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**EDGEWOOD ARSENAL
TECHNICAL REPORT**

EATR 4595

**THE COMPARATIVE EFFECTS OF CS AND VARIOUS
POLLUTANTS ON FRESH WATER PHYTOPLANKTON
COLONIES OF WOLFFIA PAPULIFERA THOMPSON**

by

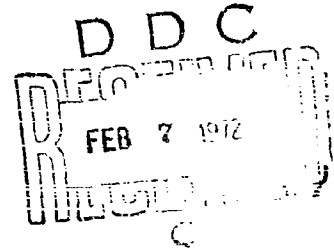
Elmer G. Worthley, Ph.D.

C. Donald Schott

December 1971



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13. ABSTRACT Varying concentrations of nine potential pollutants were tested for effects in vitro against colonies of <i>Wolffia papulifera</i> . Death was observed in colonies of <i>Wolffia</i> exposed to 100 ppm or above of CS, DDT, Malathion ^R , Diazinon ^R , and indole acetic acid (IAA) and to 1000 ppm of Aldrin ^R , Dieldrin ^R , Sevin ^R , and 2,4-D. No effects were seen in xxxxxx colonies exposed to 5 ppm or below of CS, DDT, and IAA; 1 ppm of Dieldrin, Diazinon, and Sevin; 0.1 ppm of Aldrin and Malathion; and 0.01 ppm of 2,4-D. Teratogenic effects were observed in <i>Wolffia</i> colonies exposed to Malathion at 1 ppm, of 2,4-D at 0.1 ppm and above, and Diazinon at 10 ppm. Responses of <i>Wolffia</i> colonies to all dose levels of pollutants tested, as well as pictorial evidence of teratogenicity, are included.			
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EDGEWOOD ARSENAL TECHNICAL REPORT

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Elmer G. Worthley, Ph.D.
C. Donald Schott

Medical Research Division

December 1971

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Task 1W662710AD6302

DEPARTMENT OF THE ARMY
EDGEWOOD ARSENAL
Biomedical Laboratory
Edgewood Arsenal, Maryland 21010

FOREWORD

The work described in this report was authorized under Task 1W662710AD6302, Chemical Safety Investigations, Test Area Ecology. This work was started in June 1970 and completed in May 1971.

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DIGEST

Varying concentrations of nine potential pollutants were tested for effects in vitro against colonies of *Wolffia papulifera*. Death was observed in colonies of *Wolffia* exposed to 100 ppm or above of CS, DDT, Malathion[®], Diazinon[®], and indole acetic acid (IAA) and to 1000 ppm of Aldrin[®], Dieldrin[®], Sevin[®], and 2,4-D. No effects were seen in *Wolffia* colonies exposed to 5 ppm or below of CS, DDT, and IAA; 1 ppm of Dieldrin, Diazinon, and Sevin; 0.1 ppm of Aldrin and Malathion; and 0.01 ppm of 2,4-D. Teratogenic effects were observed in *Wolffia* colonies exposed to Malathion at 1 ppm, 2,4-D at 0.1 ppm and above, and Diazinon at 10 ppm.

Responses of *Wolffia* colonies to all dose levels of pollutants tested, as well as pictorial evidence of teratogenicity, are included.

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THE COMPARATIVE EFFECTS OF CS AND VARIOUS POLLUTANTS ON FRESH WATER PHYTOPLANKTON COLONIES OF *WOLFFIA PAPULIFERA* THOMPSON

I. INTRODUCTION.

The large scale use of chemicals has not been entirely beneficial to mankind. Although they have aided man in control of disease and have increased his ability to raise food, they also have had deleterious environmental effects.¹⁻⁶

Recently much has been written about the harmful effects of various pollutants on animals and economically important plants. Little information, however, has appeared in the literature about the effects of pollutants on noneconomic species of dicotyledonous plants. Species of plants, especially phytoplankton, that are usually unknown or not considered are important components of the living network of organisms that ultimately supports man. Evaluation of the dangers to the environment after release of chemicals should be made before general use of any potential pollutant is permitted. Assessment of the danger of chemicals under controlled conditions at all trophic levels of the food web is needed before accurate estimates of the dangers of these chemicals to man can be made. In-depth studies usually are not made until the chemical has been used on a large scale.⁷⁻⁹

The purpose of this paper is to present data based on effects observed *in vitro* when colonies of *Wolffia papulifera* Thompson, a species of fresh water phytoplankton, were exposed to varying concentrations of CS, DDT, Aldrin[®], Dieldrin[®], Malathion[®], Diazinon[®], Sevin[®], 2,4-D, and indole acetic acid (IAA). Table I gives the chemical names of these compounds.

II. MATERIALS AND METHODS.

Wolffia papulifera (water-meal) is a minute member of the duckweed family Lamnaceae, order Arales. In fact, it is the second smallest flowering plant found in the world; only *W. microscopica* (Griffith) Kurz is smaller. The latter measures up to 0.5 mm in diameter, whereas *W. papulifera* is 0.9 mm in diameter. *Wolffia papulifera*, *W. punctata* Griseb., and *W. columbiana* Karst. are commonly found with the more familiar duckweeds *Lemna minor* and *Spirodela polyrrhiza* (L) Schleiden.

