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COMPILED DATA ON THE
VASCULAR AQUATIC PLANT PROGRAM: 1975-1977

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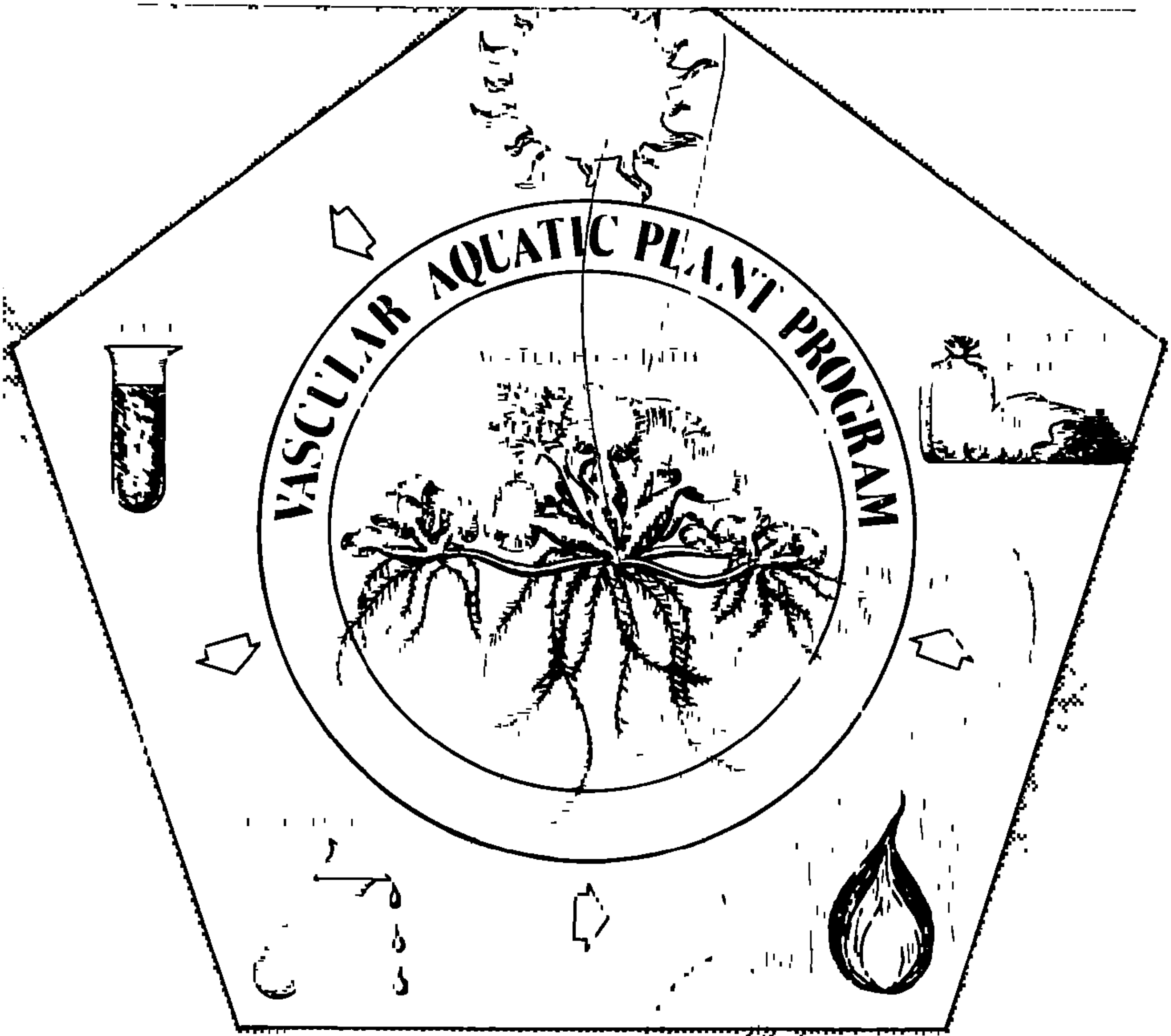

National Aeronautics and Space Administration
National Space Technology Laboratories
NSTL Station, Mississippi 39529

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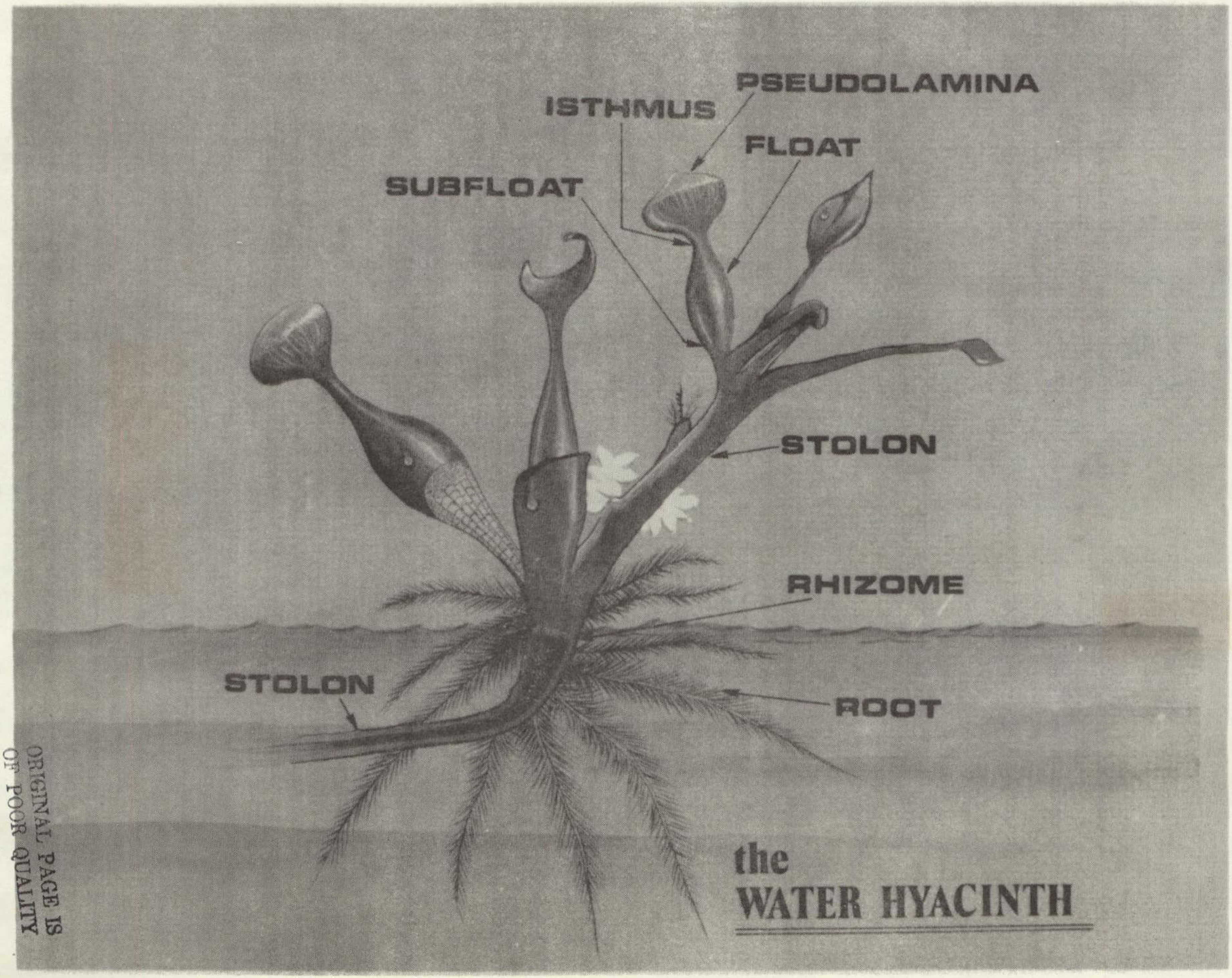

the
WATER HYACINTH

FOREWORD

The National Aeronautics and Space Administration at the National Space Technology Laboratories, Bay St. Louis, MS has been developing a unique method of using vascular aquatic plants to purify wastewater and to reclaim nutrients and metals. These investigations have focused on the free-floating water hyacinth (Eichhornia crassipes) because of its tremendous growth rate. The water hyacinth has been grown in both domestic and chemical waste treatment systems. The plants harvested from these systems were used in experiments to determine their potential as a new source of food, feed, fertilizer, and energy.

The characteristics of the water hyacinth, which make it ideally suited for these applications are:

1. It floats. The root system feeds on nutrients in the water, and the leaves are exposed to the air.
2. Its harvesting accessibility. Its floating nature facilitates harvesting.
3. Its growth rate. Under ideal temperature and nutrient conditions, it will produce over 873 kg/ha/da (800 lb/ac/da) of dry plant material.
4. It grows freely in warm climates between 32°N and 32°S latitudes. Water hyacinths survive year-round in the states of Florida, Mississippi, Louisiana, Texas, and California. Water hyacinths are highly productive over a water temperature range of 22° to 35°C with optimum water temperatures being 28° to 30°C.
5. Its absorbant qualities. Water hyacinth can sorb metals, organics, and nutrients.

The following sections are comprised of some of the available reports and articles on this program.

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SECTION I

DOMESTIC WASTE TREATMENT

SECTION I
Part 1

UPGRADING FACULTATIVE WASTE STABLIZATION PONDS WITH VASCULAR AQUATIC PLANTS

By: B. C. Wolverton
Rebecca C. McDonald

ERL Report No. 172

March, 1978

ABSTRACT

The performance of a single cell, facultative sewage lagoon at NASA's National Space Technology Laboratories has been significantly improved with the introduction of vascular aquatic plants. Water hyacinth (Eichhornia crassipes) was the dominant plant from April to November; duckweed (Lemna spp.) and (Spirodela spp.) flourished from December to March. This 2 ha lagoon receives approximately 475 m^3/day of untreated sewage and has a variable BOD_5 loading rate of 22-30 kg/ha/day.

During the first 14 months of operation with aquatic plants, the average influent BOD_5 was reduced by 95% from 110 mg/l to an average of 5 mg/l in the effluent. The average influent suspended solids were reduced by 90% from 97 mg/l to 10 mg/l in the effluent.

Although this lagoon was not harvested at a rate necessary to achieve maximum nitrogen and phosphorus removal, significant reductions in both of these nutrients were effected. The monthly kjeldahl nitrogen for influent and effluent averaged 12.0 and 3.4 mg/l, respectively, a reduction of 72%. The total phosphorus was reduced on an average of 56% from 3.7 mg/l influent to 1.6 mg/l effluent.

Introduction

Human waste disposal problems have been the focus of attention for a number of years, but now, with the population increasing rapidly, more stringent controls over waste materials are urgently needed to protect our potable and recreational waters. A primary goal of waste treatment management is to develop more efficient systems of waste stabilization, leading ultimately to water purification and recycling.

The effectiveness of waste treatment systems is measured by the reduction of oxygen demanding material (biochemical oxygen demand or BOD), total suspended solids (TSS), and nutrients such as nitrogen and phosphorus that are discharged into receiving waters. The Environmental Protection Agency has recently set stricter standards on discharges from wastewater treatment facilities. As of July 1, 1977, treatment must effect an 85 percent removal of both BOD_5 and TSS. The maximum allowable level for both parameters is 30 mg/l.

In the United States, sewage lagoons are the most popular and inexpensive method of treating domestic wastewater in small communities. Providing land costs are not excessive, waste stabilization lagoons generally cost less than half that of other treatment methods (Gloyna, 1971) and require a minimum of maintenance. According to Lewis (1974), approximately 90 percent of the wastewater lagoons in this country serve communities of 10,000 or less.

Domestic sewage lagoons can be generally classified into three categories: anaerobic, aerobic, and facultative, which combines features of anaerobic and aerobic ponds. The design features of these ponds are discussed in detail by Oswald (1963) and Gloyna (1971). In the southern United States the most commonly used design is that of the facultative waste stabilization pond.

Recently, Barsom (1973) conducted a survey assessing the performance of waste stabilization ponds throughout the nation. This survey indicated that the majority of sewage lagoons are not meeting the Environmental Protection Agency's July 1977 standards. According to this survey, the five-day BOD of facultative lagoons averaged 25 to 75 mg/l and TSS ranged from 60 to 120 mg/l. Much of the suspended solids consists of algae, but the EPA standards do not differentiate between algae and other organics. Clearly sewage lagoons must be upgraded to meet EPA standards. As was stated in a recent Environmental Protection Agency symposium, "the development of relatively inexpensive methods for upgrading lagoons that do not require sophisticated and constant operation or expensive maintenance is urgently needed" (Middlebrooks, et al., 1974). According to these workers and Barsom (1973), effective reduction of algae and suspended solids in lagoon effluents is the number one research goal.

To understand why sewage lagoons might fail to function properly, it is necessary to examine the processes which occur within these ponds. The overall principle of facultative lagoon operations is simple, relying upon the conversion of complex organics into bacteria, algae, and nutrients. The processes of synthesis and endogenous respiration carried on by algae and bacteria in lagoon systems are not thoroughly understood. However, we may simplify the natural purification of wastewater in facultative lagoons as follows: anaerobic and aerobic bacteria decompose organic waste through reduction and oxidation processes, respectively, producing carbon dioxide, methane, water, energy, and free nutrients. Algae use these nutrients in photosynthesis to generate oxygen and produce organic material in a form more compatible with the environment. Some of this algae is lost from the system in the effluent, some is consumed by aquatic grazers, and some die and are naturally degraded within the system. Effective lagoon operation requires that incoming nutrients and organic matter be broken down or removed from the system so that they do not appear in the effluent.

In general, properly designed sewage lagoons operate efficiently for much of the year. During the winter, lagoon effluent is low in both five-day BOD and TSS, since there is little biological activity or algal growth during this season. In the spring, however, as rising temperatures create conditions favorable for growth, the algae respond dramatically to the high level of nutrients which have been building up in the lagoon over the winter months, resulting in tremendous algal blooms. Subsequent massive algal dieoffs create a high oxygen demand, favoring the growth of anaerobic bacteria, which in turn often cause odor problems. Thus, spring and early summer are the seasons when lagoons are most likely to produce odors and/or effluents of unacceptable quality. The obvious solution to this problem is to reduce the amount of algae present in the lagoon during the spring and summer months.

Direct harvesting of algae is a costly and complicated procedure. To date, no methods for mechanical removal of algae feasible for use in small communities have been perfected, although several are in the testing stage (see Middlebrooks, et al., 1974). Introducing a vascular aquatic plant species has been considered as one means of reducing the amount of algae in the lagoon effluent. Vascular aquatic plants could discourage erratic fluctuations in algal populations both by removing excess nutrients and by shading out the algae. Several investigators have proposed using the water hyacinth (Eichhornia crassipes) for these purposes (Wolverton, et al., 1976; Steward, 1970; Dinges, 1976, Cornwell, et al., 1977). Water hyacinths, which remove both nutrients and organics directly from the water via their extensive root systems (Wolverton, et al., 1975; Ultsch and Anthony, 1974; Boyd, 1970), can increase at the phenomenal rate of 15 percent of their surface area per day, producing at least 20 tons wet weight per hectare per day (Wolverton and McDonald, 1976). Based on measured growth rates, Rogers and Davis (1972) estimated that one hectare of water hyacinths could remove the nitrogen and phosphorus waste of over 800 people per day. These concentrated nutrients can then be removed from the system by harvesting the water hyacinths. The harvested plant material has potential economic value as a soil amendment (Parra and Hortenstein, 1974), as a livestock feed (Baldwin, et al., 1974), and perhaps even as a human protein supplement (Taylor, et al., 1971; Wolverton, et al., in preparation).

Another vascular aquatic plant capable of supplementing the role of the water hyacinth during the winter months is duckweed, (Lemna spp.) and (Spirodela spp.). Studies with these cold-tolerant plants have shown that they can thrive on domestic wastewater and also remove excess nutrients and produce a significant effect on BOD and TSS reduction rates (Sutton and Ornes, 1977; Culley and Epps, 1973; Schultze, 1966).

Since 1975, the National Aeronautics and Space Administration has been growing water hyacinths (Eichhornia crassipes) throughout most of the year and duckweed (Lemna spp.)

and (Spirodela spp.) during the winter in sewage lagoons at the National Space Technology Laboratories (NSTL), Bay St. Louis, Mississippi, to improve effluent quality. In this report, we trace the performance of one of these lagoons before and after the addition of water hyacinths and duckweed.

Description of the System

NSTL Lagoon Number 1 consists of a single cell with a surface area of approximately 2 hectares and an average depth of 1.22 meters. The average flow rate of 475 m^3/day results in a retention time of approximately 54 days. The BOD_5 loading rate in this lagoon averages 22-30 kg/ha/day, which constitutes a relatively light load (Oswald, 1963). Before the introduction of water hyacinths, the average suspended solids discharged in the effluent waters of this lagoon exceeded the EPA limit during some months of the spring and summer.

Materials and Methods

Adequate background data on Lagoon #1 without water hyacinths necessary for comparison of BOD_5, TSS, and pH were recorded in the NSTL environmental monitoring files for the period of May to September, 1974. During this period approximately two grab samples per week were analyzed. Only six samples were obtained during July, 1974. Since only one grab sample per month was analyzed as required by the EPA discharge permit effective during the intervening months of October, 1974, to March, 1976, the data were judged inadequate for comparative purposes and, therefore, omitted. During the background months used in this study, approximately 1,000 people serviced this lagoon. This population was increased to 2,000 people by the summer of 1977.

Beginning in March of 1976, influent and effluent grab samples were taken twice a week from the lagoon and analyzed for additional parameters. Water samples were analyzed for pH, dissolved oxygen (DO), temperature, suspended and dissolved solids and five-day biochemical oxygen demand according to Standard Methods. Total organic carbon (TOC) was measured with a Beckman 915 TOC analyzer. Kjeldahl nitrogen and phosphorus were determined with a Technicon Autoanalyzer II.

Water hyacinths were introduced into the lagoon in June of 1976, and by August the plants had covered approximately 90 percent of the surface area. Following the first frost in November, 1976, the plants, which are not cold-tolerant, died back during the winter months and were replaced by duckweed. In March of 1977, the surviving plants re-sprouted, achieving a 25 percent coverage by the end of this month.

Results

By comparing the quality of the lagoon's effluent during the background and experimental periods, a clear picture of the effects of water hyacinths and duckweeds can be seen. Table 1 presents all of the compiled data on TSS and BOD_5 for the available background and water hyacinth treatment periods. The data for suspended solids can be more easily examined in Figure 1. These vascular aquatic plants substantially reduced the suspended solids

Table 1. Monthly Average Data of Total Suspended Solids (TSS) and 5-Day Biochemical Oxygen Demand (BOD_5) for Background and Experimental Periods

A. Background Period				
	TSS, mg/ℓ		BOD_5, mg/ℓ	
	Influent	Effluent	Influent	Effluent
May, 1974	130	88	83	17
June, 1974	60	76	54	25
July, 1974	40	51	61	17
August, 1974	47	46	83	23
September, 1974	50	27	92	13
March, 1976	78	17	138	9
April, 1976	77	50	93	16
May, 1976	75	39	122	14
B. Initial Stocking Months (Partial Water Hyacinth Coverage)				
June, 1976	112	25	79	9
July, 1976	63	12	70	5
C. Water Hyacinth Experimental Period				
August, 1976	89	5	109	7
September, 1976	68	6	140	5
October, 1976	64	3	112	2
November, 1976	84	4	143	2
*December, 1976	113	8	90	2
*January, 1977	88	8	69	2
*February, 1977	132	14	88	7
**March, 1977	103	17	93	14
**April, 1977	116	17	97	9
**May, 1977	161	13	201	6
June, 1977	86	18	125	5
July, 1977	78	9	141	5
August, 1977	80	12	37	6
September, 1977	101	8	96	4

*Water hyacinths damaged by cold weather; duckweed treatment operative.

**Water hyacinths recover from winter with initial coverage of 20% in March, 1977.

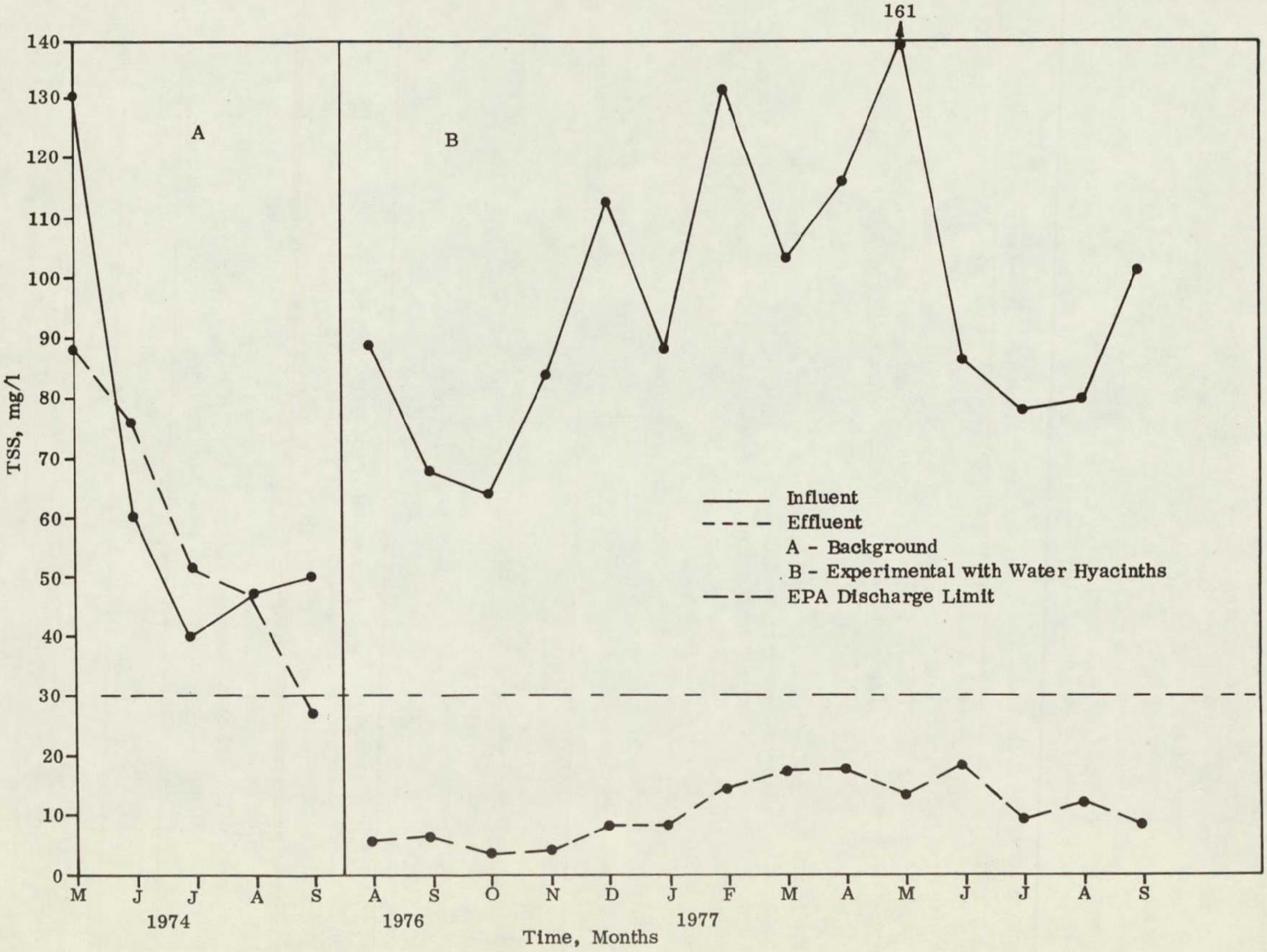

Figure 1. Monthly average total suspended solids vs. time (months)

below the 30 mg/l maximum EPA level and reliability maintained this requirement year-round. The substantial reduction of suspended solids was largely due to the virtual elimination of algae from the system. Water hyacinths are the most effective in the summer months which coincide with the maximum problem period for a lagoon. The percent reduction of TSS* was highly variable before the introduction of water hyacinths. As shown in Table 2, the TSS in the effluent was often more than those of the influent during the summer months due to periodic algal blooms. However with water hyacinths present, the total suspended solids were consistently reduced on an average of 89%.

These vascular aquatic plants also had a significant effect on the reduction of BOD_5. This reduction was not as dramatic as the one with suspended solids, since the lagoon was fairly effective at BOD_5 reduction before the introduction of aquatic plants. Figure 2 clearly shows that this aquatic plant system reliably maintains the BOD_5 year-round below the EPA discharge limit of 30 mg/l. Table 2 shows that the lagoon achieved an average of 76% reduction in BOD_5 before water hyacinths were introduced; with aquatic plants, the lagoon reduced the BOD_5 by an average of 94%.

From Table 3, the effect of these vascular aquatic plants on pH and dissolved oxygen can be ascertained. During the background period, the influent and effluent pH's averaged 7.0 and 9.3, respectively. The effluent pH often increased over 10. The EPA discharge permit limits the effluent pH to 9.0; therefore, it is clear that the lagoon rarely met this requirement. After the introduction of water hyacinths and duckweeds, the influent and effluent pH's averaged 7.1 each. This buffering effect results from an increase in CO_2 which is normally depleted during algal photosynthesis because algae derive all of their CO_2 from the wastewater, whereas most of the CO_2 required by water hyacinths is obtained from the air.

As expected, water hyacinths decreased the dissolved oxygen from an average effluent level of 6.9 mg/l without hyacinths to 2.3 mg/l with hyacinths (Table 3). This would be critical to pond operation only if the pond were heavily loaded and anaerobic conditions prevailed. Minimum aeration could be applied at the discharge point to bring the effluent DO concentration up to the normally required level of 5 mg/l.

The available background data on total Kjeldahl nitrogen, total phosphorus and total organic carbon concentrations is extremely limited (Table 4). These parameters were greatly reduced as shown in Table 5 in the summer months with somewhat less reductions during the winter when duckweed is the dominant aquatic plant. Further interpretation of these results is difficult without a direct comparison, since a significant quantity of nitrogen is normally lost from a lagoon due to natural denitrification processes. The high percentage reduction in total organic carbon is largely due to the virtual elimination of algae as demonstrated by the dramatic reduction of suspended solids.

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*$\frac{\text{INF. TSS} - \text{EFF. TSS}}{\text{INF. TSS}} \times 100\%$

Table 2. Comparative Percent Reductions of TSS and BOD_5

	Percent Reduction			
	TSS		BOD_5	
Month	Without Water Hyacinths	With Water Hyacinths	Without Water Hyacinths	With Water Hyacinths
May	*32/48	92	*80/89	97
June	-27	79	54	96
July	-28	88	72	96
August	2	*85/94	72	*94/84
September	46	*92/91	86	*96/96

* 2 Consecutive Year Reductions

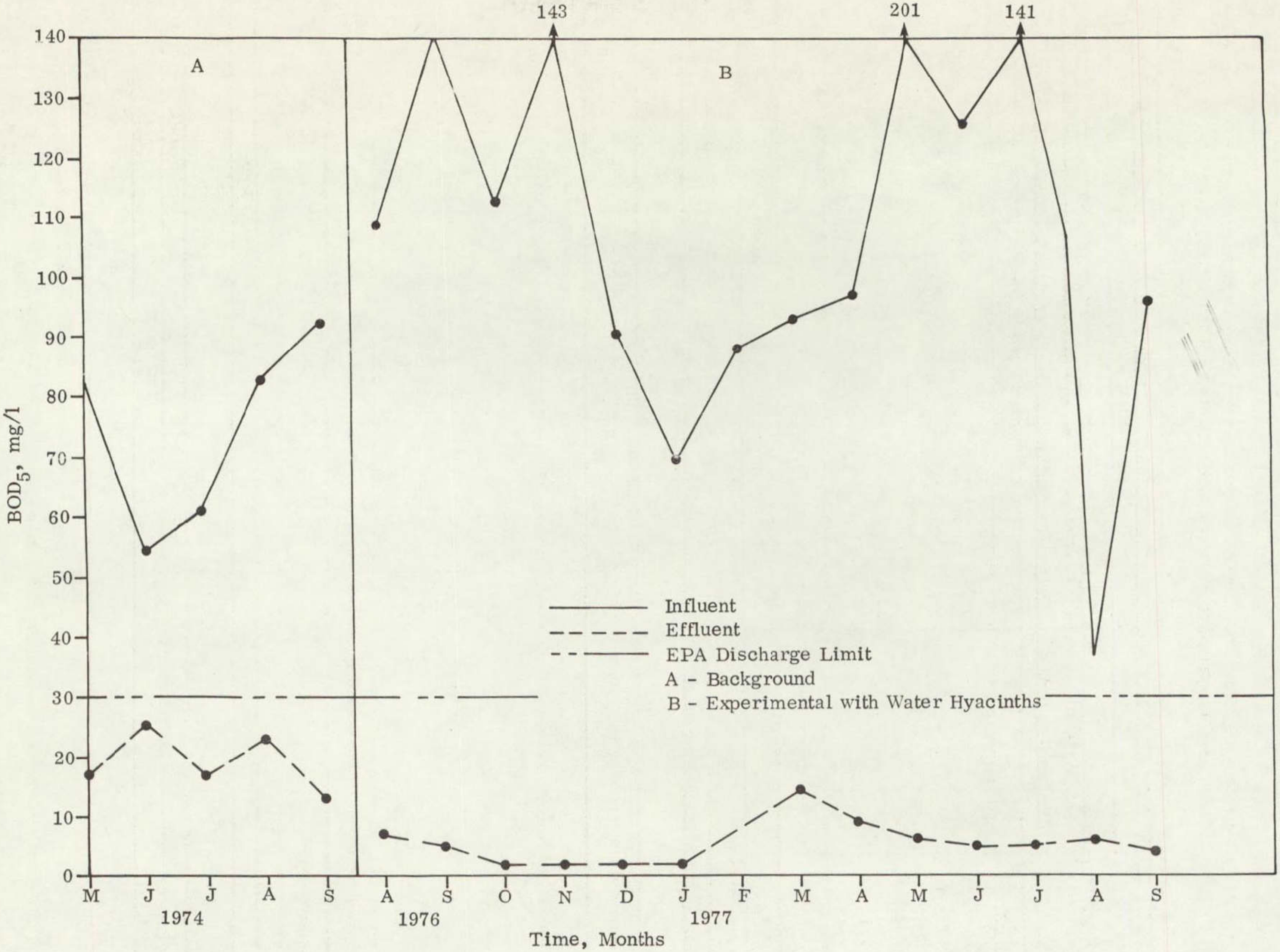

Figure 2. Monthly average BOD$_5$ vs. time (months)

Table 3. Monthly Average Data of pH and Dissolved Oxygen (DO) for Background and Experimental Periods

A. Background Period				
Date	pH		DO, mg/ℓ	
	Influent	Effluent	Influent	Effluent
May, 1974	7.1	9.8	4.3	9.6
June, 1974	7.0	10.4	3.7	6.7
July, 1974	7.0	10.0	3.2	4.2
August, 1974	7.0	9.1	2.8	4.8
September, 1974	7.0	9.2	2.4	8.2
March, 1976	6.9	7.7	2.0	3.9
April, 1976	7.0	9.1	1.1	10.8
May, 1976	7.2	8.8	1.5	7.3
B. Initial Stocking Months (Partial Water Hyacinth Coverage)				
June, 1976	7.3	7.6	1.4	6.8
July, 1976	7.2	7.3	1.0	5.0
C. Water Hyacinth Experimental Period				
August, 1976	7.4	7.2	1.1	2.4
September, 1976	7.2	7.0	1.0	0.6
October, 1976	7.2	7.0	0.9	1.5
November, 1976	7.2	7.2	1.5	2.9
*December, 1976	7.2	7.2	1.8	3.3
*January, 1977	7.0	7.1	1.7	3.5
*February, 1977	7.0	7.2	0.8	2.6
**March, 1977	7.0	7.1	1.1	2.0
**April, 1977	6.9	7.1	1.0	2.3
**May, 1977	7.1	7.2	0.7	2.7
June, 1977	7.5	7.2	1.0	2.3
July, 1977	7.1	6.9	1.0	1.8
August, 1977	7.1	6.9	1.2	1.9
September, 1977	7.0	7.1	1.1	1.8

*Water hyacinths damaged by cold weather; duckweed treatment operative.

