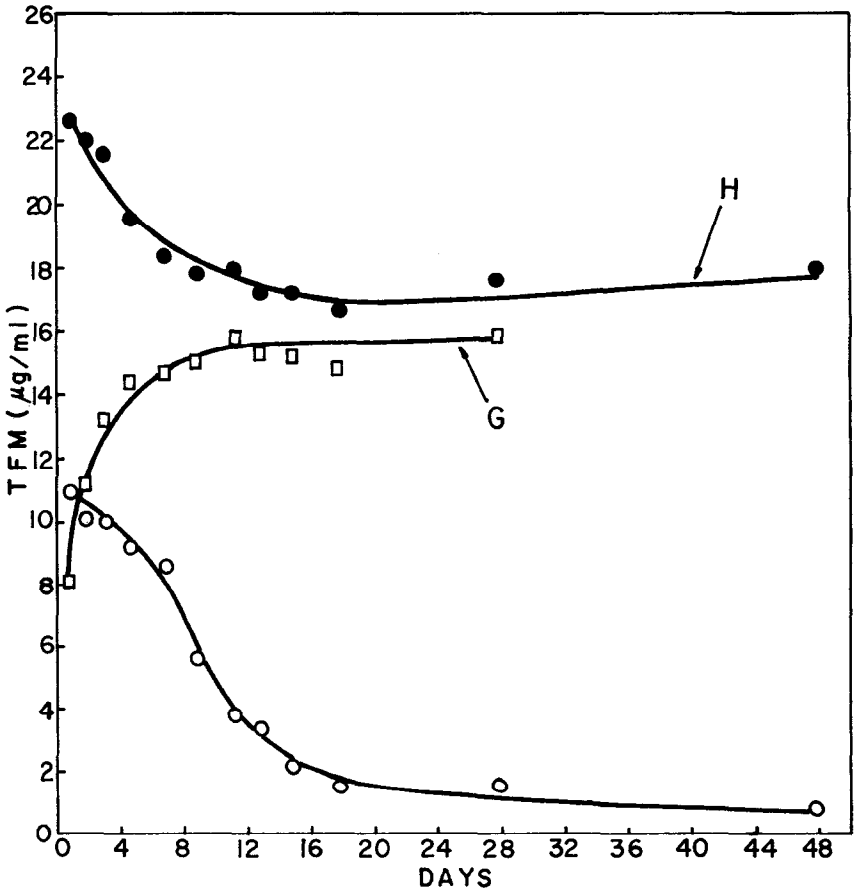

Figure 5. Prevention of TFM degradation in presence of phenol. Each of three flasks contained 50 cc of Pere Marquette River mud and 3 ml of a 1,000 µg/ml TFM solution; two flasks (G,H) contained 117 ml of a 25,000 µg/ml phenol solution. In flask H (solid circles) the mud was mixed with the phenol solution before the TFM was added and in flask G (squares) the mud was mixed with the TFM before the phenol solution was added; in the control (open circles), 117 ml distilled water were substituted for the phenol. Incubation temperature, 22 C. Curves drawn by inspection.

3 ml of a 1,000 μg/ml solution of TFM were placed in each of three flasks. In one flask (H, Fig. 5) 117 ml of a 25,000 μg/ml solution of phenol were added; in the second (G) the TFM was mixed with the mud before the same quantity of phenol solution was added; and in the third (a control) distilled water was substituted for the phenol solution.

Part of the TFM had disappeared from the water in all the flasks after 1 hour, the time at which the initial measurements were made. Although the concentration of TFM then continued to decline in the control, it approached an equilibrium level of about 16 μg/ml in flask G and 17 μg/ml in flask H. Apparently the mud first removed TFM from the water in all three flasks by sorption; the phenol then prevented degradation of TFM in the test flasks. In these test flasks the phenol appears to have first been reversibly sorbed by the muds in a competitive process with TFM. In the absence of TFM degradation, phenol and TFM ultimately equilibrated at about the same concentrations, irrespective of whether the TFM or the phenol was added first. This equilibration in flasks G and H, as well as the close similarity in the concentrations of TFM in the water of flask G and the control after 1 hour (Fig. 5) offers evidence for initial temporary removal of TFM from the water by the mud according to a sorption mechanism, as suggested earlier.

The stabilization of TFM concentrations in the water in flasks G and H at 16-17 μg/ml in about 15 days, and the decrease of TFM in the control to less than 1 μg/ml in 48 days, are results that would be expected if the TFM were being degraded by microbial metabolism in the control and microbial action were being inhibited by phenol in flasks G and H.

