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MICHIGAN DEPARTMENT OF CONSERVATION
Research and Development Report No. 43*

December 9, 1965

USE OF AS-74 TAGGED SODIUM ARSENITE IN A STUDY OF EFFECTS OF A HERBICIDE ON POND ECOLOGY[1]

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* Institute for Fisheries Research Report No. 1709.

[1] Presented by Dr. Ball at a Symposium on Use of Isotopes in Weed Research at Vienna, Austria, October 25-29, 1965. Sponsored by the International Atomic Energy Agency and the Food and Agriculture Organization of the United Nations.

Use of As-74 Tagged Sodium Arsenite in a Study of Effects of a Herbicide on Pond Ecology

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Introduction

In the lake districts of the United States aquatic weed control has become commonplace and necessary. Utilization of these waters for a variety of recreational purposes has created a demand for the elimination of nuisances associated with an overabundance of weeds.

A variety of chemicals are available for the control of higher aquatic plants and algae but sodium arsenite and copper sulfate are still widely used. These two herbicides have remained popular for over 50 years [6] despite the discovery of newer organic herbicides. Their lasting popularity can be attributed to low cost and general effectiveness. It has been assumed that they have little effect upon the aquatic biota.

Despite the fact that sodium arsenite has been used for many years, little is known about its effect upon aquatic ecosystems or the ultimate fate of the chemical within the lake or pond. Also important but poorly understood is the long-term effect of prolonged treatment. Dupree [2] studied the arsenic content of various components of a pond ecosystem following treatment with arsenite. He found that a considerable amount of arsenic was retained by the muds. This was slowly released to the water when the ponds were drained and refilled. He found that plankton concentrated the herbicide and noted concentrations as high as 7200 ppm within plankton organisms.

Lawrence [4] found that sodium arsenite markedly reduced fish production in treated ponds and killed most of the microcrustaceans and rotifers. Riggs [5] investigated the effects of several herbicides on a series of aquatic plants and described the possible ways in which sodium arsenite might attack plants.

Attempts to trace sodium arsenite through an aquatic ecosystem have been hindered by the lack of a good technique for analysis of arsenic. Conventional methods are inefficient and time consuming. In this study we utilized sodium arsenite labeled with radioactive arsenic (As-74) to trace the movement of arsenic through aquatic ecosystems following their treatment with this herbicide. The overall investigation was divided into three phases. The first was a study of the movement of sodium arsenite through the ecosystem of a small pond. The second was an approximate duplication of the pond study but was carried out by means of a series of aquaria experiments in which conditions were carefully controlled.

The third phase was a study of the effect of sodium arsenite upon the metabolism of a pond. The latter phase did not utilize the tracer (As-74). This report will deal mainly with the latter two phases although limited data are presented from the pond study.

We wish to acknowledge the assistance of Mr. Thomas Bahr, Mr. Leonard Sohacki and Mr. Jack Bails who collected data used in certain phases of this study. Research was carried out under AEC contract AT(11-1) 655. A portion of the work was done under Dingell-Johnson Project F-27-R, Michigan.

Methods

A concentration of 8 ppm of sodium arsenite was used in the treatment of all ecosystems. Enough labeled arsenic was added to the commercial sodium arsenite to give a solution with sufficient radioactivity for accurate counting. Fifty millicuries of As-74 in the form of sodium arsenite ($NaAsO_2$) dissolved in an alkaline solution, were supplied by the Oak Ridge National Laboratory, Oak Ridge, Tennessee. Initial dilutions of the tracer and the herbicide were made in a 50-gallon drum lined with polyethylene. Double distilled water was used for dilution. After diluting and mixing the isotope-herbicide solution, a period of 12 hours was allowed for equilibration of stable and radioactive arsenic. When 10 ml of this solution were dissolved in one gallon of aquarium water an activity of 6 disintegrations per second per milliliter and a concentration of 8 ppm of As_2O_3 were obtained.

Liquid samples were evaporated to dryness in an oven at 60° C and were counted in a low-background beta counter. These were the only samples that could be reduced to thin films. Arsenic sublimes at 80° C and treatment of the samples had to be kept below this temperature, hence ashing procedures could not be used. Gamma counting was employed for non-liquid samples. A well-type scintillation counter was used. The system consisted of a 3-inch NaI crystal embedded in a shielded well. The crystal and photomultiplier were connected to a variable, single-channel spectrum analyzer. This allowed only those emissions of desired energy to be recorded on the scaler, and excluded emissions from sources other than As-74. Samples were corrected for background and decay to time of entry of As-74 into the ecosystem.