**Water hyacinths recover from winter with initial coverage of 20% in March, 1977.

Table 4. Monthly Average Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), and Total Organic Carbon (TOC) for Background and Experimental Periods (Background data on these parameters not available for May, 1974-September, 1974.)

Date	TKN, mg/ℓ		TP, mg/ℓ		TOC, mg/ℓ	
	Influent	Effluent	Influent	Effluent	Influent	Effluent
A. Background Period						
March, 1976	9.9	7.4	3.1	1.9	62	20
April, 1976	8.8	4.4	2.9	2.1	65	37
May, 1976	10.8	3.8	2.8	2.2	38	38
B. Initial Stocking Months (Partial Water Hyacinth Coverage)						
June, 1976	8.2	2.9	2.3	1.7	43	34
July, 1976	7.8	1.9	2.2	0.9	33	19
C. Water Hyacinth Experimental Period						
August, 1976	12.1	3.0	3.6	1.1	51	15
September, 1976	10.0	1.4	5.8	1.0	54	14
October, 1976	11.3	2.0	3.1	1.1	35	14
November, 1976	13.3	3.5	3.5	0.7	59	15
*December, 1976	10.7	3.0	1.8	1.0	36	15
*January, 1977	12.7	4.1	2.6	1.2	56	14
*February, 1977	15.5	5.4	4.0	2.6	72	29
**March, 1977	13.7	5.1	4.6	2.8	59	24
**April, 1977	14.3	3.4	4.5	2.1	61	19
**May, 1977	15.2	1.9	4.9	1.8	62	12
June, 1977	13.2	2.3	3.7	1.8	49	15
July, 1977	8.4	2.6	3.3	1.8	44	17
August, 1977	8.5	3.9	3.3	1.4	34	17
September, 1977	9.3	5.3	3.6	1.5	89	19

*Water hyacinths damaged by cold weather; duckweed treatment operative.

**Water hyacinths recover from winter with initial coverage of 20% in March, 1977.

Table 5. Average Summer and Winter Percent Reductions in Total Kjeldahl Nitrogen (TKN), Total Phosphorus (TP), and Total Organic Carbon (TOC) with Water Hyacinths in Summer and Duckweeds in Winter

	Average Percent Reduction	
Parameter	Summer	Winter
TKN	73	67
TP	63	43
TOC	69	59

Summer Months - April through November

Winter Months - December through March

Discussion

Results indicate that when water hyacinths assume the role of major primary producer in a sewage lagoon, the operation of the system is significantly altered. The algal community, with its fast turnover rate and rapid succession, is replaced by a rapidly growing macrophyte that continuously converts dissolved organics and nutrients into a standing biomass which is not rapidly recycled and does not contribute to the total organic carbon (TOC) of the system. The hyacinth plant biomass, which remains within the system, is not present in the effluent. As a result, effluent from a hyacinth-covered lagoon will be lower in suspended solids, BOD_5 and nutrients.

Although water hyacinths are far superior to algae as the major primary producers in a lagoon system, there are several disadvantages which should be recognized in the use of this plant in sewage treatment. Since oxygen produced by the water hyacinth in photosynthesis does not significantly contribute to the oxidation process occurring within the pond, the anaerobic portion of a facultative lagoon may increase and under conditions of heavy BOD loading become total. Although water hyacinths are not affected by these conditions, odor problems may result. Therefore, when water hyacinth coverage is complete and BOD loading heavy, mechanical aeration of the lagoon may be necessary during photosynthetically inactive periods to prevent foul odors.

Another limitation of using water hyacinths is that their use without protection is restricted to the warmer months of the year. In late fall the effectiveness of the water hyacinth is greatly reduced unless the plants are protected by greenhouses or by heating the influent. If unprotected, water hyacinths should be harvested following the first hard frost in the fall. In our lightly loaded lagoon system, it was not necessary to harvest the hyacinths after one season of operation; however, for moderately to heavily loaded lagoons, the accumulated dead plant material would impose a large additional organic load, and the plants should be harvested each fall. Fortunately, the water hyacinth's seasonal demise corresponds with general periods of low biological activity within the lagoon, in which TSS and organics are at the lowest concentrations. During cooler weather, winter-resistant primary producers such as duckweed (Lemna spp.) and (Spiridela spp.) have taken over in our ponds, and perform a certain amount of purification (Sutton and Ornes, 1975).

Although the necessity of periodic harvesting of the water hyacinths adds to the cost of operation of the lagoon system, these floating plants are much more easily harvested than submerged or rooted aquatics. We are optimistic over the prospect of selling the harvested hyacinths to recover at least a part of harvesting cost. Particularly promising is the use of hyacinths for cattle feed, plant compost and biogas production. Nutrient analyses conducted at out laboratory, for example, indicate that crude protein content of hyacinths grown in sewage lagoons compares favorable with soybean and cottonseed meal, averaging 32.9% dry weight of leaves (Wolverton and McDonald, in preparation). Mara (1976) estimated the value of selective water hyacinth by-products and concluded that the market was not sufficient to help defray the cost of mechanical control of water hyacinths. However, his analyses may not be applicable to water hyacinths harvested from sewage lagoons for several reasons. Confinement of harvesting activities to a single location would minimize transportation and handling costs, thus increasing the economic feasibility of utilizing water hyacinth by-products. Locating dryers, choppers and other water hyacinth processing operations near the lagoon would further reduce processing and transportation costs. Also, when grown in nutrient-rich lagoon influent, the water hyacinth's growth rate is greatly enhanced. Scarsbrook and Davis (1971) report a 15-fold increase in dry matter production when hyacinths were grown in 25 percent sewage effluent.

In summary, these experiments have shown that substantial coverage of water hyacinths significantly upgrades effluent from a primary sewage lagoon treating the waste of approximately 2,000 people. The addition of water hyacinths to a sewage lagoon system not only reduces suspended solids and BOD_5, but also significantly decreases the nutrient and organic carbon content in the lagoon effluent. The use of vascular aquatic plants appears promising as an economical and efficient way of upgrading sewage lagoon systems in small communities.

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SECTION I
Part 2

WATER HYACINTHS FOR UPGRADING SEWAGE LAGOONS TO MEET ADVANCED WASTEWATER TREATMENT STANDARDS: PART II

By: B. C. Wolverton
Rebecca C. McDonald

Technical Memorandum X-72730

October, 1976

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ABSTRACT

Field tests using water hyacinths as biological filtration agents were conducted in the Mississippi Gulf Coast Region. The plants were installed in one single cell and one multiple cell sewage lagoon systems. Water hyacinths demonstrated the ability to maintain BOD_5 and total suspended solid (TSS) levels within the Environmental Protection Agency's (EPA) prescribed limits of 30 mg/l BOD_5 and 30 mg/l TSS.

A multiple cell sewage lagoon system consisting of two aerated and one water hyacinth covered cells connected in series demonstrated the ability to maintain BOD_5 and TSS levels below 30 mg/l year-round. A water hyacinth covered lagoon with a surface area of 0.28 hectare containing a total volume of 6.8 million liters demonstrated the capacity to treat 437,000 to 1,893,000 liters of sewage influent from 2.65 hectares of aerated lagoons daily and produce an effluent that met or exceeded standards year-round.

TECHNICAL MEMORANDUM X-72730

WATER HYACINTHS FOR UPGRADING SEWAGE LAGOONS TO MEET ADVANCED WASTEWATER TREATMENT STANDARDS: PART II

INTRODUCTION

NASA's National Space Technology Laboratories (NSTL), along with most of the small Mississippi communities, utilize sewage lagoons to treat their domestic sewage. These lagoons will have to be upgraded or replaced by more expensive treatment plants to meet the more stringent secondary treatment standards by July 1, 1977, as prescribed by State and Federal Pollution Control Laws.

For several years, NASA has been experimenting with the use of water hyacinths (Eichhornia crassipes) (Mart.) Solms, a floating, freshwater plant, as an inexpensive, natural biological waste filtration system. (1, 2, 3, 4, 5, 6, 7) The objective of these experiments has been to design and perfect a system utilizing water hyacinths to upgrade sewage effluent from existing lagoon systems. This is highly preferable to the alternative of installing an entirely new waste treatment system.

This report describes the results to date from two of NASA's on-going experimental field studies being conducted on the Mississippi Gulf Coast in the vicinity of the NSTL. Experiments of a preliminary nature were conducted at the lagoon system of Bay St. Louis, Mississippi. Findings and techniques resulting from the Bay St. Louis experiments were then applied and more rigorously tested in a second experimental lagoon system at Orange Grove, a community in north Gulfport, Mississippi.

Both experimental systems were designed to determine the following parameters:

A) Growth characteristics of water hyacinths in raw sewage.
B) The efficiency of water hyacinths in purifying sewage effluents.
C) The minimum surface area coverage requirements for efficient operation of water hyacinths.
D) Maximum sustained flow rate at which water hyacinths are effective.
E) Any gross effects of water hyacinths on the lagoon environment.
F) Any problems affecting hyacinth growth which might inhibit the plants' efficiency as waste-removing agents.

Each system is described and discussed separately, both for ease of evaluation and for comparative purposes.

ANALYSES AND SAMPLING METHODS

Sampling procedures and analyses, described below, were identical for both experimental systems. Grab samples were taken two times per week on influent and effluent wastewater from all systems. Twenty-four hour composite samples were taken monthly and results correlated well with grab sample data. Influent and effluent samples were analyzed for dissolved oxygen (DO), temperature, pH, total suspended solids (TSS), total dissolved solids, total phosphorus, total Kjeldahl nitrogen (TKN), total organic carbon (TOC), and five-day biochemical oxygen demand (BOD_5). All sample analyses were performed according to Standard Methods. (8) Values for the measured parameters were averaged for each monthly period. These monthly average values are contained in this report. Raw data of individual samples are maintained on file.

Limited plant harvesting was performed at the Bay St. Louis lagoon only. For maximum sustained nutrient removal, plants should be harvested on a regular basis. However, at this time efforts were directed at establishing minimum surface area coverage of water hyacinths necessary to meet the 1977 permit limitations rather than at purifying the effluent maximally. The purpose of the harvesting which was performed was to test experimental harvesting equipment designed for use in NASA's Vascular Aquatic Plant Program. Evaluation and cost studies for harvesting equipment and up-keep of water hyacinth sewage systems will appear in future reports.

EXPERIMENT 1. BAY ST. LOUIS LAGOON SYSTEM

A. Introduction and Description

The Bay St. Louis lagoon system (Figure 1) consists of a 17.5 hectare (42-acre) single cell lagoon which receives the domestic waste from approximately six thousand residents of Bay St. Louis, Mississippi. This lagoon receives approximately 3.79 million liters per day of domestic wastewater diluted from excessive ground water infiltration.

The massive size of the lagoon promotes the excessive growth of algae. Particularly in the summer months, excess algal growth and decomposition increase the effluent total suspended solids and cause anaerobic conditions, resulting in offensive odors that contribute to air pollution and affect nearby residents.

In March 1975, NASA's National Space Technology Laboratories entered into a joint program with the City of Bay St. Louis in which NASA's Experimental Water Hyacinth

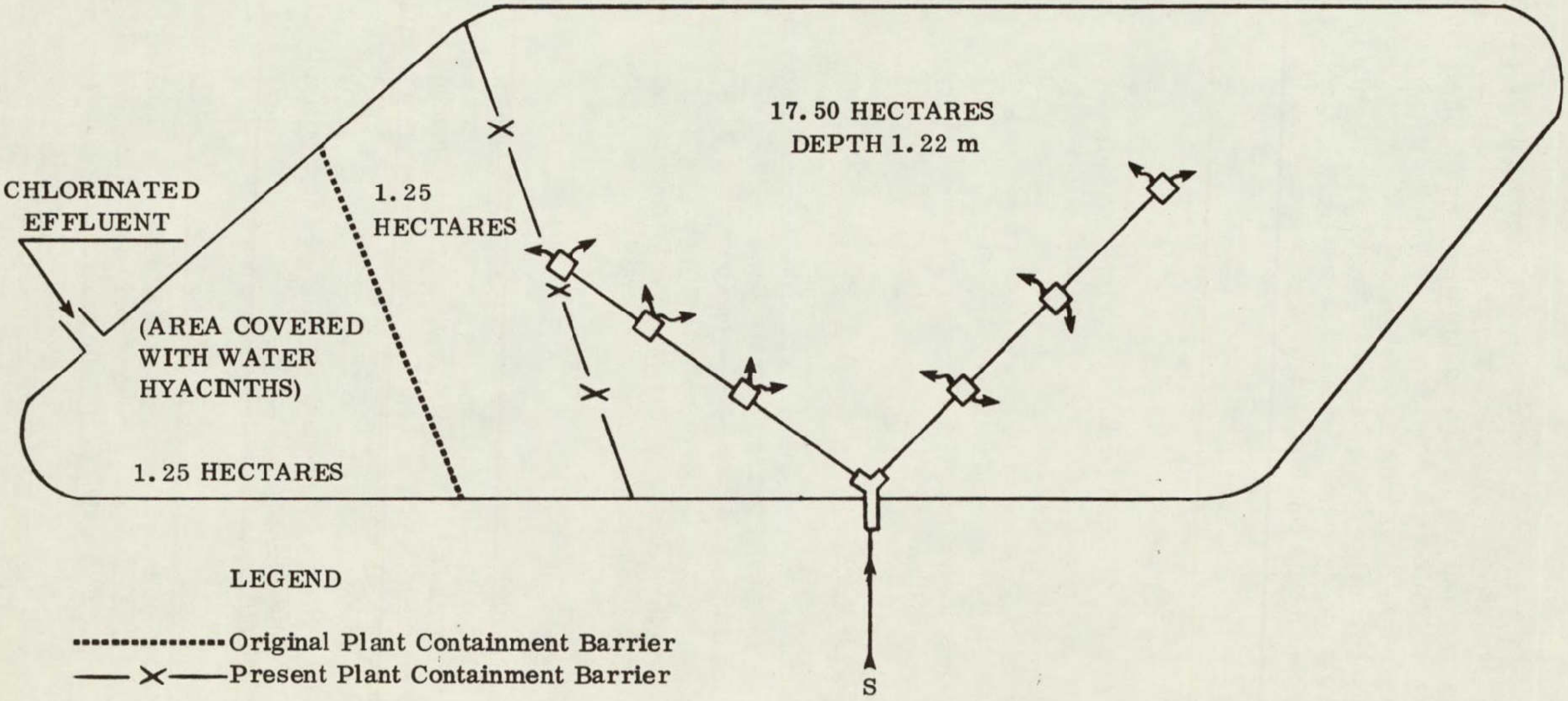

Figure 1. Bay St. Louis Sewage Lagoon

Sewage Purification System would be evaluated utilizing a portion of the Bay St. Louis sewage lagoon. A 1.25 hectare area of the lagoon was fenced off around the effluent point. Enough water hyacinths were initially introduced into the system to cover approximately 0.10 hectare. The plants were very prolific when grown in this sewage system, and the 1.25-hectare (3-acre) area was completely covered by late June. This area proved to be too small to be effective in treating this large lagoon; therefore, the retainer fence was moved, increasing the size of the enclosed area to 2.5 hectares (6 acres). By September, water hyacinths had achieved total coverage of this increased area.

B. Results

1. Growth Rate

The growth rate of the water hyacinths was monitored on a weekly basis from April to June 1975. As shown in Figure 2, significant growth occurred during the months of May and June. During this two-month period, the water hyacinths increased in surface coverage approximately six percent to ten percent (average eight percent) a day. One hectare of water hyacinths contained approximately 218 metric tons of biomass. These data indicated an average growth rate of approximately 17.5 metric tons of wet biomass per day during ideal growing conditions.

2. Other Parameters

Once the water hyacinths had achieved a coverage of 2.5 hectares, their effects on decreasing the BOD_5 and TSS levels in the sewage effluent became obvious (Table 1). Over a four-month period, the BOD_5 dropped from 22 mg/l to an average of 16 mg/l, and the total suspended solids were reduced by 88 percent in the effluent from a background level of 125 mg/l to 15 mg/l. The substantial reduction in BOD_5 and total suspended solids is graphically depicted in Figures 3 and 4, respectively.

C. Problems and Discussion

In December, approximately 5,000 coots (Fulica americana) invaded the open waters of this lagoon and proceeded to consume the plants. These water hyacinths, badly damaged by the coots and by the unusually cold weather during December and January (refer to temperature chart, Figure 5), were no longer capable of effectively treating these wastewaters and were removed from the lagoon.

Data from this experiment suggest that a single cell lagoon containing four to five surface hectares covered with water hyacinths and a retention time of 12 days or longer should be capable of meeting the sewage effluent standards of the City of Bay St. Louis without producing offensive odors.

The present lagoon is much too large and will require diking off four to five hectares, leaving approximately 12.5 hectares which could be de-eutrophied with water hyacinths or drained. Decreasing the lagoon size would help to retain the heat from the raw sewage which is rapidly dissipated in the present large lagoon. Complete coverage of a smaller sewage lagoon hopefully would discourage coots from using this lagoon by eliminating free space for landing and surface feeding. If the coot problem were to persist, noise devices

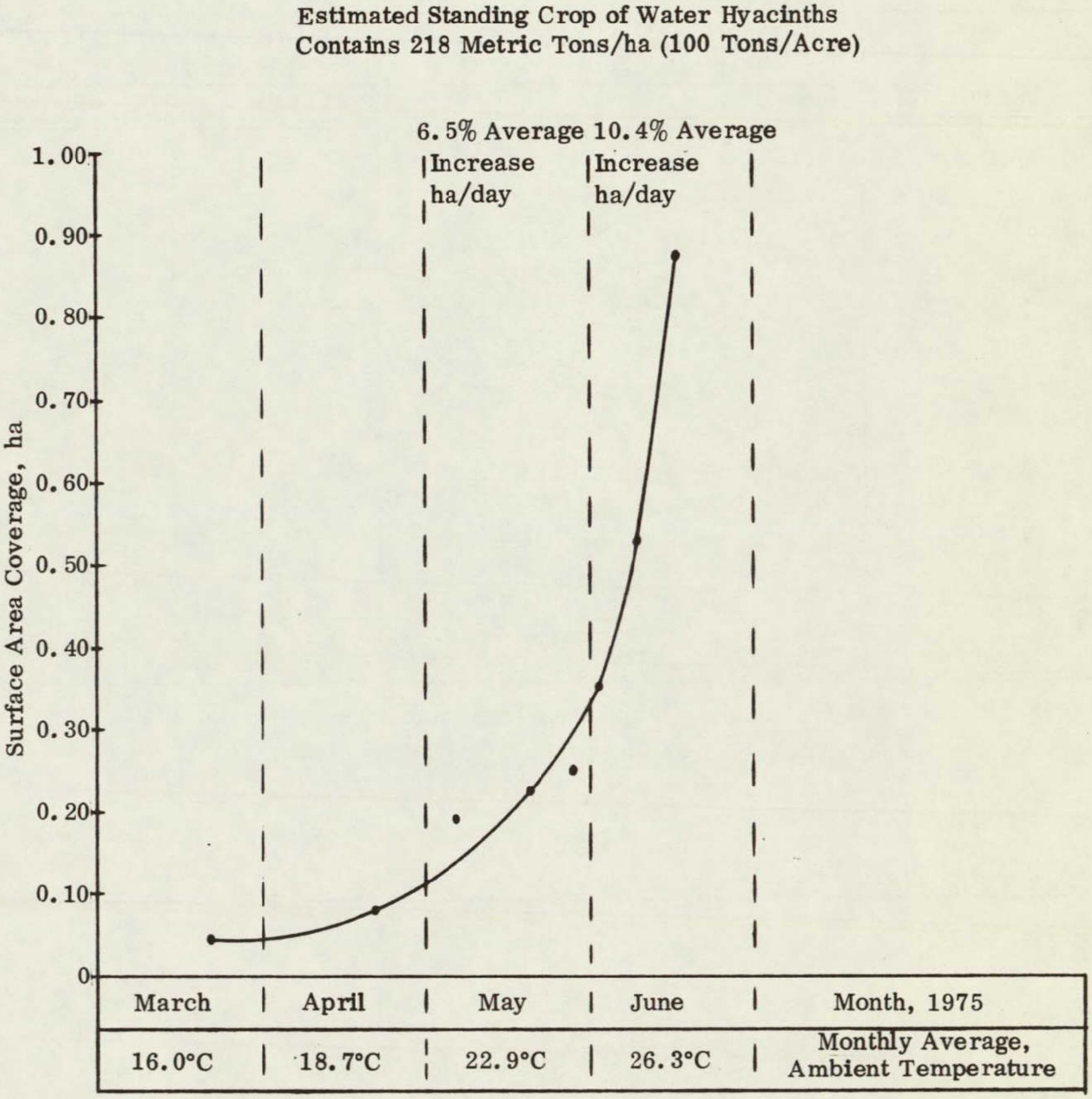

Figure 2. Data on growth rate of water hyacinths after initial stocking of plants in Bay St. Louis lagoon until testing of experimental harvesting equipment began

Table 1. Bay St. Louis, MS Sewage Waste Treatment Lagoon
(17.5 Hectare Surface Area)

Month 1975	BIOCHEMICAL OXYGEN DEMAND, BOD_5 (mg/ℓ) Influent	BIOCHEMICAL OXYGEN DEMAND, BOD_5 (mg/ℓ) Effluent	TOTAL SUSPENDED SOLIDS, TSS (mg/ℓ) Influent	TOTAL SUSPENDED SOLIDS, TSS (mg/ℓ) Effluent
April	40	22	74	125
May	75	16	62	148
*June	73	17	52	37
July	69	19	58	80
August	53	20	104	68
**September	58	20	47	9
October	27	11	42	8
November	20	18	48	18
December	52	14	60	26

NOTE: 0.10 Hectare surface area stocked with water hyacinths during last week of March, 1975

*1.25 hectare coverage

**2.50 hectare coverage

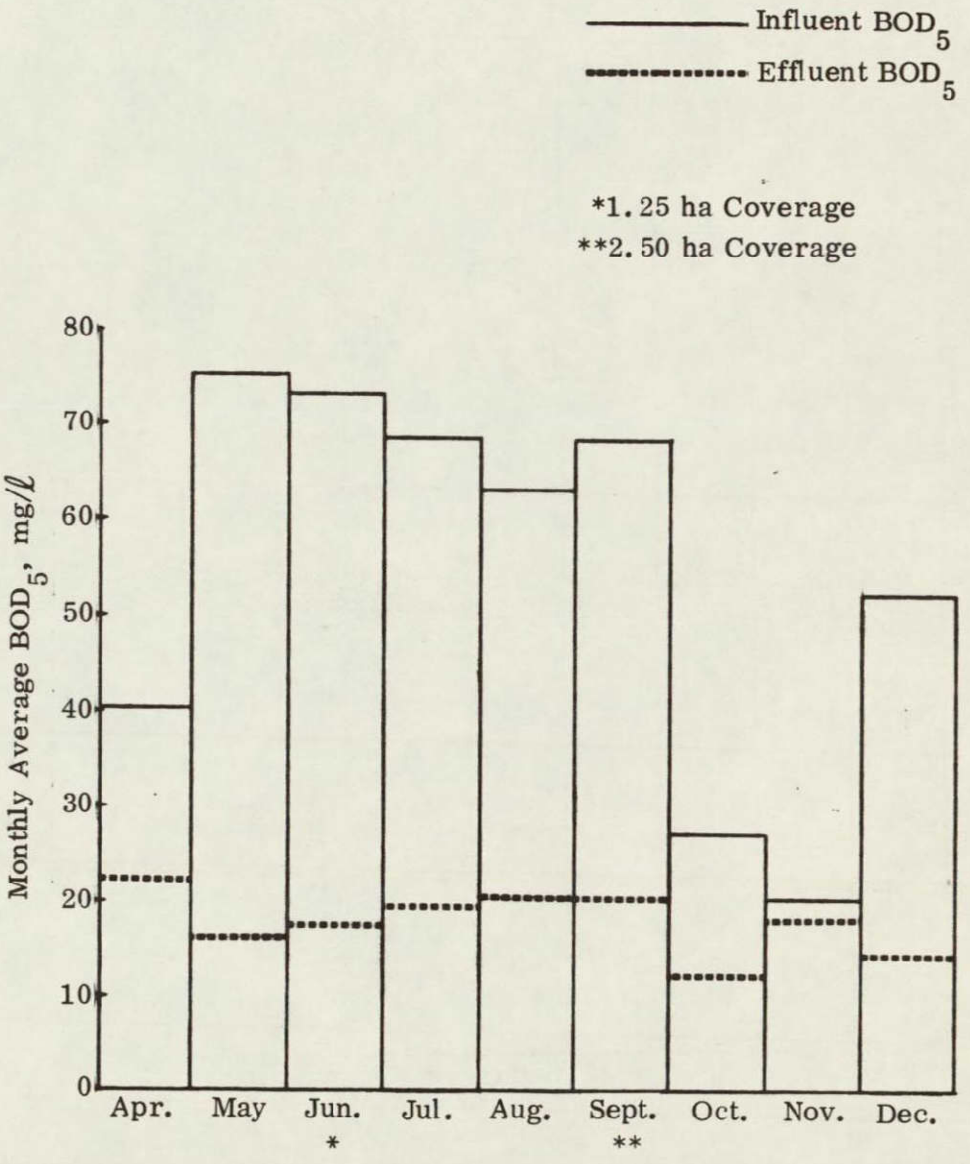

Figure 3. BOD_5 versus time for the Bay St. Louis lagoon

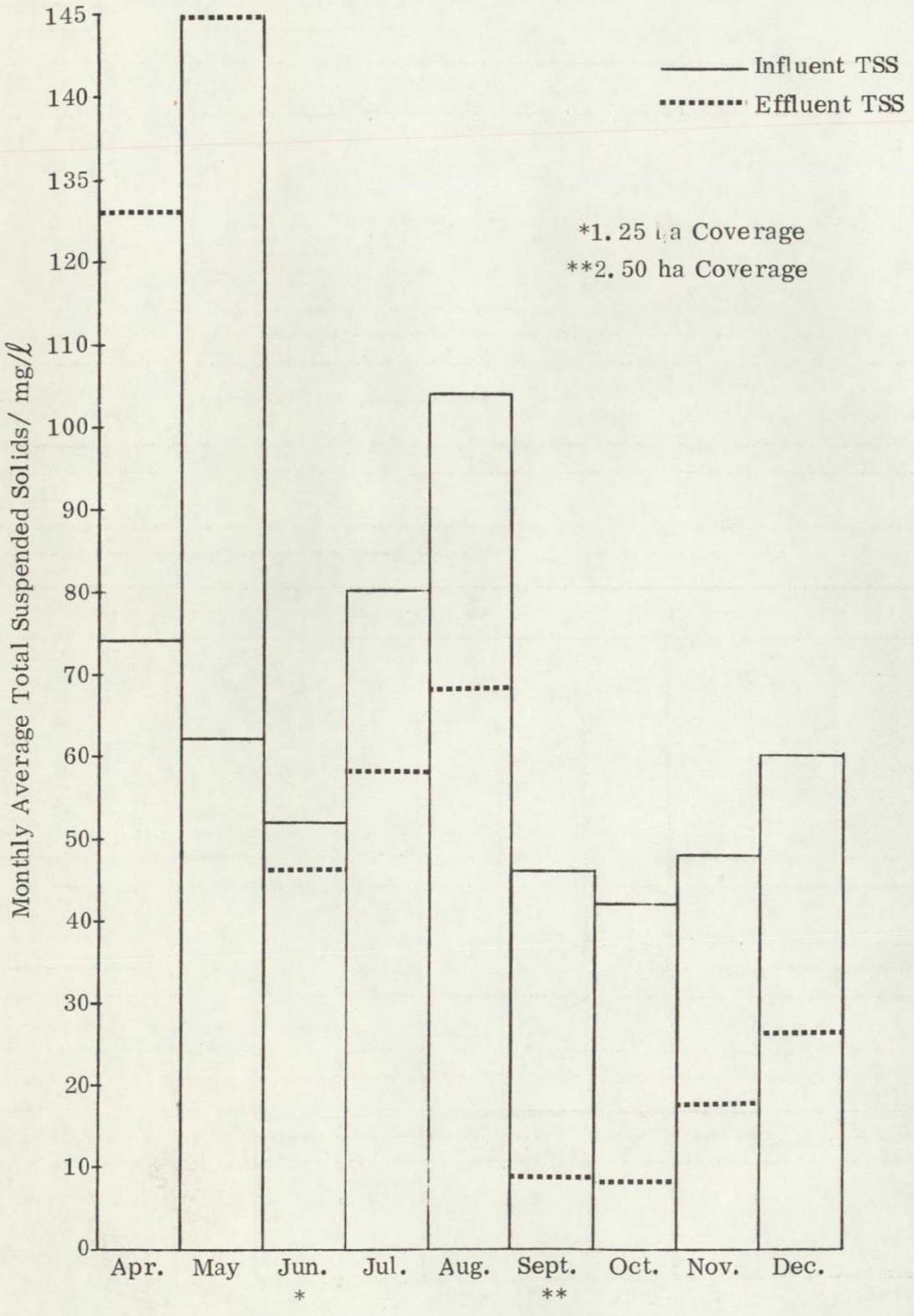

Figure 4. Total suspended solids versus time for the Bay St. Louis lagoon

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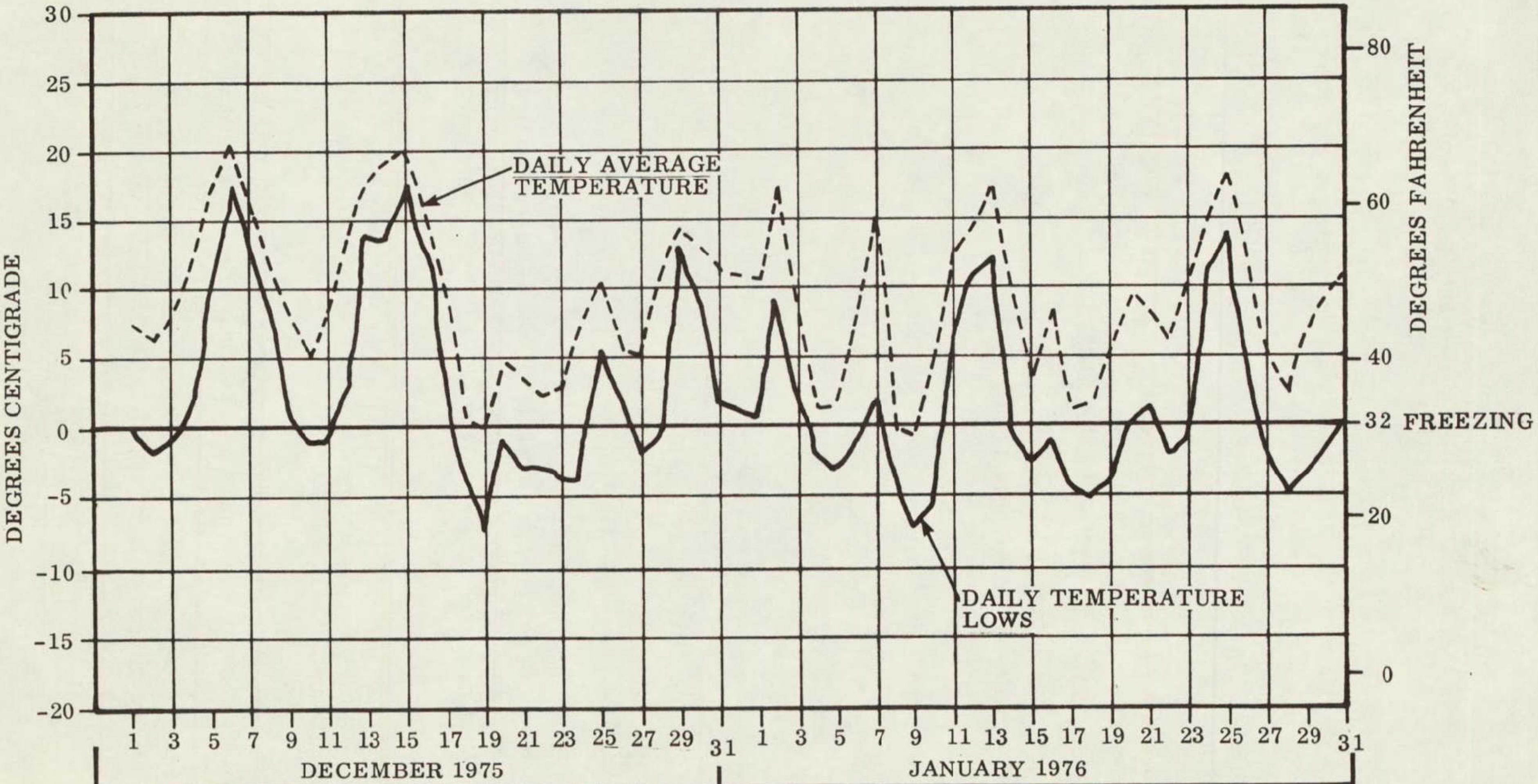


HISTORICAL DATA		HISTORICAL DATA	
13-YEAR AVERAGE TEMPERATURE	= 11.3°C	13-YEAR AVERAGE TEMPERATURE	= 10.3°C
DECEMBER '75 AVERAGE TEMPERATURE	= 9.5°C	JANUARY '76 AVERAGE TEMPERATURE	= 8.0°C
13-YEAR AVERAGE MINIMUM TEMPERATURE	= 5.3°C	13-YEAR AVERAGE MINIMUM TEMPERATURE	= 3.7°C
DECEMBER '75 AVERAGE MINIMUM TEMPERATURE	= 3.8°C	JANUARY '76 AVERAGE MINIMUM TEMPERATURE	= 0.5°C*

* Lowest on NSTL Records (records maintained since 1963)

Figure 5. NSTL climatological data for December, 1975, and January, 1976

similar to ones used by the military to keep birds from military air fields could be installed.

