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DETERMINATION OF CHEMICAL THRESHOLD CONCENTRATIONS USING 2,4-D TO CONTROL SELECTED AQUATIC MACROPHYTES—A PILOT STUDY TO EVALUATE A LABORATORY SYSTEM

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) The objectives of this pilot study focused on the evaluation of a modified diluter system used to determine the minimum sustained (threshold) herbicide concentrations required to control nuisance aquatic macrophytes. The herbicide 2,4-dichlorophenoxy acetic acid (2,4-D) was used during this pilot study to provide data for evaluating the diluter system operation as well as to provide initial estimates of the threshold 2,4-D concentration required to (Continued)		

20. ABSTRACT (Continued).

control Eurasian watermilfoil (*Myriophyllum spicatum* L.) and Sago pondweed (*Potamogeton pectinatus* L.). The experimental design included three replicate aquaria containing each of the aforementioned plant species planted in beakers containing a standard hydrosoil, and one reference aquarium without plants exposed for 6 weeks to each of several 2,4-D concentrations: 0.00, 0.01, 0.04, 0.10, and 0.20 mg/l. Results of this pilot study showed that the modified diluter system was completely satisfactory for this work. Moreover, estimates of the threshold 2,4-D concentrations needed to control the aforementioned plants were: 0.1 mg/l for *M. spicatum*, and 0.2 mg/l for *P. pectinatus*. These results will be verified following completion of recommended changes in the diluter system.

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DETERMINATION OF CHEMICAL THRESHOLD CONCENTRATIONS USING 2,4-D
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PART I: INTRODUCTION

Background

1. Excessive growth of nuisance aquatic plants is becoming a serious problem for resource managers of recreational lakes, waterways, and other aquatic environments. Management of aquatic plants is presently being achieved using chemical, biological, mechanical, environmental management, and integrated techniques. Chemical control techniques are most commonly used by the CE field offices with active aquatic plant control programs (Dardeau and Hogg 1983). Traditionally, the application rates of selected aquatic herbicides are based primarily on the treatment area without considering water volume, plant standing crop, or water quality effects. Therefore, aquatic herbicide application rates may result in unnecessarily high levels of herbicides being applied to the water body. Although an applied herbicide will usually be rapidly diluted to below the maximum allowable concentration, many public organizations have challenged the maximum concentrations legally permitted for use in aquatic environments in the United States. The increased environmental awareness over the past 10 years, the strict environmental acts proposed by the Environmental Protection Agency (EPA), and the recent development of controlled-release (CR) herbicide formulations require research to determine the minimum sustained (threshold) herbicide concentrations required under short- or long-term treatment conditions for controlling select aquatic plants.

2. Changes in select water quality parameters associated with chemical application, including decreased dissolved oxygen, plant material decay, and eutrophication promoted by nutrient releases, have curtailed the aquatic use of some effective plant herbicides. The effectiveness of chemical control on select target aquatic plants is

dependent upon many factors, e.g., light, temperature, pH, water hardness, suspended solids, and nutrient status, as well as on the physical state and growth phase of the aquatic plants. Herbicides are most often applied at maximum allowable rates in an attempt to ensure plant control under even the most difficult circumstances.

3. The logical approach to improving water quality and maximizing the effectiveness of aquatic herbicides is by incorporating the respective herbicide in a CR formulation. The CR formulations are designed to provide adequate plant contact through timed release of the herbicide, thus improving the chances for plant uptake. Laboratory data show control of aquatic plant regrowth for time periods of 4 months to 1 year.

4. Controlled-release herbicide formulations have been developed under contract and evaluated over the past several years by the Corps of Engineers (CE) Aquatic Plant Control Research Program (APCRP). The concept of the CR formulations is to allow a prolonged exposure of target aquatic plants to a sustained low concentration of a given herbicide. The effective use of CR formulations for aquatic plant control will be achieved at relatively low cost when considering the longevity of the treatment.

5. Determination of the threshold herbicide concentration required for controlling different nuisance aquatic plants is necessary for developers of CR formulations to produce or modify existing CR products to provide the best release rates. This information will assist developers in improving their product's designed release rate and ultimately pilot plant operation. Similarly, the release rate of each herbicide from the CR formulation and threshold herbicide concentration in the water are important considerations for determining field application rates and treatment costs associated with CR herbicide formulations.

Purpose and Scope

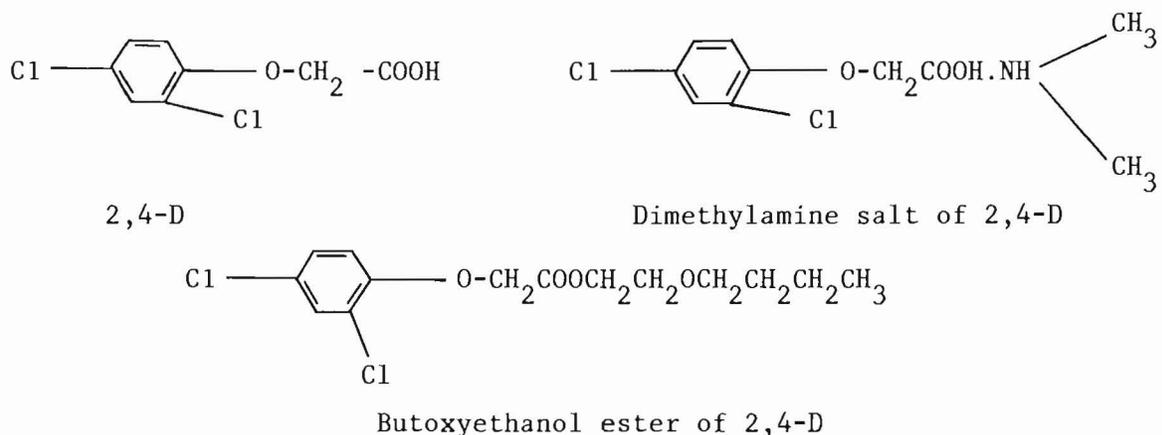
6. The purpose of this study was to evaluate a diluter system, originally developed by Mount and Warner (1965) and Mount and Brungs (1967), to be used to estimate the threshold 2,4-dichlorophenoxyacetic

acid (2,4-D) concentrations that are required to control the growth of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and Sago pondweed (*Potamogeton pectinatus* L.).

7. Construction and performance characteristics of the WES-modified diluter system are presented herein, along with preliminary test results, using this system to estimate the threshold 2,4-D concentration required to control the aforementioned plant species. Four 2,4-D acid concentrations, 0.01, 0.04, 0.10, and 0.20 mg/l, were tested using three replicate aquaria at each 2,4-D concentration. Each 2,4-D concentration was applied to aquaria containing only water to assess the extent of 2,4-D adsorption to the glass. A set of four test aquaria containing plants and receiving no 2,4-D was used as references for evaluating herbicide efficiency through comparison with treated aquaria containing plants. Efficiency of 2,4-D on *M. spicatum* and *P. pectinatus* was evaluated based on physical deterioration of the plants and statistical comparison of shoot and root biomass following completion of the pilot study, i.e., 6 weeks. Estimates of the 2,4-D threshold concentrations were made following analysis of test results.

2,4-D

8. The structural formula for 2,4-D acid and the two chemical formulations used most commonly in aquatic plant control programs to control waterhyacinth (*Eichhornia crassipes*), Eurasian watermilfoil, and Sago pondweed species are:



The uptake of 2,4-D by plants has been extensively reviewed by Jansen (1964a, 1964b, 1965a, 1965b) in relation to selection of various surfactants to enhance herbicidal activity. He concluded that herbicides move into the plants by two pathways, one hydrophilic and the other lipophilic. Foliar penetration was found to be directly related to the external herbicide concentration (Bukovac 1976).

9. Even after nearly 40 years since its discovery, the specific mode of action of 2,4-D is still unclear. The chemical is a synthetic plant growth regulator. Typically, 2,4-D is known to cause old cells to rejuvenate and to overstimulate young cells, preventing normal differentiation and maturation. Moreover, 2,4-D is known to affect enzyme activity, respiration, nucleic acid metabolism, and protein synthesis (Ashton and Bayer 1976). At relatively low doses, 2,4-D translocates throughout the above- and below-ground plant tissue thereby being able to kill root systems. Too much 2,4-D will cause excessive contact injury to the plant, resulting in little 2,4-D translocation to other parts of the plant (Ashton and Crafts 1981). The major effects of low 2,4-D levels on nucleic acid synthesis appear to be the stimulation of ribonucleic acid (RNA) polymerase and subsequent RNA production, resulting in increased protein synthesis. Low levels of 2,4-D induce cell enlargement by increasing the activity of autolytic and synthetic enzymes responsible for cell wall loosening and synthesis of new cell wall material, thereby causing abnormal and uncontrolled cell growth. High levels of 2,4-D inhibit these processes and subsequently inhibit growth.