Aquaria experiments

Organisms representing several trophic levels of the pond ecosystem were included in the study. These were species of higher aquatic plants, fish, and invertebrates. In one series of experiments groups of similar organisms were placed in separate aquaria by themselves so that uptake of the tracer-labeled herbicide could be observed in the absence of other factors. In addition, three aquaria were set up as "complete" ecosystems. These aquaria contained organisms from several trophic levels. Experimental systems studied are as follows:

Substrate experiments

Four centimeters of soil were added to the bottom of each of three aquaria and the aquaria were then filled with pond water. Precautions were taken to avoid mixing the soil and water.

Plant experiments

Three species of aquatic plants found in the ponds were used; they were *Potamogeton praelongus*, *Elodea canadensis*, *Isoetes* sp. Twelve plants of each species were planted in each of the three aquaria. In two of the aquaria plants were rooted in sand, in the third gravel was used. Two weeks were allowed for the plants to become established before aquaria were treated with the herbicide.

Fish experiments

Each of three aquaria were stocked with 25 small green sunfish (*Lepomis cyanellus*). The same number of black bull-heads (*Ictalurus melas*) were introduced into two similar aquaria. Each of two aquaria were stocked with 100 golden shiner (*Notemigonus crysoleucas*) fry. Washed gravel was used as the substrate in these experiments and all aquaria were aerated throughout the study.

Invertebrate experiments

Each of two aquaria were stocked with 25 small crayfish; each crayfish weighed about 2 grams. Twenty snails of the genus *Physa* and 15 dragonfly naiads (*Gomphus* sp.) were added to separate aquaria.

Complete ecosystem experiments

This designation was used for aquaria containing an ecosystem consisting of producers, consumers, and decomposers. These three aquaria were identical to the plant aquaria in size and contained the same number and size of plants. In addition the following organisms were added: 6 green sunfish, 6 bullheads, 6 fathead minnows, and 6 of each of the invertebrates mentioned above. Organisms were replaced which showed symptoms of distress during the two-week conditioning period that preceded the treatment.

Water experiments

Three aquaria containing only pond water were used in these tests. Several glass slides were placed in each of these tanks and were used to determine whether or not the herbicide was adsorbed to the surface of the glass. The possibility of settling out of the isotope within the aquaria was also carefully checked.

Aquaria were housed in a room in which temperature was maintained at the same level as the outside ponds (77° F). After a two-week equilibration and adjustment period each aquarium was treated with the As-74 sodium arsenite mixture. The composition of the aquaria experiments is shown in Table I.

Prior to application of the tagged herbicide radioactivity measurements were made on organisms of the same species and from the same source that was used to stock the experimental

Table I. Composition of experimental aquarium systems.

Experiments	Number of aquaria	Substrate	Plants	Animals	Organisms per aquarium
Substrate	3	Mud			
Plants	3	2 sand 1 gravel	*Elodea* *Isoetes* *Potamogeton*		12 12 12
Fish	3 2 2	Gravel Gravel Gravel		Sunfish Bullheads Shiners	25 25 100
Invertebrates	1 2 1	None Gravel Gravel		Snails Crayfish Dragonflies	10 25 10
Complete ecosystem	3	Sand	*Elodea* *Isoetes* *Potamogeton*	Sunfish Bullheads Fathead minnows Snails Crayfish Dragonflies	12 12 12 6 6 6 6 6 6
Water	3	Pond water and glass slides only			

aquaria. The counting procedures used were the same as were used in the actual experiment. None of these organisms showed an activity significantly greater than background.

Plants from the complete ecosystem and plant experiments were collected and processed 2 hours, 24 hours, and 1 week after addition of the labeled herbicide. Two entire plants of each species were removed from each aquarium and processed. Processing consisted of blotting, weighing, and rinsing with 0.01 N HCl. The acid rinse was evaporated to dryness in an oven and counted. Plants were placed in plastic vials and counted.

Movement of arsenic into the substrata was followed in the substrate, complete ecosystem, and plant experiments. Samples were taken on the first and second day after introduction of the labeled sodium arsenite and thereafter once a week for the duration of the study. A core was collected through the entire depth of the sediment. The core was then frozen and sliced into 2 cm sections and activity was measured.

