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WATER HYACINTHS FOR REMOVAL OF PHENOLS FROM POLLUTED WATERS

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16. ABSTRACT

Removal of phenol by water hyacinths (Eichhornia crassipes (Mart.) Solms) in static water was investigated. 2.75 g dry weight of this aquatic plant demonstrated the ability to absorb 100 mg of phenol per plant per 72 hours 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 hours from waters polluted with this chemical.

EDITOR'S NOTE

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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 (1, 2); prevents proper drainage of land (1); interferes with aquatic recreation (1); restricts the supply of sunlight to submerged plant and fish life (3); and increases the evaporation rate of water bodies by 3.2 to 3.7 times through evapotranspiration through the leaves (4, 5).

The water hyacinth propagates both by seed germination and by vegetative means wereby mature plants produce rosettes of leaves and fibrous roots at each node of the growing stem (6). Under proper conditions a given community of water hyacinths can double in number every two weeks (7). A single plant can produce approximately 65,000 offspring during a single season (8). Due to this phenomenal growth rate, one acre (0.40 hectare) of plants can produce approximately 240 kg of dry weight per day which far exceeds the yield of the most productive agricultural crops (9). 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 (10-15). 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 (16-17). Recently, the ability of vascular aquatic plants to remove organic chemicals, heavy metals, and pesticides from polluted waters has been demonstrated (18-24).

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 (25).

MATERIALS AND METHODS

Water hyacinths, Eichhornia crassipes (Mart.) Solms, were collected in the spring and summer of 1974 from a bayou adjacent to Louisiana Highway 190, approximately 0.40 km 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 Ortho-Gro(R) liquid plant food containing 480 ppm total nitrogen, 240 ppm available phosphoric acid (P_2O_5), 240 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 four-week and older plants. Individual plants averaging 2.75 grams 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 one-liter glass beakers. The beakers were painted black in order to inhibit algae growth. Phenol (Mallinckrodt, lot AEK, analytical reagent 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 hours of exposure. Three plant controls which were free of phenol and three phenol controls free of 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 (26). 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 25 hours 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 seconds in 35-50 ml of chloroform using a Sorvall Omni-Mixer(R). The plant material was then allowed to remain in contact with chloroform for a minimum of 48 hours. 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 percent.

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. Water hyacinths were placed in one-liter beakers containing 100-150 ppm phenol in distilled water and placed inside a 65 x 85 x 87 cm closed chamber with two 34 x 48 cm transparent windows. The atmosphere within the chamber was exhausted after 24, 48 and 72 hour 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 five-foot by one-eighth inch stainless steel column containing Chromosorb W, 70/80 mesh, coated with 5 percent free fatty acid, and conditioned at 180°C for 24 hours was employed. A retention time of 3.88 minutes was recorded for phenol with the inlet temperature at 160°C, the detector temperature at 180°C and chart speed at 20 cm/hr. Gas flow rates in cubic centimeters per minute were nitrogen 60, hydrogen 35, and air 235. Water-phenol injection sample sizes were 5 micro-liters. 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 DISCUSSION

The ability of water hyacinths to remove phenol from the three water systems employed in this investigation is presented graphically in Figure 1 (25 ppm), Figure 2 (50 ppm), and Figure 3 (100 ppm). The assimilation rates are depicted as percent 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 1. The rate of removal of phenol is very similar from the distilled water-nutrient solution and the river water. 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 (27). An extensive comparative study of the growth rate of Eichhornia crassipes (Mart.) Solms. in water culture by Chadwick and Obeid report an optimum growth rate at pH 6.9-7.0 with a decrease in plant production at either higher or lower pH values (28). The pH values measured for the three test water systems are presented in Table II. 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 like differential. The slowest removal rate occurred with the bayou water, and the mean pH value (6.1) is that furthest removed from the optimum level. In addition, Boyd observed that aquatic plants absorb mineral nutrients more slowly as the plants age (29). The age of the water hyacinths used in this phenol removal study were sufficiently variable to contribute somewhat to the observed variations in phenol removal rates both within each system and between the three separate systems.

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-hour removal sequence was repeated several times in order to confirm the reproducibility of the data. 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 toxicity 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 III for the 24-hour 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 bacteriacide. The large number of bacterial counts obtained were also predictable since considerable microbial activity exists beneath mats of water hyacinths (27). The absence of dramatic increases in bacterial counts suggests that the particular bacteria present in the system studied 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 water 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 to 10 peaks and/or shoulders after a 20-minute elution period. The phenol peak which appears at 3.88 minutes 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.

The failure of these recovery experiments indicates that phenol is removed by the water hyacinth and rapidly metabolized to other components. Peroxidases and phenol oxidases present in both plants and animals could serve as catalyzing agents for this process (26). Translocation studies are currently in progress using carbon-14 labeled phenol in order to determine the identity and final location in the water hyacinth of metabolites produced by phenol assimilation.