10. In summary, 2,4-D interferes with the normal physiological and growth processes of plants to the extent that it causes the death of susceptible plants. The degradation of 2,4-D by higher plants and microorganisms, as well as degradation and depuration by animals, have been extensively reviewed by many researchers (Loos 1975; Mumma and Hamilton 1976; and Ashton and Crafts 1981).

PART II: MATERIALS AND METHODS

Diluter System

11. The purpose of the diluter system is to deliver different concentrations of a specific herbicide to 24 aquaria. Each herbicide concentration is maintained in four aquaria. The automatic and reliable production and maintenance of prescribed herbicide dilutions permits testing to determine the chronic herbicide concentration required to control each target aquatic plant.

12. The following description, with reference to Figure 1, will explain the basic components of the diluter system and their function. The Data Trak controller (1) is a programmable microprocessor that controls all of the key components of the system. The diluter system is operated via a 24-V direct current (DC) battery supply and, therefore, is unaffected by power interruptions to the facility. The influent water (2) is pumped (3) from a stainless steel tank to the acrylic compartmented chamber (4). Following the filling of each compartment, selected quantities of the concentrated herbicide solution (5) are pumped, using Valcor, Inc., SV-500 series metering pumps (6), to the mixing chamber (12). The vacuum-starting solenoid valve (7) is opened via an electrical impulse from the float valve (8) when the overflow compartment (9) is filled. This allows water from the overflow compartment to drain and thereby exert a reduced pressure on the vacuum manifold (10) and siphon locks (11), which causes the water within each compartment (4) to be pulled up and over the outlet of each automatic siphon. The siphon breaks after each compartment empties. The water from each compartment flows into a 4-l stainless steel mixing chamber (12). Four automatic siphons within each mixing chamber operate simultaneously when the can (12) fills with water. The previously added concentrated herbicide solution was completely mixed by the inflowing water to this can. The desired herbicide dilution flows out of the overflow siphons into four 1-l stainless steel cans comprising the distribution system (13). The desired herbicide dilution proceeds to each aquarium (14),

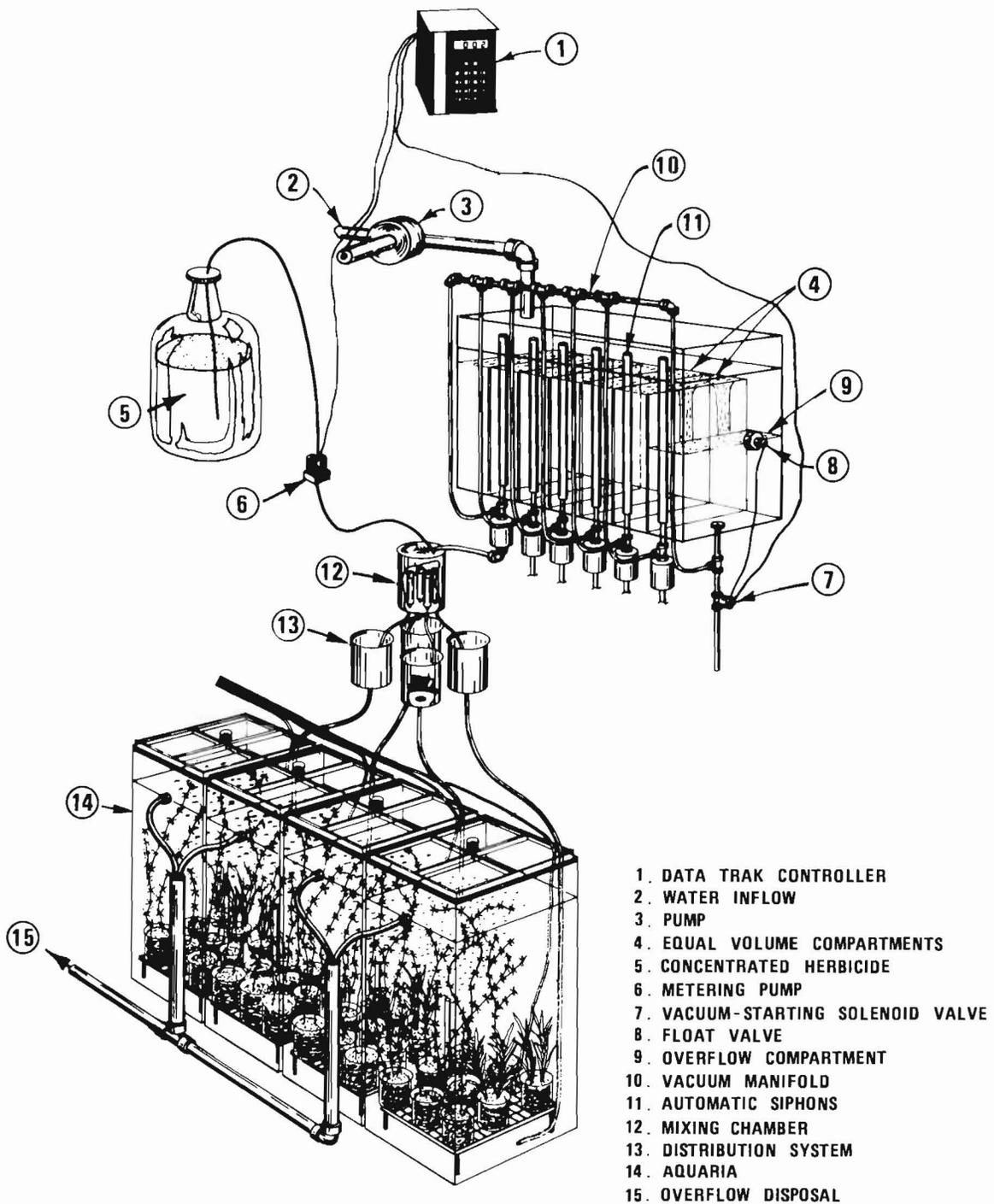


Figure 1. Schematic of the modified diluter system

respectively, via gravity flow out of these containers.

13. Currently, the modified diluter system is capable of delivering 1-ℓ water volumes to each of the 24 glass aquaria, each of which has a volume of 50 ℓ (76 cm high × 30 cm long × 30 cm wide).

14. The entire cycle repeats every 30 min, 24 hr per day, for a designated time period. Longer cycle times can be programmed into the Data Trak controller. The amount of concentrated herbicide added to the mixing cans can be varied by programming the desired number of pumping strokes into the Data Trak controller; however, adequate recycling time for the system must be allowed, i.e., 10 min.

15. The modified diluter system and aquaria (Figures 2 and 3) are located in a controlled-environment greenhouse with supplemental lighting provided by a light bank suspended 1.3 m above the aquaria (Westerdahl and Skogerboe 1982).

16. The herbicide solution enters each of the aquaria at the bottom and is circulated throughout by a pumping action created by bubbling air up an acrylic standpipe 30 cm long × 1.7 cm diameter (Figure 4). Air is injected through an air stone diffuser at the bottom of the standpipe, causing water and the injected herbicide solution to be circulated up through the standpipe. Continuous recirculation of water up the standpipe ensures thorough herbicide mixing in each aquarium, based on preliminary dye studies. Overflow from each aquarium is passed through a carbon absorption tank to remove the residual herbicide prior to disposal (15, in Figure 1).

Experimental Design

Aquaria

17. A simple randomized experimental design was used to assign each of the four 2,4-D treatments to the 24 aquaria as well as to designate which aquaria would receive glass beakers containing hydrosol and plants or only the hydrosol. The latter were to account for 2,4-D losses in the water through adsorption to glass and hydrosol. Similarly, four aquaria were set up without hydrosol or plants to determine