D. Conclusions

The Bay St. Louis sewage lagoon experiment established or reaffirmed the following points:

1. Water hyacinths will thrive on raw sewage; the high nutrient levels present in this medium stimulate rapid growth.

2. A smaller (four to six hectares) water hyacinth-covered lagoon would be most efficient in serving the residents of Bay St. Louis. A system of this size would produce a sewage effluent of excellent quality, well within the 1977 standards.

3. If water hyacinths are to be used in only a section of a large lagoon, the section(s) not containing water hyacinths should be aerated to eliminate excess algal growth.

Coots, if they are permitted to feed on water hyacinths, may greatly decrease the efficiency of the system. In any permanent sewage treatment system utilizing water hyacinths, procedures must be taken to eliminate or minimize invasion by these birds.

EXPERIMENT 2. ORANGE GROVE LAGOON SYSTEM

A. Introduction and Description

Before the introduction of water hyacinths, the existing system at Orange Grove, Mississippi, was free of offensive odors, but it did not meet the standards imposed by the State of Mississippi Air and Water Pollution Control Commission. (Effluent quality before the introduction of water hyacinths is presented in Part I of this paper, October 1975). (3)

In June 1975, NASA's National Space Technology Laboratories entered into a joint experimental program with Orange Grove to evaluate the use of a water hyacinth-covered lagoon as a simple, economical way of reducing suspended solids, BOD_5, and nutrient levels from aerated lagoon effluent.

A 0.28 hectare (0.7-acre) surface area lagoon containing a total volume of 6.8 million liters (1.5 million gallons) had been newly constructed to receive the effluent from a secondary, aerated lagoon. The present system consists of two large aerated lagoons followed by three parallel unaerated lagoons (Figure 6). The daily flow rate into the third lagoon varied from 437,000 l (116,000 gallons) to 1,893,000 l (500,000 gallons) as shown in Figure 7. This lagoon was initially stocked with sufficient water hyacinths to cover one-half of the surface area.

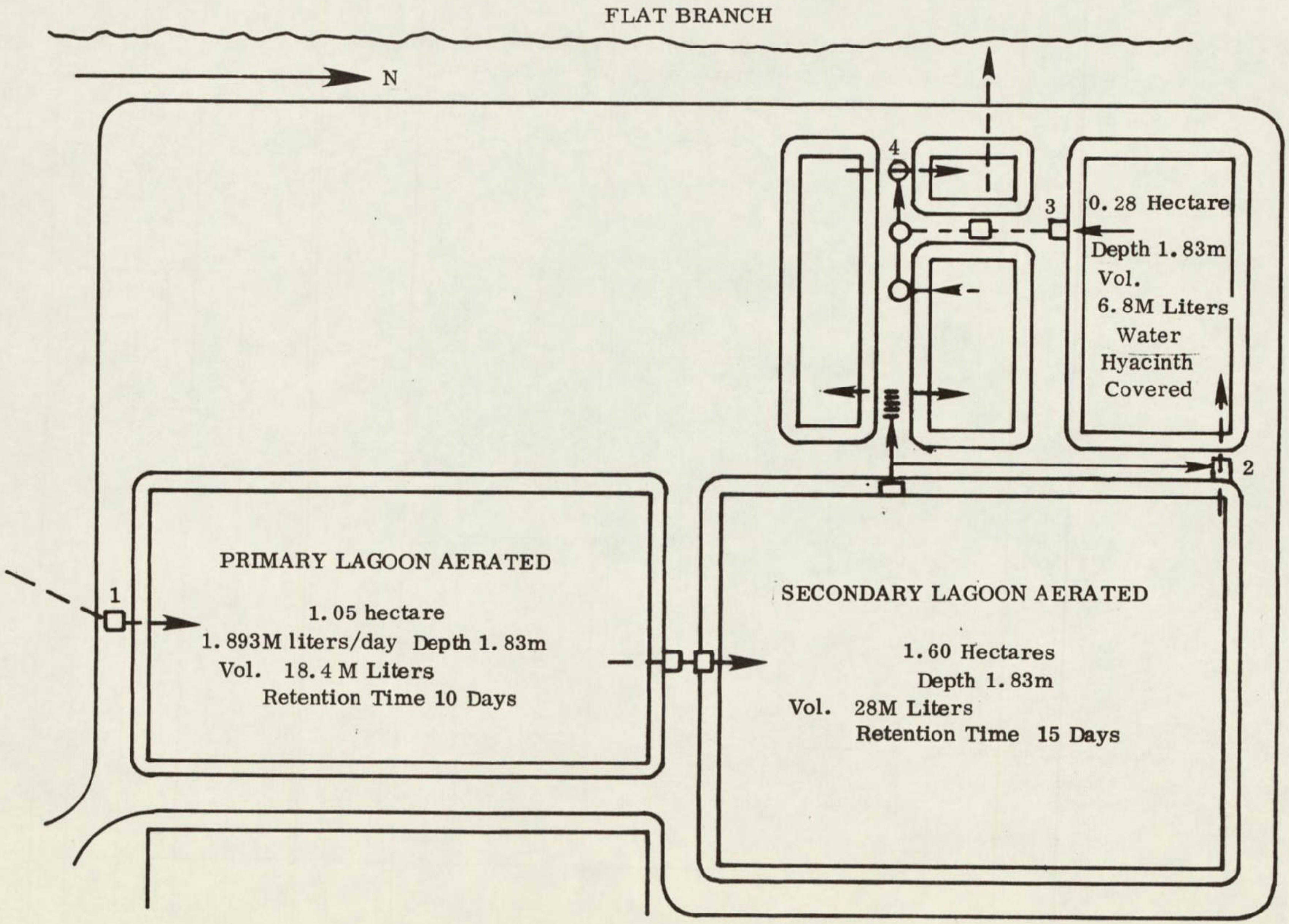
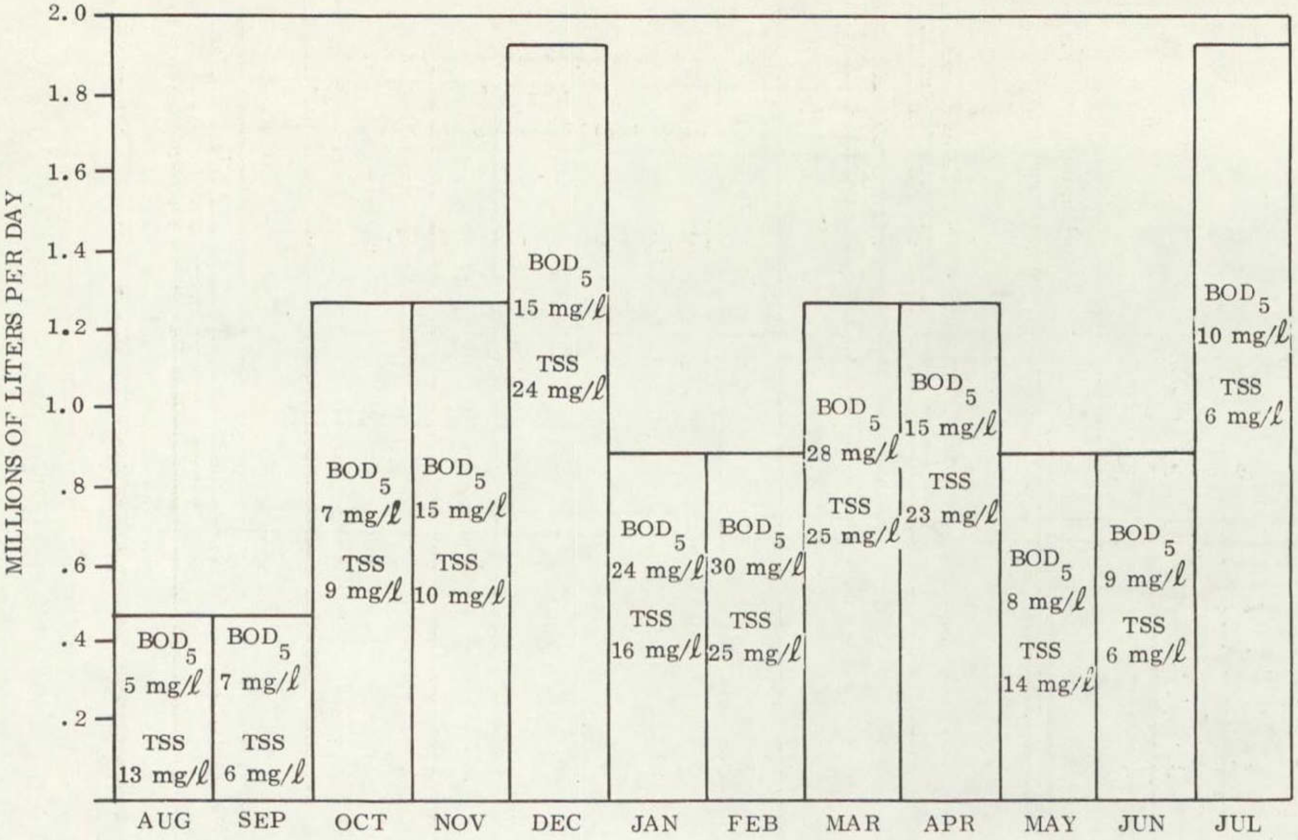


Figure 6. Orange Grove sewage lay-out



1975

August	437,000 Liters
September	437,000 Liters
October	1,059,000 Liters
November	1,059,000 Liters
December	1,892,500 Liters

1976

January	870,000 Liters
February	870,000 Liters
March	1,059,000 Liters
April	1,059,000 Liters
May	870,000 Liters
June	870,000 Liters
July	1,892,500 Liters

Raw Sewage:

Avg. BOD_5 = 150 mg/ℓ

Avg. TSS = 142 mg/ℓ

Figure 7. Sewage flow rates into Orange Grove water hyacinth covered lagoon and BOD_5 and total suspended solid (TSS) effluent average from eight analyses per month

B. Results

As in the Bay St. Louis Lagoon System, water hyacinths again demonstrated the ability to purify the sewage effluent substantially. Each parameter measured is discussed below.

1. Total Suspended Solids

The water hyacinth-covered lagoon demonstrated the ability to reduce the total suspended solids year-round from a yearly average influent level of 49 mg/l to a yearly average effluent level of 14 mg/l. This level, constituting a 71 percent reduction, is well within the 30 mg/l maximum set by the Mississippi Air and Water Pollution Control Commission. Average monthly data are presented in Table 2 and depicted graphically in Figure 8.

2. BOD_5

The BOD_5 of the wastewaters entering the lagoon averaged 50 mg/l on a year-round basis. After the introduction of water hyacinths, this level was reduced by approximately 70 percent (Table 2). The 15 mg/l maximum allowable standard was achieved on a yearly average basis. BOD_5 values of effluent waters were well within this value for all months except January through March 1976. Freezing temperatures occurring during this period killed the tops of the water hyacinths, and the decay of this large amount of biomass elevated the BOD_5 levels to a high of 30 mg/l during the month of February. However, this does not indicate that water hyacinths are incapable of dealing with high influent BOD_5 levels. After the system regained equilibrium in the spring of 1976, the plants effected a 90 percent reduction of BOD_5 influent levels during May 1976 (Figure 9).

3. Total Kjeldahl Nitrogen

As shown in Figure 10 and Table 2, water hyacinths successfully reduced the level of this nutrient below the maximum allowable level of 6 mg/l for all months except February through March 1976. The reason for excess nutrient levels during this period was described in the above section. Excluding these two months, the yearly average level for total Kjeldahl nitrogen was 2 mg/l. Even when the data from these months are taken into account, the yearly average of 3.02 mg/l is well below the prescribed limits.

4. pH

As shown in Table 2, water hyacinths maintained the pH of the effluent within the prescribed limits of 6.0 to 7.8 during all months. In addition, the plants created a "buffer" effect, reducing the magnitude of pH fluctuations.

5. Dissolved Oxygen

As expected, water hyacinths substantially reduced the concentration of dissolved oxygen, from an average of 5.3 mg/l to an average of 2.1 mg/l. However, due to natural aeration in mixing, the DO concentration reached or exceeded the minimum

Table 2. Average Monthly Data of Orange Grove Sewage Lagoon System

	Total Suspended Solids, mg/l		Total Dissolved Solids, mg/l		Biochemical Oxygen Demand (BOD$_5$), mg/l		Total Kjeldahl Nitrogen, mg/l		Total Phosphorus mg/l		Total Organic Carbon, mg/l		pH		Dissolved Oxygen, mg/l		Temperature °C	
Raw Sewage (#1)* Monthly Average	142		319		150		25.73		9.27		94		7.06		1.0		25	
Data from Water Hyacinth Lagoon	#2*	#3*	#2*	#3*	#2*	#3*	#2*	#3*	#2*	#3*	#2*	#3*	#2*	#3*	#2*	#3*	#2*	#3*
Monthly Averages	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
July, 1975	35	9	296	201	23	7	2.23	1.03	5.80	4.41	31	22	7.8	6.5	5.8	1.8	27	26
August, 1975	35	13	294	278	26	5	2.47	1.17	5.34	4.79	24	18	7.6	6.6	6.0	2.0	27	26
September, 1975	43	6	187	183	22	7	4.44	1.07	5.03	3.77	24	15	7.2	6.7	5.5	2.2	22	22
October, 1975	48	9	195	189	25	7	3.38	1.00	4.70	3.85	29	19	7.3	6.6	6.4	2.0	21.4	20.8
November, 1975	50	10	153	155	29	15	3.27	2.00	5.18	4.53	37	23	7.4	6.7	8.0	2.1	15.2	14.4
December, 1975	52	24	154	159	32	15	2.60	2.22	5.41	5.84	33	24	7.3	6.7	7.2	2.2	15.3	14.4
January, 1976	47	16	227	239	57	24	---	---	---	---	34	27	7.3	6.7	7.4	2.6	13.3	11.7
February, 1976	67	25	239	216	135	30	8.88	7.87	6.81	7.88	34	34	6.9	6.8	4.7	2.1	17.2	16.0
March, 1976	50	25	295	241	70	28	6.86	6.34	7.22	7.79	36	38	7.0	6.8	5.3	2.4	17.7	17.1
April, 1976	88	23	320	220	65	15	9.37	3.60	7.04	5.77	42	28	7.7	6.7	4.1	2.5	21.3	19.3
May, 1976	84	14	354	246	81	8	8.50	2.62	8.24	5.85	37	21	7.2	6.4	2.2	2.2	22.5	20.1
June, 1976	42	6	243	209	60	9	8.86	2.31	6.87	5.24	40	25	7.2	6.4	2.4	2.3	25	23
July, 1976	28	6	210	189	30	10	7.75	5.10	5.91	5.46	29	17	7.3	6.7	3.4	1.2	28	27

* For location of sampling stations 1, 2, and 3, see Figure 2

Note: Total Kjeldahl nitrogen and total phosphorus data not obtained during the month of January due to difficulty with necessary equipment.

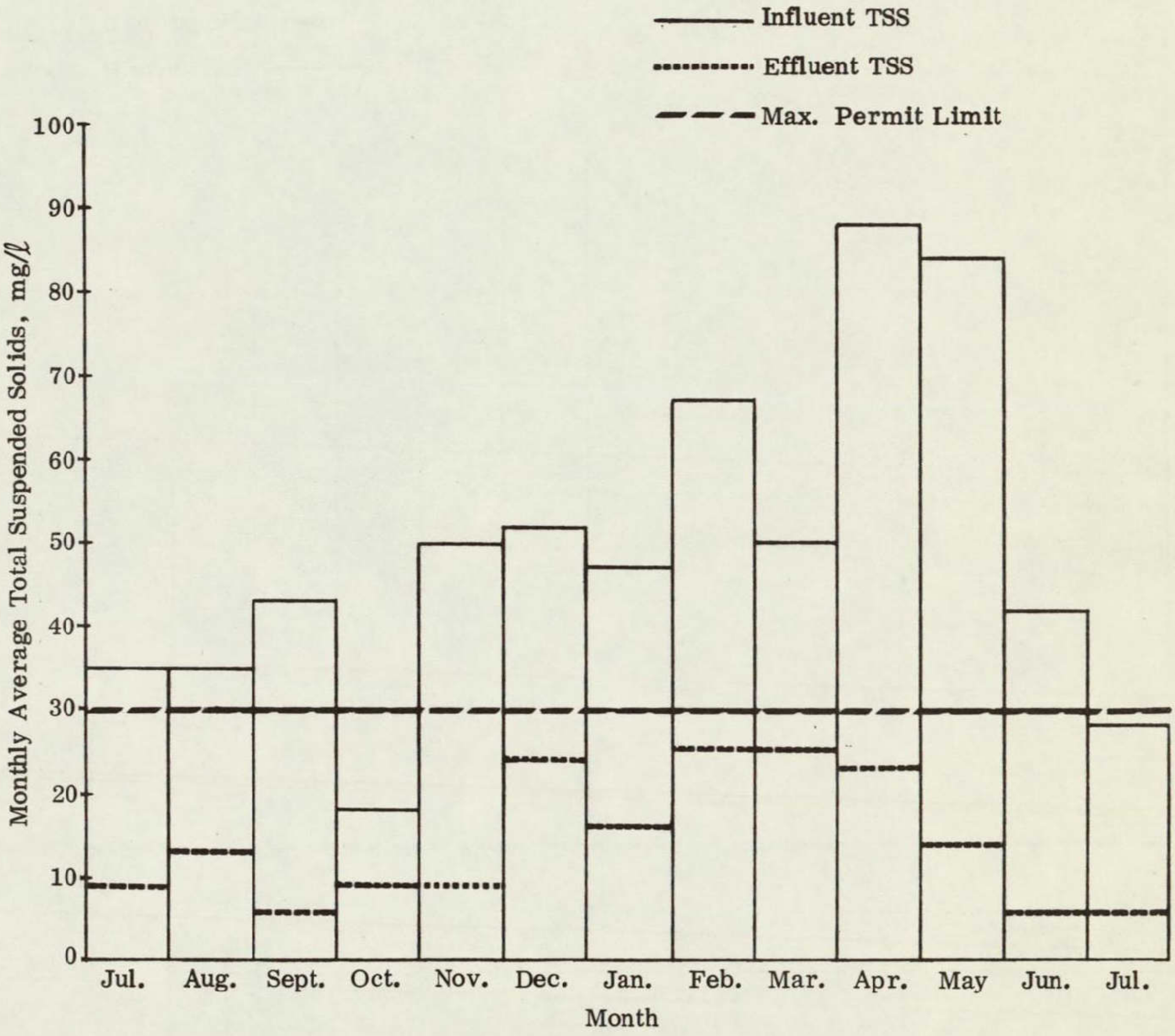


Figure 8. Total suspended solids versus time for the Orange Grove secondary lagoon covered with water hyacinths

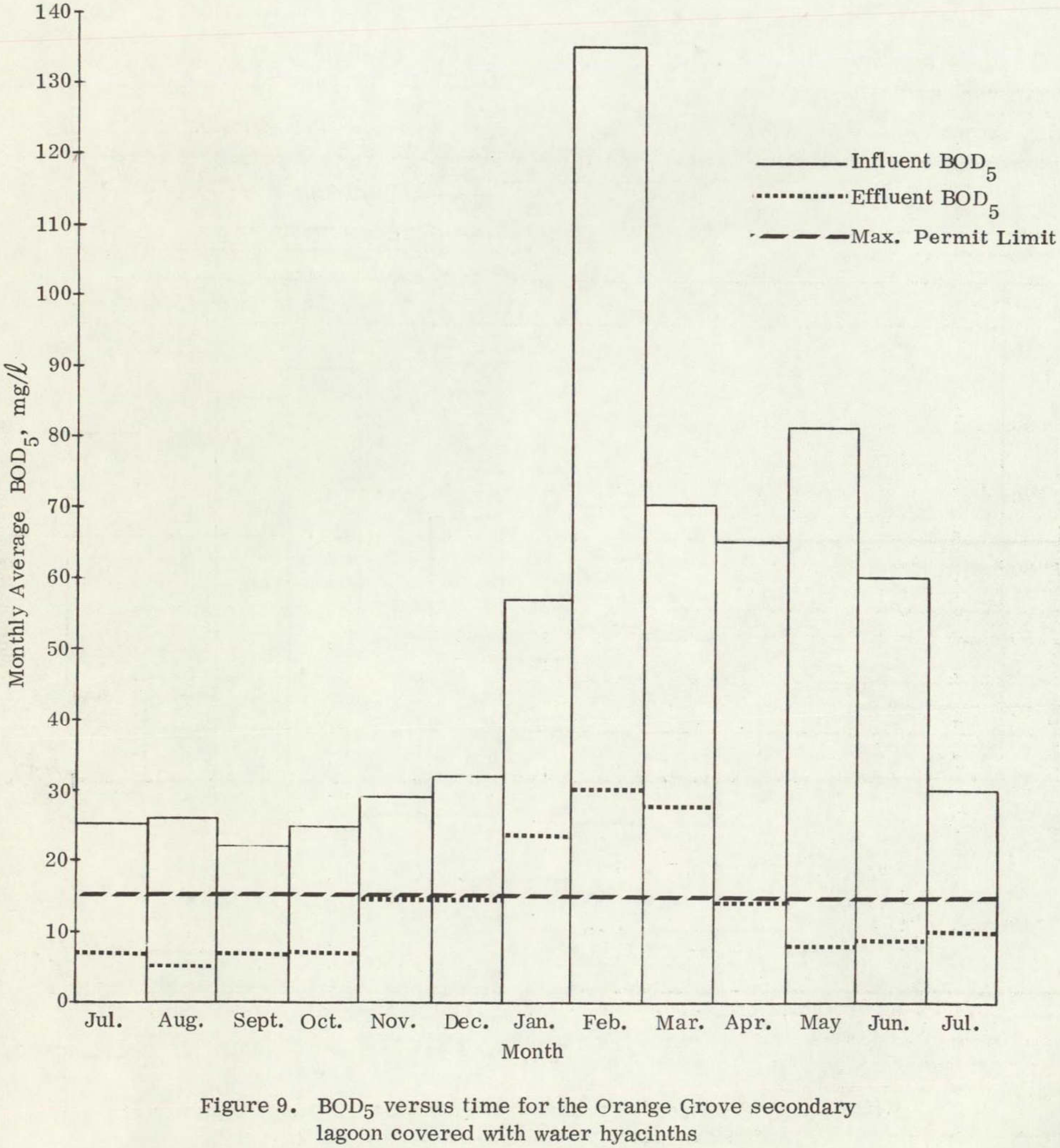
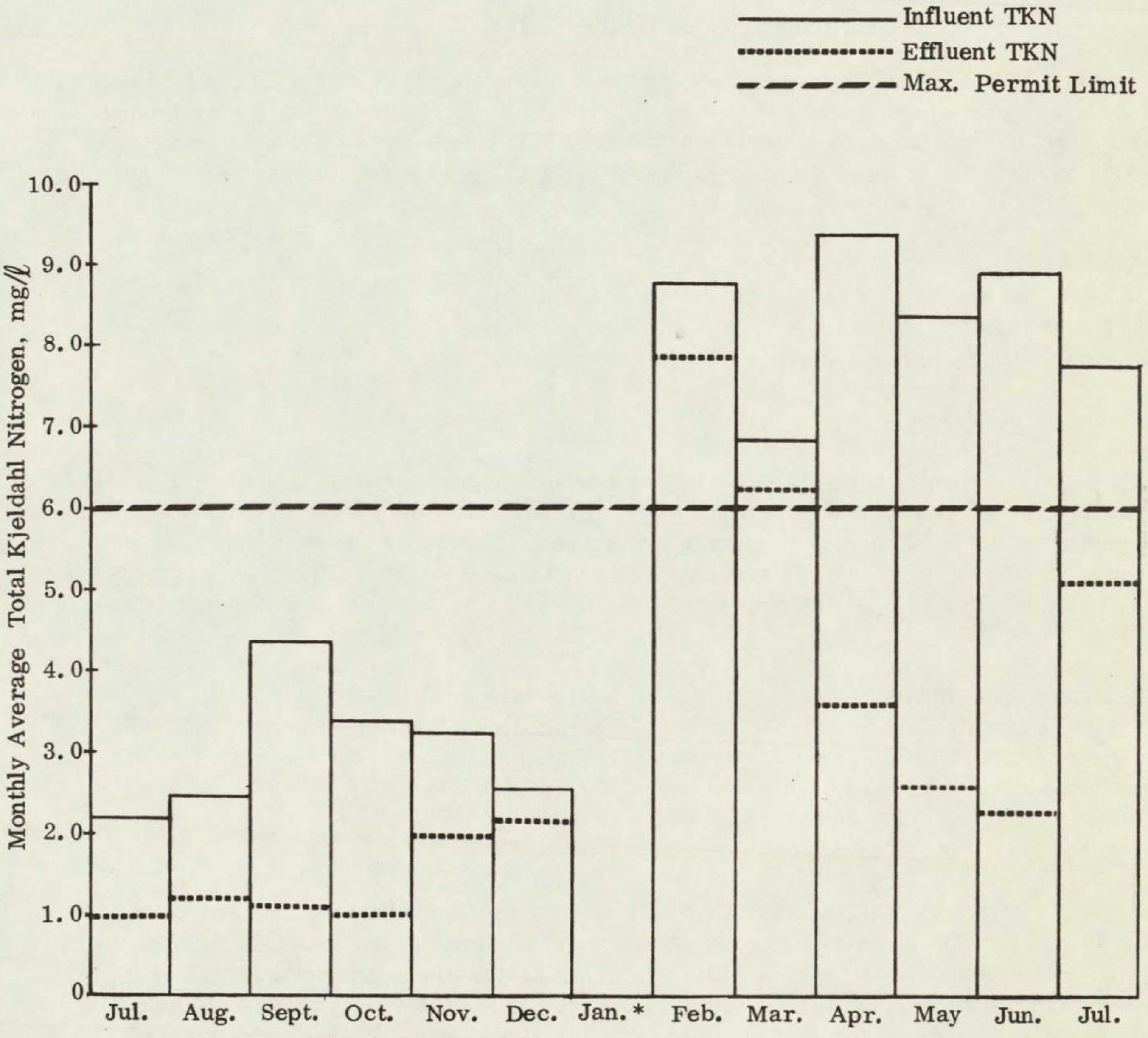


Figure 9. BOD_5 versus time for the Orange Grove secondary lagoon covered with water hyacinths



*No data collected due to difficulty with laboratory equipment

Figure 10. Total Kjeldahl nitrogen versus time for the Orange Grove secondary lagoon covered with water hyacinths

specified level of 5.0 mg/l by the time the effluent enters the drainage ditch. There was no noticeable odor of hydrogen sulfide, the "rotten egg" by-product produced under extreme anaerobic conditions.

6. Water Temperature

Water temperature (Table 2) was slightly but not significantly lowered throughout the year. This effect is perhaps due to shading and evaporative cooling.

7. Dissolved Solids and Other Nutrients

Although there are no maximum levels specified for total dissolved solids, total organic carbon and total phosphorus, a decrease in these nutrients will have a beneficial effect in retarding eutrophication of the receiving bodies of waters. The percent reduction of these nutrients in the effluent is presented in Table 2 and summarized below:

	Influent	Effluent	% Reduction
Total Phosphorus, mg/l	6.13	5.43	11%
Total Dissolved Solids, mg/l	244	210	14%
Total Organic Carbon, mg/l	33	23	30%

C. Discussion

The results from the Orange Grove experimental system substantially increased understanding of the efficiency and limitations of water hyacinths as agents for sewage effluent purification. In particular, experimental evidence indicates that water hyacinths in a nonaerated lagoon are capable of reducing TSS and BOD_5 of aerated effluent wastewaters by 74 percent and 90 percent, respectively, year-round in a system with the following specifications:

Surface Area of Hyacinth Lagoon	1.0 hectare
Total Capacity	24.3 million liters
Depth	1.83 meters
Flow Rate	2.03 million liters/day
Retention Time for Hyacinth Lagoon	12 days
Retention Time for Entire System	37 days

Even when the flow rate is as high as 97.2 million liters/day, so that retention time in the hyacinth lagoon is reduced to three days, the system is capable of maintaining BOD_5 and TSS levels of the sewage effluent well within the limits prescribed by the EPA and local authorities all but three months of the year if no protective cover is utilized through the winter months.

One major limitation of this system of sewage purification is that its efficiency is greatly reduced by long bouts of below-freezing temperatures. In order to be utilized on a year-round basis in locations where winter freezes occur regularly, the hyacinths will require protection with a greenhouse or other heat-conserving device. Other general problems and potential solutions are enumerated in the following paragraphs:

1. Aeration

When water hyacinths completely cover lagoon surfaces, natural aeration from the surrounding atmosphere is minimized. During that portion of the diel cycle when photosynthetic activity is at a minimum, dissolved oxygen concentration within the lagoon can drop precipitously. These conditions favor activity by anaerobic bacteria, which produce large quantities of the highly odorous hydrogen sulfide as a metabolic by-product. In order to minimize undesirable production of hydrogen sulfide, lagoon waters must be kept aerated. This may be achieved through the installation of mechanical aerators which operate during the night, when the water hyacinths are not actively photosynthesizing. A more economical and ecologically sound alternative is to harvest water hyacinths periodically during the peak growth months, to keep the lagoon partially open to natural aeration. Harvested water hyacinths can be composted and used as organic fertilizers and soil conditioners. On a larger scale, the harvested water hyacinths can also be processed into food, feed products, and biogas.

2. Plant Pests

a. The spider mite, (Bryobia praetiosa), often infests water hyacinths and can produce extensive damage if left untreated. Normally, these plants require spraying with insecticides such as malathion one to four times a summer to free the plants of this common pest.

b. Coots, (Fulica americana), mentioned previously, are potentially very damaging to water hyacinths in locations which provide winter nesting areas. They can perhaps be best discouraged from consuming these plants by installing inexpensive noise devices that generate sound frequencies (inaudible to humans) which repel the birds.

3. Chlorination

Although water hyacinths have proven to be highly effective in removing excess nutrients from sewage influents, they do not remove certain micro-organisms known to be present in wastewater, such as fecal coliform bacteria. Chlorination should be used to treat the effluent for elimination of these organisms.

A final treatment with water hyacinths following chlorination could possibly eliminate any of the carcinogenic chlorinated hydrocarbons formed during the chlorination process. Such a system is presently being designed for installation and evaluation at Orange Grove.

D. Conclusion

In conclusion, water hyacinths provide a means of sewage treatment which is sound both economically and ecologically. In this time of increasing ecological awareness and tightening purse strings, such a system is not only desirable, but essential.