These species occur in both woodland and pastureland ponds, are easily found in the summer months, and overwinter on the bottoms of ponds and lakes. The specimens of *W. papulifera*

¹Enderson, J. H., and Berger, D. D. Pesticides: Eggshell Thinning and Lowered Production of Young in Prairie Falcons. *Bioscience* 29, 355 (1970).

²Metcalf, R. L. *Organic Insecticides*. Interscience Publishers, Inc., New York, New York. 1955.

³Negherbon, W. O. *Handbook of Toxicology*. Vol III. Insecticides. W. B. Saunders Company, Philadelphia, Pennsylvania. 1959.

⁴West, T. F., Hardy, J. E., and Ford, J. H. *Chemical Control of Insects*. Chapman and Hall, Ltd., London, England. 1951.

⁵Rudd, R. L. *Pesticides and the Living Landscape*. The University of Wisconsin Press, Madison, Wisconsin. 1962.

⁶Porter, R. D., and Wiemeyer, S. N. Dieldrin and DDT: Effects on Sparrow Hawk's Eggshell and Production. *Science* 162, 271 (1967).

⁷Secretary's Commission Report on Pesticides and Their Relationship to Environmental Health. Department of Health, Education and Welfare. Superintendent of Documents, Washington, D.C. 1969.

⁸Jones, G. R., and Israel, M. S. Mechanism of Toxicity of Injected CS Gas. *Nature* 228, 1315-1317 (1970).

⁹Himsworth, H., Dornhorst, A. C., and Thompson, R. Report of the Enquiry Into the Medical and Toxicological Aspects of CS. Command 4173, Dd. 152898 K40. Her Majesty's Stationary Office, London, England. 1969.

Table I. Chemical Identification and Aqueous Solubility of the Compounds

Compound	Chemical name	Purity	Water solubility
		%	ppm
CS	Orthochlorobenzylidene malononitrile	100	a
DDT	Dichloro-diphenyl-trichloroethane	77.2	0.001 ^b
Aldrin [®]	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo, exo-5,8-dimethanonaphthalene	90	0.01 ^b
Dieldrin [®]	1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo, exo-5,8-dimethanonaphthalene	85	0.1 ^b
Malathion [®]	0,0-Dimethyl S-(1,2-dicarboethoxyethyl) dithiophosphate	—	c
Diazinon [®]	0,0-Diethyl 0-2-isopropyl-4-methylpyrimidyl-(6)-thio nophosphate	97	40 ^b
Sevin [®]	1-Naphthyl N-methyl-carbamate	—	d
2,4-D	2,4-Dinitrophenoxyacetic acid	100	Soluble
IAA	Indole-3 acetic acid	100	Soluble

^aCompound present after ad mixture with water is malononitrile, the water soluble breakdown product of CS. From Feinsilver, L., Chambers, H. A., Jr., Vocci, F. J., Doasch, L., and Berkowitz, L. M. EATR 4521. Some Metabolites of CS From Rats. May 1971. UNCLASSIFIED Report.¹³

^bData from Lichtenstein, E. P., and Schulz, K. R. Volatilization of Insecticides from Various Substrates. Agr. Food Chem. 18, 814-818 (1970).¹⁴

^cObtained from Octagon Process, Inc., in a 57% emulsifiable concentration.

^dObtained from Alco Chemical Corp. as a 21.5% aqueous suspension.

used in this study were collected in Harford County, Maryland. *Wolffia papulifera* (henceforth referred to as *Wolffia*) is found in the eastern and southern United States, Mexico, and Argentina.

Wolffia cultures were grown in 125-ml Erlenmeyer flasks, each of which contained 40 ml of medium. Hutner's medium*, one-fifth the recommended concentration (X/5), proved to be best for growth of *Wolffia* under the present experimental conditions.¹⁰ Before introduction of *Wolffia*, the flasks were closed with a cotton stopper and autoclaved for 45 minutes at 20 pounds pressure. *Wolffia* were sterilized by soaking for 5 minutes in 1% chlorine (sodium hypochlorite), after which they were transferred to the flasks. Subcultures of sterile colonies were made later by standard transfer methods.

All pesticides (see table I) used in this study (except Sevin, which was commercially available in a 21.5% aqueous suspension from Alco Chemical Corporation, and Malathion, which was obtained from Octagon Process, Inc., in a 57% emulsifiable concentration) were dissolved in 1 to 2 ml ethanol and added to enough Hutner's medium so that the pesticide concentration was 1000 ppm. A 1000-ppm solution of Sevin and Malathion was made by direct transfer of the water-miscible preparation to Hutner's medium.

*Hutner's medium is a combination of the basic elements needed by plants for growth. See Hillman¹⁰ for specific ingredients.

¹⁰Hillman, W. S. The Induction of Flowering. L. T. Evans, ed. The Macmillan Company. New York, New York. 1969.

The 1000-ppm stock solution for each pesticide then was used to prepare final test solutions in the Hutner medium of 0.01, 0.1, 1.0, 5, 10, 20 or 50, and 100 ppm. Although control tests showed that as much as 1% ethanol was harmless to the *Wolffia* cultures, the maximum concentration in any of the test solutions was no more than 0.1%.

All test cultures and controls were set up in triplicate in open 10-ml beakers. Each beaker contained 7 ml of the control medium or the various pollutant concentrations. Three *Wolffia* were put into each beaker to preclude the inconsistency in early growth rates that occurs when only one individual is used. They were checked daily (up to 1 month) for increase or decrease in numbers, for abnormal growth, and for death.