Effects of heat sterilization

In tests of the effects of heat sterilization, 25 cc of Pine River mud and 114 ml of distilled water were placed in each of 10 flasks, and a test tube containing 6 ml of a 1,000 μg/ml solution of TFM was placed in an upright position in each flask. The flasks were covered with snugly fitting Pyrex beakers and autoclaved at 121 C for 2.5 hours. After the materials were equilibrated with air at 50 C, the 6 ml of sterile TFM solution in each of the test tubes was mixed with its corresponding sterile mud-water system by tipping the flask to about a 45" angle. This procedure provided a sterile mixture of TFM, water, and mud, without the possibility of the TFM being degraded during autoclaving. An unsterilized control flask contained a mixture of 114 ml distilled water, 25 cc Pine River mud, and 6 ml of the 1,000 μg/ml TFM solution. All of the mixtures were incubated at 50 C. At intervals of 2 to 6 days after the initial sampling, the contents of a sterile flask were sampled and discarded. Successive samples were taken from the single control flask.

Heat sterilization prevented the loss of TFM from the system (Fig. 6). Although some TFM was initially sorbed from the water in the sterile systems, no further removal occurred; the TFM concentration remained at about 31-34 μg/ml from the 5th day until the experiment was terminated on the 19th day. In the nonsterile systems the initial sorption was followed by nearly complete disappearance of TFM from the water within a week.

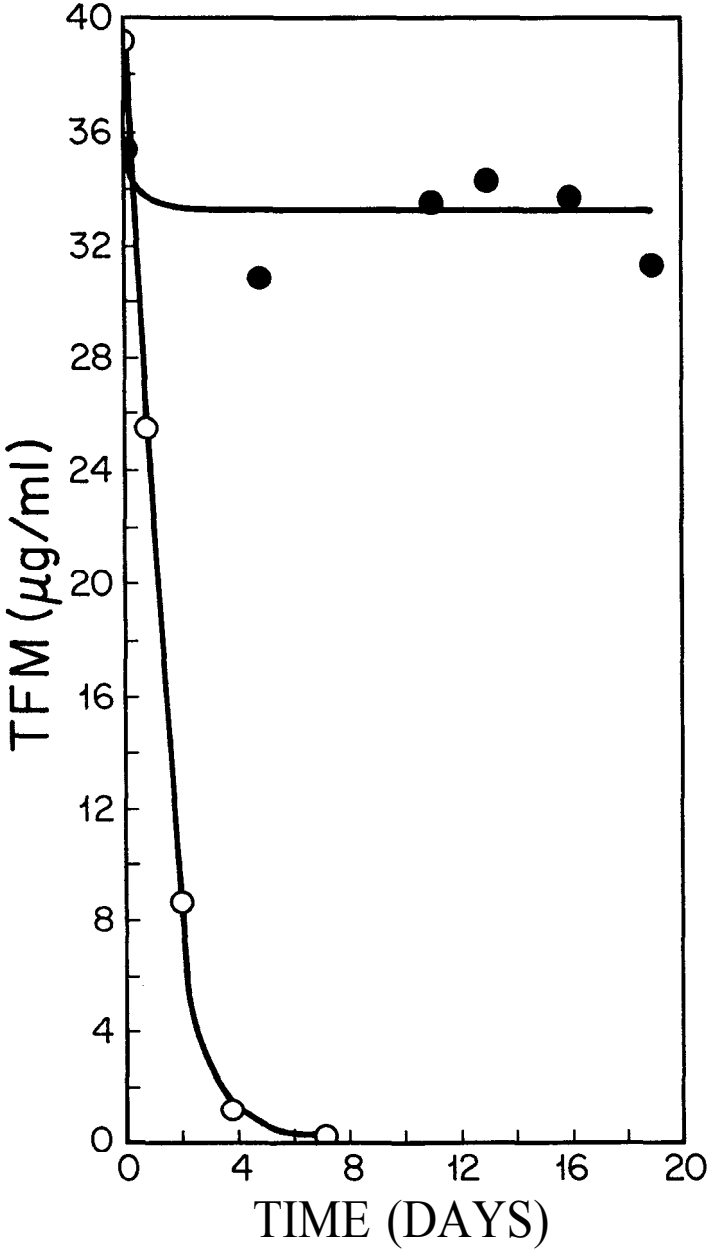

Figure 6. Prevention of TFM degradation by heat sterilization. Each point along the upper curve (solid circles) represents a different flask containing 114 ml of distilled water, 25 cc of Pine River mud, and 6 ml of a 1,000 µg/ml solution of TFM, autoclaved at 121 C for 2.5 hours. Points along the lower curve (open circles) represent successive samples from a flask containing the same mixture, unsterilized. Incubation temperature, 50 C. Curves drawn by inspection.