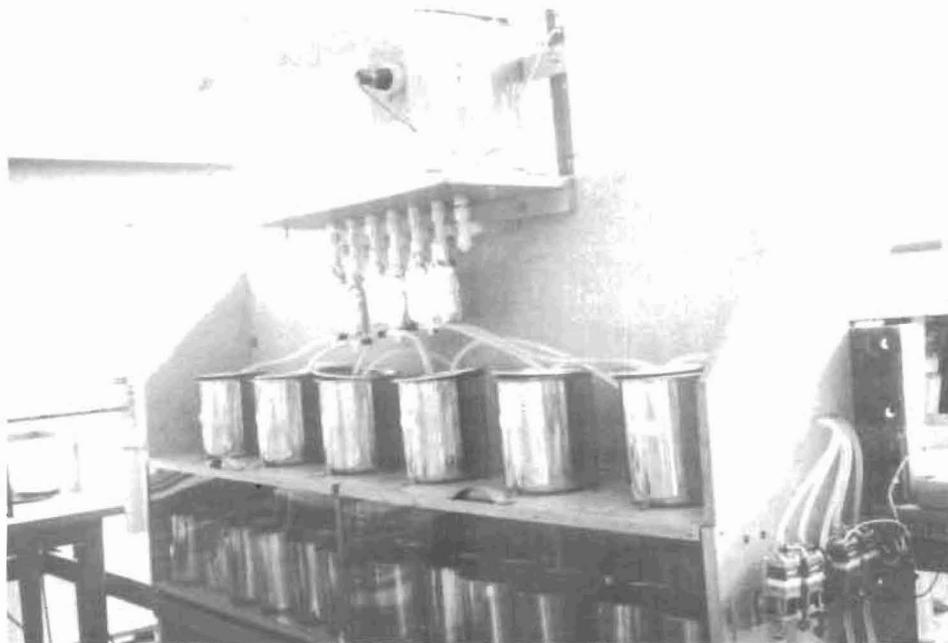
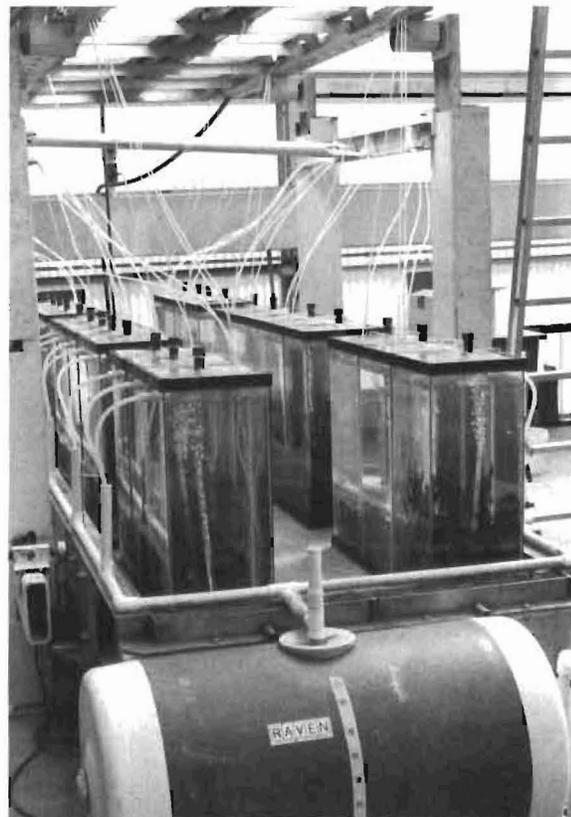


Figure 2. Diluter system located in a controlled-environment greenhouse

Figure 3. Test aquaria located in a controlled-environment greenhouse



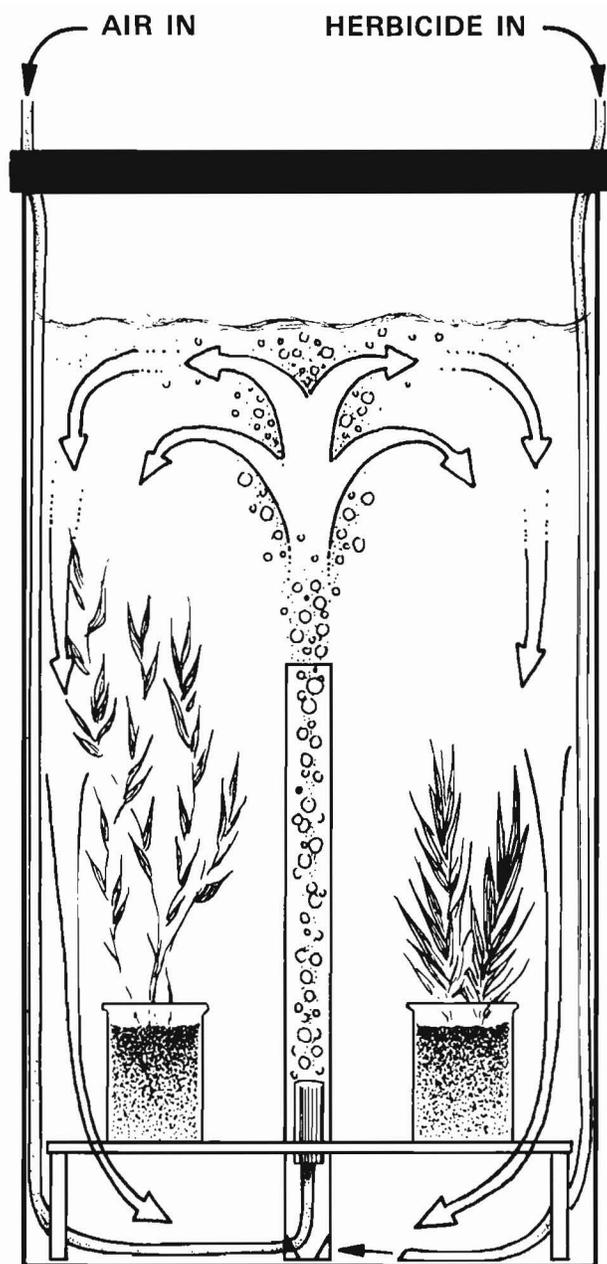


Figure 4. Schematic of a test aquarium illustrating the internal mixing pattern

2,4-D adsorption to the aquaria glass. The 2,4-D treatment rates and aquaria contents are given in Table 1. The selected 2,4-D acid concentrations bracketed the control range, based on previous laboratory and field research, i.e., 0.01, 0.04, 0.10, and 0.20 mg/l. Randomization was necessary to offset potential bias resulting from differences in insolation from the north-to-south and east-to-west exposures of the aquaria. The aquaria were lined up in an east-west orientation in two rows, each containing 12 aquaria.

18. The light bank was used to supplement existing lighting in the greenhouse and to reduce the variation in light intensity received by the aquaria (Figures 5 and 6). The light bank was controlled by a timer which was set to be on from 8:00 a.m. to 4:00 p.m. Ambient light was monitored periodically using a Lambda, Inc., Photosynthetically Active Radiation (PAR) meter.

Water

19. The water used for operating the modified diluter system was uncontaminated, filtered tap water that had been passed through activated carbon and a 0.45- μ cartridge filter. The water supply was checked periodically to determine its chemical composition (Table 2) and to ensure that significant changes did not occur throughout the experiment.

20. Each aquarium was monitored closely throughout the experiment. The 1-l flow of water every 30 min to each aquarium was measured periodically as a check on the diluter system operation. Moreover, 2,4-D residue in the water flowing into and out of each aquarium was monitored to determine losses of 2,4-D within the aquarium. Water temperature was maintained at $25^{\circ} \pm 2^{\circ}\text{C}$ throughout the study period.

Aquatic plants

21. Mature plants of *M. spicatum* were obtained from Lake Washington in Seattle, Wash., and Lake Seminole near Chattahoochee, Fla. Germinated tubers of *P. pectinatus* were obtained from Wildlife Nurseries, Inc., in Oshkosh, Wis.

22. These two aquatic macrophytes were selected as target plants because of their known susceptibility to 2,4-D, and, more importantly, because *M. spicatum* is a major problem in waterways of the Corps of