Water samples were taken from the aquaria used in plant, substrate, water and complete ecosystem experiments every day for the first week and then three times a week for the duration of the study. The water of all other aquaria was sampled only at times when some other component of that aquarium was sampled. Activity measurements were made on all samples.

Fish were removed from the complete ecosystem experiments for activity measurements twice during the study; two days and six weeks after application of the labeled herbicide. In other aquaria fish were collected once a week during the first month and then every two weeks until the end of the study. Three fish were taken from each aquarium; they were weighed, rinsed with 0.01 N HCl and their activity measured. Activity of the fish and the rinse were measured separately.

Crayfish were removed from aquaria and their activity measured three times each week beginning three days after the addition of the labeled herbicide. Snails were collected at 2-week intervals, counted alive, and returned to the same aquaria. Snails were not rinsed in dilute acid. Three times during the study glass slides were removed from the aquaria containing only water. No activity was found on any of these slides indicating that little if any of the arsenical was adsorbed by the glass of the aquaria.

Pond study

The pond treated with labeled sodium arsenite was one of a series of similar ponds that had been studied intensively for the previous three years as part of a study of aquatic herbicides [1]. An extensive biological and chemical background was available on this pond. The pond has a surface area of 0.135 hectares (1/3 acre) and a maximum depth of 2 meters. Approximately one-third of the pond bottom was covered with *Chara* and higher aquatic vegetation. The water was of moderate hardness (80 ppm total alkalinity).

The pond was treated with the same concentrations of stable and radioactive arsenic used in the aquarium experiments and data were collected on the activity of the same organisms and on the activity of water and substrate materials. In addition water chemistry and pond metabolism were monitored and quantitative measurements were made of the abundance of plants, plankton, periphyton, invertebrates and fish. One hundred pots containing the same species of plants that were used in the aquarium experiments were placed in the pond. Pots were supported on a wooden platform at the median pond depth. This permitted precise observation and measurement of the effect of the herbicide.

Because of differential changes in weight caused by bacterial decay, data on the activity of plants collected after the first few days are not comparable when expressed in terms of radioactivity per unit weight of plant. They do however show that activity was retained by the decaying plant.

Results

Aquaria experiments

Aquaria that contained either sand, gravel, or mud substrates showed significant decreases in radioarsenic during the sixty days of the study. Aquaria without a substrate material showed little change in radioactivity. Changes in the water activity of the three aquaria having sand or gravel bottoms are shown in Figure 1. The initial activity was about 200 pc/ml. Activity decreased to about half this amount in approximately two weeks, and within sixty days it was 25 per cent of the original value. The shape of the time-concentration curves suggests that an equilibrium was reached or approached between the arsenic of the sediments and that of the water.

Measurements of the activity of the sediments themselves demonstrate that activity passed into the substrata from the water (Figure 2). Each point on the curves of Figure 2 represents the mean of the corrected activity of one core from each of three aquaria. The upper curve shows the activity density (pc/gm dry wt) of the top 2 cm of the core, i.e., that portion immediately below the mud-water interface. The bottom curve shows the activity density of the bottom 2 cm of the core. The upper layers of the sediments were the first to receive a countable level of activity. After one week radioarsenic penetrated to a depth of 2 cm. Arsenic was easily stripped from the soil and plant samples by dilute HCl. This finding, together with Dupree's observation [2] that the greater the arsenic content of the mud the easier it is to leach arsenic from the soil with distilled water, indicate that arsenic is not tightly bound to the plants or muds.

Table II summarizes the rates at which activity disappeared from the water in the plant, complete ecosystem, and substrate experiments. Results indicate that activity is lost to the water and taken up by the mud substrates much more actively than by either sand or gravel (Table III); gravel showed the least interchange.

Figure 1. Water radioactivity of three aquaria used in substrate experiments following treatment with labeled sodium arsenite. Each point is the activity of one 10 ml sample. Data corrected for background and decay. Bottom substrates were sand and gravel.