Stability of TFM in the sterile systems was also indicated by the fact that, after the initial equilibration, no TFM was lost from the water solution by evaporation, azeotropic distillation, or other processes during the incubation period.

Effects of addition of nutrients to a system after TFM loss had ceased

The stabilization of TFM concentration at some level above zero, which was regularly observed in mud-water-TFM systems, suggested that if microbiological action caused the degradation of the TFM, perhaps the responsible microorganisms had depleted a nutrient that was necessary for their continued activity. If so, it should be possible to establish the stabilization level of TFM at a desired point by adjusting the ratio of the volume of mud to volume of TFM solution in the test systems, and to reactivate stabilized systems by adding nutrients to the TFM solution after TFM degradation ceased, but when TFM was still present in the water. Both of these hypotheses were tested.

A small quantity (10 cc) of Pine River mud was placed in each of four flasks; 120 ml of a solution containing 25 µg/ml TFM plus 25,000&ml phenol in distilled water were added to two flasks (A and B, Fig. 7), and the same amount of a 25 µg/ml TFM solution in distilled water (but no phenol) was added to two others (C and D).

After 22 days of incubation, little if any TFM was lost from the mixtures containing phenol (A and B, Fig. 7), whereas TFM in the flasks with no phenol reached limiting levels of 16 and 19 µg/ml within 13 days-in comparison with a level of about 3 µg/ml in 13 days when a larger quantity (50 cc) of Pere Marquette River mud was mixed with 120 ml of 25 µg/ml TFM solution (Fig. 5). On the 22nd day of the experiment, 2.75 ml of single-strength TSB were added to flasks A and C. The TFM concentration remained constant in flask A, which contained phenol, but immediately began to fall in C and reached a level of about 1 µg/ml by the 38th day (Fig. 7). This experiment further supported the hypothesis that living microorganisms degraded TFM and that the mud supplied both the inoculum of microorganisms and the nutrients required for their growth.

EFFECTS OF MICROBIAL CULTURES ON TFM

When a single-strength TSB solution containing 70 µg/ml TFM was inoculated with one loopful of an actively growing enrichment culture prepared from Pine River mud, the TFM concentration dropped to 3.4 µg/ml in 9 days. This procedure yielded closely similar results when cultures obtained from muds of other rivers and Lake Erie were used, or when fluid thioglycollate medium containing added TFM was used instead of TSB.

Although these cultures were probably not pure, aerobic growths on plating media indicated only colonies of Pseudomonas. An occasional isolate degraded TFM on transfer, but most did not. Possibly the active cultures obtained from a single colony contained a mixture of species of *Pseudomonas,* or a few survivors of other microorganisms from the liquid culture that did