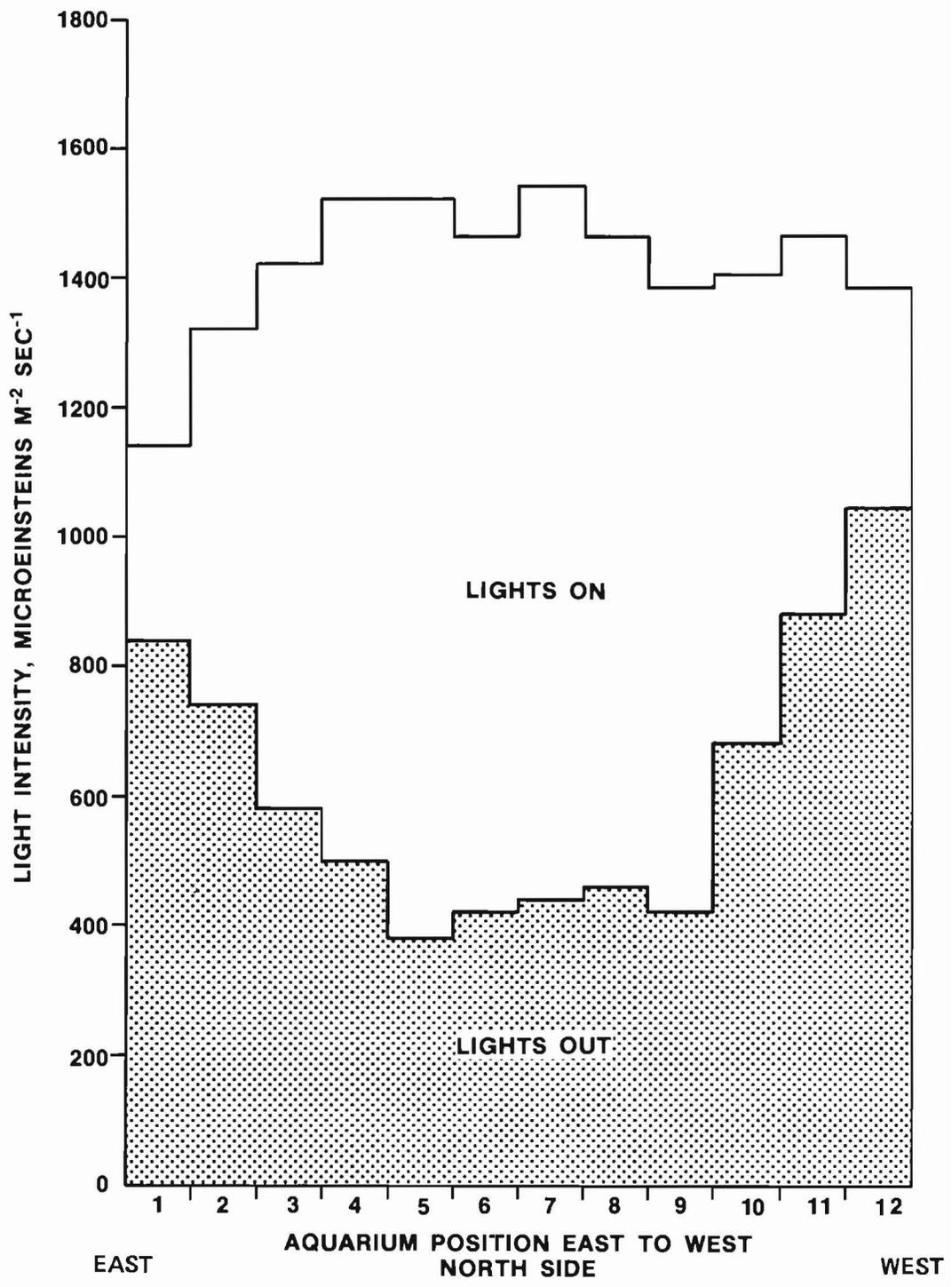


Figure 5. Distribution of light intensity received by the row of aquaria positioned on the north side of the light bank at approximately 1:00 central daylight time (CDT)

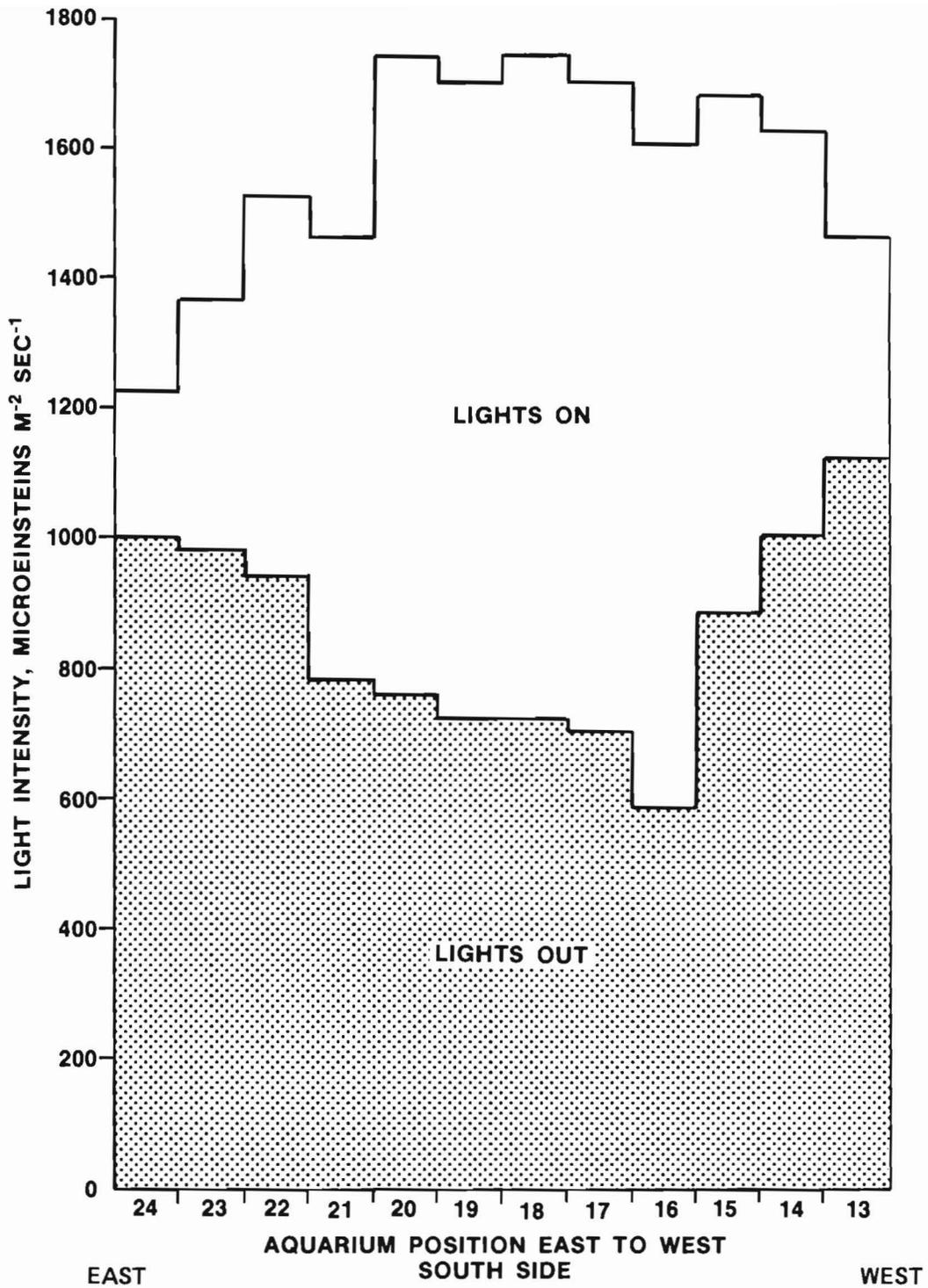


Figure 6. Distribution of light intensity received by the row of aquaria positioned on the south side of the light bank at approximately 1:00 p.m. CDT

Engineers and *P. pectinatus* is of equal concern to the Bureau of Reclamation in western United States irrigation systems. An interagency cooperative effort involving the Corps of Engineers, U. S. Department of Agriculture (USDA) Aquatic Plant Management Laboratory, and Bureau of Reclamation was initiated approximately 2 years ago to standardize and coordinate laboratory herbicide evaluation programs for aquatic plant control.

23. *Myriophyllum spicatum* was maintained as a stock culture in reconstituted natural hard water (U. S. EPA 1975) using a standard hydrosoil (Steward 1981) containing by volume 70 percent washed sand, 25 percent Michigan peat, and 5 percent processed cow manure. Approximately 4 weeks prior to testing, five 15-cm meristematic cuttings of *M. spicatum* were planted in each 250-ml glass beaker by burying the cut end of the plants approximately 5 cm into the hydrosoil. The surface area of each beaker was 34 cm². A finely sieved, washed sand was placed over the hydrosoil to an approximate depth of 2 cm to prevent the peat fragments from floating into the overlying water. Five germinated tubers of *P. pectinatus* were planted in each beaker and covered with 3 cm of hydrosoil and 2 cm of fine sand, respectively. Five beakers each of *M. spicatum* and *P. pectinatus* were placed in the designated aquaria. During the next 4 weeks only water flowed through the aquaria to permit acclimation of the plants and the development of adequate root structure prior to introducing the 2,4-D.

Laboratory Analysis

24. Herbicide residue determinations were performed using approved, standard procedures (American Public Health Association (APHA) 1976). Herbicide residue analyses in water were performed by the Tennessee Valley Authority in Chattanooga, Tenn. Water samples for 2,4-D analysis were obtained before treatment and at 1, 3, 6, 14, 21, 28, and 34 days following initiation of the experiment. At the end of 6 weeks, the aquaria were dismantled and *M. spicatum* was carefully removed from each beaker by washing the hydrosoil from the beaker with deionized

water. Shoots and roots of each plant group within a beaker were separated, dried at 70°C to constant weight, and the weight was recorded (accuracy: ± 0.5 mg/l). It was decided not to separate *P. pectinatus* into shoots and roots because additional rhizomes were formed within the same and adjacent beakers, thus making it impossible to identify which plants were the original ones in most cases.