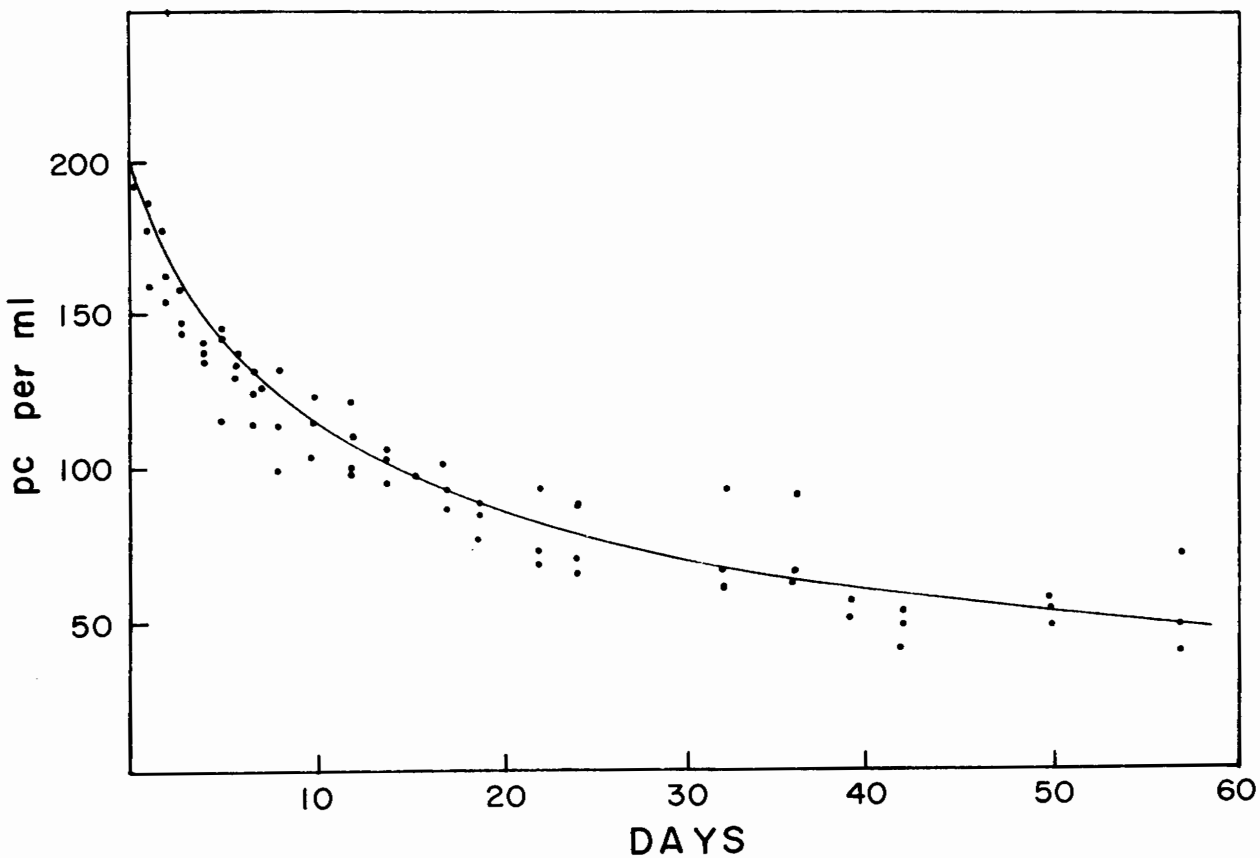
200
150
100
50
pc per ml
10
20
30
40
50
60
DAYS

Figure 2. Activity-density (pc/g) of top and bottom 2 cm fractions of mud cores from three aquaria used in substrate experiments. Each point is a mean of three samples.