Counting was facilitated by placing the beakers over a grid. Partially or fully developed individuals were counted as one. A new individual, when clearly discernible in the brood pouch (figure 1), was counted as well as the parent individual. Each combination of fully and partly developed *Wolffia* (figures 2B through 2F) would be counted as two regardless of size. There are 17 individuals in figure 3A and 6 in figure 3B counting by this method. Figure 3A also depicts the appearance of individual *Wolffia* as they were counted under 7X magnification.

The mean numbers per colony obtained in this manner were plotted versus time to obtain growth curves. Doubling time was calculated by using the method of Hillman¹¹ as follows:

$$k = \frac{M.R.}{1000} = \frac{\text{Log}_{10}(Fd) - \text{Log}_{10}(FO)}{d}$$

$$= \frac{\text{Log}_{10} \frac{(Fd)}{(FO)}}{d}$$

where

- k = growth constant
- $M.R.$ = multiplication rate
- Fd = number of individuals on day d
- FO = number of individuals on day 0

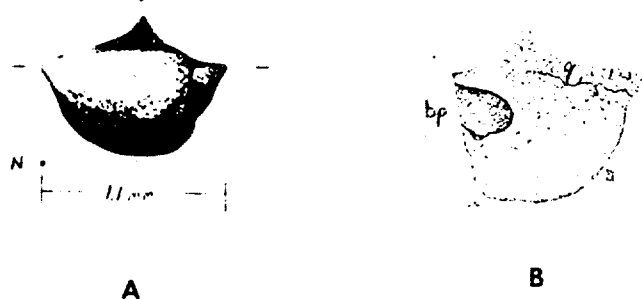


Figure 1. Lateral View of Budding *Wolffia* (A) and Tangential View of Mature *Wolffia* (B)

Dashed lines (A) indicate water level; bp indicates the brood pouch, which gives rise to new individuals; a indicates inflated cells or aerenchyma; and N indicates the actual size of one individual *Wolffia*.

¹¹Hillman, W. S. The Lemnaceae, or Duckweeds. Bot. Rev. 27, 221-287 (1961).