Research presently being conducted will provide solutions to minor problems such as ensuring year-round operation, and discouraging insect and bird pests.

For their capabilities of purifying wastewaters, for their high nutrient level and resulting potential to be converted to food, feed, fertilizer, and energy sources, water hyacinths must surely be recognized as The Plant of the Future.

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SECTION I

Part 3

WATER HYACINTH (Eichhornia crassipes) PRODUCTIVITY AND HARVESTING STUDIES

By: B. C. Wolverton
Rebecca C. McDonald

ERL Report No. 171
March, 1978

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ABSTRACT

Water hyacinth growth rates were monitored from May through October in two sewage lagoons with different nutrient loading rates. The lagoon receiving the heaviest load sustained the highest average growth rates throughout the summer. The lightly loaded lagoon averaged a 29% increase in weight per week over the six month period with the highest growth rate occurring during June with an average weekly weight gain of 71%. The heavily loaded lagoon sustained an average growth rate of 46% per week for the same six month period with the highest measured growth rate of 73% increase in weight per week also occurring in June.

In addition, the performance of three harvesters was evaluated. One harvester, consisting of a chopper and conveyor, was capable of picking up and chopping approximately 2.3 m.t. of plants per hour and delivering them to a waiting truck. The second harvester was a single 1.52 m (5 ft.) wide conveyor, and the third one was a modified clam-shell bucket attached to a dragline. The average harvesting rate of each of these harvesters was approximately 9.3 m.t. of water hyacinths per hour.

WATER HYACINTH (*Eichhornia crassipes*) PRODUCTIVITY AND HARVESTING STUDIES

Introduction

Using vascular aquatic plants to treat wastewaters has proven to be a very promising method to treat domestic sewage, particularly for small communities of 10,000 people or less that already use lagoons. The aquatic plants studied to date involving waste treatment include the water hyacinth (1-5), duckweed (6,7), bullrush (8), and submersed plants (9). Most researchers recognize the water hyacinth as the most prolific of these aquatic plants.

Future wastewater treatment facilities using aquatic plants to upgrade their discharge may be designed in two manners. One design where nutrient removal is not important may minimize biomass production in order to reduce harvesting and plant disposal costs. The other alternative design where nutrient removal is desired should maximize biomass production, and therefore make the conversion of plant material into methane, fertilizer, or feeds a practical and profitable by-product of waste treatment. Two areas of study necessary to design a plant for maximum biomass production include plant growth rates and harvesting techniques.

The National Aeronautics and Space Administration at the National Space Technology Laboratories has been evaluating the effectiveness of water hyacinths to upgrade existing domestic sewage lagoons. Water hyacinth growth rate measurements and evaluation of harvesting techniques with a variety of harvesting equipment were a part of this effort. The results of these productivity and harvesting studies are presented in this paper in two parts.

Previous growth rate studies by Dymond (10) and Penfound and Earle (11) were used by Westlake (12) in estimating that the annual productivity of the water hyacinth is 11-33 m.t./ha, dry weight. A later study by Wooten and Dodd (13) found a production of 30 m.t. organic matter/ha in only 105 days. Westlake (12) projected that possible maximum annual production rates of 110 to 150 m.t. organic matter/ha/yr could be obtained if the plants were regularly thinned out to reduce self-shading and grown in tropical or sub-tropical climates.

Part I represents our water hyacinth productivity studies in two, one cell oxidation ponds with different loading rates located in a sub-tropical climate. Part II is an evaluation of three harvesting systems which can aid in removing the plants from oxidation ponds as necessary in order to encourage maximum biomass production.

PART I. PRODUCTIVITY STUDIES

Procedure

Both lagoons used in these productivity studies are single cell, facultative ponds, commonly referred to as oxidation ponds, which receive only domestic sewage. These lagoons are located at the National Space Technology Laboratories in Hancock County, MS and at Lucedale, MS. Both of these locations are in the Gulf Coast Region. The normal range of influent nutrient concentrations and loading rates, based on 5-day biochemical oxygen demand (BOD_5), total Kjeldahl nitrogen (TKN), and total phosphorus (TP), for these lagoons are presented in Table 1, A and B. These values were obtained from estimated flow rates and the analysis of two influent grab samples per week for a period of one year, including the growth rate study months.

Two galvanized wire mesh baskets, 2m W X 2m L X 0.5m D, supported by pontoons were placed in the NSTL lagoon. One basket was partially harvested at one to three week intervals to encourage maximum growth rates. The other basket was partially harvested every eight to ten weeks in order to collect data of plant length versus mass/surface area. An identical basket was placed in the Lucedale lagoon and partially harvested every three to six weeks.

Results

The NSTL sewage lagoon loaded at a rate of 16-34 kg BOD_5/ha/day (15-31 lb BOD_5/ac/day) receives a relatively light nutrient and organic load. The Lucedale lagoon receives almost two times the BOD_5 load and four times the nutrient load. This difference in nutrient loading is directly reflected in the measured growth rates shown in Table 2.

Water hyacinths grew the fastest in the early summer months of May and June in both systems. This observation correlates with that of Scarsbrook and Davis (14). The highest growth rates averaged 71% and 73% per week for NSTL and Lucedale, respectively. At this rate the plants doubled their mass every 9 to 10 days.

In order to use the growth rates more effectively, one must also use the data graphically represented in Figure 1. These data were collected from the basket at the NSTL lagoon which was maintained at 100% coverage. The average root and stalk heights were measured, and the plants were weighed. The straight line of best fit was obtained by least squares analysis for the plot of total plant length (stalk height plus root length) versus wet weight (metric tons) per hectare. We found that the total weight was more linearly related to the total plant length than just the stalk height alone. This relationship also held true with the plants from the Lucedale lagoon, although these plants had a much higher ratio of stalk height to root length as compared to those grown in the less fertile environment at NSTL.

Table 1 (A) Influent Nutrient Concentrations, Estimated Flow Rates, and Surface Areas of the Two Lagoons

Parameter	NSTL	Lucedale
5-day Biochemical Oxygen Demand, mg/1	108 ±41	127 ±39
Total Kjeldahl Nitrogen, mg/1	12.2 ±2.4	29.3 ±4.3
Total Phosphorus, mg/1	3.6 ±0.75	8.6 ±1.4
Flow Rate, m^3/day	475	1,140
Surface Area, ha	2.08	3.75

(B) Normal Range of Nutrient Loading Rates

Parameter, kg/ha/day	NSTL	Lucedale
5-day Biochemical Oxygen Demand	16-34	26-50
Total Kjeldahl Nitrogen	2.2-3.4	8.6-11.6
Total Phosphorus	0.65-1.09	2.2-3.0

Table 2. Average Growth Rates of the Water Hyacinth at NSTL and Lucedale

Month, 1977	Average Percent Increase by Weight			
	NSTL		Lucedale	
	Daily	Weekly	Daily	Weekly
May	6	52	7	61
June	8	71	8	73
July	3	27	5	42
August	2	15	5	39
September	1	8	4	35
October	0	0	3	24

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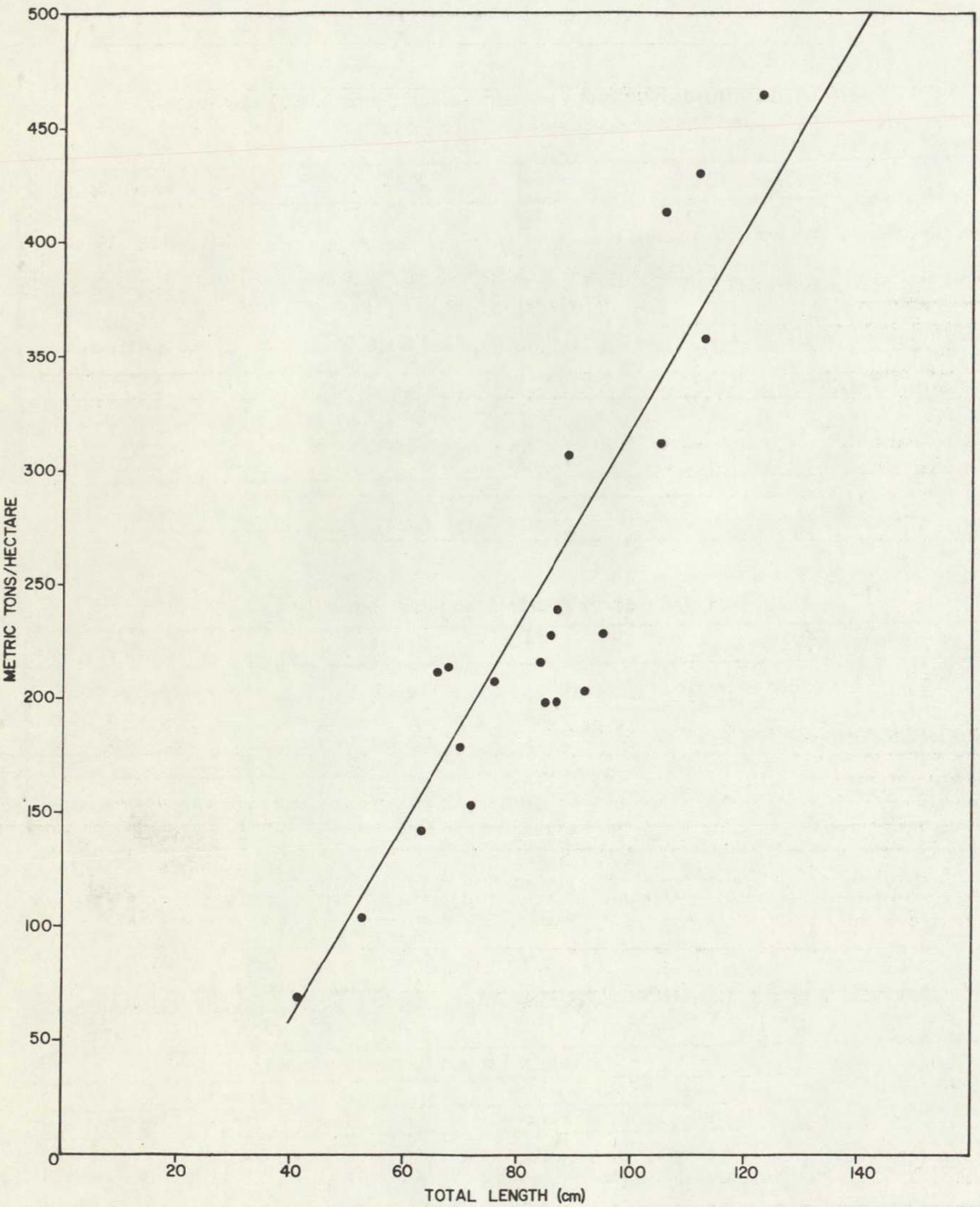

Figure 1. Chart for estimating water hyacinth wet weight/hectare based on total plant length. The straight line of best fit was obtained by least squares analysis.

PART II. HARVESTING

Harvesting Equipment

A. The first harvester designed under contract for NASA is shown in Figure 2. This harvester received plants pushed by a boat. The plants were picked up by a 1.83m (6') wide rotary head, coarse chopped and loaded into a dump truck via a 46 cm (18") conveyor belt. The chopper harvester required a four cylinder gasoline engine, and the small conveyor used a 5 hp gasoline engine.

The pusher boat shown in Figure 3 was a 4.27m (14') aluminum boat driven with a 20 hp outboard motor. A heavy wire mesh scoop (0.64 m W x 2.84 m L) was mounted on the front of the boat. This scoop was easily lowered or raised by hand by the boat operator.

B. The second harvester designed by NASA and fabricated at NSTL consisted of a single large conveyor belt (see Figure 4). The conveyor belt was 1.52 m W x 8.53 m L and was driven by a four cylinder diesel engine. The plants were pushed to the conveyor by the same pusher boat described in paragraph A above.

C. The third harvester evaluated by NASA was a modified clam shell bucket attached to a standard drag line (shown in Figure 5). The bucket was expanded to 3.05 m long and could pick up a 1.52 m wide area.

Results

Because the mass of water hyacinths per hectare is dependent on the size of plants, the efficiencies of the three harvesters are best compared by estimating surface area harvested per hour and correlating this value with the size of the plants to be harvested using Figure 1. Table 3 gives the maximum and realistic harvesting capabilities of the three machines.

The limiting factor for both the single conveyor and the conveyor-chopper was the pusher boat. At best a single pusher boat can only keep plants on the conveyors 25% of the time. These systems could be further optimized by using two or more pusher boats or by devising another system that could continuously feed the harvesters.

The conveyor-chopper was subject to more mechanical breakdowns than the simple conveyor due to its more complex nature. The rotary pickup head was easily clogged with sticks and other rigid objects. The single large conveyor was far more reliable because its moving parts could not be clogged by floating debris.

The original design for the single conveyor called for wing conveyors to extend out into the water at the base of the central conveyor belt. These wing conveyors would form a funnel and greatly aid the pusher boat in channeling plants up to the central harvester. However the size of the lagoon at NSTL was not large enough to warrant the extra expenditure of funds necessary to enlarge the conveyor.

The modified clam shell bucket proved to be the easiest harvester to use continuously. Its overall harvesting capacity of 418 m^2/hr was comparable to that of the single conveyor.

Figure 2. Chopper-conveyor harvester

Figure 3. Pusher boat

Figure 4. Single conveyor harvester

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Figure 5. Modified clam-shell bucket

Table 3. Comparative Efficiencies of the Three Harvesters Used to Harvest Water Hyacinths From Wastewater Lagoons

Harvester	Av. % Time Harvesters Loaded With Plants	Surface Area Harvested Per Hour (m^2/hr)		*Mass Harvested Per Hour (met. tons/hr)	
		Max. Possible	Average	Max. Possible	Average
Conveyor-chopper	25	414	104	9.1	2.3
Single conveyor	25	1670	418	36.7	9.2
Modified clamshell bucket	75	558	418	12.3	9.2

*Based on an average standing crop of 220 met. tons/ha

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This harvester was very efficient in harvesting the plants from the water hyacinth-chemical waste treatment system which was constructed in a zig zag configuration that was 6.4 m wide (15). It could be easily moved around the lagoon and harvest specific areas of plants. This flexibility was very important in the chemical waste system due to its configuration. Plants at the upper end of the canal required harvesting more frequently than those at the discharge point because they became saturated with heavy metals more quickly and had to be replaced periodically.

Many people have built their own aquatic plant harvesters from equipment that they already owned in order to economize. One example is shown in Figure 6. This harvester consists of a modified bucket attached to a suitable piece of heavy equipment. The cost of modifying the bucket was nominal. For small water hyacinth treatment systems where harvesting requirements are minimal, devising harvesters out of existing back hoes and related equipment can be very practical. Other water hyacinth harvesters are compared in a comprehensive survey prepared by the U. S. Army Corps of Engineers (16).

Discussion

Overall, the water hyacinth in the enriched environment at Lucedale, MS averaged a 46% increase in weight per week during the months of May to October. The growing season also includes April for the Gulf Coast region, although the growth rate studies were not begun in time to include this month.

The number of metric tons per week will fluctuate due to monthly growth rate differences. More biomass can be harvested during May, June, and early July than during the other months. However, on an average 101 wet metric tons biomass can be harvested per hectare per week from April through October based on maintaining an average standing crop of 220 m.t./ha. Over the seven months, 3,080 wet metric tons or 154 dry metric tons (based on an approximate solids content of 5% of wet weight) per hectare can be obtained. This value is amazingly close to Westlake's prediction of an annual productivity of 150 m.t. organic matter/ha/yr under ideal conditions (12).

This potential yield of 154 m.t./ha/yr far exceeds the dry biomass yields of any terrestial, saltwater, or freshwater plant, except algae, yet recorded. For example, sugar cane and sweet sorghum, which are considered potential candidates for bioconversion due to high growth rates, can yield 44.8 m.t./ha./yr (18). In California average yields of Eucalptus sp., a woody plant, have been as high as 53 m.t./ha/yr (17).

Macroscopic red algae (Rhodophyceae) can produce 41.8 to 55.0 m.t./ha/yr when grown in enriched seawater (19). The giant algae or kelp (Macrocystis) that has been considered a prime candidate for bioconversion produces an average yield of 14.5 m.t. organic matter/ha/yr or approximately 26.1 total m.t. of mass per ha/yr (20). These yields have been reported as high as 30.6 and 55.1, respectively.

Algae is considered among the most prolific of the freshwater plants. Maximum obtainable growth rates for this plant vary greatly. Oswald reports yields of 35.2 to 70.4 m.t./ha/yr for algae harvested from enriched sewage lagoons (21). However McGarry and Tongkasome report that yields of 157 m.g./ha/yr are obtainable when algae is grown year-round (22).

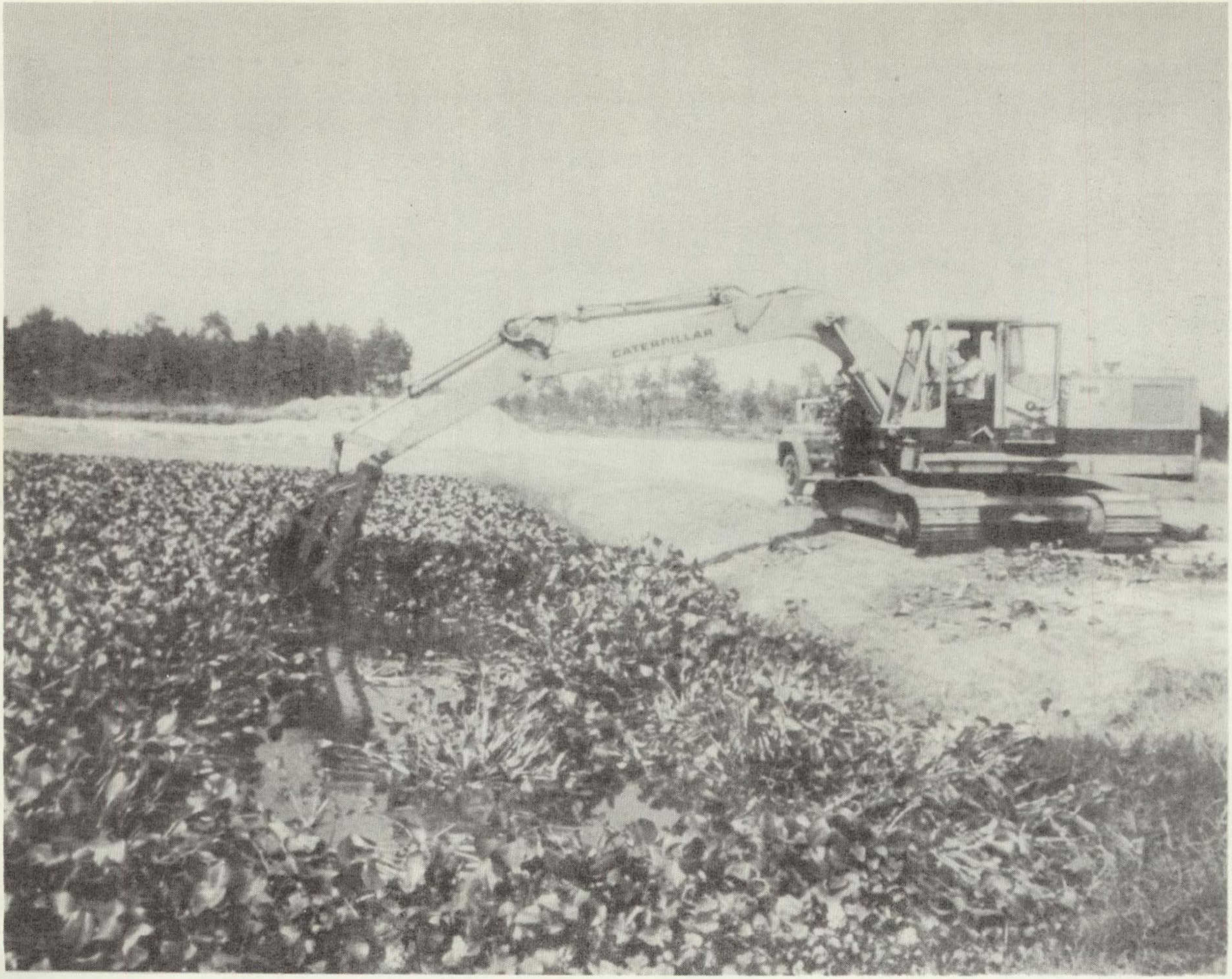

Figure 6. Modified bucket attached to a back hoe.

This potential water hyacinth crop of 154 m. t./ha produced during seven months of the year has shown promise for feeds and fertilizers. On a dry weight basis, the crude protein averaged 22.3% (32% in the leaves) (23). The phosphorus and ash content averaged 0.89% and 15.1% of dry weight, respectively. The plants are also rich in potassium, calcium, iron, manganese, magnesium, etc., along with many vitamins such as thiamine, riboflavin, niacin, and B-12 (23).

Water hyacinths are also a major candidate for bioconversion to produce methane for energy. In batch studies of anaerobically digesting water hyacinths, NASA has found that 350 to 411 liters bio-gas per kg dry weight (5.7 to 6.6 scf per dry lb) can be obtained (24 and unpublished data). This bio-gas contains approximately 60% methane. Therefore, one hectare of water hyacinths grown in an enriched environment in a warm climate for seven months of the year can be used to produce approximately 58,400 m^3 (2,290,000 scf) of bio-gas containing 35,100 m^3 (1,370,000 scf) methane.

These potential products are presently being explored by NASA as well as other investigators. This phenomenal annual production of organic matter per hectare that can be obtained as a by-product of domestic waste treatment contributes to the economic attractiveness of using the water hyacinth as a new source of feed, fertilizer, and energy.

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SECTION II

CHEMICAL WASTE TREATMENT

SECTION II
Part 1

WASTEWATER TREATMENT UTILIZING WATER HYACINTHS (*Eichhornia crassipes*) (Mart.) SOLMS

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and Disposal of Industrial Wastewaters and Residues

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WASTEWATER TREATMENT UTILIZING WATER HYACINTHS (*Eichhornia Crassipes*) (Mart.) Solms

Introduction

In the last two decades, the practice of dumping either untreated or partially treated waste into rivers and streams has become a major source of conflict between industry and groups of citizens concerned about protecting our environment. Consequently, the U. S. Environmental Protection Agency was chartered to impose and enforce regulations on the quality of the wastewater that industries can discharge into receiving water. This agency is slowly imposing stricter standards on industrial wastewater effluents with the aim of eventually achieving "zero discharge" of any industrial pollutants into receiving waters (Public Law 42-500).

Whether or not zero discharge is a realistic goal is a matter of debate. In any case, the discharge of industrial waste must be regulated, since its constituent components, both organic and inorganic, have been shown to have deleterious effects. Some organic compounds may act directly as toxins or carcinogens. Others may increase the biochemical oxygen demand (BOD) and consequently lower the dissolved oxygen in receiving waters, causing suffication and death of many aquatic species. Still others may impart objectionable taste and odors to drinking waters, a less harmful but certainly undesirable effect (8).

Inorganic compounds also have many adverse effects on man and the environment. Toxic heavy metals tend to concentrate in the fauna and flora of the aquatic environment and produce a variety of effects in man once they are ingested. For example, cadmium, besides being a carcinogen, has been linked to kidney ailments, hypertension, and other cardiovascular conditions; hexavalent chromium is toxic and carcinogenic to both man and organisms found in the aquatic environment; mercury concentrates in the human fetus and causes permanent fetal brain damage; and silver produces a permanent blue-gray discoloration of the skin and becomes toxic if allowed to accumulate (4, 6, 10).

The National Space Technology Laboratories (NSTL), Bay St. Louis, Mississippi, has the problem of treating chemical and photographic waste products that contain a variety of organic compounds as well as silver and trace amounts of such metals as cadmium and chromium. Public Health Service (PHS) recommendations for maximum discharge levels of some heavy metals are presented in Table 1.

Table 1. Public Health Service Recommendations for Maximum Discharge Levels of Heavy Metals

Metal	Maximum Discharge Level, mg/ℓ
Lead	0.05
Silver	0.05
Cadmium	0.01
Chromium	0.05

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Present techniques for treating photographic wastewater include package activated sludge plants and aerobic lagoons (6). Heavy metals can be removed with varying degrees of efficiency by chemical precipitation, electrodeposition, solvent extraction, ultrafiltration, ion exchange and activated carbon absorption (3). Mixed wastes such as the wastes discharged at NSTL would require a combination of these treatment techniques. All of these methods are expensive to install and maintain and do not always meet EPA standards.

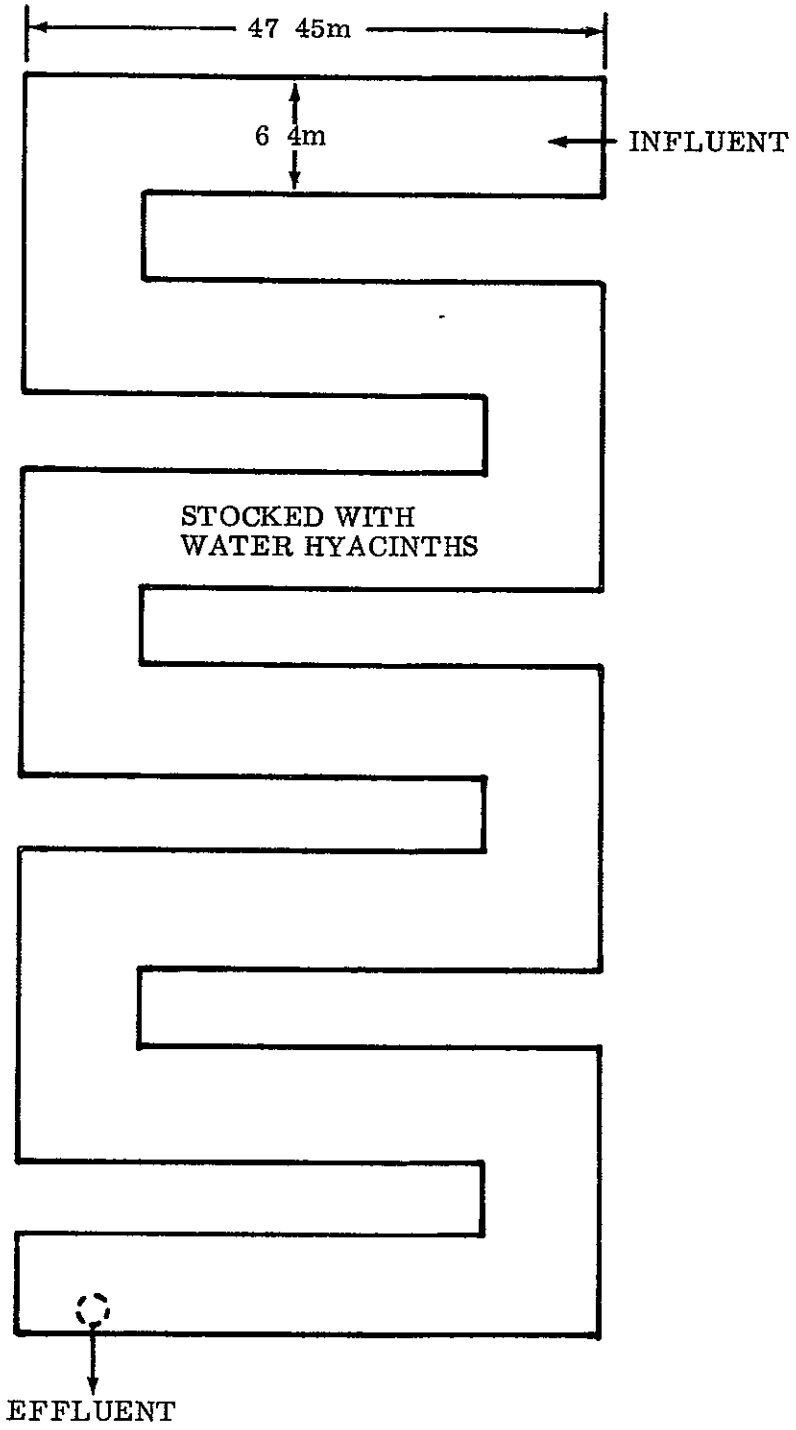
In an effort to develop a relatively inexpensive and effective means of treating the chemical and photographic waste at NSTL, the National Aeronautics and Space Administration (NASA) has installed a water hyacinth filtration system. The water hyacinth, (Eichhornia crassipes) (Mart.) Solms, is an excellent candidate for a biological filtration system for a number of reasons. Water hyacinths possess an extensive root system which allows them to feed directly from the aqueous medium, extracting chemicals and nutrients rapidly and efficiently. In experimental sewage and chemical treatment systems, water hyacinths have demonstrated the ability to substantially reduce the concentrations of organics, minerals, and heavy metals in the effluent waters (7, 9, 12, 19). Another feature is the plant's tremendously high growth rate. Capable of producing 17.5 metric tons of wet biomass per hectare per day under ideal growing conditions (18), the water hyacinth is believed by many botanists to be the most productive plant on earth (1). These features, which make the water hyacinth such a successful pest species, can also be of great potential benefit to man when the plants are properly utilized.

Description of the Water Hyacinth Treatment System

A specially designed lagoon was constructed at the National Space Technology Laboratories by NASA for the treatment of photographic and chemical laboratory waste. The lagoon was constructed in a zig-zag configuration with the following specifications: length, 332 m; width, 6.4 m; depth, 0.78 m; total volume 1,675,000 liters, total surface area, 0.22 ha (See Figure 1). The zig-zag design promotes efficient filtration by maximizing the lagoon's length within a relatively small area. In addition, this design facilitates access of harvesting machines to the water hyacinths.

This lagoon receives approximately 95,000 liters per day. A minimum retention time of 20 days was built into the system, assuming that this would be the maximum time during the winter months in which the plants would be metabolically inactive.

In May 1975, this system was stocked with sufficient water hyacinths to cover approximately 20 percent of the surface area, and the waste from the chemical photographic laboratories was diverted into the lagoon. Although chemical waste was the sole source of nutrients available to the plants, they grew rapidly, multiplying to 75 percent coverage within four weeks. During the summer months, the water hyacinths were sprayed with malathion to control spider mites, (Bryobia praetiosa). The plants thrived during all months of the experiment with the exception of January and February, when freezing temperature caused the tops of the plants to die back.



0. 78m DEEP
1,041,000 l TOTAL VOLUME
0 22 ha TOTAL SURFACE AREA

Figure 1. NASA/NSTL water hyacinth chemical waste filtration system

Methods

Daily grab samples were taken from the wastewater before it entered the lagoon and from the effluent waters. Water samples were analyzed for pH, dissolved oxygen (DO), total suspended solids (TSS), total organic carbon (TOC), 5-day biochemical oxygen demand (BOD_5), total phosphorus, and chemical oxygen demand (COD), according to Standard Methods (11). Heavy metal content of water samples was determined with the aid of an IL Model 253 Atomic Absorption/Flame Emission Spectrophotometer.

Over a six-week period, sample water hyacinths were taken from the lagoon weekly and analyzed for heavy metals. Roots, stems and leaves were analyzed separately to determine whether the metals migrated to upper parts of the plants. These plants were washed, dried in an oven at 110°C for 48 hours and ground to an even, fine consistency in a Waring commercial blender. All glassware was acid-washed prior to use. One gram samples were weighed out and transferred to 100 ml Kjeldahl flasks. To the flask was added 10 ml concentrated nitric acid, approximately 60 ml distilled water and boiling chips. The samples were digested until only a clear solution and a fine residue remained. The supernatant was filtered into 100 ml volumetric flasks and diluted to volume. The solution was analyzed by atomic absorption. A blank was digested with all samples and used as a correction factor for any contaminants in the reagents that might have been introduced.

Results

Table 2 shows a complete yearly analysis by month of the influent and effluent waters of this system. Silver was the only metal present in quantities sufficient to be noted. Traces of other metals were occasionally detected in the influent waters, but no other metals were found in the effluent. The water hyacinths maintained the effluent pH between 6.8 and 7.8. The dissolved oxygen remained above the generally accepted standard of 5 mg/l all but one month. No algal blooms were observed during these twelve months as indicated by the relatively low suspended solids. The reduction in dissolved solids varied from 29 percent to a high of 75 percent.