The water hyacinth (Eichhornia crassipes (Mart.) Solms) effectively removed 36 milligrams of phenol from distilled water, river water, and

bayou water systems per gram dry weight of plant material in 72 hours. Since one hectare contains approximately 1.62×10^6 plants (7) and the average dry weight per plant was determined to be 2.75 grams, one hectare of water could conceivably remove 160 kilograms of phenol in a 72-hour 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 (8). 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.

Table I. Percent of phenol concentration remaining at indicated sampling times 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	86.6	0.4	86.6	0.4	*
Distilled	50 ppm	54.5	98.9	2.1	93.9	0.4	*
Distilled	100 ppm	60.0	99.3	13.3	86.7	0.5	85.0
River	25 ppm	16.4	62.3	0.4	44.5	0.4	*
River	50 ppm	34.9	89.0	0.4	61.0	0.4	58.0
River	100 ppm	82.4	96.6	16.1	84.3	2.8	84.8
Bayou	25 ppm	45.9	94.3	5.87	56.7	0.4	55.7
Bayou	50 ppm	58.8	91.7	2.05	84.5	0.4	71.3
Bayou	100 ppm	61.4	93.3	14.1	83.1	0.4	70.2

* Samples not collected; phenol concentration less than or equal to detection limit (0.4%).

Table II. Measured pH values for test water systems. The reported variation of ± 0.2 is that observed within a particular set of experiments.

WATER SYSTEM	pH VALUE
Distilled Water plus Nutrients	7.6 ± 0.2
East Pearl River Water	6.3 ± 0.2
Bayou Water	6.1 ± 0.2

Table III. Average bacterial counts expressed as bacteria per milliliter (BPM) for 24-hour sampling time.

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	

Table III. Average bacterial counts expressed as bacteria per milliliter (BPM) for 24-hour sampling time (continued)