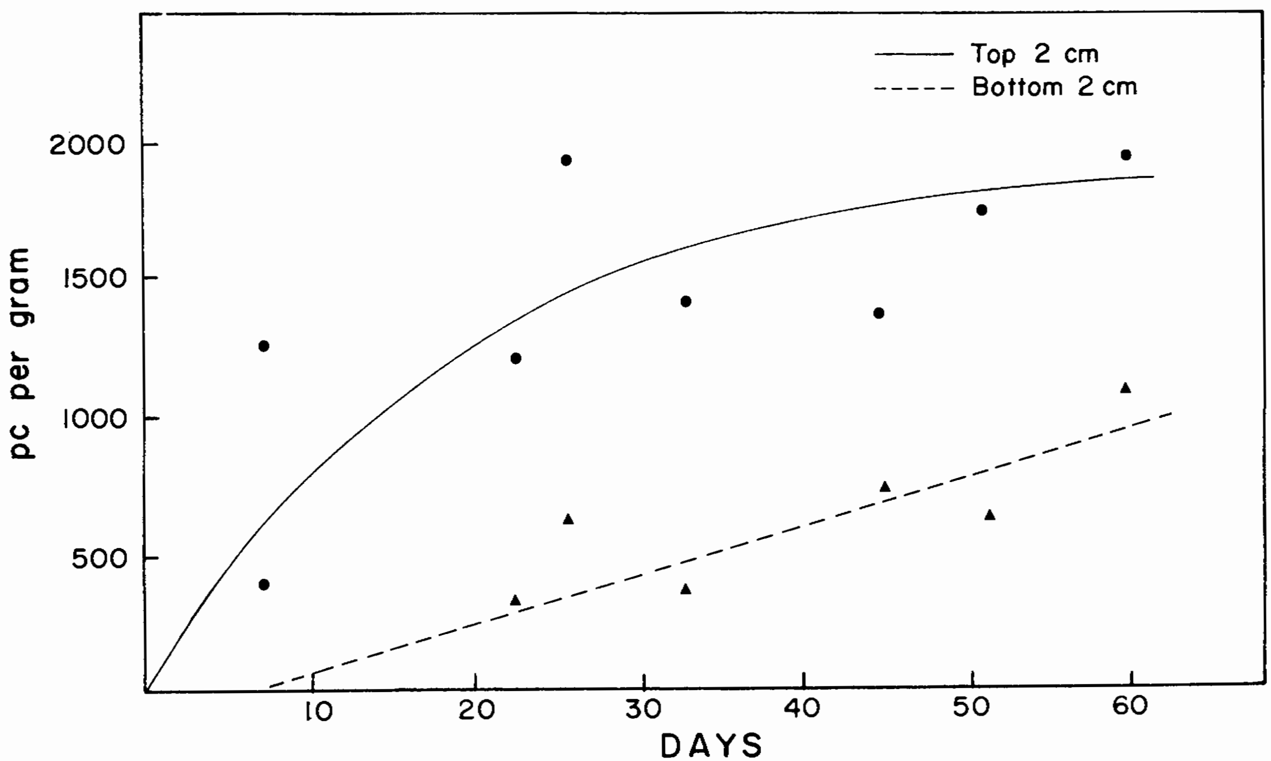
Top 2 cm
Bottom 2 cm
2000
1500
1000
500
pc per gram
10
20
30
40
50
60
DAYS

Table II. Rate of disappearance of water radioactivity in aquarium experiments following treatment with labeled sodium arsenite. Rates are the slopes of tangents of the activity-time curve at the times indicated.

Type of experiment	Number of experiments	Average rate (pc/ml/day) 1 day	30 days	60 days
Mud bottom	3	20.0	1.0	0.25
Potamogeton praelongus Sand bottom	2	8.0	1.5	0.75
Potamogeton praelongus Gravel bottom	1	2.5	1.5	0.25
Complete ecosystem Sand bottom	3	10.0	1.5	0.25

Table III. Rate of uptake of radioactivity by the top 2 cm and bottom 2 cm of the substrata of aquarium experiments. Rates are the slopes of the tangents to the activity-time curves at the indicated times. Values given for the bottom 2 cm layer are average rates for the 60-day period.

Type of experiment	Number of experiments	Average rate (pc/ml/day)			
		Top 2 cm			Bottom
		1 day	30 days	60 days	2 cm
Mud bottom	3	9.5	2.0	1.00	2.0
<u>Potamogeton praelongus</u> Sand bottom	2	3.0	2.0	0.75	0.5
<u>Potamogeton praelongus</u> Gravel bottom	1	2.0	0.5	0.00	0.0
Complete ecosystem Sand bottom	3	4.5	3.0	0.50	1.3

<u>Uptake by plants</u>

The initial uptake of radioarsenic by aquatic plants was rapid and within two hours after application over half of the plants tested showed levels of activity above 1100 pc/gm. Uptake rates for three periods during the first week following application are shown in Table III. These data are for *Potamogeton praelongus* but similar rates were obtained for other species tested.