Data Analysis

25. The threshold 2,4-D concentration was defined to be the minimal concentration required to inhibit plant growth and produce 100-percent control, resulting in plant death after an extended time period. The effects of various 2,4-D concentrations on the growth of *M. spicatum* and *P. pectinatus* were determined using percent plant injury (0 = no control and 100 = total kill) and a set of qualitative factors, which is currently in use at the Aquatic Plant Management Laboratory (APML) in Fort Lauderdale, Fla. (Hoeppel and Westerdahl 1981). These qualitative factors include: heavy algal cover; roots evident; no meristems on stems or branches; leaf loss; evidence of solarization; stem flaccidity; degree of node or internode decomposition; stem and branch tip decomposition; general decomposition of plants; advanced decomposition (only a few stems remaining intact); complete disintegration of plant material; and subsequent regrowth. Plant height measurements of the tallest *M. spicatum* plant per beaker in each aquarium were taken initially and at 3, 4, and 6 weeks. The purpose was to measure growth effects on the plants resulting from constant exposure to several 2,4-D concentrations. For *M. spicatum* the mean shoot:root ratio, expressed as dry weight and representing the three replicate aquaria, was computed for each 2,4-D concentration and compared to the reference. These results assisted in determining the estimated threshold 2,4-D concentration when considered with the plant injury data.

PART III: RESULTS AND DISCUSSION

26. The major emphasis of the pilot study was to assess the reliability and maintenance requirements of the diluter system over extended periods of operation and to identify necessary improvements. However, estimates of the threshold 2,4-D concentration required to control *M. spicatum* and *P. pectinatus* were also determined.

Diluter System Operation

27. The overall reliability and accuracy in delivering pre-programmed water volumes (± 10 percent) with specific 2,4-D concentrations to the test aquaria every 30 min were found to be within the design limits as described by Benoit and Puglisi (1973). However, as the duration of the experiment increased, so did the required maintenance resulting from attached filamentous algal growth in the teflon tubing (0.48 cm inside diameter) leading from the distribution cans to the respective aquaria. Whenever this tubing was not lying flat in the trough carrying the lines to the vicinity of each test aquarium, water was trapped. The entrained air and water in the tubing acted as a valve by preventing water from flowing through the tubing to the aquaria, creating environmental conditions conducive to the growth of algae. These problems required considerable attention to ensure that each test aquarium received the appropriate water volume every 30 min.

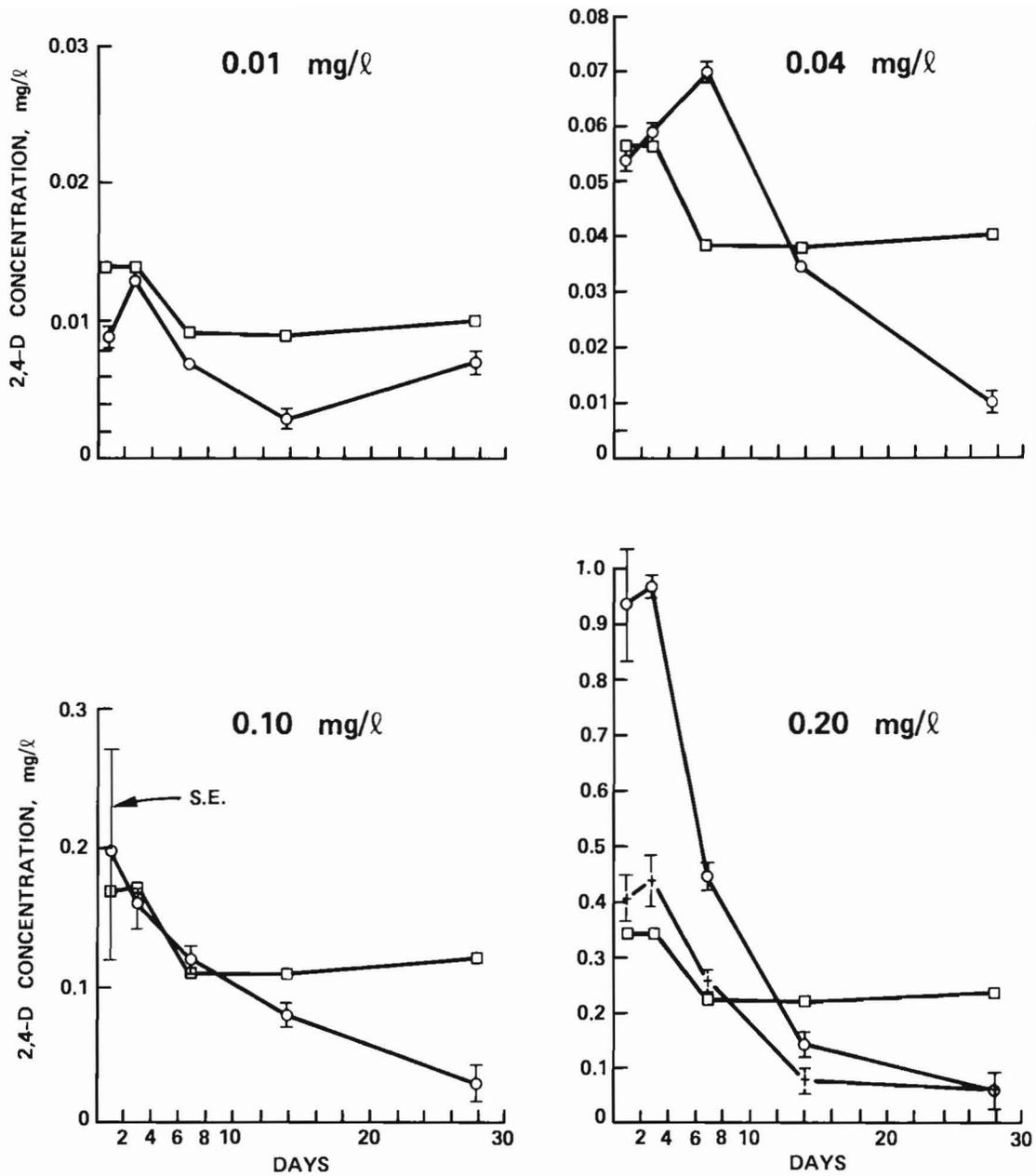
28. The automatic siphons in some of the mixing cans required readjustment on an intermittent basis to ensure delivery of equal water volumes. This problem was a result of a mechanical defect in the construction of some of the automatic siphons. An adjustable neoprene rubber disk, used in each siphon to preset the initiation of siphon action, did not fit properly. The disk would shift slightly resulting in the siphon not starting in conjunction with the others. This problem occurred sporadically during the study period, but it did not significantly change the 2,4-D concentration in the test aquaria. Approximately 2 percent of the water volume in each aquarium was replaced

every 30 min. Hence, daily short-term interruptions for the purpose of adjusting the neoprene disk and checking calibration of water flow and inflow herbicide concentration to each aquarium had negligible effects on the herbicide concentration in the test aquaria.

29. Excessive fungal and algal growth in components of the diluter system became apparent approximately 2 to 3 weeks after initiating the study. Fungal growth was a significant problem only in the mixing and distribution cans and associated teflon tubing receiving the 2,4-D acid. The 2,4-D acid was probably being used as a carbon source for the fungi. No detectable variation in the inflow 2,4-D concentration to each aquarium was observed or expected since the 2,4-D contact time with the cans and tubing during each cycle was approximately 5 min. A residual 50 to 75 ml of water remained in each mixing and distribution canister following each cycle and may have provided sufficient 2,4-D required as a carbon source for supporting fungal growth. Residual moisture and trapped dilute herbicide in the teflon tubing also provided an adequate growth environment for fungi. Excessive algal growth in the acrylic, multicompartmented chamber (Figure 1) and all associated tubing in the diluter system was a result of high moisture conditions and ambient lighting in the greenhouse. Routine maintenance was initiated to keep the system clear of algae through the remainder of the study period.

2,4-D Residue in Water

30. Periodic monitoring of the 2,4-D concentration in the inflow and outflow of each aquarium permitted evaluation of the fate of 2,4-D passing through the aquaria. Figure 7 summarizes these data for each 2,4-D concentration, i.e., 0.01, 0.04, 0.10, and 0.20 mg/l. The initially high 2,4-D concentration delivered to each of the test aquaria during the first 4 days of the study simulated the release expected from controlled-release 2,4-D formulations currently under development. Additional adjustments were made in the diluter system during the first few days of operation to ensure that an equal water volume with the correct 2,4-D concentration was delivered to each of the test aquaria.



LEGEND

- 2,4-D CONCENTRATION IN INFLOW (PLANTS & SOIL)
- 2,4-D CONCENTRATION IN OUTFLOW (PLANTS & SOIL)
- +—+—+ 2,4-D CONCENTRATION IN OUTFLOW (SOIL ONLY)

Figure 7. Mean 2,4-D residue concentrations in inflow and outflow water of aquaria treated at 0.01, 0.04, 0.10, and 0.20 mg/l

During the first week of operation, problems were encountered with the delivery of water and 2,4-D to test aquaria programmed to receive 0.04 and 0.20 mg/ℓ 2,4-D. These problems resulted from mechanical difficulties with the metering pumps and the neoprene disks in the overflow siphons. Once these problems were corrected, the inflow 2,4-D concentration remained constant. The 2,4-D concentrations in the outflow from all of the aquaria were lower than those measured in the inflow after the first week, suggesting that some of the 2,4-D was being removed by the plants and soil. Reference aquaria, containing neither plants nor soil, showed no appreciable decline in 2,4-D concentrations from inflow to outflow. The disappearance of 2,4-D in aquaria containing both plants and hydrosol and only hydrosol suggested that the macrophytes, algae, fungi, and organic debris were the most probable sinks for the 2,4-D. The hydrosol, comprised mostly of sand with minor amounts of peat and processed cow manure, would not be expected to absorb large quantities of 2,4-D from the water. Also, the peat was below a 1-cm-thick cover layer of sand, thereby reducing its effect on 2,4-D absorption. The additional nutrients contributed by processed cow manure to the hydrosol and the available high light intensity provided adequate conditions for extensive development of filamentous algae and fungi on the sediment and plants. These microorganisms were considered to be an important sink for the herbicide.