TEST SYSTEM	ADDITIVE	BACTERIAL COUNTS (BPM) SPECIMEN	CONTROL
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	

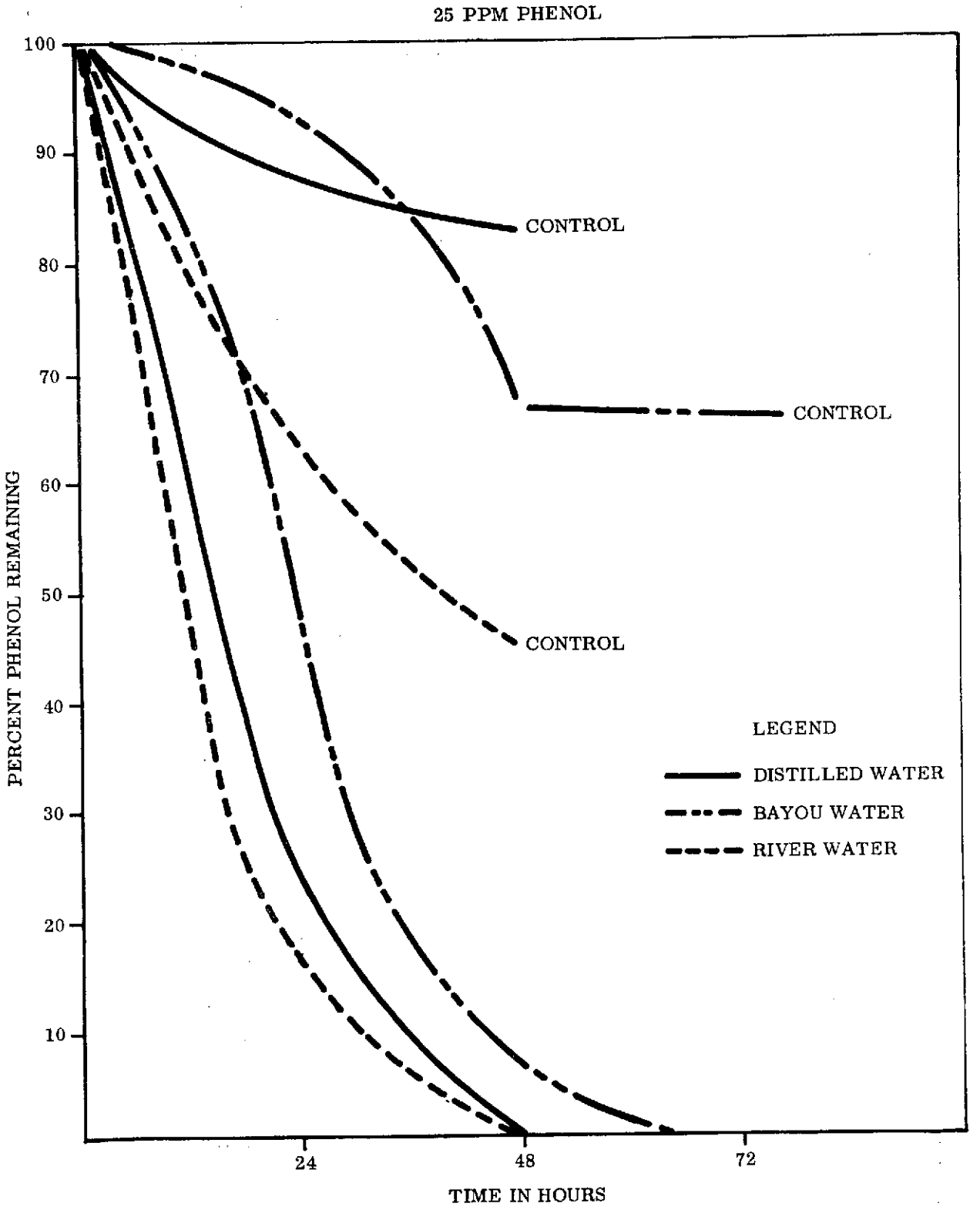

FIGURE 1. Graphic Representation of Removal Rate of 25ppm Phenol From Phenol Controls (No Plants) and Phenol Exposed Plants.

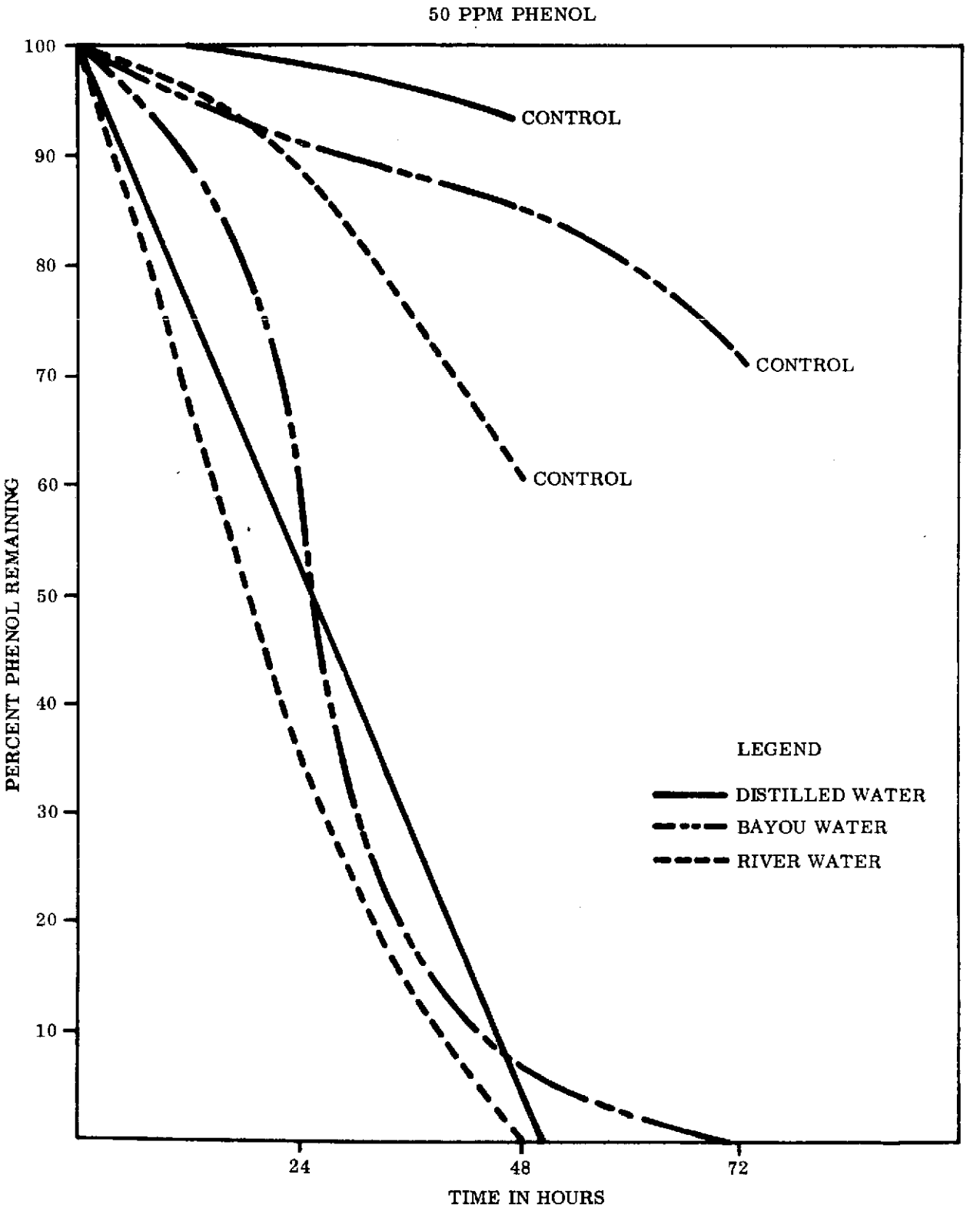

FIGURE 2. Graphic Representation of Removal Rate of 50ppm Phenol From Phenol Controls (No Plants) and Phenol Exposed Plants.