Three days or less after treatment leaves started to turn brown and the external cells began to slough off. Plants started to lose turgidity in about a week and shortly thereafter they became almost transparent. Leaves which dropped off early in the process retained their shape and measurements of their activity showed levels as high as 15,000 pc/gm. This high activity-density may have been due to decomposition and loss of tissues low in activity and retention of tissues of high activity. Retention of arsenic by tissues for a considerable period has the effect of delaying the recycling of the herbicide.

The activity adsorbed on the plant surface appeared to decrease as the activity incorporated in the plant cell increased. This apparent trend may also have been due to the sloughing off and decay of the outer cell layers. The rapid initial uptake by plant tissues indicates that the arsenic was incorporated through the leaves rather than through the roots since little activity was found in the soil at the time the leaves of *Elodea* showed a high activity (Table IV).

Table IV. Activity density (pc/gm) of *Elodea* after treatment with labeled sodium arsenite.

Time (Hours after treatment)	Complete ecosystem experiments	Plant experiments
2	1736	850
24	2610	1686
168	5367	----

Fish

Little activity was found in the fish. Of more than 100 fish sampled only 10 per cent showed an activity significantly greater than background. Activity values ranged between 300 and 800 pc/gm, live weight. Some fish were observed eating decaying vegetation; fish with detectable activity may have obtained their arsenic in this way, although the possibility of direct uptake of the isotope from the water cannot be entirely excluded. In any case it appears that the fish used in this study, which are representative of two trophic levels, received very little activity.

Pond studies

Metabolism

Study of the effects of the arsenical upon pond metabolism and productivity was considered to be of equal importance to the tracing of arsenic through the food chain of the ecosystem. To study metabolism oxygen measurements were made in 1962 and 1963 [1], and at the time the labeled herbicide was applied.

The oxygen of a pond arises from aquatic macrophytes, periphyton, phytoplankton, and from interchange with the oxygen of the atmosphere [10]. From earlier experiments [7] with sodium arsenite and copper sulfate it was evident that in these ponds most of the oxygen was produced by the higher aquatic macrophytes. Lesser amounts were produced by *Chara*, phytoplankton, and periphyton in that order.

The effect of the eradication of a heavy growth of aquatic plants within the pond in 1963 upon the dissolved oxygen concentration is shown in Figure 3. Soon after treatment production of oxygen came to a complete stop for a short time, and a minor fish-kill was noted. Dissolved oxygen did not return to normal levels for approximately two weeks. With cessation of oxygen production, fixation of energy by plants stopped. Thus the treatment had an important effect upon the productive capacity of the pond.

Phytoplankton and zooplankton

Prior to and following the treatment plankton samples were taken from the experimental pond and from a control pond. The 16 liter samples were centrifuged and pigment extracts were made of the concentrate. Previous work on these ponds [7] indicated that measurements of pigment were well correlated with microscopic counts of algae. Accordingly phytopigment measurements were considered a valid index of the standing crop of phytoplankton during the experiment. The immediate effect upon phytopigment is shown by two samples taken on the day of treatment. A sample taken in the morning, just prior to application, had 0.051 phytopigment units while an afternoon sample collected after treatment had only 0.031 units. Sodium arsenite apparently destroyed a major portion of the phytoplankton. Phytopigment did not return to pretreatment levels for about four days (Figure 4). Uptake of C-14 on the day of application gave another indication of the immediate effects upon phytoplankton production. These results also indicate a great reduction in rate of fixation of energy immediately following treatment (Table V). The pronounced

Figure 3. Oxygen production and solar energy received by Pond C after treatment with sodium arsenite in 1963.