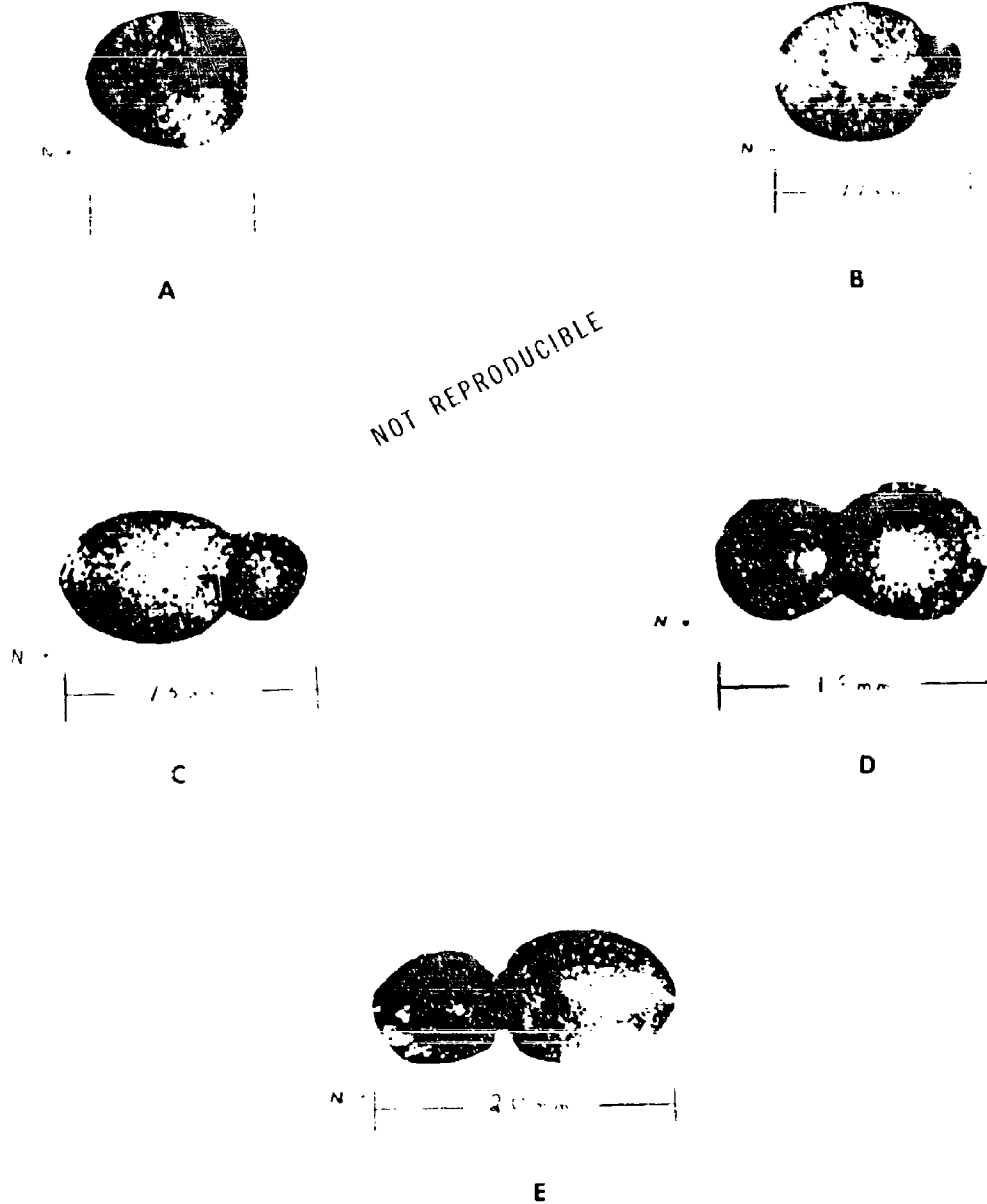


Figure 2. Vegetative Reproduction of *Wolffia*
 A - mature individual; B - 12 hours after appearance
 in A; C - 24 hours later; D - 36 hours later; and
 E - 48 hours later. N indicates the actual size of
 one individual *Wolffia*.

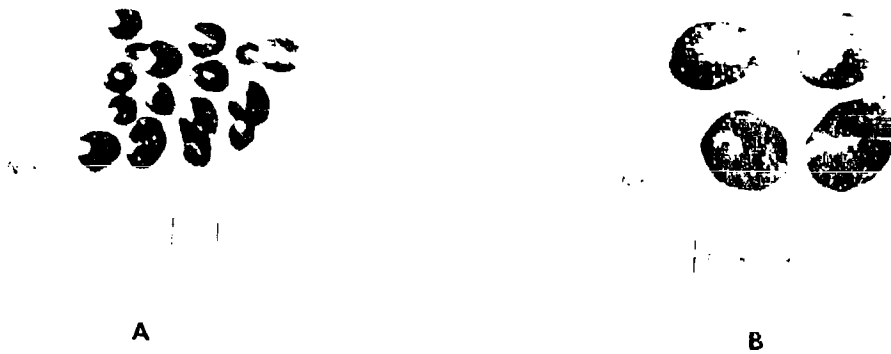


Figure 3. Appearance of a *Wolffia* Colony When Counted Routinely Under 7X Magnification (A) and a 2- to 3-Day Old Colony (B)

There are 17 individuals present in A and 6 in B. N indicates the actual size of one individual *Wolffia*.

Doubling of individuals each day yields an *M.R.* of 301 ($\text{Log}_{10} 2 = 0.301$). Control cultures in this experiment yielded an *M.R.* of 152 ± 6 ; therefore, control cultures doubled $301/152$, or every 2.0 days.

III. RESULTS.

Table II summarizes the effects on *Wolffia* of various concentrations of the compounds tested. Concentrations of 1000 ppm of all compounds killed all *Wolffia*. At 100 ppm only CS, DDT, Malathion, Diazinon, and IAA killed the entire colony. The lowest dose that produced some abnormal effect in the *Wolffia* (increased or decreased growth rate or teratogenic effects) was 10 ppm for CS, DDT, and IAA; 5 ppm for Dieldrin, Diazinon, and Sevin; 1 ppm for Aldrin and Malathion; and 0.1 ppm for 2,4-D.

Figures 4 to 13 show the effects of various concentrations of each compound on growth rate of *Wolffia*. Each figure includes a control curve obtained by plotting the mean values for the three control samples. Although most concentrations of compounds resulted in depressed growth rates, 1 and 5 ppm of Aldrin, 5 ppm of Dieldrin, and 5 and 10 ppm of Diazinon caused significant increase in growth rates.

Teratogenic effects also were observed in *Wolffia* exposed to 1 ppm Malathion, 10 ppm Diazinon, and 0.1 to 100 ppm 2,4-D. Figure 14 shows the teratogenic changes observed in *Wolffia* exposed to 0.1 ppm 2,4-D. The first manifestation was abnormal elongation. This was followed by loss of the ability to separate and excessive growth. The resulting individuals were abnormally large and grossly out of shape. Figure 14A-C shows examples of this. *Wolffia* exposed to 1 ppm Malathion and 10 ppm Diazinon did not lose the ability to separate. At these dose levels, only abnormal elongation (as shown in figure 14A for 2,4-D) occurred.

Table II. Summary of Effects Observed When Colonies of *Wolffia papulifera* Were Exposed to Varying Concentrations of Nine Pollutants

Compound	Effects*								
	Concentrations tested (ppm)								
	1000	100	50	20	10	5	1	0.1	0.01
CS		D	X	-	X	0	0	0	0
DDT	D	D	-	X	X	0	0	0	0
Aldrin	D	X	-	X	X	1	1	0	0
Dieldrin	D	X	X	-	X	1	0	0	0
Malathion	D	D	-	X	X	-	X,E	0	0
Diazinon	D	D	X	-	I,F	1	0	0	0
Sevin	D	X	X	-	X	X	0	0	0
2,4-D	D	X,E	X,E	-	X,E	X,E	X,E	F	0
IAA	D	D	X	-	X	0	0	0	0

*D = death; X = decrease in test population; I = increase in test population; F = teratogenic effects observed in test population; 0 = no change in individuals or colony appearance or growth; - = not tested.

IV. DISCUSSION.

The compound of primary interest in this study has been CS, but so many pesticides are in common use that it is valuable to have comparative data on some of these agricultural compounds to be able to recognize potential damage from their appearance in the local ecosystem. This can happen either from area spraying for the suppression of mosquitoes or from chance windborne contamination or water runoff from nearby agricultural areas.