The concentrations of total Kjeldahl nitrogen and total phosphorous were also reduced by large percentages, as indicated in Table 2. The most significant demonstration of water hyacinths' biological filtration capabilities was the reduction of BOD_5. The chemical oxygen demand has also been reduced by 83 percent to 92 percent.

Table 3 shows the systemic uptake of the heavy metals that were routinely detected in the influent wastewaters. Over a 6-week period, water hyacinths accumulated these heavy metals to concentrations several hundred times the initial levels. The highest concentrations of heavy metals were found in the roots, the site of uptake of these substances, but there was also a significant accumulation in the plant stems and leaves.

Table 2 Monthly Average Data of the Water Hyacinth Chemical Waste Filtration System

Months	pH		Dissolved Oxygen mg/ℓ		Total Suspended Solids mg/ℓ		Total Dissolved Solids mg/ℓ			Silver mg/ℓ		Total Organic Carbon mg/ℓ			Biochemical Oxygen Demand mg/ℓ			Total Kjeldahl Nitrogen mg/ℓ			Total Phosphorus mg/ℓ			Chemical Oxygen Demand mg/ℓ		
1975	Inf	Eff	Inf	Eff	Inf	Eff	Inf	Eff	% Red	Inf	Eff	Inf	Eff.	% Red	Inf	Eff	% Red.	Inf	Eff	% Red	Inf	Eff	% Red	Inf	Eff	% Red
May	7.79	8 88	-	-	7	33	568	246	57	-	-	65	23	65	-	-	-	4 97	2 16	57	0 73	0 14	81			
June	7 66	7 36	2 57	5 47	4	16	560	186	67	0 74	<0 02	81	16	80	-	-	-	6 61	0 55	92	1 38	0 18	87			
July	7 57	6 89	3 59	3 18	6	12	380	212	44	0 99	<0 001	75	13	83	33*	4	88	2 36	0 43	82	0 48	0 08	83			
August	7 42	6 81	7 45	6 75	12	9	622	271	56	0 45	<0 001	61	7	89	73	1	99	3 32	0 19	94	1 84	0 04	98			
September	7 61	7 14	4 19	6.28	9	6	454	277	39	2 40	<0 001	53	8	85	69	1	99	4 19	0 16	96	0 95	0 04	96			
October	7 47	7 19	2 89	5 61	7	5	454	238	48	0 32	<0 001	27	11	59	153	1	99	9 38	0 44	95	0 54	0 02	96			
November	7 74	7 35	4 58	8 31	6	2	484	287	41	0 49	<0 001	47	15	68	71	2	97	8 11	1 29	84	1 06	0 02	98			
December	7 52	7 52	7 10	9 78	8	8	391	224	43	0 59	<0 001	46	14	70	89	1	99	11.70	1 37	88	0 47	0 03	94			
1976																										
January	7 90	7 79	2 30	9 90	8	10	386	273	29	1 19	0 04	45	13	71	156	3	98	13 60	3 73	73	1 06	0 05	95	192	33	83
February	7 47	7 59	1 15	9 62	9	5	856	297	65	1 00	0 03	64	16	75	151	2	99	16 80	5 44	68	1 88	1 29	31	385	29	92
March	7 79	7 73	0 79	10 50	9	28	1302	328	75	2 38	0 06	146	22	85	97	5	95	62 00	4 18	93	3 88	0 81	79	527	41	92
April	7 30	7.85	0 78	9 96	13	11	852	387	55	2 79	0 05	142	26	82	171	7	96	27 50	3 27	88	3 77	1 29	66	545	45	92
May	7 54	7 63	1 23	10 60	7	25	640	386	40	1 99	0 06	83	27	67	150	9	94	27 20	3 38	83	2 15	0 87	60	301	49	84
June	7 75	7 47	1 14	8 77	12	31	527	352	33	2 06	0 02	57	29	49	138	5	96	12 31	2 56	79	1 51	0 46	70	356	57	84

NOTE Data partially presented for May and June 1975 due to insufficient data to average for BOD_5, DO, and Ag Laboratory not equipped to do routine COD analysis until January 1976

Table 3. Analyses of Water Hyacinths Before Introduction into the Chemical Waste System and After Six Weeks Exposure

Metal	Concentrations, ppm (Dry Weight)					
	Leaves		Stems		Roots	
	Initial	Six Weeks Exposure	Initial	Six Weeks Exposure	Initial	Six Weeks Exposure
Copper	17.5	32	10.9	48	24.0	594
Lead	8.4	33	2.1	45	40.0	297
Silver	0.8	9	<0.1	4	36.0	113
Cadmium	<0.1	2	<0.1	10	<0.1	164
Chromium	<0.1	4	<0.1	12	<0.1	286

Discussion

The water hyacinths proved to be a very effective filtration system for cleaning wastewater containing a complex chemical mixture. Organics, heavy metals and other elements were effectively removed from the wastewater by plant root sorption, concentration and/or metabolic breakdown (Table 2). Trace elements entering the lagoon system were effectively removed to levels which comply with PHS recommendations.

Even the hardy water hyacinth is not immune to heavy metal pollutants. Approximately every eight weeks during the summer, the leaf tips began to turn brown and curl, indicating that the plants had sustained permanent metabolic injury from the environmental pollutants. The damaged sections of water hyacinths were harvested and piled nearby, since it is believed that plants in this condition are no longer maximally efficient at purifying wastewaters. When water hyacinths are used in permanent chemical waste treatment systems, periodic harvesting of damaged and/or saturated plants may be necessary if the discharge of toxic heavy metals is very high.

Since the plant stems and leaves, as well as roots, were found to contain heavy metals, no part of the harvested plants can be used as feed or fertilizer. However, the harvested plants can be used safely for the production of biogas. Whole harvested plants (or remaining sludge, if biogas is produced) should be put in a pit specially designed to eliminate ground water infiltration. Such a pit is planned to be utilized at the NSTL zig-zag lagoon. Over a period of years, the heavy metals in the pit may accumulate to levels high enough that their extraction becomes economically feasible. Such small "mining" operations--particularly of silver--may prove to be an efficient method of recycling valuable metals for industrial use.

Determining the optimal retention time for a system designed to remove heavy metals is complicated by the fact that these substances readily undergo chelation in the presence of the organic chemicals also discharged into the system (2,5). Although plants will rapidly take up metals in the ionized form, chelated metals are not readily sorbed by the plant roots. Some chelates are very stable and can be broken down only by active microbial degradation. Once degradation has occurred, the plant roots will readily sorb the free metal ions. More research is needed to understand the time lag engendered by the process of chelation/microbial degradation and the effect of this process on determining the proper retention time for maximum removal of heavy metals from the system.

Conclusions

As a result of the water hyacinth's demonstrated ability to treat chemical waste effectively, the experimental lagoon system has been permanently installed at NSTL.

In combination with microorganisms, aquatic plants such as water hyacinths must be seriously considered in developing filtration systems for removing trace toxic chemicals such as heavy metals and carcinogenic organics. For large industrial systems, use of the water hyacinth may be limited to warm climates, but small volume operations should consider greenhouse techniques for maintaining these plants. Additional research and screening should be conducted with the numerous chemicals found in industrial waste to establish chemical concentration levels that the water hyacinth and other aquatic plants can tolerate and remove.

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SECTION II

Part 2

WATER HYACINTH SORPTION RATES OF LEAD, MERCURY AND CADMIUM

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ABSTRACT

Experiments were performed to test the ability of the water hyacinth (Eichhornia crassipes) to remove lead, cadmium and mercury from solution both individually and in combination. The plants were exposed to 10 ppm lead and one ppm each of cadmium and mercury for a period of 96 hours. The sorption of the heavy metals was monitored by periodic water sampling and plant tissue analysis at the termination of the experiment. Results indicated no significant interactive effect among the three metals tested. Within one hour, water hyacinths removed approximately 65 percent of the lead, 50 percent of the cadmium and 65 percent of the mercury, whether the plants were exposed to these metals individually or in combination. Almost all of the heavy metals were concentrated in the root tissue of the plants, although there was some translocation, particularly of cadmium, into the upper plant parts.

These results are discussed in the light of previous investigations. It is concluded that the water hyacinth could be useful both as an agent for reducing heavy metal pollution and as an indicator or monitor of chronic metal contamination of aquatic systems.

Introduction

Through mining and industrial activities and through the combustion of fossil fuels, man spews thousands of tons of metallic pollutants into the air and water each year (10). Toxic metal pollution is not only a problem for exposed workers, but is one of global concern. Heavy metals constitute a serious form of pollution since they do not degrade as do organic pollutants. Many metallic ions form stable complexes or chelates, which tend to concentrate in the food chain and may function as cumulative poisons in high-level consumers such as man. Leland (12) has recently reviewed the literature on the sources and effects of metal pollutants in aquatic environments.

The toxic effects of lead, cadmium, and mercury are well documented, both in the laboratory and, unfortuanately, under natural conditions. Mercury is severely toxic and can produce loss of vision, paralysis and even death in low concentrations. Excess levels of lead can cause anemia, kidney and liver disease, paralysis, brain damage, convulsions and death. Low levels of this metal may contribute to hyperactivity, learning disabilities, night blindness and suppression of the body's immune responses (19, 25). Cadmium can produce kidney and liver damage as well as certain forms of cancer, pulmonary disease and death. According to a recent review by Doyle (4), chronic low levels of cadmium may cause decreased growth, hypertension, and alterations in blood cholesterol and trace element metabolism.

In view of these facts, it is understandable that the Environmental Protection Agency sets a strict upper limit to the amounts of heavy metals permissible in drinking water. According to the 1977 regulations, the maximum levels for lead, cadmium, and mercury are 0.05 ppm, 0.01 ppm and 0.002 ppm, respectively. However, despite these regulations, there remains the potential danger of aquatic pollution from accidental spills and cumulative effects of industrial discharges.

Certain plants are known to concentrate heavy metals to high levels. Mosses and lichens strongly sorb metal ions present in air and water and have often been used as monitors of atmospheric and aquatic concentrations of lead and other metals (7, 13, 23). The metal content of the leaves and twigs of woody plants has also been used to indicate atmospheric pollution levels (20).

Plant uptake of metals from soils and solution has been investigated for a variety of species (see, for example, 1, 15, 18). The majority of these studies involve the phytotoxic effects of heavy metals on crop species over a relatively long term exposure period; the concept of using plants as biological agents to remove heavy metal pollutants has received little attention.

Experiments conducted at NSTL and others have shown that the water hyacinth (Eichhornia crassipes) is capable of removing considerable quantities of excess organic nutrients from sewage lagoon systems (3, 28, 29). The water hyacinth, with its extensive root system and rapid growth rate, seems an ideal candidate for detection and removal of heavy metals from aquatic systems. The plants grow extremely well in lagoon systems, producing an average standing crop of 17.5 tons per hectare per day wet weight (3, 28). Preliminary work at NSTL indicated that water hyacinths are capable of concentrating certain heavy metals under laboratory and field conditions (26, 27). In this paper we report further results of the water hyacinth's ability to remove lead, cadmium and mercury from aquatic systems.

Many cases of aquatic trace element contamination involve more than one heavy metal. Therefore, although knowledge of the effects of individual metals is essential, it is also important to understand the interactive or competitive action of metal ions in solution and their combined effects upon biological systems. The interaction of metals in solution can alter the pattern of plant uptake of individual metals. For example, Miller, et al. (14) found that uptake of soil cadmium by corn was enhanced in the presence of lead, while the presence of cadmium depressed lead uptake by these plants. In this investigation, we compare the water hyacinth's removal potential for dissolved lead, cadmium and mercury individually and in combination.

Materials and Methods

Water hyacinth plants were collected from a sewage lagoon on the National Space Technology Laboratories Facility. Tests were conducted in five-gallon plexiglass aquariums, each containing 15 liters of water taken from the East Pearl River. (The average nutrients and physical parameters of this water are presented in Table 1.) The experimental design consisted of four treatment groups with three replicates per treatment. Treatments included exposure to 1 ppm cadmium, 10 ppm lead, 1 ppm mercury and finally, a combination of these three metal concentrations in each test container. Metal solutions were prepared by diluting Fisher Scientific standard solutions to the desired concentration.

Four mature plants of approximately equal size were rinsed and placed in each of these metal-containing aquariums. A control aquarium contained water hyacinths in 15 liters of river water with no metals; other controls consisted of smaller jars containing four liters of river water polluted with each metal or combination of metals present in the experimental aquariums. Plants were exposed to approximately 400 foot-candles of fluoescent light for 14 hours daily and maintained at 27±°C throughout the experiment. Water samples collected periodically from 0 through 96 hours from each aquarium were acidified and analysed for the appropriate metal with an IL 253 Atomic Absorption/Flame Emission Spectrophotometer. After 96 hours of experimental run, all plants in each container were removed, rinsed and grouped according to experimental condition. The roots were separated from the tops, and all plant parts were dried at 60°C for 48 hours. The dried plant parts were weighed to the nearest 0.1 gram and ground in a Waring commercial blender to a fine powder.

For cadmium and mercury analysis, 0.500 grams of the plant material was digested at 350°C with 10 ml of nitric acid and five ml of sulfuric acid in volumetric Technicon digestion tubes. The digested samples were diluted to volume with deionized water. Cadmium was analyzed by atomic absorption. Mercury was analyzed by a flameless emission method (9).

For lead analysis, two grams of dry plant material was dry-ashed at 550°C in a muffle furnace overnight. The ash was dissolved in 10 ml of nitric acid and five ml of sulfuric acid with heating, transferred to a volumetric flask, and made to volume with deionized water. Lead was analyzed by atomic absorption.

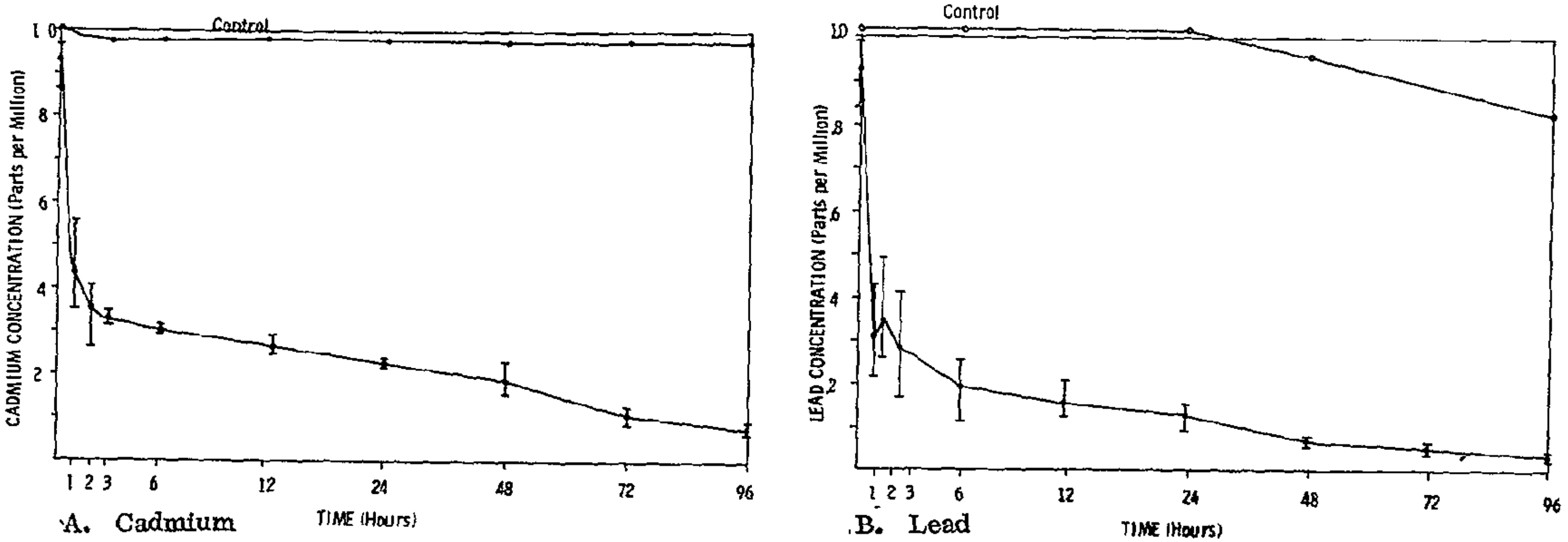
Table 1. Composition of River Water Used in Experiments

Parameter	Value
pH	7.6
Total Organic Carbon	7.0 mg/l
Total Kjeldahl Nitrogen	0.1 mg/l
Total Phosphorus	1.25 mg/l
Total Suspended Solids	8 mg/l
Total Dissolved Solids	72 mg/l

Table 2. Percent Reduction of Heavy Metals by Water Hyacinths

Metal	Condition	Percent 1 Hr.	Reduction 24 Hrs.	Significance level of percent reduction at 24 hours in comparison with control.
Cd	Alone	53	70	$.01 < p < .025$
Cd	Combined	45	81	$.01 < p < .025$
Pb	Alone	67	87	$p < .005$
Pb	Combined	65	87	$p < .005$
Hg	Alone	56	92	$.05 < p < .1$
Hg	Combined	75	91	$.05 < p < .1$

* Students Paired T Test (Freund, 1960)



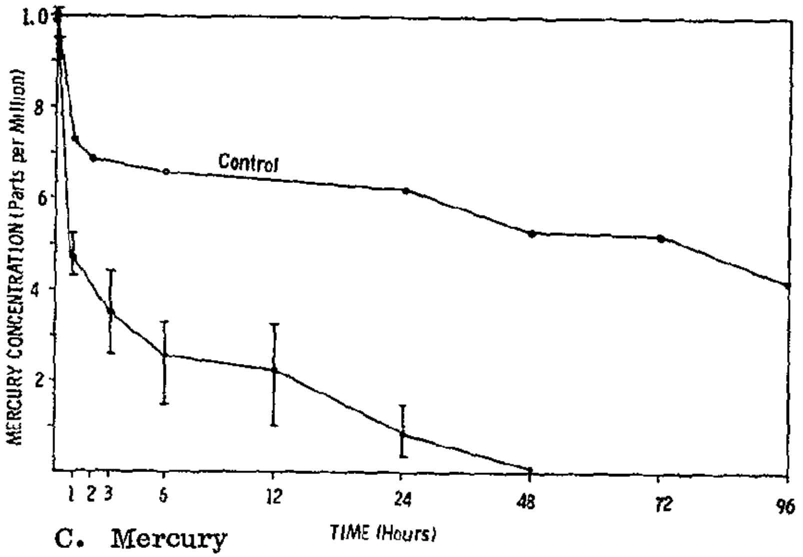
A. Cadmium

B. Lead

A. Average Reduction of Cadmium by Water Hyacinths over 96 Hour Period

B Average Reduction of Lead by Water Hyacinths over 96 Hour Period

C Average Reduction of Mercury by Water Hyacinths over 96 Hour Period



C. Mercury

Figure 1. Average reduction by water hyacinths over 96 hour period

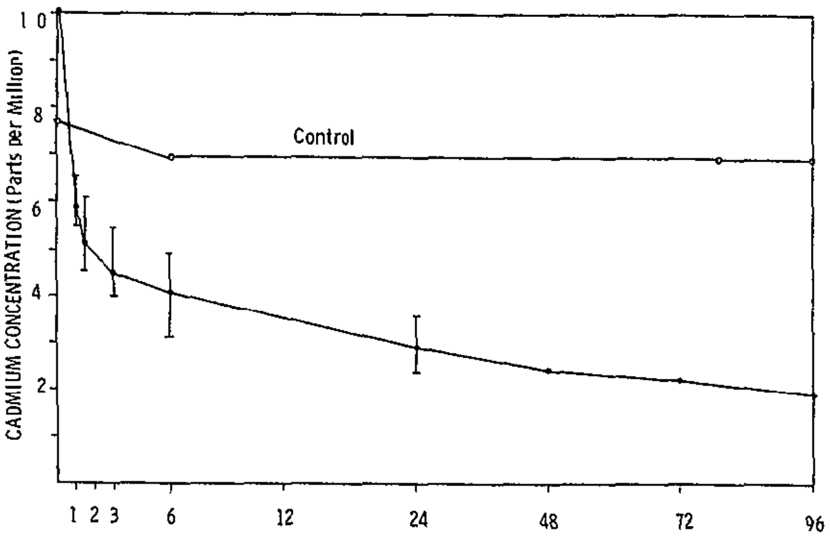


Figure 2A

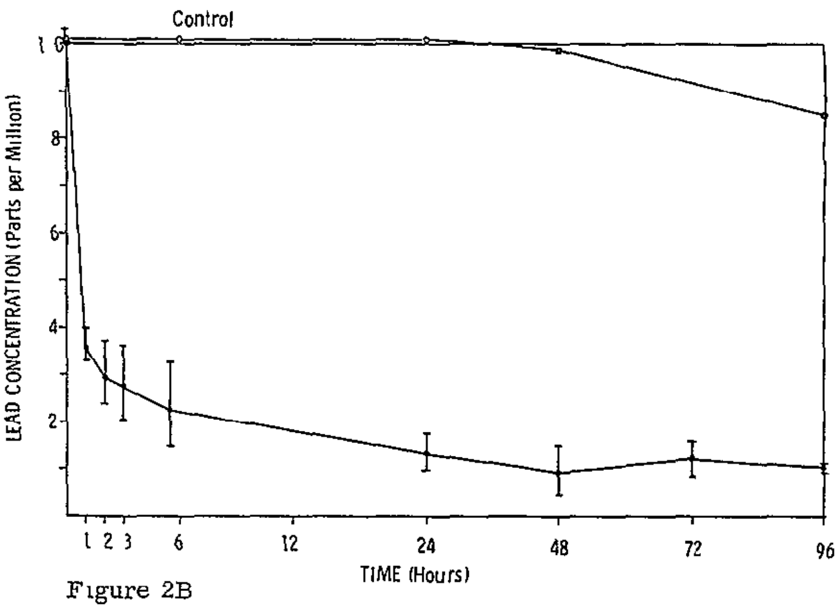


Figure 2B

Figure 2A Average Reduction by Water Hyacinths of Cadmium when in combination with Lead and Mercury

Figure 2B Average Reduction by Water Hyacinths of Lead when in combination with Cadmium and Mercury

Figure 2C Average Reduction by Water Hyacinths of Mercury when in combination with Lead and Cadmium

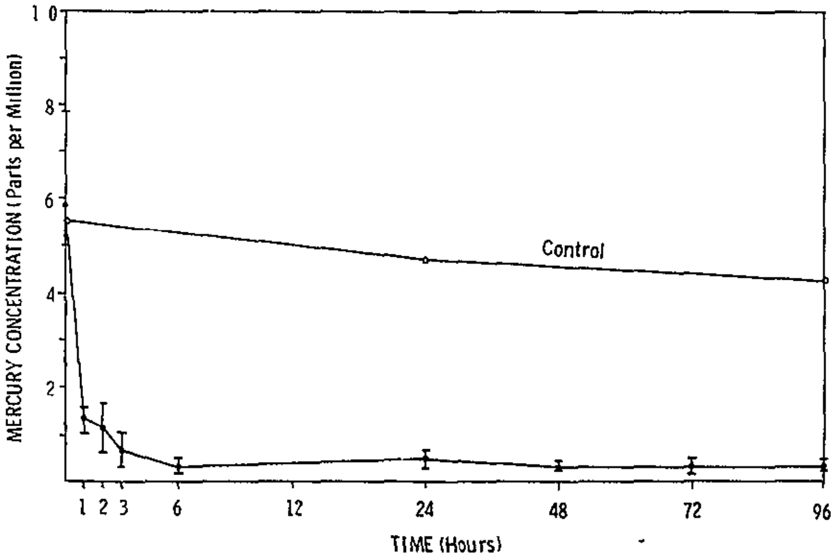


Figure 2C

Blanks were prepared with all digestions and used to correct all samples for any metals introduced from the reagents.

Results and Discussions

The effect of water hyacinths on the metal concentrations of the solutions is indicated in Figures 1 and 2. As these figures show, the pattern of metal removal is similar for all metals under both conditions; there is an initial precipitous drop in metal concentration over the first several hours, followed by a leveling off or slow decline for the remainder of the experimental period. After 24 hours, the rate of metal reduction appears to have stabilized for all three metals tested.

The percent metal reduction at one and twenty-four hours is shown for each metal and condition in Table 2. In comparison with the control, the water hyacinths effected significant reductions of lead and cadmium; mercury reduction approached but did not quite attain significance at the 0.05 level. This was due to an interaction of the small sample size and the evaporative losses of this volatile metal from the control.

As indicated in Table 2 and Figures 1 and 2, the general pattern of metal removal by water hyacinths does not appear to be affected by the presence of other metals. At no time throughout the experimental run was there a significant difference between concentrations of individual and combined metals for any metal tested.

The absolute amount of metal removed and percent recovery from the plant tissue is shown in Table 3. For lead and cadmium, it is apparent that most of the metal remains in the plant tissue. The results for mercury were extremely erratic; as can be seen, the mean percent recovery for this metal is very low. Since mercury is highly volatile, it may be that much of this metal was lost during the digestion process.

Distribution of lead and cadmium within the plant tissue is shown in Table 4. (Mercury was excluded from this analysis due to the highly variable results with this metal.) The levels of metals present in the plant tissue indicate that these plants accumulate concentrations of heavy metals which are several hundred times greater than those present in solution. Note that there is a trend for cadmium to be more concentrated in the roots when this metal occurs in solution in combination with lead and mercury, and there is a slight reversal of this trend for lead. Miller et al. (14) found the opposite of these trends to occur in corn plants, where the presence of soil lead enhances cadmium uptake and translocation. Species variability and the differential behavior of metallic ions in soil and solution could account for these differences.

From our experiments, the effects of water hyacinths on heavy metals present in the water are apparent. But are the plants themselves affected by the metals? At the termination of the four-day experimental period, the water hyacinths showed no obvious signs of heavy metal poisoning; however, the long-term toxic effects of heavy metals on water hyacinths remain to be determined. Most crop species tested have exhibited decreased growth and yield and/or chlorosis in response to prolonged heavy metal treatment (8, 18). However, the susceptibility of different crop species to heavy metal poisoning varies widely. Turner (22), for example, reports that tomatoes are much more sensitive to low levels of cadmium

Table 3. Recovery of Heavy Metals by Water Hyacinths From Polluted Waters (96 Hour Exposure Period)

	Average Amount Removed				mg Average Amount in Plants		% Recovery From Plant Tissue	
	Alone		Combined		Alone	Combined	Alone	Combined
	mg	%	mg	%				
Cd	12.8	92	13.3	82	10.5	10.4	82	78
Pb	134	96	138	90	97	117	72	85
Hg	15.8	100*	8.3	96	0.53	0.33	8	7

*48 hours

Table 4. Distribution of Cadmium and Lead in Dry Plant Tissue

	Whole Plants ppm	Roots ppm	Tops ppm	% In Tops	% In Roots
Cd Alone	300	667	60	8	92
Cd In combination with Pb & Hg	300	840	15	2	98
Pb Alone	3200	6500	92	2	98
Pb In combination with Cd & Hg	3330	9280	200	4	96

than are other vegetables. Carlson, et al. (2) found heavy metals to cause a greater growth inhibition in corn than in sunflower. Irrespective of their tolerance or sensitivity to the long-term effects of heavy metals, water hyacinths demonstrated the ability to reduce initially high concentrations of heavy metals within a matter of hours, before any phytotoxic symptoms would be likely to appear.

Crop species vary widely in their ability to concentrate heavy metals. Page, et al. (16) found that for a given concentration of cadmium in solution, the leaf cadmium concentration of different crop species could vary as much as 3000 percent. Considering their relatively short-term exposure periods to heavy metals, water hyacinths appear to compare favorably with crop species in their ability to accumulate heavy metals. Unfortunately, our results are not directly comparable with those of most other workers, since the heavy metal accumulation of most crop species has been measured in soil or in nutrient solutions containing high levels of nitrogen and phosphorus which may alter the plants' uptake patterns (11, 24). Our experiments were run in river water for the express purpose of simulating conditions in which an actual hazardous materials spill might occur.

Conclusions

Water hyacinths have demonstrated the ability to concentrate lead, cadmium and mercury to levels several hundred times those present in the aquatic medium. Based on the observed rate of uptake, we estimate that one hectare of water hyacinths (which equals approximately 16.4 tons dry weight) could potentially purify two million liters of water polluted with 1 ppm cadmium, 2.2 million liters polluted with 10 ppm lead, and 3.4 million liters of water polluted with 1 ppm mercury in 96 hours. A one-acre lagoon with an average depth of three feet could adequately handle this load.

Since these plants grow and photosynthesize very rapidly and since they are adapted to an aquatic mode of existence, water hyacinths could prove very useful in assessing and rectifying heavy metal pollution. The plants could be useful to environmentalists in two primary ways. First, water hyacinths might actually be used as agents to aid in the removal of spilled heavy metals. Second, since a rapid uptake of metallic ions occurs even during the first hour of exposure, water hyacinths could be used as indicator or monitor organisms to assess chronic pollution levels at factory out-flows, in high-risk areas, or even in drinking water, lakes and rivers.

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SECTION II
Part 3

BIO-ACCUMULATION AND DETECTION OF TRACE LEVELS OF CADMIUM IN AQUATIC SYSTEMS USING *Eichhornia Crassipes*

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Rebecca C. McDonald

Presented at:
The National Institute of Environmental Health Sciences
Workshop on
Higher Plant Systems as Monitors of Environmental Mutagens

Marineland, Florida
January 16-18, 1978

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ABSTRACT

The water hyacinth (Eichhornia crassipes) may be used as a sensitive biological indicator for continuously monitoring trace quantities of toxic heavy metals in aquatic systems. A river water system polluted with cadmium was simulated while other factors of temperature, day-night cycle, water quality, and light intensity remained constant. When the water hyacinth is maintained in river water containing 0.001 mg/l of cadmium chloride, the plant's root system will concentrate this element at an average rate of 0.9, 1.4, and 3.0 μg Cd/g root dry weight (D.W.) after 24, 48, and 72 hour exposure periods, respectively. At a higher cadmium concentration of 0.01 mg/l, cadmium was concentrated in the roots much faster to levels of 6.8, 13.6, and 39.1 μg/g root (D.W.) after 4, 8, and 24 hour exposure periods, respectively. At initial concentrations of 0.05 mg/l cadmium, the roots contained 29.5, 48.8, and 156 μg/g root (D.W.) following 4, 8, and 24 hour exposure periods, respectively. During these same time intervals, the water hyacinth sorbed 56.7, 153, and 281 μg/g root (D.W.) when the initial cadmium concentration was increased to 0.10 mg/l.

The water hyacinth tops can also assist in the monitoring process when cadmium contamination levels are 0.10 mg/l and greater. At this initial cadmium concentration, cadmium is translocated into the tops. After 8 hours, the tops averaged 1.1 μg/g top (D.W.). After 24 hours, this concentration was increased to 6.1 μg/g top (D.W.).

Introduction

Cadmium is a toxic, heavy metal which can present a serious threat to human health. Toxic effects from this heavy metal are well documented. Excess levels of cadmium can cause kidney and liver damage, pulmonary disease, and cancer in experimental animals (1-4). Chronic low levels of cadmium may contribute to hypertension, decreased growth, and alterations in blood cholesterol and trace element metabolism (5).

Heavy metals present a serious form of pollution in aquatic systems since they do not degrade as do most organics. Even trace quantities of toxic metals in water systems are serious potential health problems because of the ability of certain aquatic plants to concentrate heavy metals which are then consumed by fish that form a part of man's diet (6, 7).

Using biological indicators such as plants for monitoring both air and water pollution has been recognized and used to a limited extent over the years. Mosses and lichens strongly sorb metal ions from the air and water and are useful for detecting atmospheric and aquatic lead and other metal contamination (8-10). Leaves and twigs of woody plants have also been used to indicate atmospheric pollution (11).

One of the most promising candidates for biological indicators of trace levels of heavy metals in aquatic systems is the water hyacinth (Eichhornia crassipes). In static laboratory experiments, this plant demonstrated an amazing ability to sorb and concentrate cadmium, as well as other metals such as mercury, lead, and nickel (12, 13). Water hyacinths have been used successfully by NASA at the National Space Technology Laboratories to remove organics and heavy metals from its chemical waste prior to discharge (14). In the course of this system's evaluation, water hyacinths were found to contain detectable levels of heavy metals, especially in the roots, although these same heavy metals in the waters were below normal detection limits by atomic absorption-flame spectrometry. The study presented in this paper is an outgrowth of this observed phenomenon. Water hyacinths are used to develop a rapid, biological monitoring system for establishing cadmium pollution in the aquatic environment.