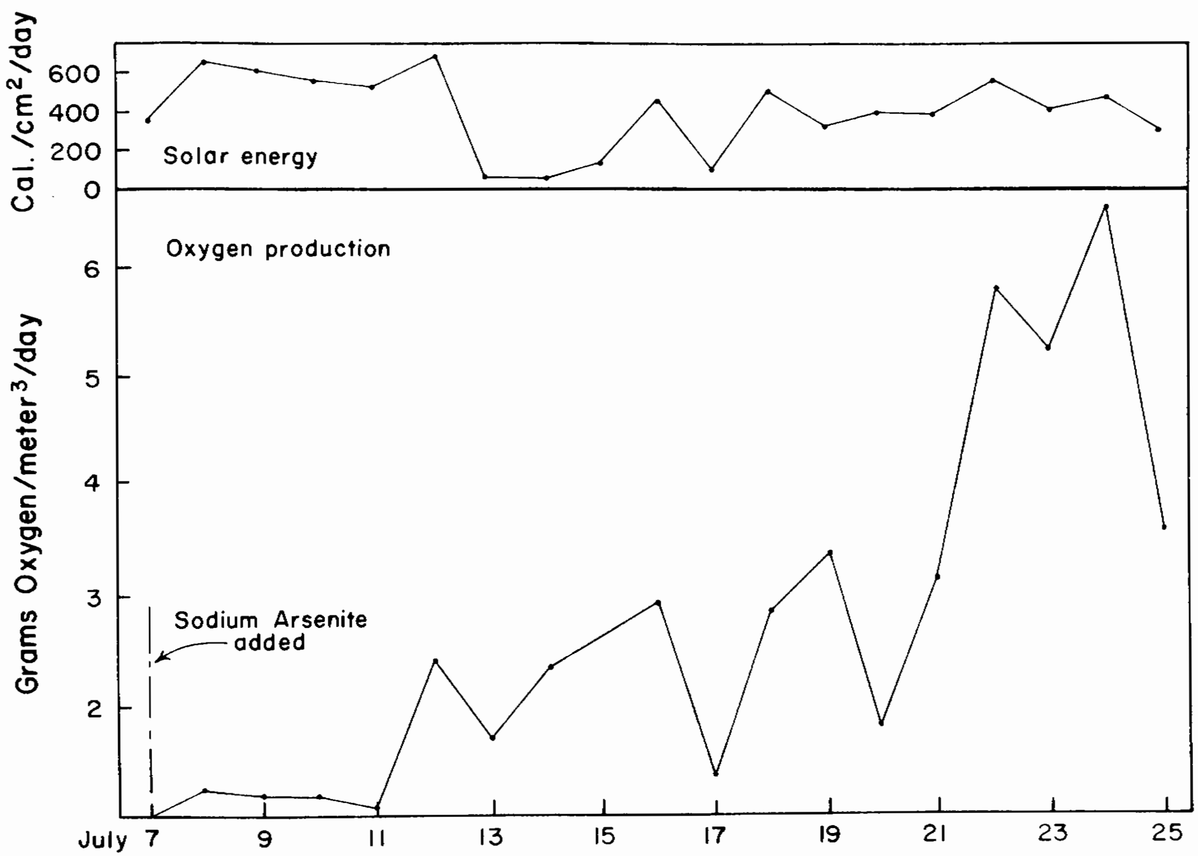
Cal. /cm2/day
600
400
200
0
Solar energy
Oxygen production
Grams Oxygen/meter3/day
6
5
4
3
2
Sodium Arsenite
added
July 7
9
11
13
15
17
19
21
23
25

Figure 4. Phytopigment of experimental and control ponds before and after treatment of experimental pond with sodium arsenite in 1964. Phytopigment extracted from 16 liter water samples.

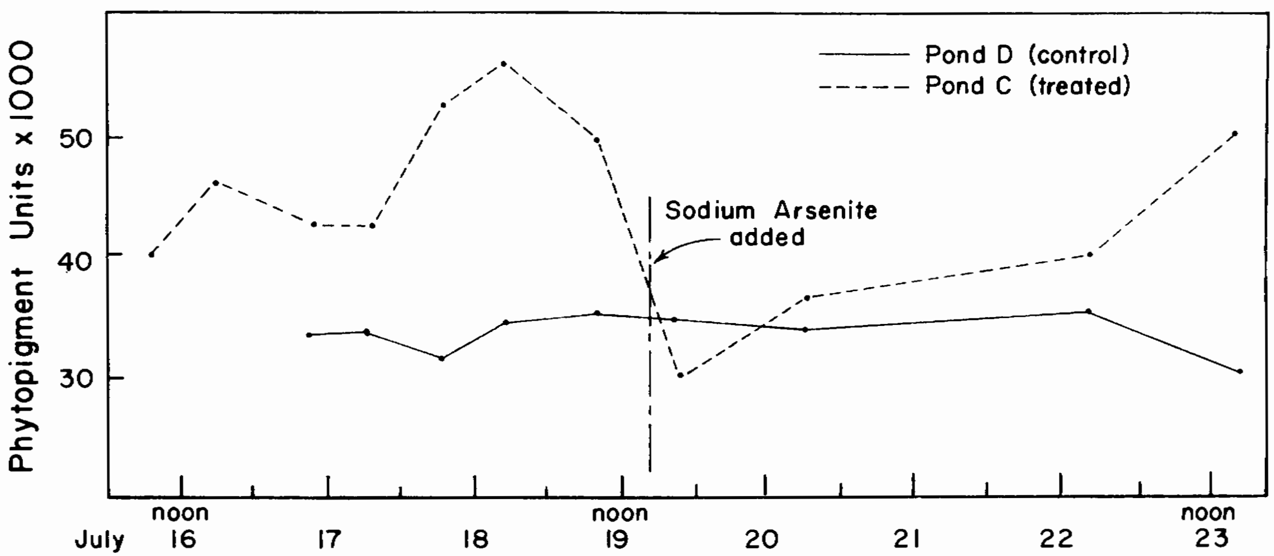
Pond D (control)
Pond C (treated)
Phytopigment Units x 1000
50
40
30
Sodium Arsenite
added
noon
noon
noon
July
16
17
18
19
20
21
22
23