Growth Response of the Select Plants

31. Weekly measurements of the maximum shoot length per beaker in each aquarium provided an estimate of growth stimulation and inhibition resulting from exposure to different 2,4-D concentrations. *Potamogeton pectinatus* could not be measured accurately to determine maximum shoot heights because the presence of new plants resulting from numerous rhizomes made growth response evaluation impossible. From Table 3, the growth responses were very similar for *M. spicatum* growing in the reference aquaria and in aquaria exposed to 0.01 mg/ℓ 2,4-D. There was visual indication during the first 3 days of operation that increased

milfoil growth and abnormal internode elongation occurred in aquaria receiving 0.01 mg/l 2,4-D. Growth inhibition of milfoil was evident at the 0.04-mg/l 2,4-D exposure. The *M. spicatum* exhibited no growth in those aquaria treated at 0.10 and 0.20 mg/l 2,4-D.

32. Table 4 illustrates the effect of various 2,4-D concentrations on shoot and root biomass of *M. spicatum* following a 6-week continuous exposure to 2,4-D. At the 0.04-mg/l 2,4-D concentration, there was a 2 to 1 reduction in shoot and root biomass compared to the reference and 0.01 mg/l 2,4-D treatments, resulting in the shoot to root ratio remaining unchanged. From the 0.04- to 0.20-mg/l 2,4-D exposures, there was a 4 to 1 reduction in shoot biomass while root biomass remained unchanged. Likewise, the shoot to root ratios decreased. No attempt was made to differentiate living and dead root tissue; however, at the higher treatment rates, the root mass contained more brown root tissue. Therefore, it was not possible to determine quantitatively the effects of 2,4-D concentrations on the root tissue. However, continuous exposure to 2,4-D at concentrations exceeding 0.04 mg/l resulted in no measureable increase in root biomass. Perhaps an extended exposure of root tissue to 2,4-D at 0.04 mg/l would have resulted in root death and decomposition as indicated by Ashton and Crafts (1981).

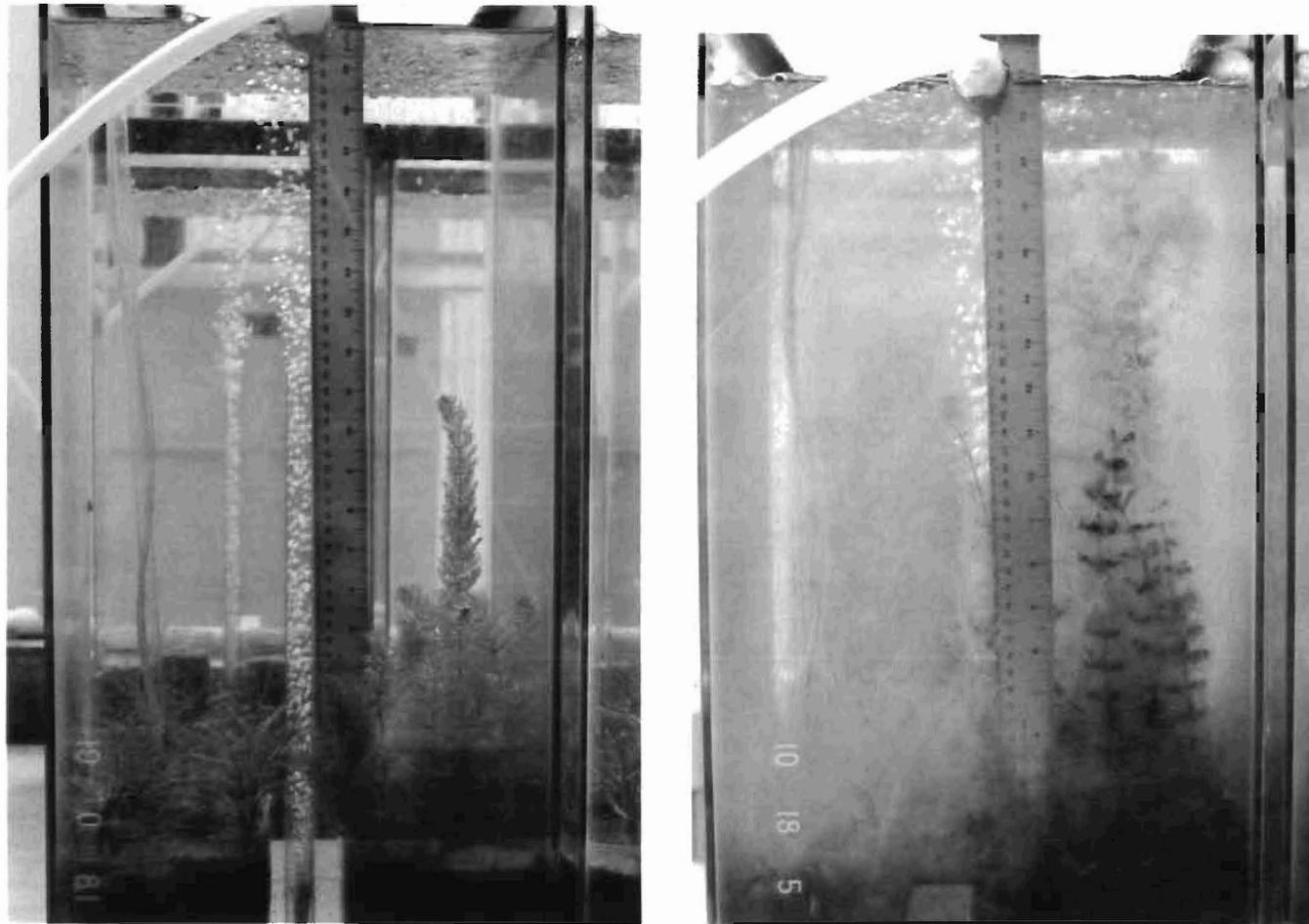
33. Figures 8-12 show physical changes in *M. spicatum* and *P. pectinatus* over the 6-week study period during which they were under constant exposure to 2,4-D at the respective concentrations of 0.00, 0.01, 0.04, 0.10, and 0.20 mg/l. The threshold 2,4-D acid concentration for *P. pectinatus* was estimated to be around 0.20 mg/l based on the extensive physical damage to the plant. The chlorotic condition of this plant was clearly evident at the 0.20-mg/l 2,4-D concentration (Figure 12), indicating that plant growth was controlled at this concentration. However, slight chlorosis was noted at the 0.10 mg/l 2,4-D concentration (Figure 11), suggesting significant tissue damage. It was not clear from these results whether or not a constant chronic exposure to 0.10 mg/l 2,4-D for more than 6 weeks would control *P. pectinatus*.



BEFORE

AFTER

Figure 8. Growth response of *M. spicatum* (right) and *P. pectinatus* (left) in reference aquaria before and after 6 weeks. (Note filamentous algae on plant tissue)



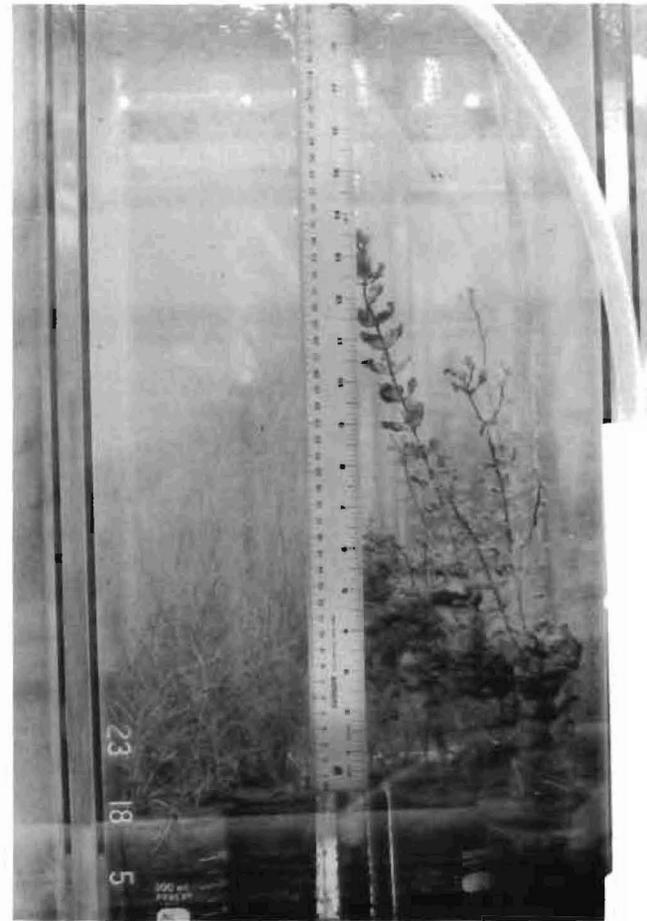
BEFORE

AFTER

Figure 9. Growth response of *M. spicatum* (right) and *P. pectinatus* (left) before and after a 6-week exposure to 0.01 mg/l 2,4-D. (Note enhanced growth of *M. spicatum* and attached filamentous algae on plant tissue)