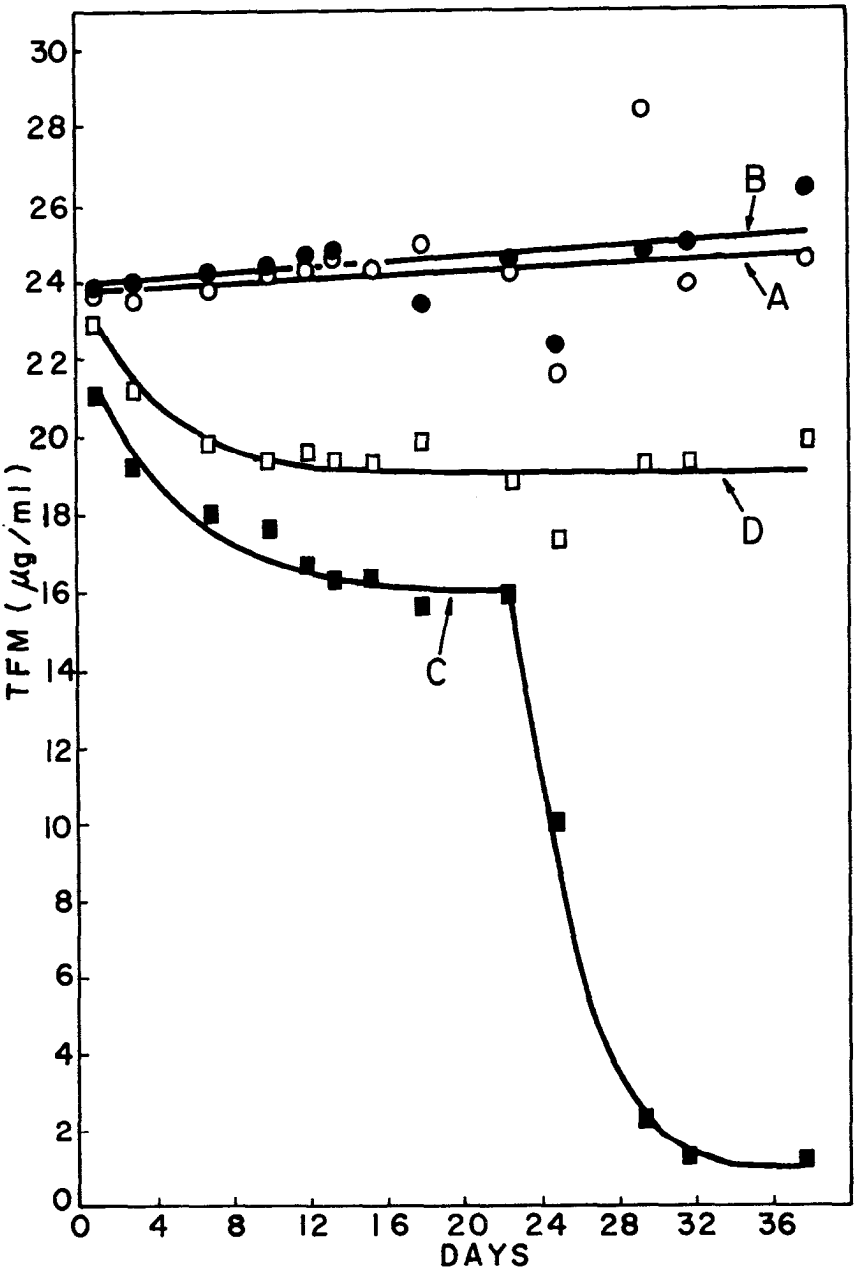

Figure 7. Reinstatement of TFM removal from a mud-water system after addition of a nutrient medium. Flasks A (open circles) and B (solid circles) contained 10 cc of Pine River mud mixed with 120 ml of a solution containing 25 µg/ml TFM and 25,000 µg/ml phenol; flasks C (solid squares) and D (open squares) contained 10 cc of mud and 120 ml of a 25 µg/ml TFM solution. On the 22nd day, 2.75 ml of single-strength TSB were added to flasks A and C. Incubation temperature, 22 C. Curves drawn by inspection.

not grow aerobically on solid media. A further possibility is that deadaptation occurred. Whatever the reason for the inactivity of many of the single-colony isolates, the organisms in the original liquid cultures (and occasional single-colony isolates) unquestionably degraded TFM rapidly.

SUMMARY

This study is an inquiry into the degradation of TFM in natural waters, and the role of microorganisms in that breakdown. When sufficient quantities of bottom muds from rivers of the Great Lakes drainage basin and from Lake Erie were incubated with TFM solutions, the TFM disappeared from the water or was drastically reduced in 2 to 4 weeks at 22 C. The disappearance involved two stages: In the first few hours, some TFM was reversibly sorbed by the mud; but also, beginning almost immediately, irreversible degradation occurred. The TFM that was sorbed by the mud could be almost completely removed by leaching with water during the first hour of an experiment. Less and less could be leached out as time progressed, and within 2 to 4 weeks all or nearly all the TFM had disappeared. The mud-water system was then no longer toxic for lamprey larvae or goldfish. Fluoride ion accumulated in the supernatant water during incubation, suggesting that at least some of the TFM degraded to its atomic constituents.

Considerable evidence indicated that the degradation might be caused by microbiological activities: The rate increased progressively from 6 to 60 C, but decreased from 60 to 85 C; the process was prevented by heat sterilization or by the addition of phenol; and, in systems in which degradation had ceased while some TFM was still present, the process resumed when nutrients were added. When enrichment cultures of microorganisms, obtained by inoculating bacteriological culture media containing TFM with some of the muds used in the incubation studies, were reinoculated into bacteriological culture media containing TFM, the TFM was degraded rapidly.

CONCLUSION

Microorganisms in sediment-water systems degrade TFM.

ACKNOWLEDGMENTS

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