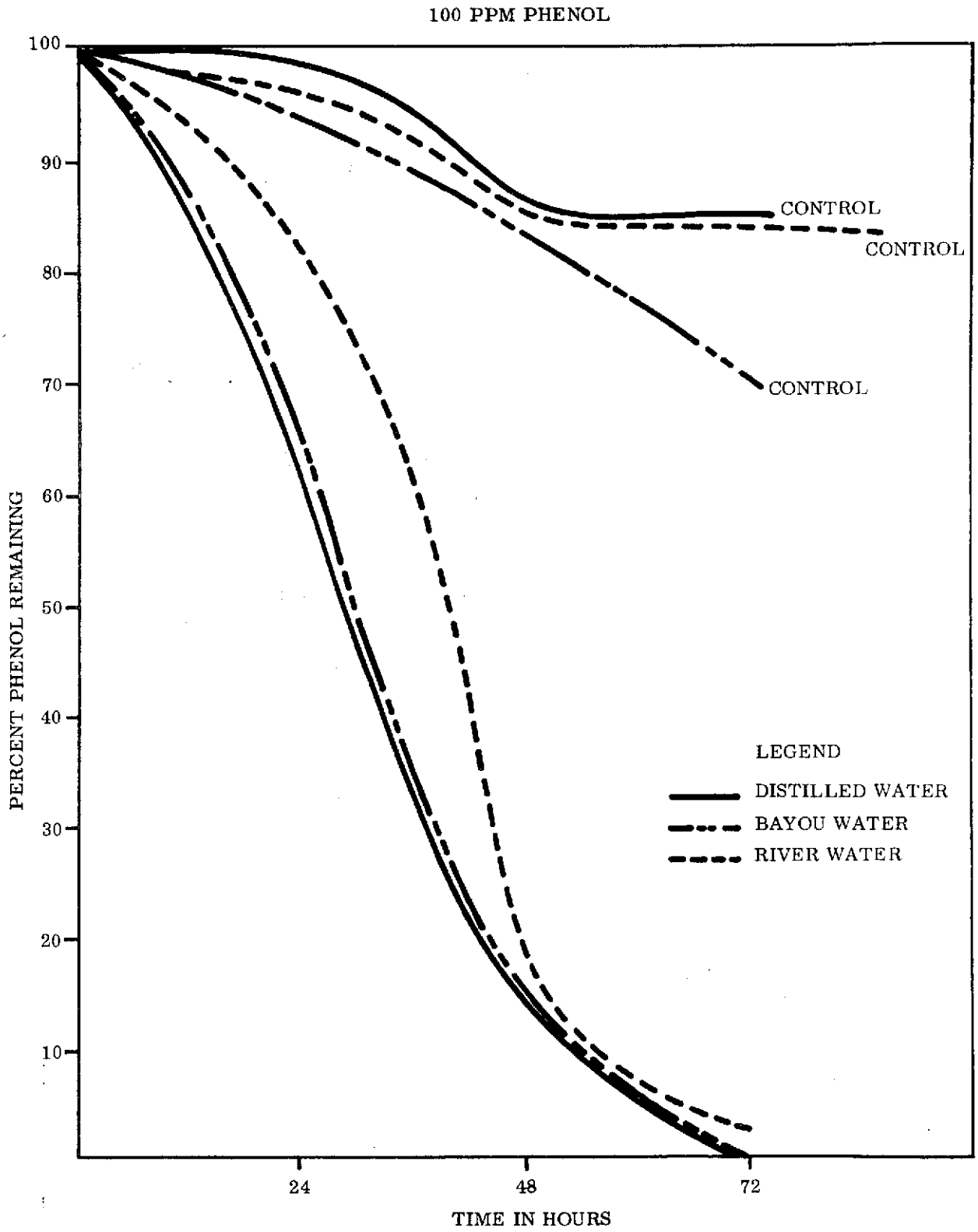

FIGURE 3. Graphic Representation of Removal Rate of 100ppm Phenol From Phenol Controls (No Plants) and Phenol Exposed Plants.

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APPROVAL

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The information in this report has been reviewed for security classification. Review of any information concerning Department of Defense or Atomic Energy Commission programs has been made by the NSTL Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

This document has also been reviewed and approved for technical accuracy.

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Manager, National Space Technology Laboratories