Procedure

For each different cadmium concentration, twelve glass aquariums were filled with 15 liters of river water. Nine of the aquariums were polluted with sufficient 1000 mg Cd/l standard solution to produce an approximate initial cadmium concentration of 0.1 mg/l for run #1, 0.05 mg/l for run #2, and 0.01 mg/l for run #3. Three aquariums were left unpolluted. Four groups of nine water hyacinths were thoroughly washed and placed in three of the polluted containers and one of the unpolluted containers. After four hours, three plants from each aquarium were removed for analysis, and the remainder of the plants were transferred to four fresh aquariums. This procedure was repeated again after eight hours. Each experiment was terminated after 24 hours.

For run #4, twelve glass aquariums were filled with 30 liters of river water. Nine containers were polluted to an approximate cadmium concentration of 0.001 mg/l. The experiment was conducted in the same manner as outlined above, except the plants were removed and transferred at 24, 48, and 72 hour intervals.

During each experiment, the plants were maintained with growth lights supplying approximately 500 FC to the plants during a 16 hour photoperiod and 8 hour nyctoperiod. The air temperature was 23° ± 5°C. The initial unpolluted river water samples were analyzed according to Standard Methods (15) and found to contain the following average concentration: 0.25 mg/l total Kjeldahl nitrogen;<0.13 mg/l phosphorus, 52 mg/l dissolved solids; 9 mg/l total organic carbon; pH 6.9. The initial cadmium concentration of 0.1 μg/l was determined with an IL 555 flameless atomizer and an IL 351 AA/AE spectrophotometer.

The roots and tops of the plants to be analyzed were separated, washed, dried at 60°C to a constant weight, ground and homogenized in a Waring blender. 0.500 grams of each plant sample was weighed and transferred to a 75 ml volumertric digestion tube. The plant samples were charred at 400°C with 10 ml conc. H_2SO_4 for two minutes and then digested for 20 minutes at 400°C with an additional 10 ml conc. HNO_3. The samples were allowed to cool, and then 2 ml 30% H_2O_2 was added. The tubes were again heated to 400°C for 10 minutes. Following the digestion process, the samples were diluted to volume with deionized, distilled water, and the cadmium content determined by flame spectrometry using an IL 351 AA/AE spectrophotometer. A reagent blank was also digested in the same manner, and any cadmium introduced into the plant samples from the reagents was subtracted from cadmium concentrations in the plants.

Discussion

The experiments for assessing the potential of using water hyacinths as biological indicators for estimating the level of cadmium pollution in aquatic systems were designed to simulate real conditions. A fresh volume of polluted river water was supplied to the plants at regular intervals. The cadmium concentrations were varied while other factors of temperature, day-night cycle, water quality, and light intensity remained constant. The data in Tables 1 and 2 are the results of this series of four experiments.

The first experiment conducted with 0.1 mg Cd/l was a relatively high cadmium concentration for potable or recreational water systems. The water hyacinths were found to average 56.7 μg Cd/g root (D.W.) after only 4 hours of exposure. The concentration in the roots continued to increase to an average of 153 μg/g root (D.W.) after 8 hours and 281 μg/g root (D.W.) after 24 hours. At this high cadmium level, cadmium was first detected in the leaves after only 8 hours of exposure.

The concentration in the second experiment was decreased to 0.05 mg Cd/l. The quantity of cadmium sorbed per gram dry root weight over the same time intervals was almost exactly half of the concentrations found at the 0.1 mg Cd/l level. The cadmium was concentrated to average levels of 29.5, 48.8, and 156 μg/g root (D.W.) after 4, 8, and 24 hours, respectively. No cadmium was detected in the leaves of these plants, nor was any cadmium detected in the leaves of any of the later experiments.

Table 1. Average Cadmium Uptake Within 24 Hours

Initial Cd Conc in River Water, mg/l	µg Cd/g Dry Weight					
	After 4 Hours		After 8 Hours		After 24 Hours	
	Tops	Roots	Tops	Roots	Tops	Roots
0 1	< 0.15	56 7±16 9	1.1±0 9	153±14 0	6 1±1 9	281±9 0
0 05	< 0.15	29.5±16 1	< 0 15	48 8±17.1	< 0 15	156±4 2
0 01	< 0.15	6 8±1.9	< 0.15	13 6±1 9	< 0 15	39 1±5 6
0.001	—	—	—	—	< 0 15	0.9±0.3

Note Tops and Roots of Plant Controls Contained < 0 15 µg Cd/g dry weight after 24 and 72 Hours

Table 2. Average Cadmium Uptake in 0.001 mg Cd/l

Exposure Time, hours	µg Cd/g Dry Weight	
	Tops	Roots
24	< 0 15	0 9±0 3
48	< 0 15	1 4±0 2
72	< 0 15	3 0±0.6

This same trend was also observed in the third experiment when the cadmium concentration was decreased to 0.01 mg/1. The cadmium concentrations in the roots averaged 6.8, 13.6, and 39.1 μg/g root (D.W.) after 4, 8, and 24 hours, respectively.

The exposure time in the fourth experiment was increased in order for the water hyacinths to accumulate sufficient cadmium at the 0.001 mg/l level to be detected by the procedure outlined above. This very low concentration of cadmium in the water had to be determined by atomic absorption using a flameless atomizer. The cadmium in the water could not be detected without concentrating it if the normal method of atomic absorption-flame spectrometry had been used. The first root samples analyzed after 24 hours of exposure contained an average of 0.9 μg/g root (D.W.). This concentration was far less than the expected value of one-tenth of the 39.1 μg/g root (D.W.) found after 24 hours of exposure in 0.01 mg Cd/1. However, the 24, 48, and 72 hours samples demonstrated a fairly consistent linear relationship of μg Cd/g root (D.W.).

Conclusions

The data from Table 1 was plotted in Figure 1. This figure demonstrates how a family of curves can be used to estimate low levels of cadmium in river water utilizing water hyacinths. At very low levels of cadmium this graph must be expanded as in Figure 2.

The leaves were found to be useful for estimating high cadmium concentrations. At the highest level of cadmium in this study, the leaves contained detectable levels of cadmium even after 8 hours of exposure.

The sorption rates of cadmium as well as other toxic heavy metals will vary from one system to another depending on environmental factors. However, the data necessary to obtain a family of curves such as Figure 1 for a particular aquatic system can be obtained without much difficulty.

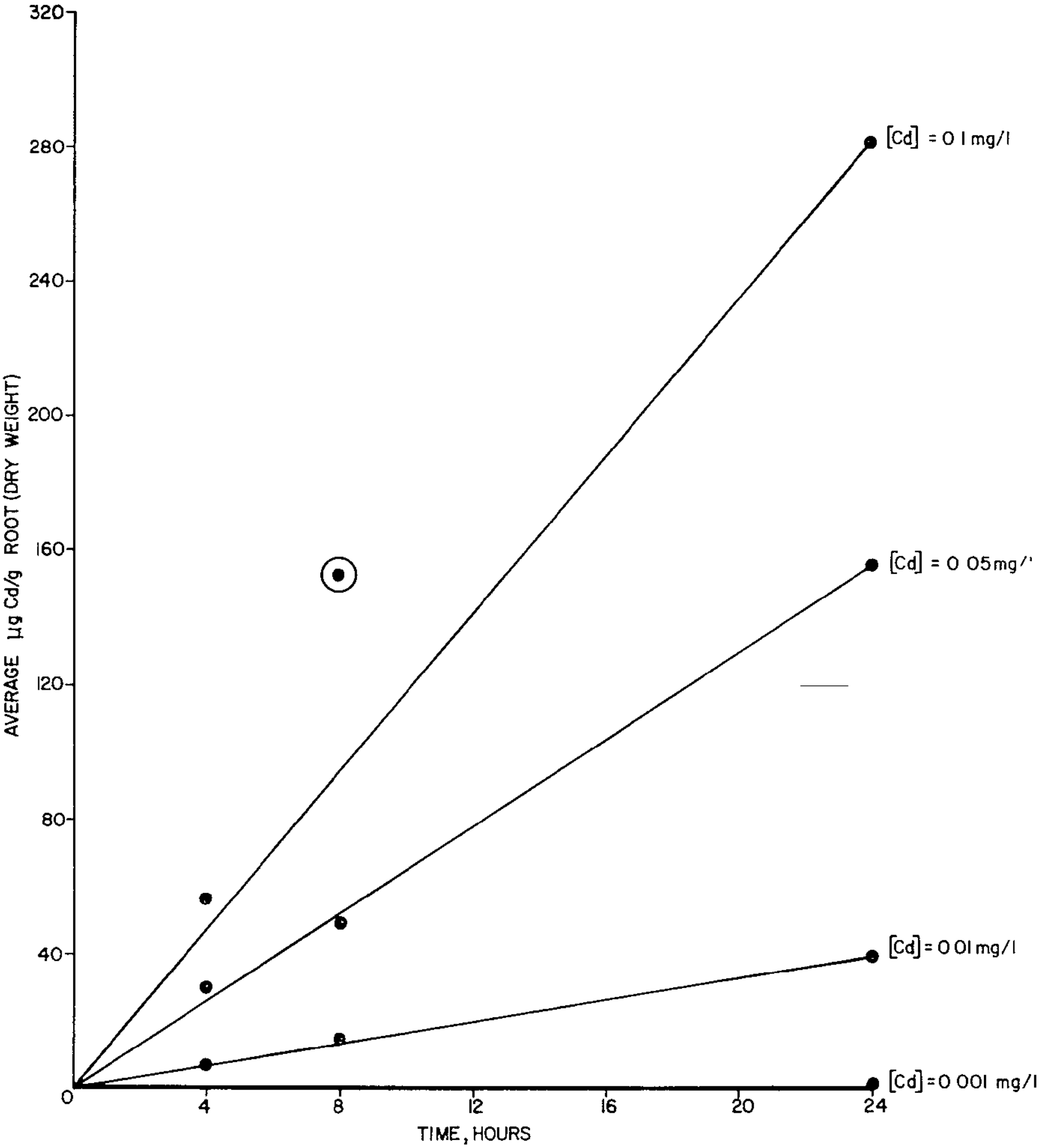

Figure 1. Average amount of Cd sorbed by the roots under different Cd concentrations and exposure times. (The circled point representing the amount of Cd sorbed after 8 hrs when exposed to 0.1 mg/1 Cd was not used in the least squares analysis. The standard deviation for this point varied by 35%.)

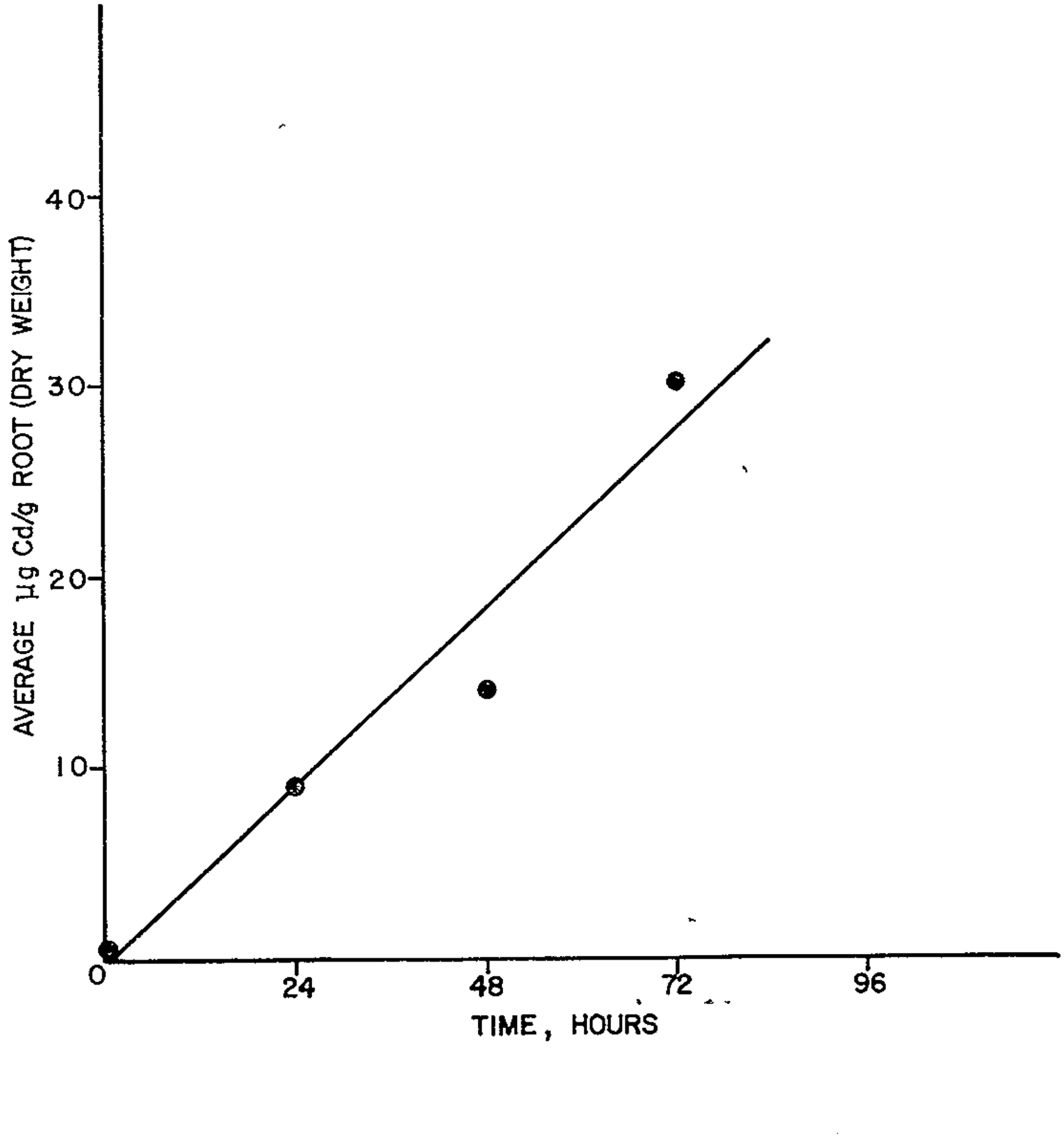

Figure 2. Average amount of Cd sorbed per hour when exposed to a Cd concentration of 0.001 mg/l in river water

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SECTION II
Part 4

WATER HYACINTHS FOR REMOVAL OF PHENOLS --FROM POLLUTED WATERS

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ABSTRACT

Removal of phenol by water hyacinths, (Eichhornia crassipes) (Mart.) Solms, in static water was investigated. A quantity of 2.75 g dry weight of this aquatic plant demonstrated the ability to absorb 100 mg of phenol per 72 h from distilled water, river water and bayou water. One hectare of water hyacinth plants is potentially capable of removing 160 kg of phenol per 72 h from water polluted with this chemical.

Introduction

Water hyacinths, (Eichhornia crassipes) (Mart.) Solms, grow profusely throughout the subtropical and tropical regions of the world and have been the subject of many scientific investigations. Most of the earlier studies of this vascular aquatic plant were directed toward eradication since the rapid growth rate of mat-forming water hyacinths obstructs navigable waterways (Penfound and Earle, 1948; Gay, 1960), prevents proper drainage of land (Penfound and Earle, 1948), interferes with aquatic recreation (Penfound and Earle, 1948), restricts the supply of sunlight to submerged plant and fish life (Unni, 1971), and increases the evaporation rate of water bodies by 3.2 to 3.7 times through evapotranspiration through the leaves (Holm et al., 1969; Brezay et al., 1973).

The water hyacinth propagates both by seed germination and by vegetative means whereby mature plants produce rosettes of leaves and fibrous roots at each node of the growing stem (Das, 1969). A single plant can produce approximately 65,000 offspring during a single season (Rogers and Davis, 1972). Due to this phenomenal growth rate, 1 acre (0.40 ha) of plants can conceivably produce approximately 240 kg of dry weight per day in subtropical climates which far exceeds the yield of the most productive agricultural crops. Consequently, the water hyacinth is widely recognized as one of the most serious aquatic weed problems known to exist in warm climates.

Ironically, the water hyacinth is also one of the most promising candidates for solving many serious problems in areas of food supply, energy requirements and water pollution control. Boyd and others have shown that vascular aquatic plants such as the water hyacinth are a possible food source for animals and humans in studies examining the amino acid, protein, caloric, and mineral nutrient content of these plants (Gonzales et al., 1968; Taylor and Robbins, 1968; Boyd, 1970 a and c; Boyd and Vickers, 1971; Gossett and Norris, 1971). The conversion of plant material to usable products such as compost and methane gas through anaerobic fermentation is a promising approach to the problems of depleted energy sources (Piree, 1060; Aboul-El-Fadl et al., 1968). Recently, the ability of vascular aquatic plants to remove organic chemicals, heavy metals, and pesticides from polluted waters has been demonstrated (DeMarte and Hartman, 1971; Sutton and Blackburn, 1971; Sutton et al., 1971; Reay, 1972; Bingham, 1973; Haghiri, 1973; Wolverton and Harrison, 1973). Seidel conducted an extensive series of experiments using Scirpus lacustris L. for elimination of 10--100 ppm phenol from water (Seidel, 1963, 1965, 1966; Seidel et al., 1967a). The inflow and effluent of a test system installed in Urach was investigated and the following parameters were markedly reduced after being exposed to Scirpus lacustris: biological oxygen demand; phosphate (PO_4); ammonia (NH_3); organic nitrogen; total nitrogen; detergent content; and bacterial content (Seidel, 1966). The use of water hyacinths and other aquatic plants for removal of chemicals from photographic and chemical laboratory waste waters at the NASA National Space Technology Laboratories (NSTL), Bay St. Louis, Mississippi, is presently being investigated as part of a pollution abatement program at this facility.

The purpose of this study is to examine the ability of water hyacinths to remove phenol from natural waters. Phenol and phenolic derivatives were chosen for this investigation since they are common organic pollutants found in domestic and industrial waste water and in drinking water supplies. In addition, chlorophenols, which have an extremely objectionable odor and taste, are produced by chlorination of drinking water contaminated with phenolic compounds (Standard Methods for the Examination of Water and Wastewater, 1971, p. 501).

Materials and Methods

Water hyacinths were collected in the spring and summer of 1974 from a bayou adjacent to Louisiana Highway 190, approximately 400 m north of U. S. 90 intersection in St. Tammany Parish, Louisiana. Lush, green adult plants were selected, some of which were in the flowering stage and contained offshoots produced by vegetative reproduction. These plants were transferred in plastic bags both to a greenhouse, where they were maintained between 26°C and 32°C, and to a cooler location where they could be maintained between 24°C and 25°C. All plants were kept in metal troughs containing tap water and a commercial OrthoGro* liquid plant food containing 480 ppm total nitrogen, 240 ppm available phosphoric acid (P_2O_5), 250 ppm soluble potash (K_2O), 20 ppm iron and 4 ppm zinc. The tap water contained 19 ppm silica, 0.02 ppm iron, 0.10 ppm manganese, 3.7 ppm calcium, 0.5 ppm magnesium, 91 ppm sodium, 1.1 ppm potassium, 194 ppm bicarbonate, 11 ppm carbonate, 17 ppm sulfate, 12 ppm chloride, 0.3 ppm fluoride, 0.6 ppm nitrate and 252 ppm dissolved solids.

Studies to determine the capacity of water hyacinths to remove phenol were conducted with 4-week old and older plants. Individual plants averaging 2.75 g dry weight were exposed to phenol concentrations by placing them either in distilled water containing liquid plant food, water from the East Pearl River at NSTL, or water from the sampling site, contained in 1-l glass beakers. The beakers were painted black in order to inhibit algal growth. Phenol (Mallinckrodt, lot AEK, analytical regent grade) in concentrations of 25 ppm, 50 ppm, and 100 ppm was used. Phenol concentrations and bacteria contamination levels were determined immediately after initiation of the experiment and after 24, 48, and 72 h of exposure. Three plant controls free of phenol and three phenol controls without plants were established with each set of experiments.

Bacterial counts were determined for all testing water systems to investigate bacterial influence, if any, on phenol assimilation by the water hyacinth since micro-organisms have been identified that utilize phenol by the process of oxidation (Committee on Bacteriological Technique, Society of American Biologists, 1957). The culture media used for determining bacterial counts in the experimental solution in East Pearl River water, bayou water and distilled water was Difco Nutrient Agar. The plates were incubated at 25°C for 24 h and colonies counted and reported as bacteria per milliliter (BPM).

Two studies were performed in an effort to recover phenol removed from the water test containers by water hyacinths. The large, fibrous root system was extracted separately from the leaves and floaters in order to determine if phenol was transported upward in the plant to the leaves. The first investigation was an extraction of water hyacinth tissue using A.C.S. grade chloroform. The plants were removed from the beakers after 25 ppm, 50 ppm and 100 ppm concentrations of phenol had been removed from the water. The plants were rinsed with distilled water and pulverized for 60 sec in 35--50 ml of chloroform using a Sorvall Omni-Mixer*. The plant material was then allowed to remain in contact with

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* Registered trademark

chloroform for a minimum of 48 h. The chloroform layer was analyzed for phenol by gas chromatography. One chloroform extraction of phenol from a standard aqueous solution of 100 ppm has a recovery efficiency of 78%.

The second recovery experiment was designed to determine whether water hyacinths could absorb phenol from water solutions and release it into the atmosphere through the process of evapotranspiration as suggested by Seidel (1966). Water hyacinths were placed in 1-l beakers containing 100--150 ppm phenol in distilled water and placed inside a 65 cm x 85 cm x 87 cm closed chamber with two 34 cm x 48 cm transparent windows. The atmosphere within the chamber was exhausted after 24, 48 and 72 h periods through a sodium hydroxide solution which would trap phenol as sodium phenoxide. Following acidification of the sodium hydroxide solution, which reconverts sodium phenoxide to phenol, analysis for phenol was performed.

All phenol analyses were performed with a Model 2100 Varian Aerograph Gas Chromatograph equipped with a hydrogen flame ionization detector. A 5-ft by 1/8-inch stainless steel column containing Chromosorb W, 70/80 mesh, coated with 5% free fatty acid, and conditioned at 180°C for 24 h was employed. A retention time of 3.88 min was recorded for phenol with the inlet temperature at 160°C, the detector temperature at 180°C and chart speed at 20 cm/h. Gas flow rates in cm^3/min were nitrogen 60, hydrogen 35, and air 235. Water-phenol injection sample sizes were 5μl. The concentration of the phenol was determined by comparing the peak height of the injection sample to freshly prepared standards containing 25 ppm, 50 ppm, and 100 ppm of phenol. The detection limit was 0.1 ppm of phenol.

Results and Disscussion

The ability of water hyacinths to remove phenol from the three water systems employed in this investigation is presented graphically in Fig. 1 (25 ppm), Fig. 2 (50 ppm), and Fig. 3 (100 ppm) respectively. The assimilation rates are depicted as a percentage of initial phenol concentration remaining as a function of time for the phenol controls and phenol-exposed plant systems. The exact experimentally determined values used for these plots are listed in Table I. The rate of removal of phenol from the distilled water-nutrient solution and the river water is very similar. A slightly slower rate of phenol assimilation was observed when bayou water from the plant collection site was used. It is possible to offer an explanation for the different removal rates if phenol removal can be compared in a general sense to removal of mineral nutrients by vascular aquatic plants. It is known that mineral uptake rates per unit of dry matter are greater for plants in a rapid growth phase (Boyd, 1970). An extensive comparative study of the growth rate of water hyacinths in water culture by Chadwick and Obeid (1966) reports an optimum growth rate at pH 6.9--7.0 with a decrease in plant production at either higher or lower pH values. The pH value measured for the three test-water systems ranged from 7.6 ± 0.2 (distilled water plus nutrients) to 6.3 ± 0.2 (river water) and to 6.1 ± 0.2 (bayou water). It is observed that the distilled water and river water systems differ in pH from the optimum growth value by equivalent amounts, and the similar rates of phenol uptake may be explained by this difference in pH. The slowest removal occurred with the bayou water, and the mean pH value (6.1) is that deviating most from the optimum level. In addition, Boyd (1970) observed that aquatic plants absorb mineral nutrients more slowly as the plants age. The age of the water hyacinth used in this phenol removal study was sufficiently variable to contribute somewhat to the observed variations in phenol removal rates both within each system and between the three separate systems.

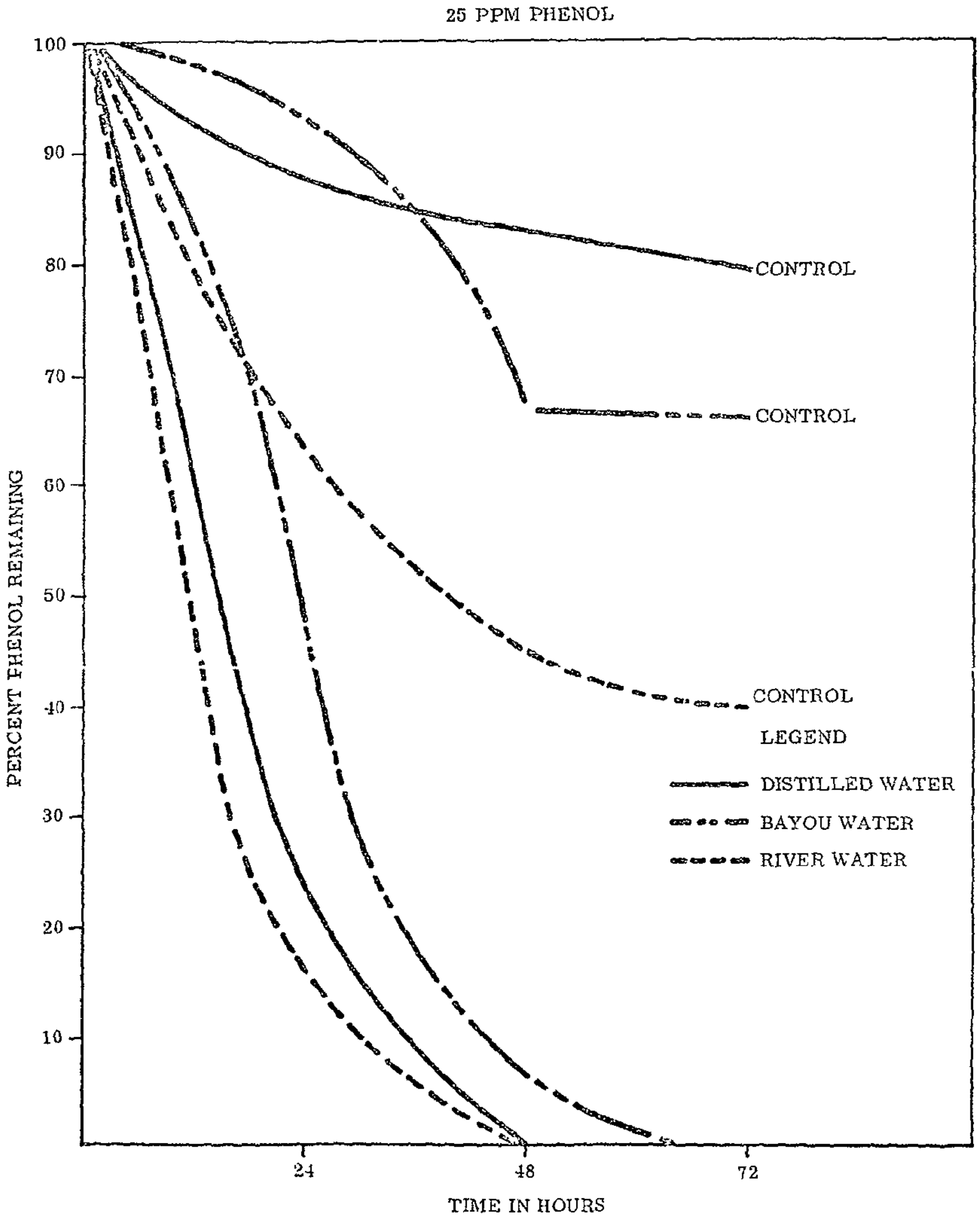

Figure 1. Graphic representation of removal rate of 25 ppm phenol from phenol controls (no plants) and phenol exposed plants

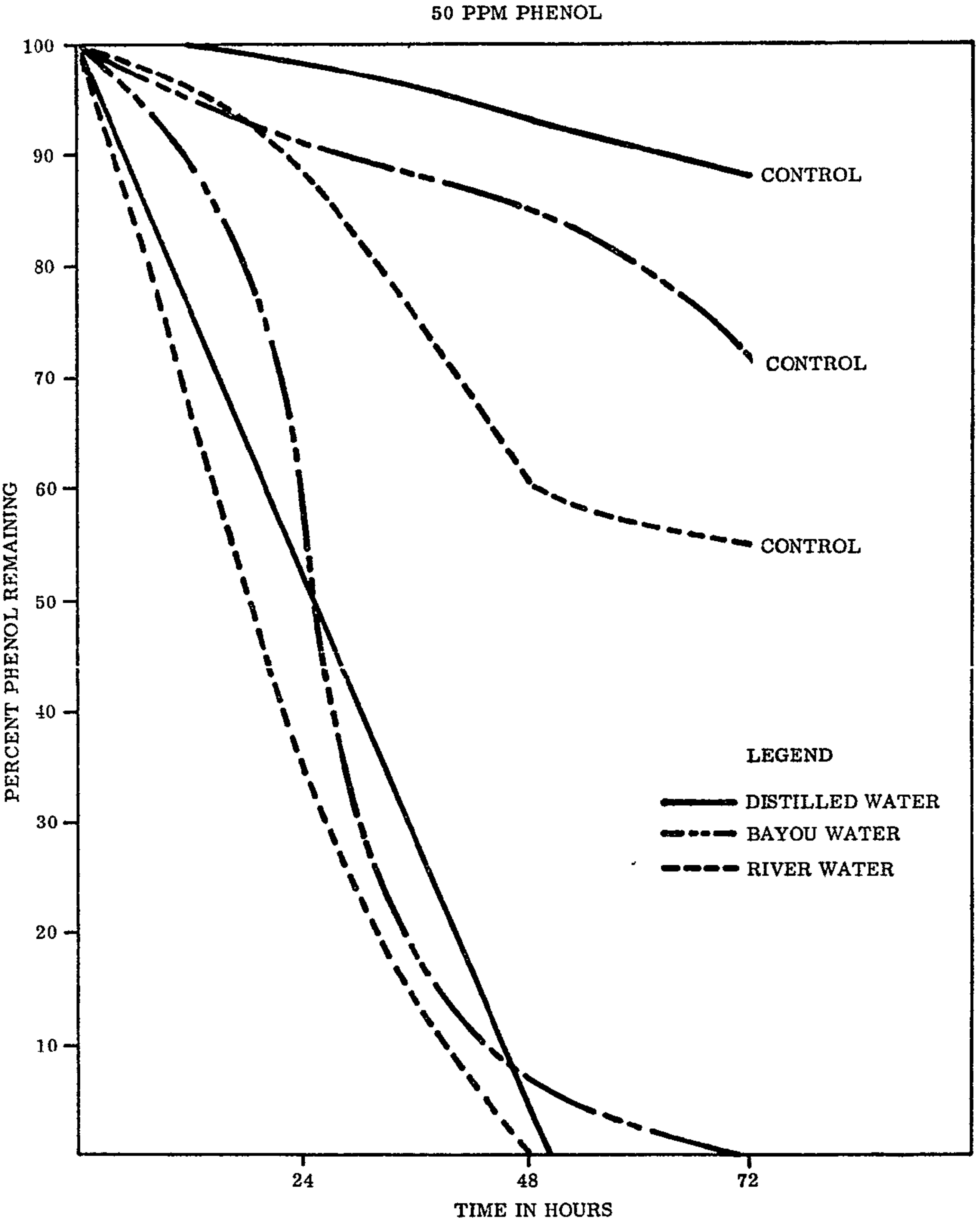

Figure 2. Graphic representation of removal rate of 50 ppm phenol from phenol controls (no plants) and phenol exposed plants