All the compounds tested were harmful to *Wolffia* in some degree. CS proved to be moderately toxic, producing death at 100 ppm, and causing measurable reduction in growth at 10 ppm. In terms of lethal effects, none of the compounds seemed to be extremely toxic to *Wolffia*. Malathion, CS, DDT, IAA, and Diazinon all caused death at 100 ppm; 2,4-D, Sevin, Aldrin, and Dieldrin caused death only at 1000 ppm. *Wolffia*, however, was sensitive to 2,4-D and Malathion at the very low level of 1 ppm, at which growth was inhibited. Sevin was effective in this way at 5 ppm and the others only at 10 ppm except Diazinon, which showed inhibition at 20 ppm.

A few compounds had teratogenic effects: these are 2,4-D at 0.1 ppm; Malathion at 1 ppm; and Diazinon at 10 ppm.

Photosynthesis is a vital function in algae and higher plants. It can be inhibited by DDT and Dieldrin at 0.1 ppm¹² and this, although not measured in these experiments, may be the function that was affected, producing the inhibition of growth that was observed. Comparison of the toxicity of these compounds to *Wolffia* with toxicity to some other aquatic organisms (table III) shows that *Wolffia* is much less sensitive than mosquito larvae, shrimp, and certain small fish.

¹²Menzel, D. W., Anderson, J., and Radtke, A. Marine Phytoplankton Vary in Their Response to Chlorinated Hydrocarbons. *Science* 167, 1724-1726 (1970).