BEFORE

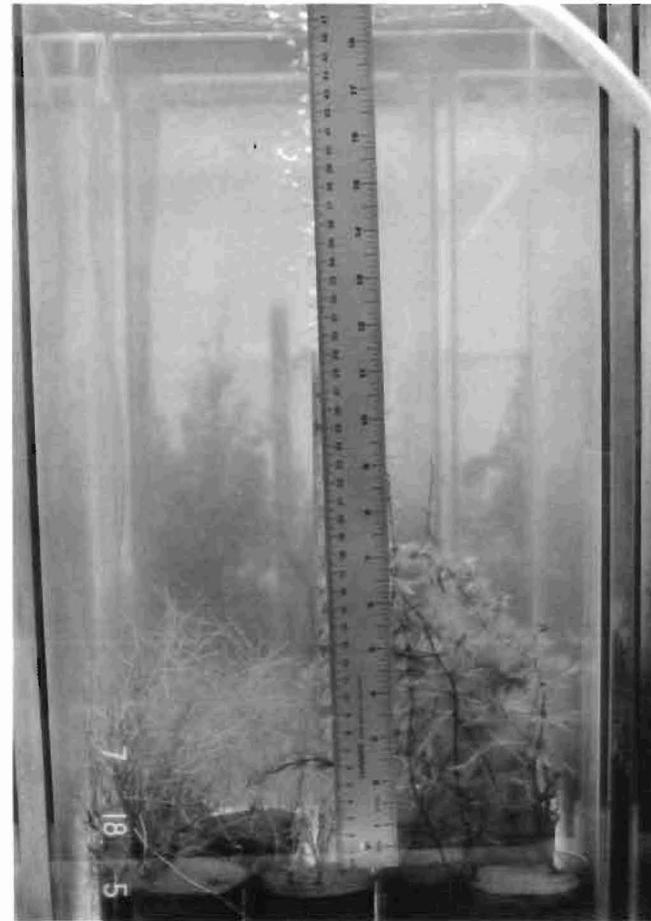


AFTER

Figure 10. Growth response of *M. spicatum* (right) and *P. pectinatus* (left) before and after a 6-week exposure to 0.04 mg/l 2,4-D



BEFORE

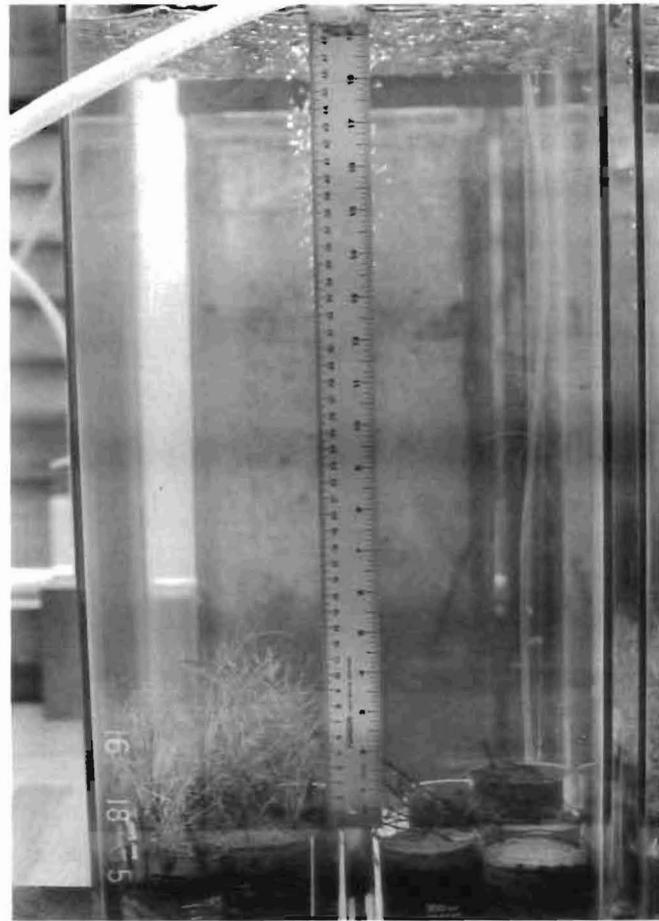


AFTER

Figure 11. Growth response of *M. spicatum* (right) and *P. pectinatus* (left) after a 6-week exposure to 0.10 mg/l 2,4-D



BEFORE



AFTER

Figure 12. Growth response to *M. spicatum* (right) and *P. pectinatus* (left) before and after a 6-week exposure to 0.20 mg/l 2,4-D

Assessment of Plant Injury

34. Excessive blue-green algal growth was noticed approximately 3 weeks after the study was initiated, making it difficult to rate plant injury. The supplemental lighting was shut off for the duration of the study to see if the algae would be eliminated. Unfortunately, the available nutrients from the composted cow manure in the hydrosol and decomposing plant tissue enhanced algal production without the additional lighting. After reducing the light intensity, healthy *M. spicatum* and *P. pectinatus* were able to grow through a developing filamentous green algal mat attached to the plants. Blue-green algae were no longer dominant. Plant injury ratings were continued until completion of the study.

35. Plots of *M. spicatum* and *P. pectinatus* response to various 2,4-D concentrations are illustrated in Figures 13 and 14, respectively. At the lower 2,4-D concentrations, only minimal plant tissue damage was observed within 1 to 2 weeks after initiation of this study. Accumulative plant tissue damage, resulting from continuous uptake of 2,4-D beyond this initial period, was not observed. Apparently, the uptake of 2,4-D is retarded after initial plant tissue damage.

36. Regression analysis was used to compare the percent injury incurred by *M. spicatum* and *P. pectinatus* when exposed continuously to the selected 2,4-D concentrations. The regression equation ($y = bx$) was used to estimate the time required to produce 50-percent injury for each 2,4-D concentration. The minimum length of time required to produce 50-percent injury to *M. spicatum* was slightly more than 5 weeks with a continuous exposure to 0.04 mg 2,4-D/l; approximately 2.5 weeks with 0.10 mg 2,4-D/l; and 1.5 weeks with 0.20 mg 2,4-D/l. For *P. pectinatus*, this time period was 2.6 weeks with 0.10 mg 2,4-D/l and 1.7 weeks with 0.20 mg 2,4-D/l. Control, i.e., complete kill of target plants, of these macrophytes at a 2,4-D concentration of 0.01 mg/l was not observed. Most important, however, was that complete control for *M. spicatum* was achieved at the 0.10-, and 0.20-mg 2,4-D/l treatment, whereas for *P. pectinatus* complete control was achieved only at the 0.20-mg 2,4-D/l treatment.

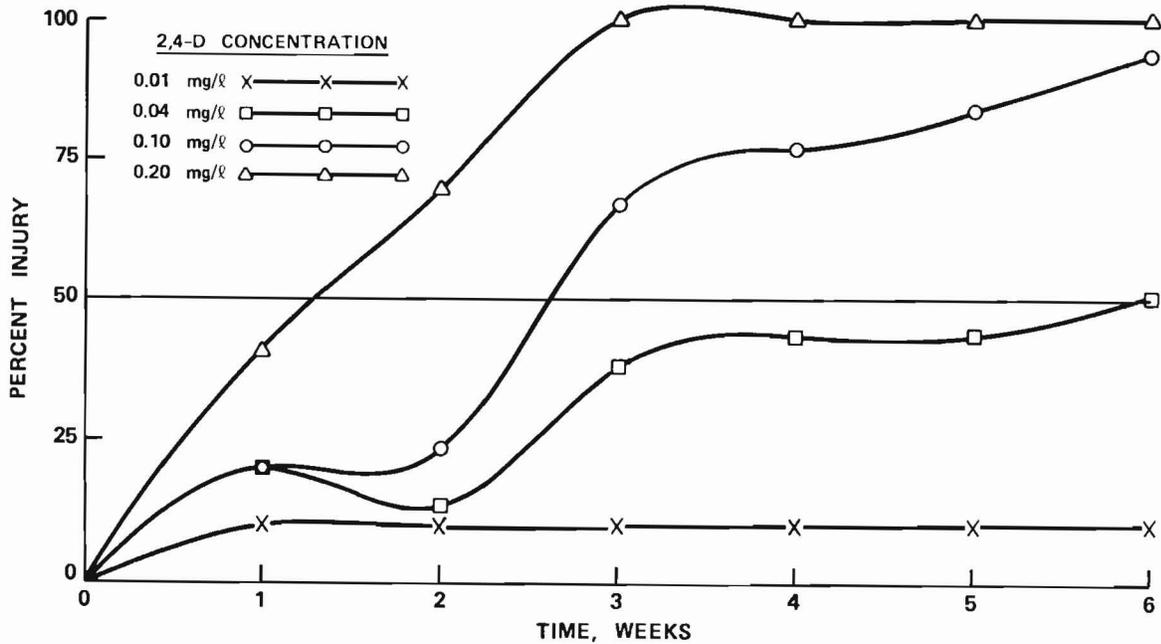


Figure 13. *Myriophyllum spicatum* response to four treatment concentrations of 2,4-D acid over a 6-week study period