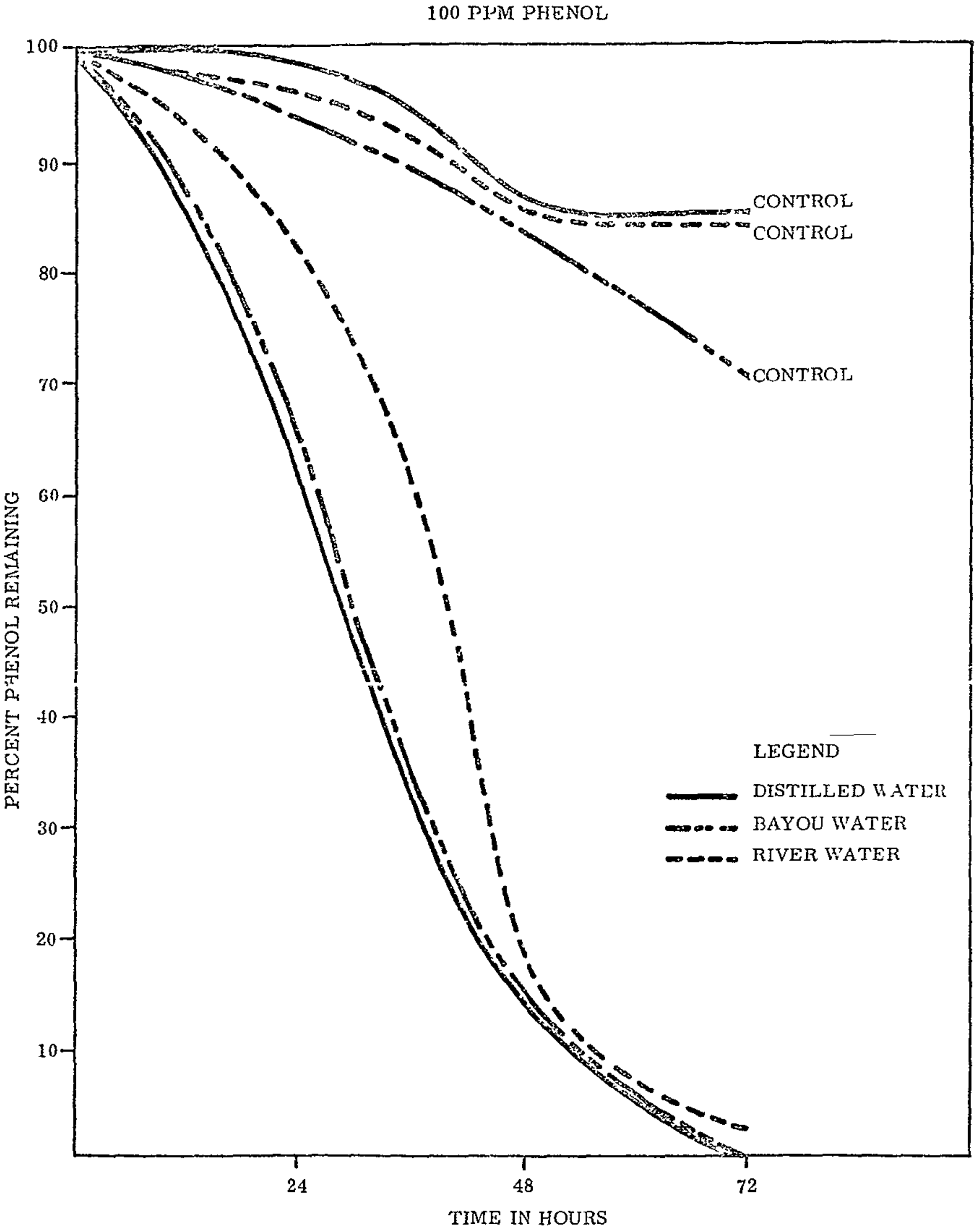

Figure 3. Graphic representation of removal rate of 100 ppm phenol from phenol controls (no plants) and phenol exposed plants

Table I. Percentage of Phenol Concentrations Remaining at Indicated Sampling Times and Confidence Limits Based on 100% at Time Zero. All Concentrations Were Measured by Gas Chromatographic Analysis as Described in Materials and Methods

Water System	Phenol Addition	24 Hours		48 Hours		72 Hours	
		Specimen	Control	Specimen	Control	Specimen*	Control
Distilled	25 ppm	23.4 ± 1.5	86.6 ± 6.0	0.4 ± 0.1	86.6 ± 6.0	0.4 ± 0.1	79.8 ± 6.0
Distilled	50 ppm	54.5 ± 1.0	98.9 ± 2.0	2.1 ± 0.1	93.9 ± 2.0	0.4 ± 0.1	88.2 ± 2.0
Distilled	100 ppm	60.0 ± 0.5	99.3 ± 0.5	13.3 ± 0.5	86.7 ± 0.5	0.5 ± 0.1	85.0 ± 0.5
River	25 ppm	16.4 ± 1.5	62.3 ± 6.0	0.4 ± 0.1	44.5 ± 3.5	0.4 ± 0.1	40.0 ± 3.5
River	50 ppm	34.9 ± 1.0	89.0 ± 2.0	0.4 ± 0.1	61.0 ± 2.0	0.4 ± 0.1	58.0 ± 2.0
River	100 ppm	82.4 ± 0.5	96.6 ± 0.5	16.1 ± 0.5	84.3 ± 0.5	2.8 ± 0.2	84.8 ± 0.5
Bayou	25 ppm	45.9 ± 1.5	94.3 ± 6.0	5.87 ± 0.3	56.7 ± 3.5	0.4 ± 0.1	55.7 ± 3.5
Bayou	50 ppm	58.8 ± 1.0	91.7 ± 2.0	2.05 ± 0.2	84.5 ± 2.0	0.4 ± 0.1	71.3 ± 2.0
Bayou	100 ppm	61.4 ± 0.5	93.3 ± 0.5	14.1 ± 0.5	83.1 ± 0.5	0.4 ± 0.1	70.2 ± 0.5

*Phenol detection limits 0.4 ppm.

All concentration determinations for a particular series of experiments were made in duplicate or triplicate. A faster rate of removal was observed for cases where the size of the plant was larger than average. Each 72-h removal sequence was repeated several times in order to confirm the reproducibility of the data. The variability in results is expressed in Table I for the 24-h sampling period and was ± 1.5 ppm (± 6.0%) for 25 ppm phenol addition, ± 1.0 ppm (± 2.0%) for 50 ppm phenol addition, and ± 0.5 ppm (± 0.5%) for the 100 ppm phenol addition. As expected, the percent error decreased with increased amount of phenol additive. The percent error also decreased proportionally for the 48-h and 72-h sampling times since the amount of phenol remaining at the specified times was very reproducible. It was noted that if one of the plants died during the course of an experiment, no further decrease in phenol concentration resulted. The health of the control plants was compared to that of those exposed to phenol in any given set of experiments; no indication was evident that phenol toxity levels had been exceeded.

The bacterial analyses of water samples taken from the plant controls, phenol controls, water controls, and plant-phenol beakers showed insignificant variation in bacterial counts. Average counts for all experiments expressed as bacteria per milliliter (BPM) are shown in Table II for the 24-h sampling time. It is apparent from this table that bacterial growth was inhibited by increasing concentrations of phenol. This growth pattern was expected since phenol is a commonly used bactericide. The high numbers obtained from bacterial counts were also predictable since considerable microbial activity occurs beneath mats of water hyacinths (Boyd, 1970). The absence of dramatic increases in bacterial numbers suggests that the particular bacteria present in the system did not utilize the phenol as an energy source. However, this type of microbial activity was probably present to a small extent since there was a decrease in phenol concentrations in the phenol controls (no plants). This possibility is further confirmed since phenol disappearance from the control solutions was greater in the river and bayou water where larger BPM values are found.

To date, attempts to recover phenol from the water hyacinth following assimilation have been unsuccessful. Gas chromatographic analyses on the chloroform extracts of the roots and the leaves/floaters of the water hyacinth showed 8--10 peaks and/or shoulders after a 20-min elution period. The phenol peak which appears at 3.88 min was not present even in trace quantities in the chromatograms of any of the extracts of either the plant controls or the plants exposed to phenol. In addition, no difference was observed between components eluted from the root section and the leaf-floater section.

Gas chromatograms of the acidified sodium hydroxide solution obtained from the evapotranspiration experiment also failed to exhibit peaks corresponding to measurable amounts of phenol. In fact, no peaks were observed other than that corresponding to water. Seidel (1966) noted that the phenol absorbed by Scirpus lacustris is not totally metabolized; a portion of the phenol was shown to be lost via evapotranspiration. Experiments using ^{14}C-labeled phenol suggested that Scirpus lacustris uses phenol to form increased concentrations of amino acids and peptides on the plant tissue (Seidel et al., 1967b).

The failure of these recovery experiments indicates that phenol is removed by the water hyacinth and primarily metabolized to other compounds. Peroxidases and phenol oxidases present in both plants and animals could serve as catalyzing agents for this process (Manual of Microbiological Methods, 1957). Translocation studies similar to those performed by Seidel et al. (1967b) are currently in progress using ^{14}C-labeled phenol in order to determine

Table II. Average Bacterial Counts Expressed as Bacteria Per Milliliter (BPM) for 24-h Sampling Time (+ 0.5 x 10 BPM)

Test System	Additive	Bacterial Counts (BPM)	
		Specimen	Control
Distilled Water	25 ppm phenol/nutrients	1.19×10^4	0
Distilled Water	50 ppm phenol/nutrients	1.70×10^3	0
Distilled Water	100 ppm phenol/nutrients	4.50×10^2	0
Plant Control in Distilled Water	Nutrients	4.70×10^3	
Distilled Water Control	Nutrients	1.90×10^3	
River Water	25 ppm phenol	9.65×10^4	7.25×10^4
River Water	50 ppm phenol	1.68×10^4	1.05×10^4
River Water	100 ppm phenol	7.50×10^3	1.00×10^3
Plant Control in River Water	None	2.78×10^5	
River Water Control	None	3.00×10^5	
Bayou Water	25 ppm phenol	2.00×10^4	1.55×10^4
Bayou Water	50 ppm phenol	2.00×10^3	3.00×10^3
Bayou Water	100 ppm phenol	5.70×10^2	5.00×10^2
Plant Control in Bayou Water	None	2.40×10^5	
Bayou Water Control	None	4.50×10^5	

the identity and final location in the water hyacinth of metabolites produced by phenol assimilation.

The water hyacinth effectively removed 36 mg of phenol from distilled water, river water, and bayou water systems per g dry weight of plant material in 72 h . Since 1 ha contains approximately 1.62 x 10^6 plants (Boyd and Vickers, 1971) and the average dry weight per plant was determined to be 2.75 g, 1 ha of water hyacinths could conceivably remove 160 kg of phenol in a 72-h period.

A water-filtering lagoon system is presently under construction at NASA/NSTL that will use water hyacinths and other species of vascular aquatic plants for pollution abatement purposes on the site. The water hyacinth is particularly well-suited for this project since it is a floating aquatic plant and equipment for removal of the plant for use as food or energy supplies has already been constructed (Rogers and Davis, 1972). It is feasible to start mats of hyacinths in the lagoons at different times so that plants in a rapid growth phase would be present throughout the growing season.

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SECTION III

WATER HYACINTH PRODUCTS

SECTION III
Part 1

NUTRITIONAL COMPOSITION OF WATER HYACINTHS GROWN ON DOMESTIC SEWAGE

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ABSTRACT

A nutrient analysis of water hyacinths grown in sewage wastewaters was conducted. Crude protein averaged 32.9% dry weight in the leaves, where it was most concentrated. The amino acid content of water hyacinth leaves was found to compare favorably with that of soybean and cottonseed meal. The vitamin and mineral content of dried water hyacinths met or exceeded the FAO recommended daily allowance, in many cases. It is concluded that in favorable climate zones, water hyacinths grown in enriched mediums, such as sewage lagoons, could potentially serve as a substantial dietary supplement or nutrient source.

Introduction

The water hyacinth, (Eichhornia crassipes) (Mart.) Solms, has perhaps been the subject of more intensive study than any other aquatic plant in recent years. A native of South America, this floating aquatic species has adapted exceedingly well to almost every area into which it has been introduced. In the southern United States, it is the number one aquatic plant pest species. Due to its vegetative reproduction and extremely high growth rate, water hyacinths spread rapidly, clogging drainage ditches, shading out other aquatic vegetation and interfering with shipping and recreation (11,14). Much effort and many dollars have been devoted to the control of this prolific weed (4).

In the last several years, many investigators have directed their research endeavors to the utilization of the water hyacinth. Several scientists (6,12,15,16,23) have considered the water hyacinth as a potential biological agent for treating sewage wastewaters and feedlot operations. The water hyacinth is particularly well-suited for this purpose, since it is extremely productive (19,23) and feeds directly from the water via its extensive root system. Wolverton and McDonald (23) reported growth rates as high as 17.5 metric tons of wet biomass per hectare per day (approximately 0.88 metric tons dry matter per hectare per day, based on an estimated 5% solids per wet weight) when water hyacinths are grown in domestic sewage lagoons during the warm summer months.

In order to maximize the efficiency of nutrient removal by water hyacinths, the plants should be periodically harvested as they become saturated with excess nutrients. Ideally, the harvested plant material should be utilized, in order to defray the costs of removal. Various investigators have proposed using harvested water hyacinths as a food supplement both for cattle (2) and humans (20), as a soil additive (9,13,21), as a source of paper and fiber (1), and as an energy source (22,24). The use of water hyacinths as a food source appears promising. Gosset and Norris (8) have demonstrated a definite relationship between nutrient availability and the nitrogen and phosphorus content of water hyacinths. Haller and Sutton (10) analyzed water hyacinths grown in nutrient solutions with different phosphorus concentrations and found that the phosphorus content in the plants increased as the phosphorus content of the water increased up to a maximum level of 40 ppm phosphorus in the water. Since it has been shown that the nutrient composition of water hyacinths is generally proportional to the nutrient content of the medium in which the plants are grown, sewage-grown water hyacinths should be particularly high in protein and minerals.

Since 1975, NASA (23) has been experimenting with the use of water hyacinths as a biological treatment method for domestic wastewaters. In this paper, we present the result of nutrient analyses of water hyacinths grown on four experimental sewage lagoons in southern Mississippi. Most workers investigating nutrient contents of the water hyacinth have analyzed whole plant tissue. We have examined the relative nutrient contributions of the roots, stems, and leaves. Analyses for vitamins and minerals were also performed.

Materials and Methods

The four sewage lagoons into which we have introduced water hyacinths are located in southern Mississippi. Two are located on NASA's National Space Technology Laboratories (NSTL), Bay St. Louis, and the other two serve small communities in the area. Nutrient loading rates are presented in Table 1.

Plants were collected in late summer on the same date from four domestic sewage lagoons that had supported the growth of water hyacinths since spring. The plants were thoroughly washed with tap water. The leaves, stems and roots were separated from half the plants, the remainder of the plants were left intact. All samples were dried in an oven at 100°C for 24 hours and ground to an even consistency in a blender. The plant powder was analyzed for vitamins, minerals, amino acids, ash, fiber, and fat.

Analyses for vitamins, sulfur, total Kjeldahl nitrogen, total phosphorus, crude fiber, ether extract (fat), pepsin digestibility, and xanthophyll were done by Research 900, St. Louis, Missouri. The crude protein was calculated as Kjeldahl nitrogen x 6.25.

The mineral analyses were determined by atomic absorption/flame emission with an IL 253 spectrophotometer following digestion of 0.50 g of plant material in 10 ml concentrated nitric acid and 2 ml 30% hydrogen peroxide. A blank was also analyzed for background correction.

Five-day biochemical oxygen demand (BOD_5), total Kjeldahl nitrogen (TKN), and total phosphorus analyses of the influent wastewater were performed according to Standard Methods (7). To determine the average nutrient loading rates, two grab samples per week were collected from the lagoon influent. The samples were collected for a period of one year from NSTL Lagoons 1 and 2 and over a six month period from the other two lagoons.

Results and Discussion

A. Gross Composition

As shown in Table 2, whole plants from different sampling locations were found to contain fairly constant amounts of fat, fiber and ash; findings for these constituents were quite comparable with those reported by other investigators (5, 13). Phosphorus content and crude protein, on the other hand, were found to vary considerably among the sampling sites. This is again consistent with the findings of other authors. Boyd (5) noted that the protein content of water hyacinths declined with plant age and varied greatly among plants taken from different locations, in general reflecting the nutrient content of the waters in which they are grown. Gosset and Norris (8) also found that both the nitrogen and the phosphorus content of water hyacinths increased with increasing concentrations of these nutrients in the culture solution. Our results corroborate these findings. Inspection of Tables 3 and 4 reveals a direct correlation between nutrient loading rate and crude protein content of the water hyacinths; that is, plants grown in lagoons with higher loading rates contain proportionally greater amounts of crude protein. At the highest nutrient loading rates, the difference in percent crude protein is less pronounced (compare, for example, Lucedale with Orange Grove, Table 1), indicating that plant protein is reaching a maximum level.

Table 1. Nutrient Loading Rates of Sample Sites
(Based on Yearly Average Nutrient Concentrations)

	5-Day Biochemical Oxygen Demand		Total Kjeldahl Nitrogen		Total Phosphorus	
	kg/ha/day	lb/ac/day	kg/ha/day	lb/ac/day	kg/ha/day	lb/ac/day
Lucedale	57	52	9.8	9.0	2.4	2.2
Orange Grove	83	76	14.9	13.6	8.3	7.6
NSTL Lagoon #1	24	22	2.5	2.3	0.7	0.7
NSTL Lagoon #2	4	4	1.1	1.0	0.3	0.3

Table 2. Composition (Dry Weight) of Whole Plants From Four Locations

Location	Crude Protein, %	Fat, %	Fiber, %	Ash, %	Kjeldahl Nitrogen, %	Phosphorus, %
Lucedale	22.3	2.04	19.5	15.1	3.56	0.89
Orange Grove	23.4	2.20	17.1	20.4	3.74	0.85
NSTL Lagoon #1	17.1	1.59	18.6	11.1	2.73	0.45
NSTL Lagoon #2	9.7	1.68	19.2	19.9	1.56	0.31

Table 3. Amino Acid Profile of the Leaves and Stolons of Water Hyacinths Collected at Lucedale, MS (g/100g) Dry Weight

Amino Acid	Leaves	Stolons
Aspartic	3.77	5.71
Glutamic	3.45	1.93
Alanine	1.94	0.67
Isoleucine	1.46	0.54
Phenylalanine	1.70	0.59
Ammonia	0.70	0.94
Threonine	1.36	0.54
Proline	1.88	0.62
Valine	1.74	0.58
Leucine	2.59	0.85
Histidine	0.69	0.23
Arginine	1.64	0.50
Serine	1.28	0.50
Glycine	1.61	0.59
Methionine	0.44	0.14
Tyrosine	1.06	0.37
Lysine	1.78	0.54
Cysteine	0.409	0.122
Tryptophan	0.309	0.167

Table 4. Amino Acid Composition of Grain Protein Compared to Dried Water Hyacinth Leaves

	FAO Reference Pattern*	Grams/100g Protein					Water Hyacinth Leaves Grown in Human Waste
		Corn	Rice	Oats	Wheat	Sorghum	
Lysine	2.2	0.8	3.5	4.0	2.6	1.8	5.7
Methionine + Cysteine	2.4	3.6	3.4	4.8	3.6	3.0	2.7
Threonine	1.3	4.1	3.3	3.6	3.0	3.6	4.3
Isoleucine	1.8	6.4	4.5	4.0	3.4	4.5	4.7
Leucine	2.5	15.0	8.0	7.1	6.8	11.6	8.3
Valine	1.8	5.3	5.4	5.1	4.6	5.4	5.6
Phenylalanine + Tryosine	2.5	13.1	10.3	8.4	7.6	5.2	8.8
Tryptophan	0.65		0.6	0.9	1.1	0.8	1.0
Histidine			2.2	2.2	2.3	2.0	2.2
Arginine			7.8	6.1	4.7	3.4	5.2

*World Health Organization Technical Report Series, No. 522, 1973, p.53.

One reason that water hyacinths are so effective at removing excess nutrients is that they exhibit luxury consumption, particularly of phosphorus. That is, they will absorb more of this nutrient than they can utilize for growth. (Excess phosphorus is stored within the plant tissue.) In a study concerning the effects of high phosphorus concentration on growth of the water hyacinth, Haller and Sutton (10) found that this plant can absorb roughly four times more phosphorus than other plants which have been previously studied. These authors found that at moderate phosphorus concentrations (5-10 mg/1) phosphorus within the plants was concentrated mostly in the leaves and stems, while at concentrations exceeding 20 mg/1, phosphorus was distributed more uniformly throughout the plant tissue. We also found roughly 66% more phosphorus in the leaves and stems than in the roots of the plants we sampled (Figure 1); phosphorus concentration of the influent water ranged from 8.47-9.70 mg/1.

Other plant constituents also showed some partitioning within the plant tissue (Figure 1). For example, leaves were found to contain the highest percentage of crude protein and the lowest percentage ash. The stems contained the largest portion of the plant fiber. Thus, the leaves of the water hyacinth would produce the greatest percent yield for protein extraction. However, this is somewhat misleading; since the stems comprise a much greater percentage of the total plant mass, they also contain a considerable amount of protein.

B. Amino Acid Composition

The amino acid composition of water hyacinth leaves and stolons collected in September from Lucedale is presented in Table 3. The relative amounts of certain amino acids differ considerably from those previously reported (5, 17, 18); apparently the ratio of the plant's constituent amino acids is strongly affected by environmental variables and perhaps seasonal variables as well.

Comparison of the amino acid composition of water hyacinth leaves with that of grain crop species and the FAO reference pattern (Table 4) reveals that the water hyacinth would make an excellent protein source or could be used as a dietary supplement to balance the amino acid intake in a predominantly grain diet. For example, water hyacinth could be used to supplement the lysine content of a corn diet for cattle or a rice diet for humans. A diet consisting mainly of wheat could be enriched in lysine, threonine, isoleucine, leucine, phenylalanine, tyrosine, valine, and arginine by the addition of water hyacinth protein. As shown in Table 5, water hyacinth leaves compare favorably with the crude protein and amino acid content of high protein crops such as cottonseed and soybean.

C. Minerals and Vitamins

The mineral composition of water hyacinths is presented in Tables 6 and 7. As shown in Table 7, most minerals are present throughout the plant tissue. However, iron and perhaps copper and sulfur are concentrated in the roots while magnesium appears to be concentrated in the leaf tissue. Note that there were no toxic levels of lead, silver, cadmium, or chromium detected in the plant tissue. When detected at all, the amounts of these substances were no greater than those present in soybean and cottonseed meal.

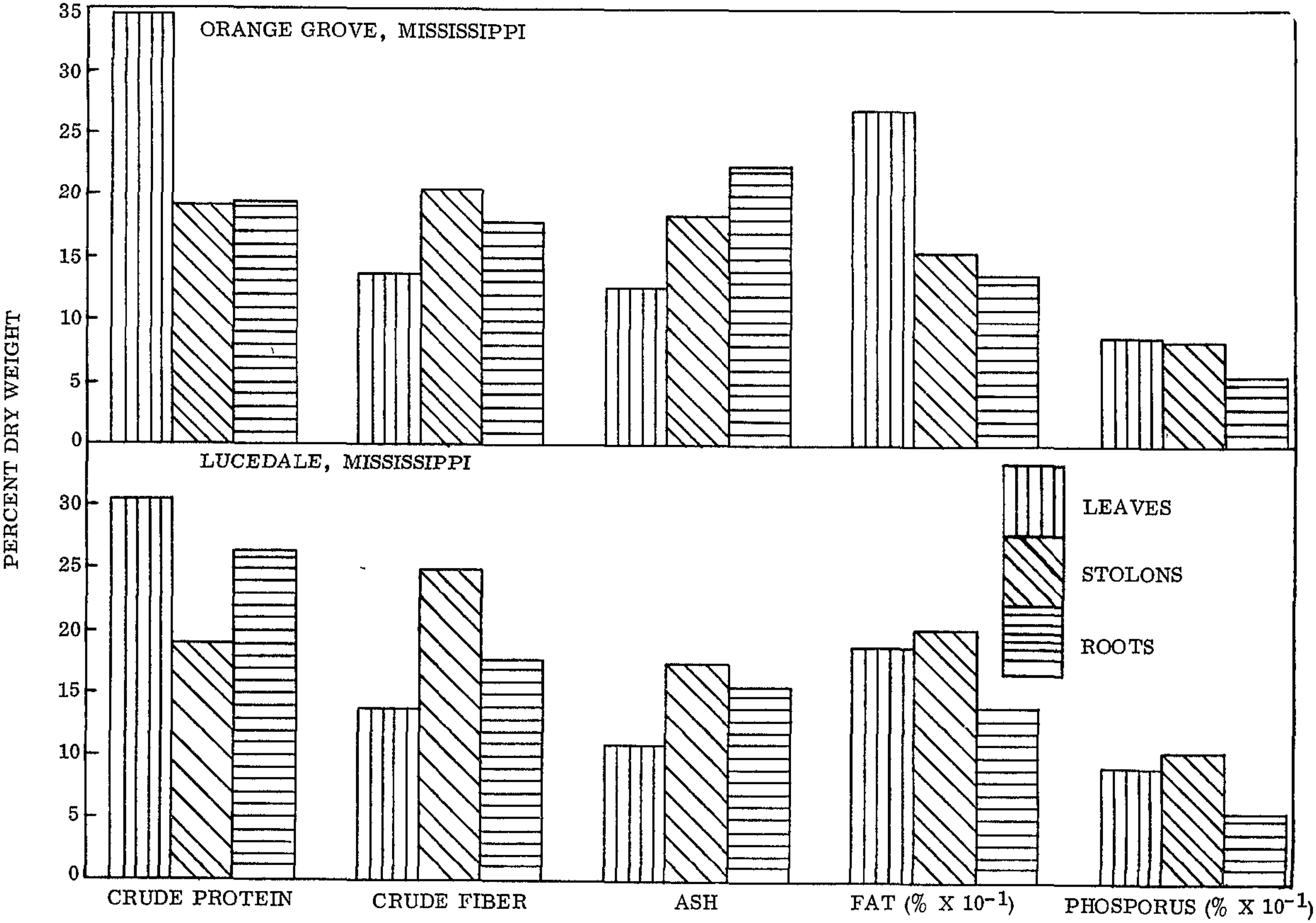

Figure 1. Partitioning of nutrients in sewage grown water hyacinths from two locations

Table 5. Amino Acid Composition of Cottonseed Meal and Soybean Meal as Compared to Dried Water Hyacinth Leaves

Concentration, g/100g crude protein

Amino Acid Analysis	Cottonseed Meal	Soybean Meal	Water Hyacinth Leaves**
Lysine*	5.40	6.49	5.68
Histidine	2.16	2.63	2.20
Arginine	5.17	6.98	5.23
Aspartic	19.22	12.18	12.03
Threonine*	4.86	4.26	4.34
Serine	4.94	5.51	4.08
Glutamic	13.66	19.36	11.01
Proline	5.02	5.29	6.00
Glycine	5.56	4.48	5.14
Alanine	6.33	4.58	6.19
Valine*	5.48	4.80	5.55
Methionine*	1.31	1.37	1.40
Isoleucine*	4.40	4.90	4.66
Leucine*	7.80	7.98	8.26
Tyrosine	3.55	3.94	3.38
Phenylalanine*	5.10	5.37	5.42
Tryptophan*			0.99
Crude Protein %	39.1	44.5	31.3

*Essential Amino Acids **Leaves collected from Lucedale

NOTE: Cottonseed meal and soybean meal analysis supplied by Mississippi State University.

Table 6. Mineral Concentration of Whole Water Hyacinth Plants Collected From the 4 Experimental Sites

Location	Concentration (ppm), (Dry Weight)											
	Sr	Li	B	K	Na	Ca	Fe	Mn	Mg	Cu	Zn	S
Lucedale	146	3	3980	27,800	18,600	7,450	1581	86	1683	10	25	5810
Orange Grove	254	4	4760	34,500	16,300	5,600	2260	291	3473	42	48	4040
NSTL Lagoon #1				16,400	16,600	17,200	2120	151	2185	21	21	4310
NSTL Lagoon #2	335	6	4780	26,600	20,200	7,920	6150	127	2944	55	68	4400

No toxic levels detected of the following heavy metals: Pb, Ag, Cd, Cr

Table 7. Partitioning of Minerals in Water Hyacinths Collected From Lucedale and Orange Grove

September 1976

Site	Concentration (ppm), Dry Weight									
	Plant Part	K	Na	Ca	Fe	Mn	Mg	Cu	Zn	S
Lucedale	leaves	36,000	18,300	7,560	143	69	8,490	8	23	4,500
	stolons	27,300	12,100	8,760	82	88	1,540	1	15	3,370
	roots	30,300	10,200	6,860	5,630	41	1,810	44	63	16,200
Orange Grove	leaves	36,000	18,300	2,890	143	69	8,490	14	19	*
	stolons	33,000	6,570	4,110	178	176	2,570	42	32	*
	roots	28,000	25,600	5,420	5,940	356	2,830	81	76	16,200

*Insufficient sample available for analysis

The relatively high mineral content of the water hyacinth, comparable to that of many crop species, suggests that this plant could make a good soil additive as well as a dietary supplement. In a study comparing water hyacinth with commercial fertilizer, Parra and Hortenstein (13) found that water hyacinth applications produced as good or better crop yields than did applications of commercial fertilizers for certain soil types. Basak (3) found that the nutrient content of water hyacinth compost was approximately four times greater than that of farmyard manure and twice as great as compost prepared from town refuse and night-soil. The mineral values of our sewage-grown hyacinths are similar to those reported by Parra and Hortenstein, except the Mississippi hyacinths contain slightly more sodium and considerably more calcium and copper than they reported.

The vitamin contents of sewage-grown water hyacinth leaves are presented in Table 8. Vitamins may be even more concentrated in other plant parts. For example, the roots contain over 50 times the amount of vitamin B_{12} than is present in the leaves. A comparison of water hyacinth content of selected vitamins and minerals with that of the U. S. recommended daily allowance is shown in Table 9.

Conclusions

Inspection of Tables 4, 5, and 9 reveals that the water hyacinth could be an excellent source of proteins, vitamins, and minerals, and could be of particular value as a dietary supplement in countries where human diets are generally deficient in these nutrients. The high water content of E. crassipes (95%) makes utilization of this species difficult on a large-scale commercial basis, however, we feel that this fast-growing plant species would be beneficial to human diets on an individual basis, since less than 3 kg of harvested fresh water hyacinth leaves could provide essentially all of the protein, minerals, and vitamins required daily in the human diet. We are currently experimenting with low-cost harvesting and processing methods which should make the utilization of water hyacinth nutrients more feasible on a large-scale basis.