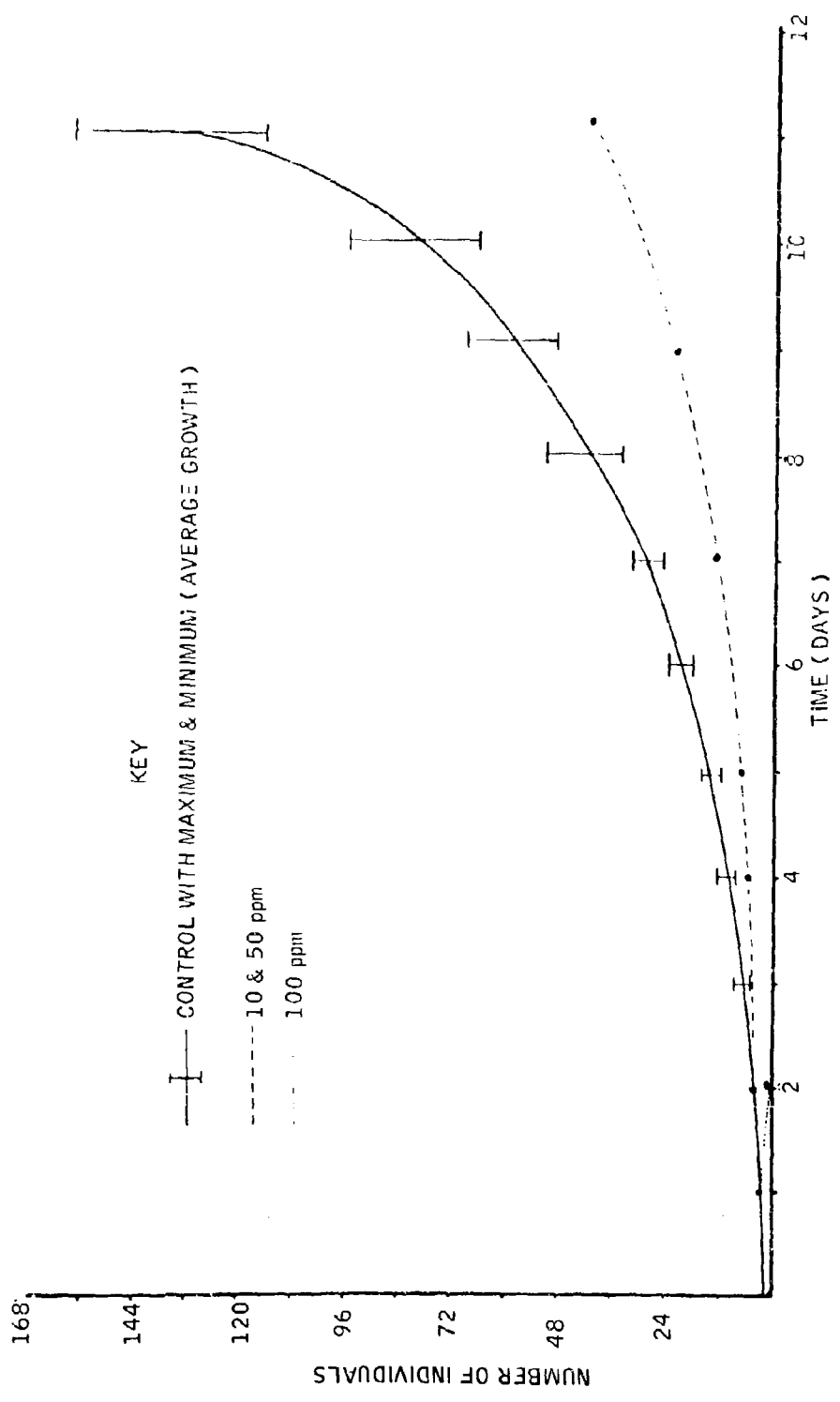


Figure 4. Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of CS

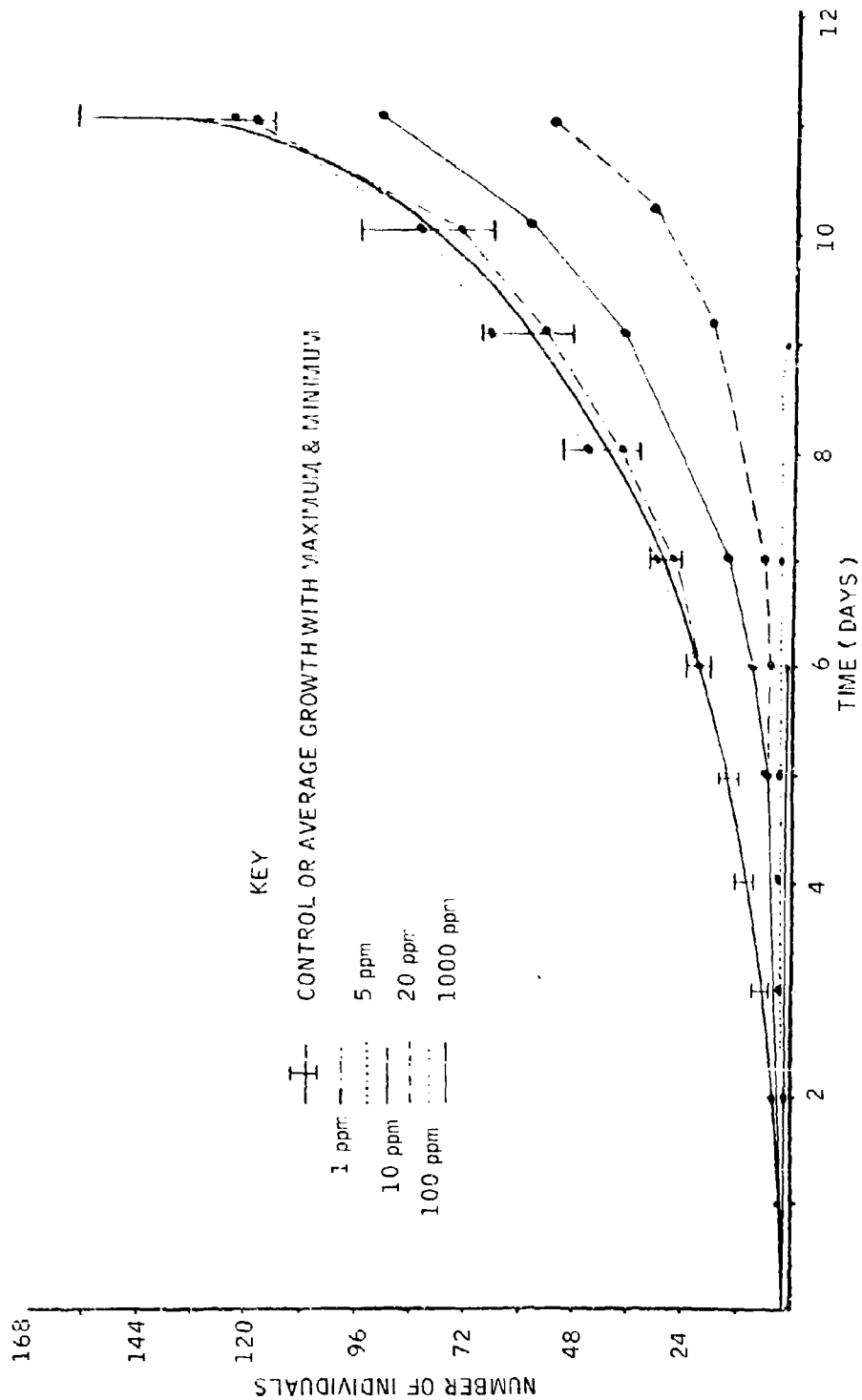


Figure 5. Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of DDT