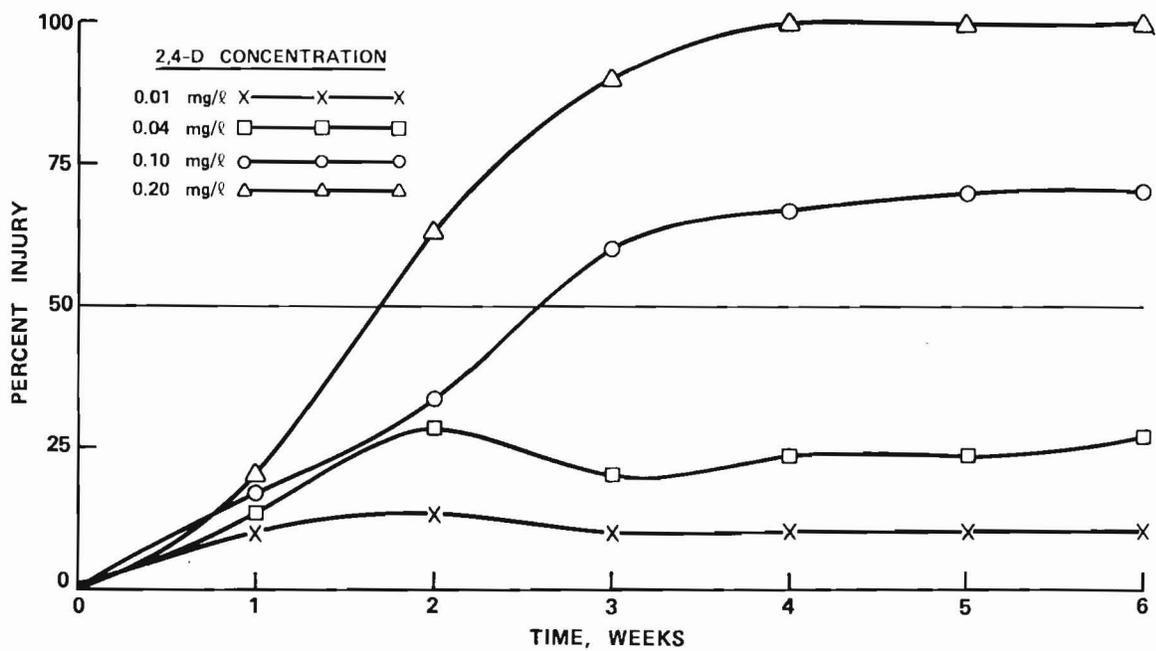


Figure 14. *Potamogeton pectinatus* response to four treatment concentrations of 2,4-D acid over a 6-week study period

PART IV: CONCLUSIONS AND RECOMMENDATIONS

Conclusions

37. As a result of this pilot study, the following conclusions were drawn:

- a. The WES-modified diluter system was reliable and accurate in dispersing and maintaining preprogrammed water volumes and 2,4-D concentrations in each of the test aquaria.
- b. Further modifications in the diluter system are necessary to prevent excessive algal and fungal growth in the tubing, cannisters, and test aquaria, which inhibited accurate efficiency assessment during the study.
- c. The threshold 2,4-D concentrations required to control *M. spicatum* and *P. pectinatus* were 0.10 mg/ℓ and approximately 0.20 mg/ℓ, respectively, in the conditions prevailing during this study.

Recommendations

38. The following items are recommended:

- a. All test aquaria should be wrapped in black plastic to allow light from the overhead light bank to enter only from the top of the aquaria.
- b. The hydrosol mixture should not contain the processed cow manure, but only sand and peat.
- c. All tubing leading from the distribution cans to the test aquaria should have a larger inside diameter to permit rapid drainage and prevent air from being trapped in the lines. All of these lines should be held in a planar, downward-sloping attitude toward the aquaria.
- d. All tubing, along with the multicompartmented acrylic chamber, should be covered with aluminum foil to reduce growth of algae during conduct of the study.
- e. Routine maintenance of the modified diluter system should include periodic calibration checks of the water delivered to each aquarium as well as herbicide delivered to each mixing can.
- f. All tubing and stainless steel cans should be periodically cleaned to prevent buildup of algae and fungi populations.

- g. The threshold 2,4-D concentration(s) required to control *M. spicatum* and *P. pectinatus* should be verified after the recommended modifications to the diluter system are completed. The study should be conducted for a 10- to 12-week period to determine accumulative toxic effects of low 2,4-D concentrations on *M. spicatum* and *P. pectinatus*.

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Table 1
Aquaria Treatment Rates and Contents for the
2,4-D Pilot Study

2,4-D Concentration mg/ℓ	Number of Aquaria	Aquarium Contents		
		Empty	Hydrosoil*	Plants**
0	4			x
0.01	1	x		
0.01	3			x
0.04	1	x		
0.04	3			x
0.10	1	x		
0.10	3			x
0.20	2	x		
0.20	3		x	
0.20	3			x

* Each aquarium contained ten 250-ml beakers of standard hydrosoil.

** Each aquarium contained ten 250-ml beakers of standard hydrosoil; each of five beakers contained five propagules of *M. spicatum* and *P. pectinatus*, respectively.

Table 2
Chemical Composition of Filtered Tap Water Used for the
Diluter System for April - May 1981

<u>Parameter</u>	<u>Concentration, mg/ℓ</u>
Total organic carbon	<1.0
Total Kjeldahl nitrogen	<0.1
Ammonia-N	<0.01
Nitrate-N	0.02
Total phosphorus	0.10
Orthophosphate	0.01
Total iron	<0.05
Total manganese	0.04
Alkalinity	62.0
Hardness	66.0
Calcium	11.5

Table 3
Mean Maximum Shoot Height of *M. spicatum* Exposed to
 Various 2,4-D Concentrations

2,4-D Concentration, mg/ℓ	Date (1981) - Mean Maximum Shoot, cm			
	3/27	4/21	4/28	5/12
Reference	13.1 ±5.4**	27.8 ±5.3	34.4 ±4.0	37.3* ±8.0
0.01	13.0 ±2.3	26.5 ±3.8	27.5* ±4.0	*
0.04	14.1 ±3.3	19.3 ±5.4	19.3* ±5.3	*
0.10	15.3 ±2.5	†	†	†
0.20	16.1 ±1.7	†	†	†

* Excessive algal growth occluded all plants; however, plants were healthy in reference and 0.01 mg/ℓ 2,4-D treated aquaria.

** ± Standard Error of Mean, n = 15.

† Plants were settling to the bottom of the aquaria.

Table 4
Biomass and Shoot:Root Ratio of *M. spicatum* Following 6-Week
Continuous Exposure to Several 2,4-D Acid Concentrations

2,4-D Acid Concentration, mg/ℓ	Biomass, g*		Shoot:Root Ratio
	Shoots	Roots	
Reference	1.08 ± 0.14**	1.41 ± 0.21	0.77
0.01	1.14 ± 0.15	1.53 ± 0.18	0.75
0.04	0.66 ± 0.09	0.86 ± 0.16	0.77
0.10	0.34 ± 0.05	0.94 ± 0.12	0.36
0.20	0.17 ± 0.05	0.82 ± 0.23	0.21

* Expressed as dry weight.

** Mean ± Standard Error, n = 15.

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Determination of chemical threshold concentrations using 2,4-D to control selected aquatic macrophytes-- a pilot study to evaluate a laboratory system / by Howard E. Westerdahl ... [et al.] (Environmental Laboratory, U.S. Army Engineer Waterways Experiment Station). -- Vicksburg, Miss. : The Station ; Springfield, Va. : available from NTIS, 1983. 33, [4] p. : ill. ; 27 cm. -- (Technical report ; A-83-4)

Cover title.

"February 1983."

Final report.

"Prepared for Office, Chief of Engineers, U.S. Army."

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