Table 8. Miscellaneous Vitamins and Nutrient Values for Water Hyacinth Leaves from Lucedale Sewage Lagoon

VITAMINS AND NUTRIENTS	CONCENTRATION, DRY WEIGHT
Thiamine HCl (B-1)	5.91 ppm
Riboflavin (B-2)	30.7 ppm
Vitamin E	206 ppm
Pyroxidine HCl (Vitamin B-6)	15.2 ppm
Vitamin A	2.45 ppm
Chemical Niacin	79.4 ppm
Pantothenic Acid	55.6 ppm
Pepsin Digestibility	67.0%
Xanthophyll	485 ppm
Vitamin B-12	0.0126 ppm (roots: 0.682 ppm)

Table 9. U. S. Recommended Daily Allowances of the Following Vitamins and Minerals Compared to Dried Water Hyacinth Leaves Grown in Domestic Sewage

VITAMIN/MINERAL	U S RECOMMENDED DAILY ALLOWANCE	CONTENT PER 100 g DRIED WATER HYACINTH LEAVES
Thiamine	1 5 mg	0 591 mg
Riboflavin	1 7 mg	3 0 mg
Niacin	20 mg	7.94 mg
Vitamin E	30 I U	20 6 I U
Pantothenic Acid	10 mg	5 56 mg
Pyroxidine HCl (Vitamin B-6)	2 mg	1.52 mg
Vitamin B-12	6 µg	1 26 µg
Calcium	1 g	0 756 g
Iron	18 mg	14.3 mg
Phosphorus	1 g	0 927 g
Magnesium	400 mg	849 mg
Zinc	15 mg	2 3 mg
Copper	2 mg	0 8 mg
Sodium	0 2-4 4 g	1 83 g
Potassium	3.3 g	3 60 g
Sulfur	0 85 g	0.45 g

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SECTION III
Part 2

BIO-CONVERSION OF WATER HYACINTHS INTO METHANE GAS

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Technical Memorandum X-72725

July, 1975

ABSTRACT

Bio-gas and methane production from the microbial anaerobic decomposition of water hyacinths (Eichhornia crassipes) (Mart.) Solms was investigated. These experiments demonstrated the ability of water hyacinths to produce an average of 13.9 ml of methane gas per gram of wet plant weight. This study revealed that sample preparation had no significant effect on bio-gas and/or methane production. Pollution of water hyacinths by two toxic heavy materials, nickel and cadmium, increased the rate of methane production from 51.8 ml/day for non-contaminated plants incubated at 36°C to 81.0 ml/day for Ni-Cd contaminated plants incubated at the same temperature. The methane content of bio-gas evolved from the anaerobic decomposition of Ni-Cd contaminated plants was 91.1 percent as compared to 69.2 percent methane content of bio-gas collected from the fermentation of non-contaminated plants.

TECHNICAL MEMORANDUM X-72725

BIO-CONVERSION OF WATER HYACINTHS INTO METHANE GAS

Two of the most pressing problems facing the United States and other industrial nations today are rapid depletion of vital natural resources and pollution of the environment. One important factor in the rise of the United States to its present high industrial level has been an abundance of fossil fuel resources. Presently, available coal, oil, and large reservoirs of underground natural gas are all produced through natural decomposition of prehistoric forms of life. Modern society is depleting these resources at an alarming rate, and renewable sources must be developed within the near future for continued industrial growth.

As we deplete our natural resources, we are also polluting our environment at the same alarming rate. Fortunately, a large number of the minerals with which we are polluting our water systems have the potential of being recovered through natural biological processes.

Recently, the ability of vascular aquatic plants to remove organic chemicals, heavy metals, pesticides, and nutrients from polluted waters has been demonstrated (1, 2, 3, 4, 5, 6). Harvested plant material from these experiments is a potential source of renewable resources, such as natural gas, fertilizers, and other valuable minerals. Vegetation can be fermented anaerobically and made to release bio-gas containing a high percentage of methane (7, 8).

Many factors can affect the actual amount of gas and fertilizer produced from the digestion of plant material. One of the most important of these factors is the carbon to nitrogen (C/N) ratios of the material used. For maximum bio-gas production the C/N ratio should be approximately 30:1.

Water hyacinths (Eichhornia crassipes) (Mart.) Solms, were chosen for this study because they have demonstrated the most promise in removing chemicals from polluted waters and producing large quantities of harvestable plant material possessing a desirable C/N ratio for maximum methane gas production. This aquatic plant has the potential of producing over 240 kg (529 lbs) of dry plant material per 0.40 hectare (acre) per day while removing undesirable chemicals from waste waters.

MATERIALS AND METHODS

Water hyacinths used in these experiments were grown by vegetative reproduction inside a greenhouse maintained between 25°C and 30°C. Several plants were selected for each experiment whose total wet mass ranged from 300g to 878g. In one of the fermentation studies, water hyacinths were contaminated with nickel and cadmium by exposing them to a known concentration of cadmium and nickel prior to fermentation. The 542.8g wet mass of water hyacinths absorbed 5.40 mg of nickel and 6.87 mg of cadmium from 2.5 liters of Ni-Cd contaminated distilled water. Metal concentration was monitored by atomic absorption.

For four of the five fermentation units, the plants were chopped into approximately one-inch long pieces. Water hyacinths were blended into a slurry form for the other fermentation study. The chopped or blended water hyacinths were transferred into three liter Erlenmeyer flasks covered with aluminum foil to prevent exposure to light.

Starter seed for the fermentation studies was prepared by allowing water hyacinths to decompose under water and mud approximately six months in an anaerobic condition. For each fermentation unit incubated at 36°C, approximately 20 g of this seed was blended with 350 ml of distilled water. Fifty grams of seed and 800 ml of distilled water were used for each experiment at room temperature. The sediment from the seed and water mixtures was allowed to settle 30 minutes, and the supernatant liquid was then decanted into each flask containing water hyacinths.

The Erlenmeyer flasks were sealed to the atmosphere with two-hole rubber stoppers. One outlet was fitted with a rubber septum for gas chromatographic sampling, and the other outlet was connected with rubber hose to a sealed container filled with water acidified with sulfuric acid. The displacement of water in the second container by the bio-gas produced in the fermentation flask provided a convenient method of monitoring the volume of bio-gas production. Mixing of the water hyacinths was accomplished by shaking the fermentation flask once each day.

Samples for gas chromatographic analysis were taken through the rubber septum. Matheson Gas Products c.p. grade methane was used as the methane standard. The methane content of the bio-gas was analyzed by gas chromatography using a Varian 2100 GC with a flame ionization detector. Gas chromatographic conditions were:

Column:	6' x 1/4" i.d. glass
Packing:	Porapak® Q 150-200 mesh

Flow Rates: ml/minute	Nitrogen 60, Hydrogen 35, and Air 235
Temperature:	Detector 155°C, injection 150°C, Column 55°C
Carrier Gas:	Nitrogen
Sample Size:	5 μl and 10 μl

RESULTS AND DISCUSSION

Five laboratory experiments were conducted in order to evaluate the effect of temperature, toxic metal contamination, and plant preparation on the production of bio-gas and/or methane from the microbial anaerobic decomposition of water hyacinths, (Eichhornia crassipes) (Mart.) Solms.

Two of the three experiments were conducted at room temperature (25°C ± 5°C) and contained water hyacinths chopped into one-inch long pieces (Figures 1 & 2). The other experiment was conducted under the same conditions except the water hyacinths were blended into a slurry form (Figure 3). According to the data presented in Tables 1 and 2, there was no significant difference in the results of these three experiments. The chopped water hyacinths produced 11.0 and 6.4 ml methane per gram wet weight, as compared to a production of 7.9 ml methane per gram weight for the blended water hyacinths. The methane content of the total bio-gas produced by the anaerobic decomposition of the slurried water hyacinths was 61.1%. This value was comparable to the 57.2% and 61.5% methane content of the bio-gas produced in the other two experiments with chopped water hyacinths at room temperature.

Temperature played an important role in the rate of bio-gas and methane production. The time lag between the production of bio-gas and the production of methane gas was reduced from an average of eight days for those maintained at 25°C ± 5°C to approximately one day for experiment 4 incubated 36°C. The methane content of the total bio-gas produced in experiment 4 at 36°C was 69.2%. This percent methane was higher than the average methane content of 59.9% for the three experiments conducted at room temperature.

Comparison of the data for experiments 4 and 5 in Table 1 in which chopped plants were incubated at 36°C showed that nickel and cadmium contamination of the water hyacinths at concentration levels of 9.95 and 12.66 mg/kg wet weight nickel and cadmium, respectively, had no adverse effect on the percent methane content, volume of methane produced per unit wet weight, or the rate of bio-gas and/or methane production. In fact, the Ni-Cd contaminated plants produced bio-gas with a 91.1% methane content, as compared to the lower value of 69.2% for the other experiment incubated at 36°C (Figures 4 & 5).

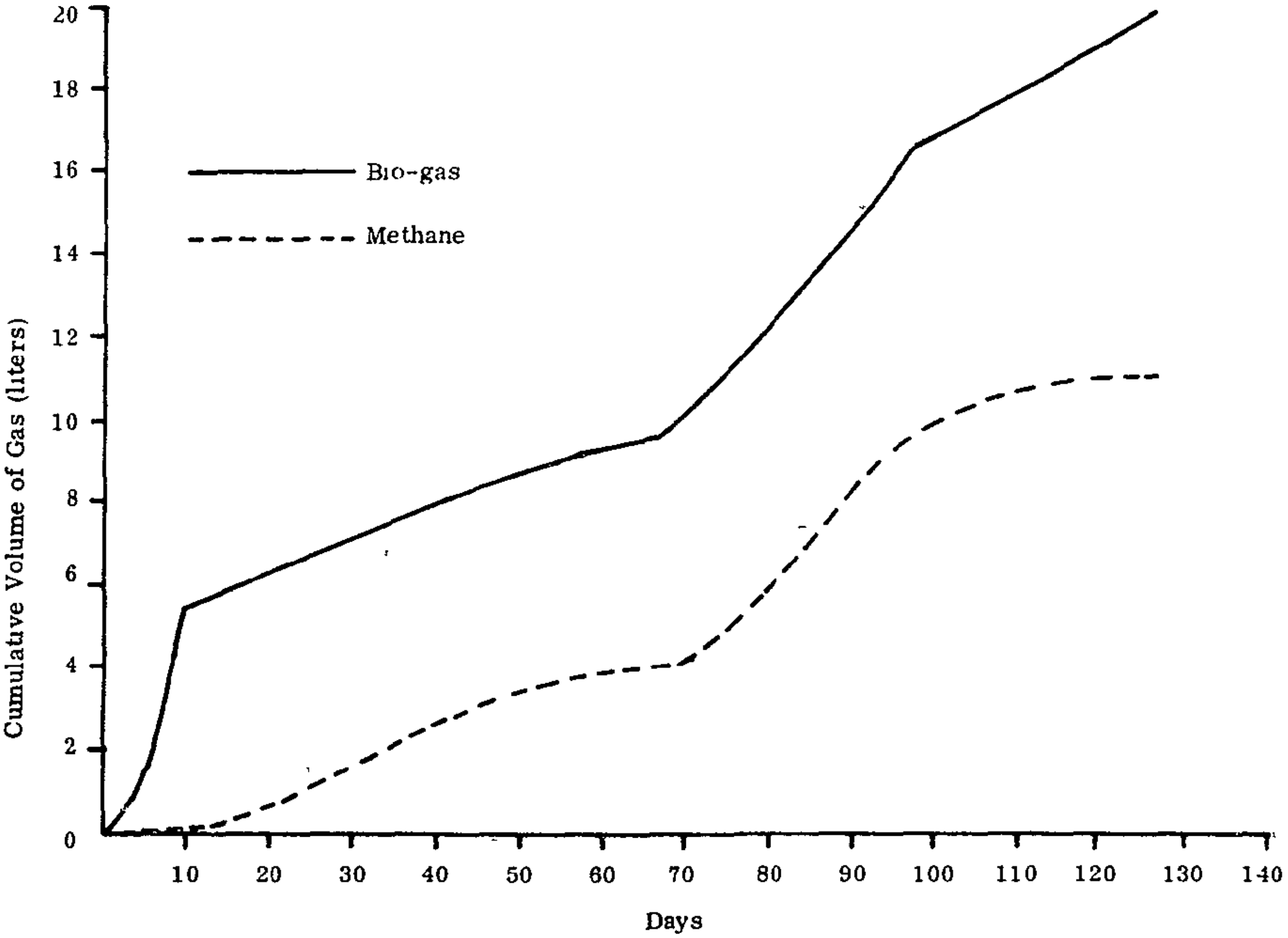

Figure 1. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths at 25°C ± 5°C versus number of days elapsed since initiation of anaerobic fermentation

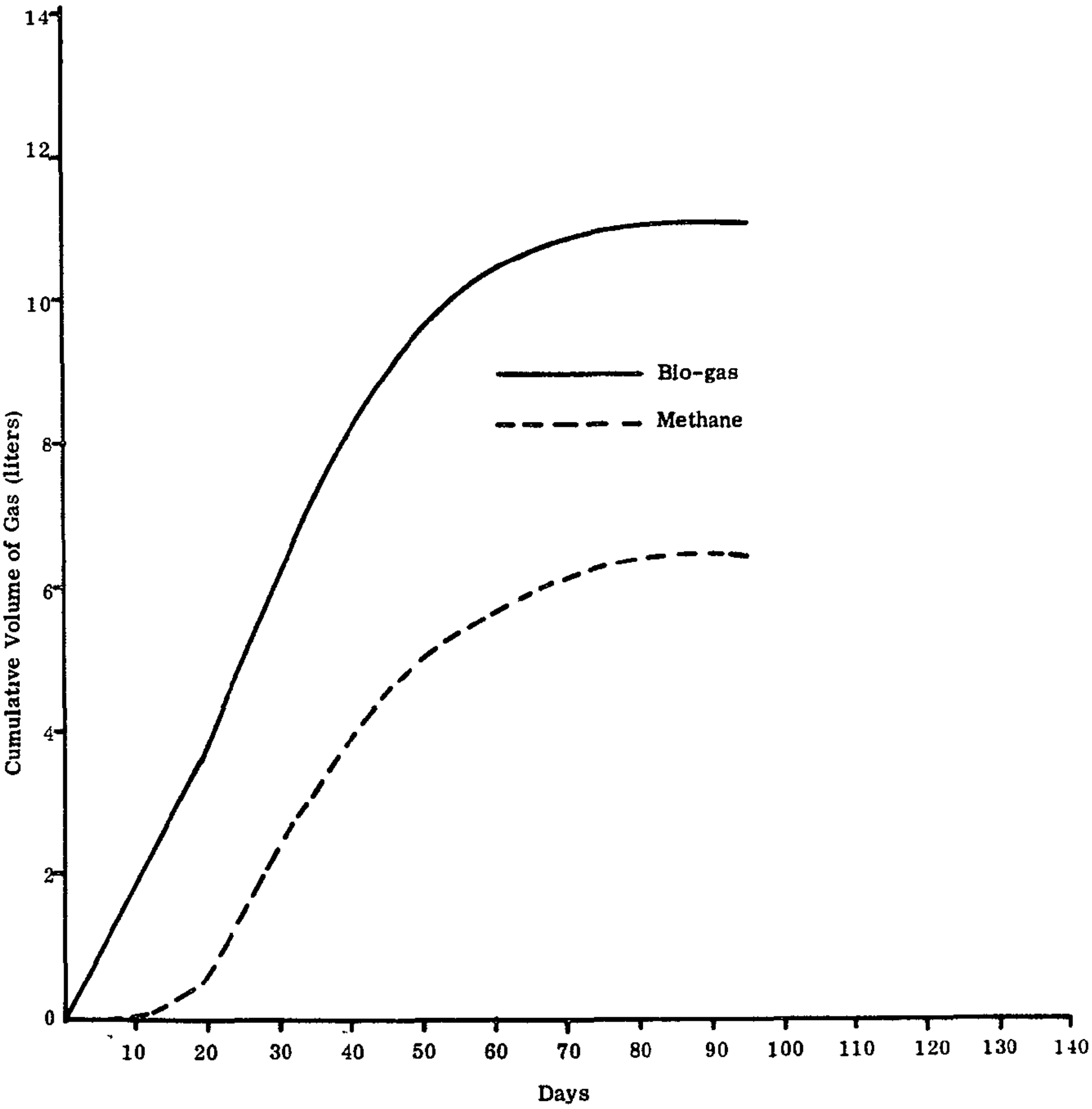

Figure 2. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths at 25°C ± 5°C versus number of days elapsed since initiation of anaerobic fermentation.

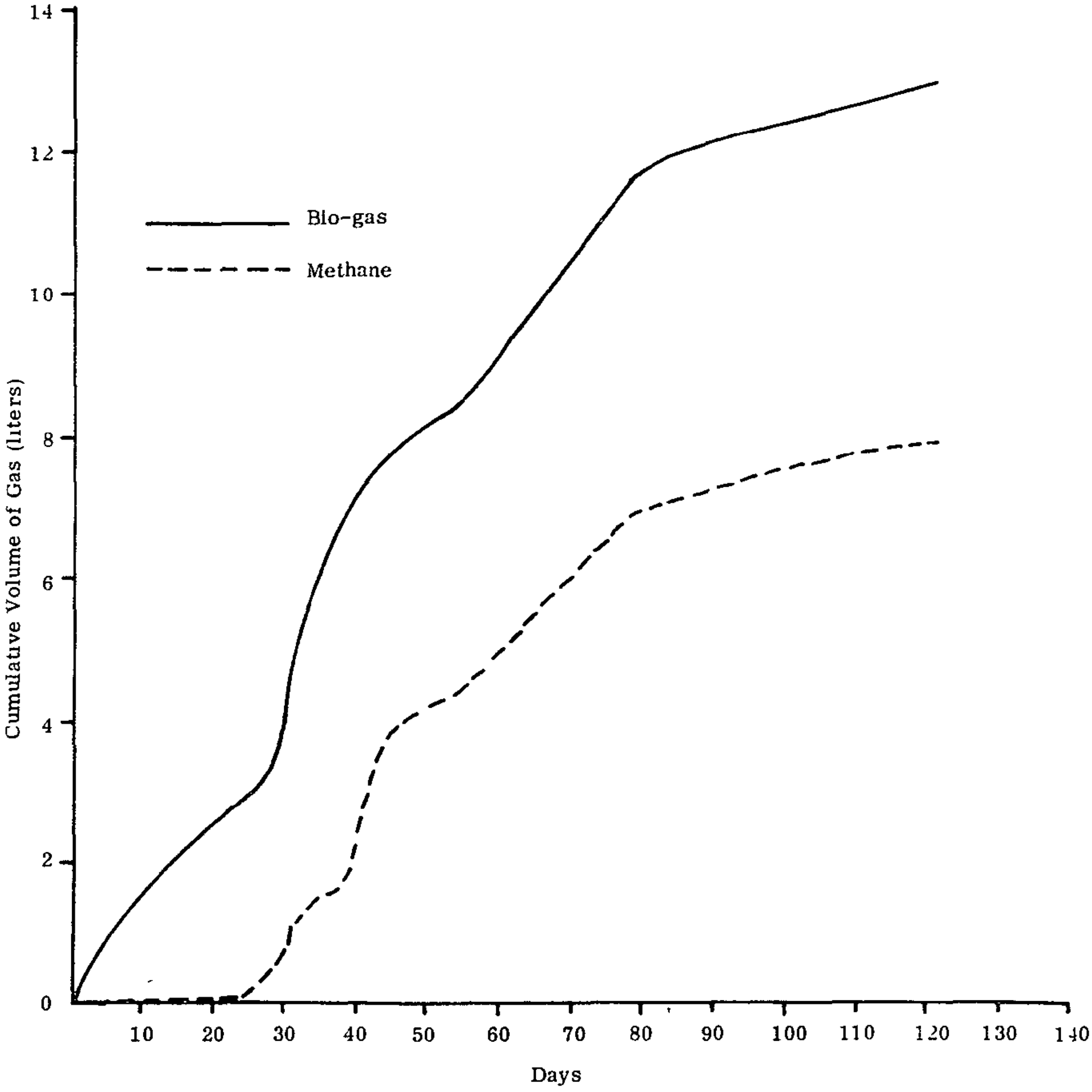

Figure 3. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of blended water hyacinths at 25°C ± 5°C versus number of days elapsed since initiation of anaerobic fermentation

Table 1. Bio-gas Data From the Anaerobic Decomposition of Water Hyacinths

EXPERIMENT 1

Wet Mass 300g
Form Chopped
Temp 25°C ± 5°C
Vol. H_2O 800 ml

No Days Elapsed	Cum Bio-gas (ml)	Cum Bio-gas per 1.0 Kg Wet Mass (ml)	Cum Methane (ml)	Cum Methane per 1.0 Kg Wet Mass (ml)
4	325	1,082	0	0
7	1,150	3,830	0	0
12	1,650	5,495	43	143
41	2,461	8,195	846	2,817
68	2,861	9,527	1,206	4,016
83	3,911	13,024	1,994	6,640
97	4,961	16,520	2,939	9,787
126	5,361	17,852	3,299	10,986

EXPERIMENT 2

Wet Mass 754 g
Form Chopped
Temp 25°C ± 5°C
Vol H_2O 800 ml

No Days Elapsed	Cum Bio-gas (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)	Cum Methane (ml)	Cum Methane per 1 0 Kg Wet Mass (ml)
4	600	798	0	0
10	1,465	1,943	0	0
12	1,745	2,314	56	74
19	2,745	3,641	356	472
21	3,345	4,436	692	918
25	4,095	5,431	1,097	1,455
27	4,495	5,962	1,497	1,985
35	5,485	7,275	2,487	3,298
39	6,010	7,971	2,891	3,834
45	6,760	8,966	3,491	4,630
52	7,460	9,894	4,051	5,373
68	8,110	10,756	4,571	6,062
95	8,410	11,154	4,811	6,381

EXPERIMENT 3

Wet Mass 800 g
Form Blended
Temp 25°C ± 5°C
Vol H_2O 800 ml

No Days Elapsed	Cum Bio-gas (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)	Cum Methane (ml)	Cum. Methane per 1.0 Kg Wet Mass (ml)
4	600	750	0	0
10	1,250	1,563	0	0
12	1,450	1,813	11	14
21	2,050	2,563	47	59
27	2,475	3,094	247	309
31	3,775	4,719	897	1,121
35	4,850	6,063	1,972	2,465
39	5,460	6,825	2,192	2,740
45	6,300	7,875	3,032	3,790
52	6,660	8,325	3,392	4,240
64	7,660	9,575	4,392	5,490
79	9,310	11,638	5,547	6,934
122	10,310	12,888	6,297	7,871

EXPERIMENT 4

Wet Mass 500 g
Form Chopped
Temp 36°C
Vol H_2O 350 ml

No. Days Elapsed	Cum. Bio-gas (ml)	Cum. Bio-gas per 1.0 Kg Wet Mass (ml)	Cum Methane (ml)	Cum. Methane per 1.0 Kg Wet Mass (ml)
3	665	1,330	86	172
22	1,425	2,850	413	826
27	2,920	5,840	1,833	3,666
34	3,900	7,800	2,470	4,940
50	4,525	9,050	3,064	6,128
65	4,990	9,980	3,366	6,732
101	6,260	12,520	4,319	8,638
103	6,460	12,920	4,469	8,938

EXPERIMENT 5

Wet Mass 500 g
Form Chopped
Temp 36°C
Vol H_2O 350 ml

No Days Elapsed	Cum Bio-gas (ml)	Cum Bio-gas per 1 0 Kg Wet Mass (ml)	Cum. Methane (ml)	Cum. Methane per 1.0 Kg Wet Mass (ml)
3	575	1,150	518	1,036
20	2,350	4,700	2,151	4,302
22	2,825	5,650	2,617	5,234
27	3,750	7,500	3,524	7,048
34	4,100	8,200	3,755	7,510
50	4,350	8,700	3,993	7,986
63	5,690	11,380	5,266	10,532
101	6,210	12,420	5,656	11,312

Table 2. Calculated Data of Bio-gas and Methane Production for Experiments 1-5

Experiment #	% Methane in Total Bio-gas	ml Bio-gas per Gram Wet Weight	ml Methane per Gram Wet Weight
1	61.5	17.9	11.0
2	57.2	11.1	6.4
3	61.1	12.9	7.9
4	69.2	12.9	8.9
5	91.1	12.4	11.3

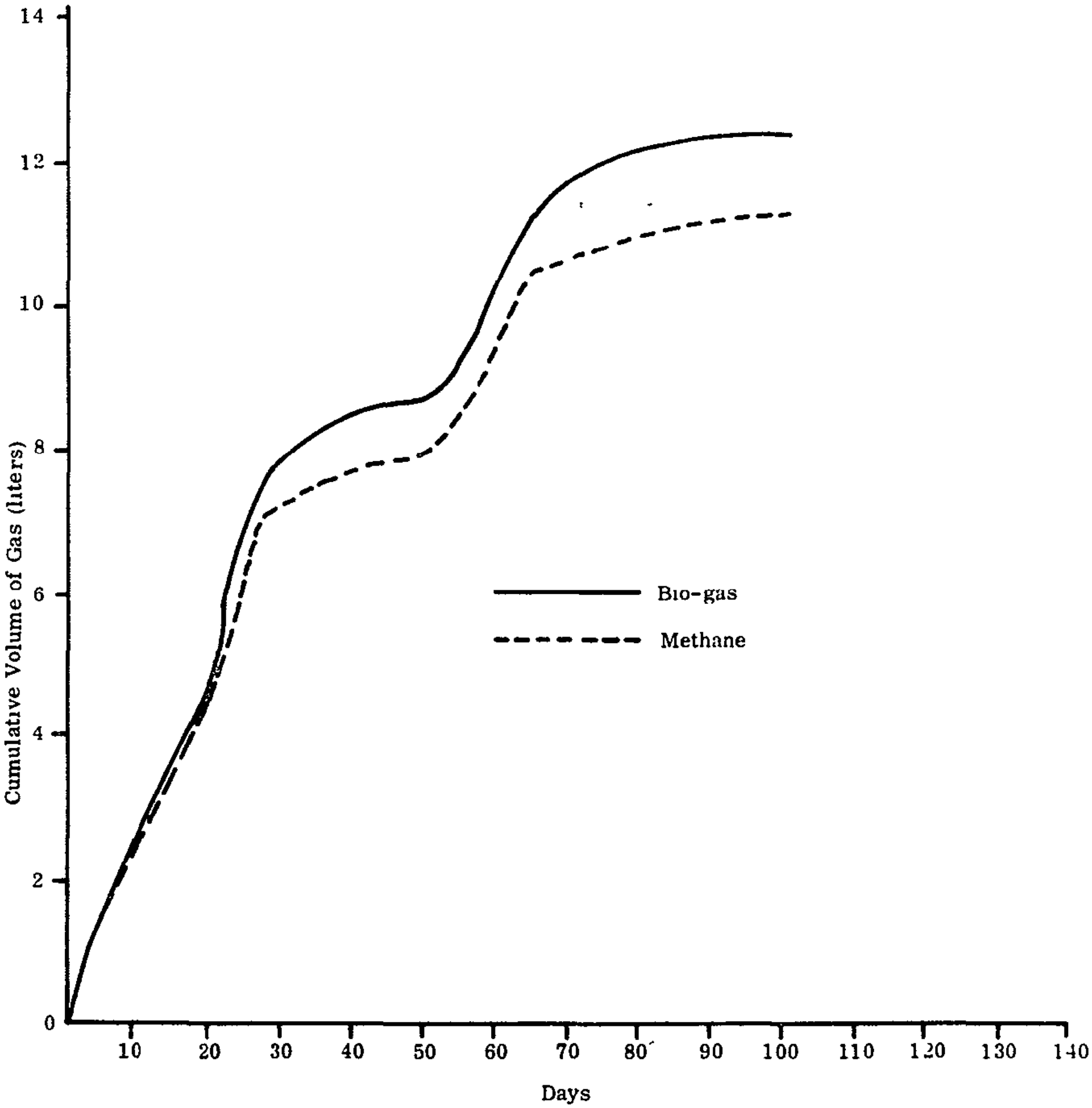

Figure 4. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths incubated at 36°C versus number of days elapsed since initiation of anaerobic fermentation.

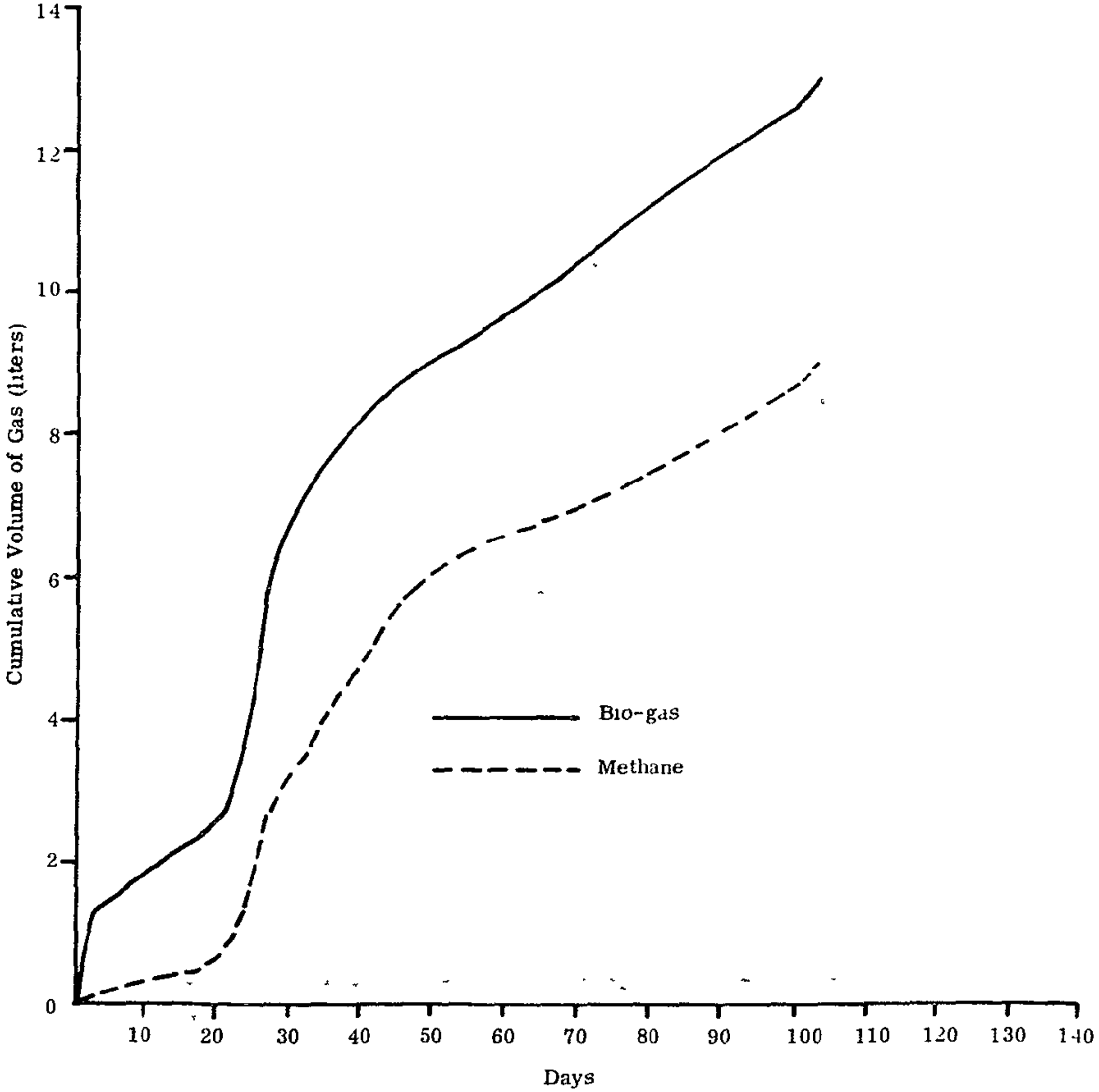

Figure 5. Cumulative volume of bio-gas and methane gas per 1.0 Kg wet mass of chopped water hyacinths incubated at 36°C versus number of days elapsed since initiation of anaerobic fermentation

The total volume of bio-gas produced from the Ni-Cd contaminated plants was less than the volume produced from the non-contaminated plants; however, due to the much higher percentage of methane in the bio-gas, the Ni-Cd contaminated plants yielded 11.3 ml methane per gram wet weight, as compared to only 8.9 ml methane per gram wet weight for the non-contaminated plants. Also, the average rates of bio-gas and methane production (87.5 and 81.0 ml/day, respectively) for the Ni-Cd contaminated water hyacinths for the first 65 days of incubation was significantly higher than the rates for the non-contaminated plants (76.8 and 51.8 ml/day bio-gas and methane production, respectively) incubated at the same temperature.

CONCLUSION

This study on the anaerobic decomposition of water hyacinths revealed that sample preparation, either chopped or blended, had no significant effect on bio-gas and/or methane production. Incubation of the experimental units at 36°C increased not only the rate of bio-gas production, but also the methane content of the total bio-gas produced in these experiments. Pollution of the water hyacinths by two toxic heavy metals, nickel and cadmium, actually increased the rate of methane production and improved the methane content of the bio-gas evolved in the anaerobic decomposition of the contaminated plants.

Further studies are planned in this area utilizing the preliminary information from these experiments. Temperature controlled, continuous feed fermentation chambers with efficient stirring devices are presently being developed by NASA at the National Space Technology Laboratories.

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SECTION IV

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