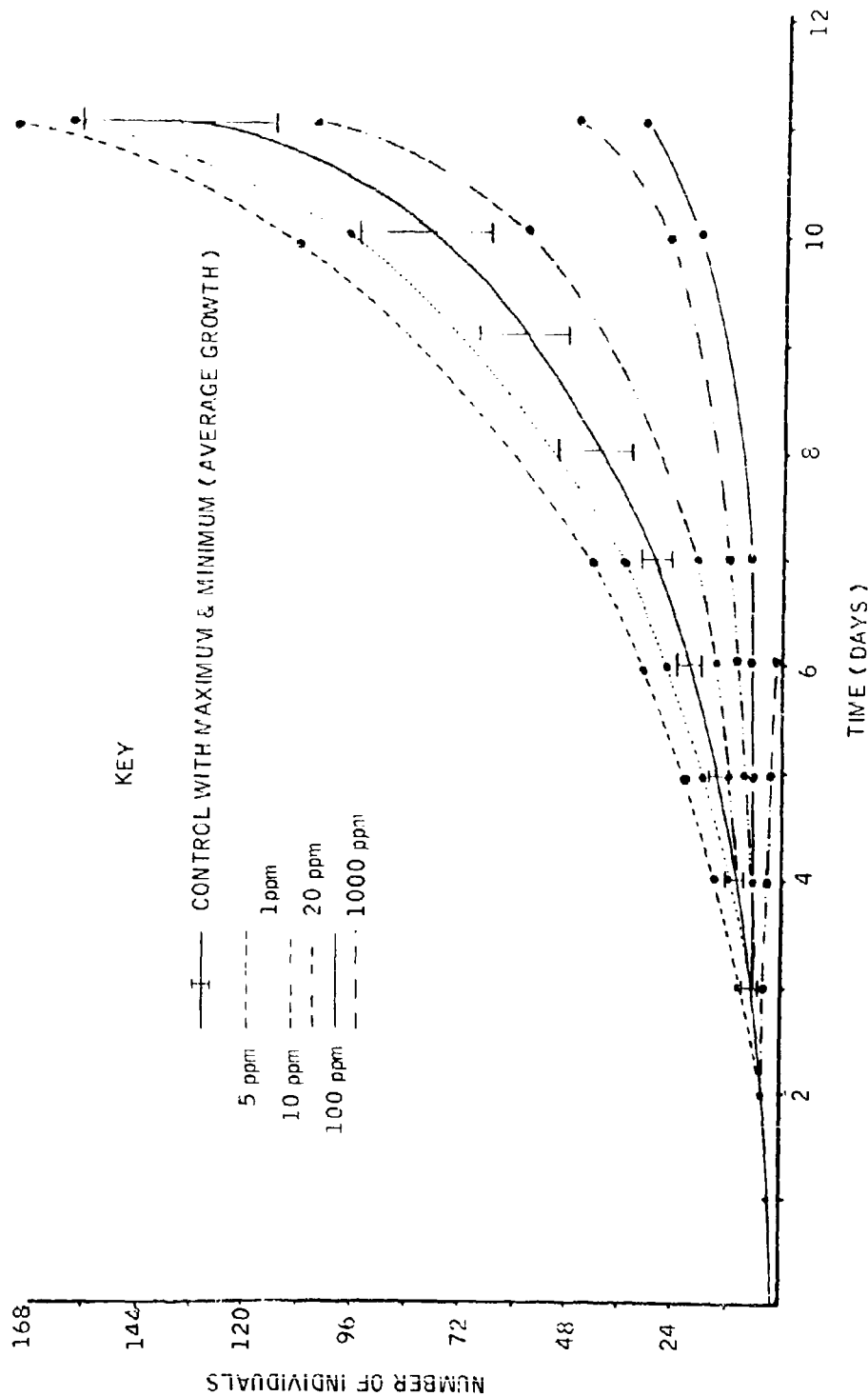


Figure 6. Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of Aldrin

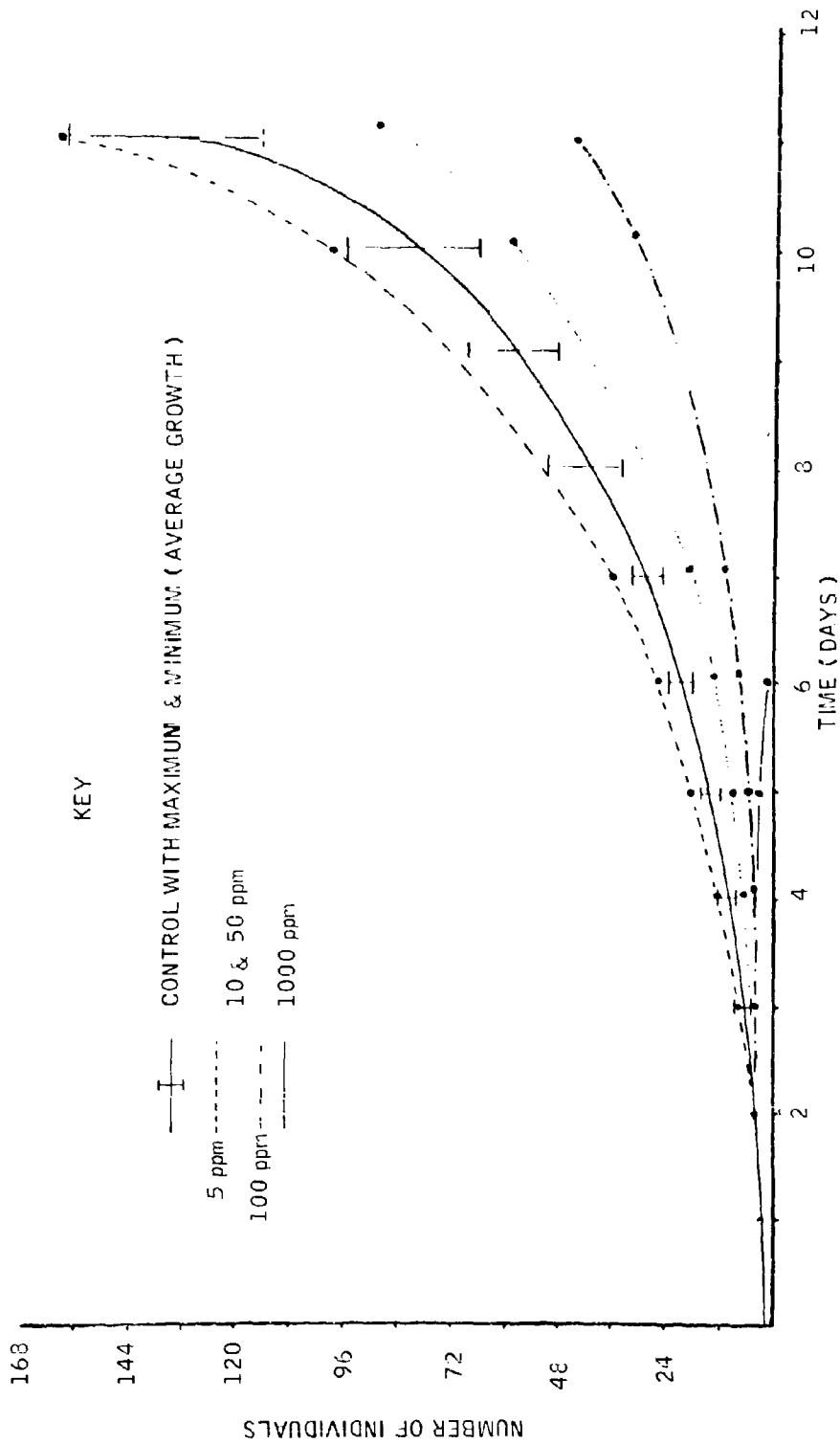