Table V. Light energy received at the pond surface and the assimilation of carbon by phytoplankton as indicated by the C-14 method during three time periods on the day of application of sodium arsenite. Arsenite applied just prior to 1400.

Time	Light cal/cm^2	Assimilated carbon mg/m^3
0600 - 1000	22.7	7.67
1000 - 1400	33.3	15.44
1400 - 1800	30.6	3.96

decrease in rate noted after 1400 took place while the light energy being received by the pond was high. Such a sharp change in rate has not been noted in earlier studies of these ponds [3].

Bottom invertebrates

Reduction in number and volume of invertebrates in the ponds was observed following the treatment (Table VI). Reduction in numbers ranged from 57 per cent in the case of dragonflies, a group known to be resistant to sodium arsenite [9], to 100 per cent in the case of mayflies. Since invertebrates are the principal food source for the fish population, their loss must be considered to be of great ecological importance. The rate of recovery of bottom invertebrates from the treatment varied according to the taxonomic group but in every group recovery was slower than for phytoplankton and zooplankton. Slower recovery of bottom fauna as compared to zooplankton was to be expected since the bottom fauna was chiefly insects which emerge and reproduce infrequently as compared to the invertebrate groups of the plankton. Many species of insects had reproduced prior to treatment; for these species a new generation did not appear until the following year.

Discussion

The pronounced effects of sodium arsenite upon phytoplankton observed in this study are surprising in view of widespread belief that this herbicide affects only aquatic

Table VI. Percentage reduction in weight of bottom invertebrates of test ponds following treatment with sodium arsenite. Differences in actual numbers of individuals of all invertebrate groups found in both post-treatment and pre-treatment samples were significant at the 5 per cent level.

Invertebrate group	Percentage reduction
Tendipedidae	79.1
Ephemeroptera	100.0
Odonata	57.3
Other insects	83.8
Molluscs	91.6

macrophytes. Nutrients liberated by the decay of macrophytes following treatments have been considered to be available to phytoplankton. It is of interest to note that post-treatment plankton blooms have not been observed following treatment of these ponds with sodium arsenite.

The conclusion that can be drawn regarding the overall effects of the herbicide is that the herbicide persists in the ecosystem at low concentration levels and that it has an adverse effect upon many components of the system. Sodium arsenite kills many organisms important to the economy of the pond and modifies the primary producers to such an extent that production is seriously affected. These losses of food organisms and productive capacity could have serious deleterious effects upon the recreational values of lakes and ponds; therefore, these losses must be weighed against benefits achieved by removal of aquatic vegetation.

Summary

The ecological effects of sodium arsenite upon ponds was studied by treating a pond and a series of aquarium ecosystems with sodium arsenite labeled with As-74. The arsenical was traced through the food webs, water and sediments of the pond and aquaria.

A steady movement of arsenic into the sediments was noted. Uptake was more rapid by mud substrates than by sand or gravel. The herbicide eliminated the higher aquatic plants. Uptake by plants was rapid and the arsenical persisted in plant tissues during decay. The herbicide reduced pond metabolism and brought about an immediate decrease in the rate of carbon fixation by phytoplankton. The reduced rate persisted for 10 days. Certain groups of invertebrates were eliminated, others were reduced in numbers, and recovery of invertebrates was slow. A low level of activity was detected in a few fish but most fish showed no activity.

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