Figure 7. Growth Profile of *Wolffia papuujera* When Exposed to Varying Concentrations of Dieldrin

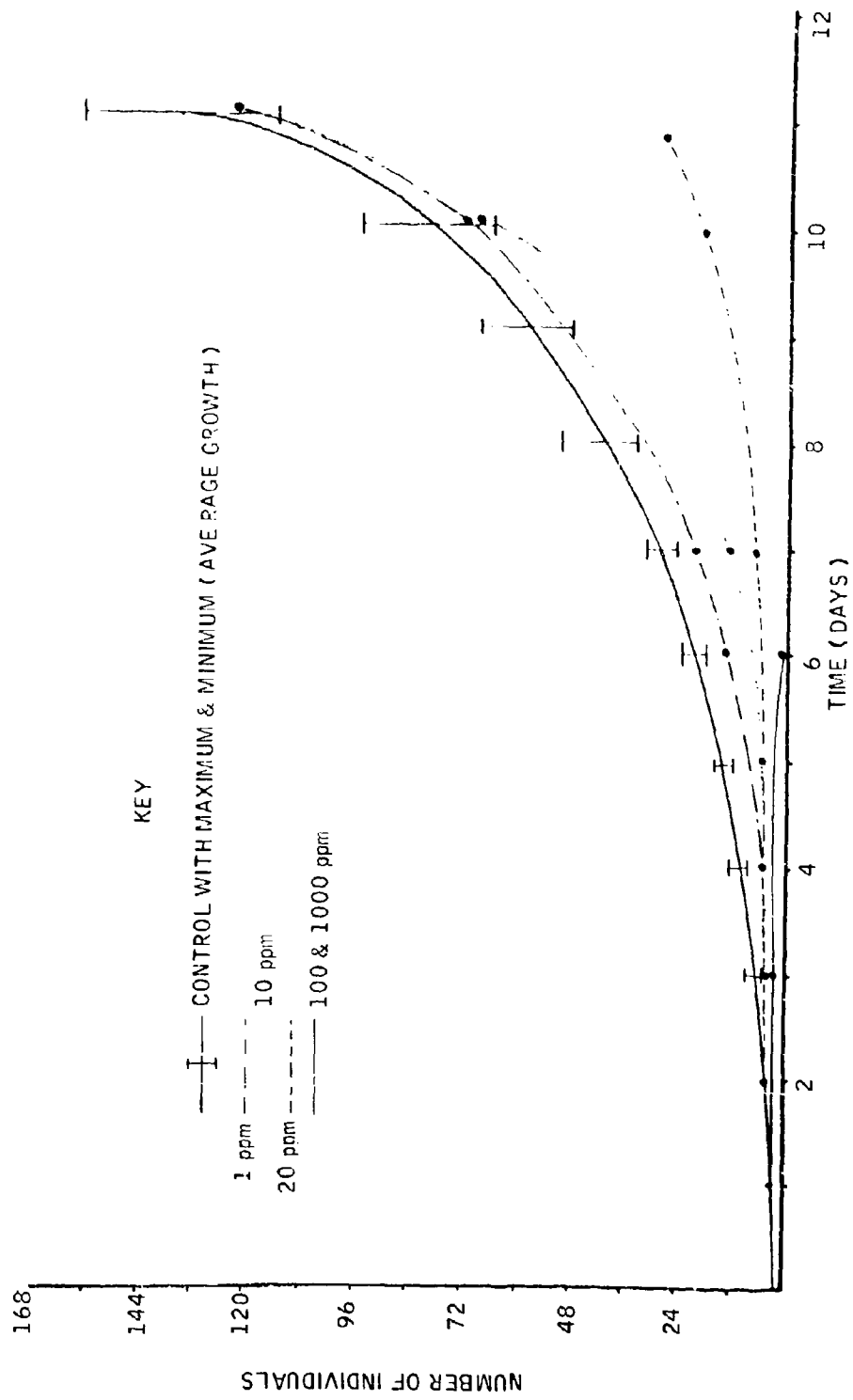


Figure 8 Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of Salinity

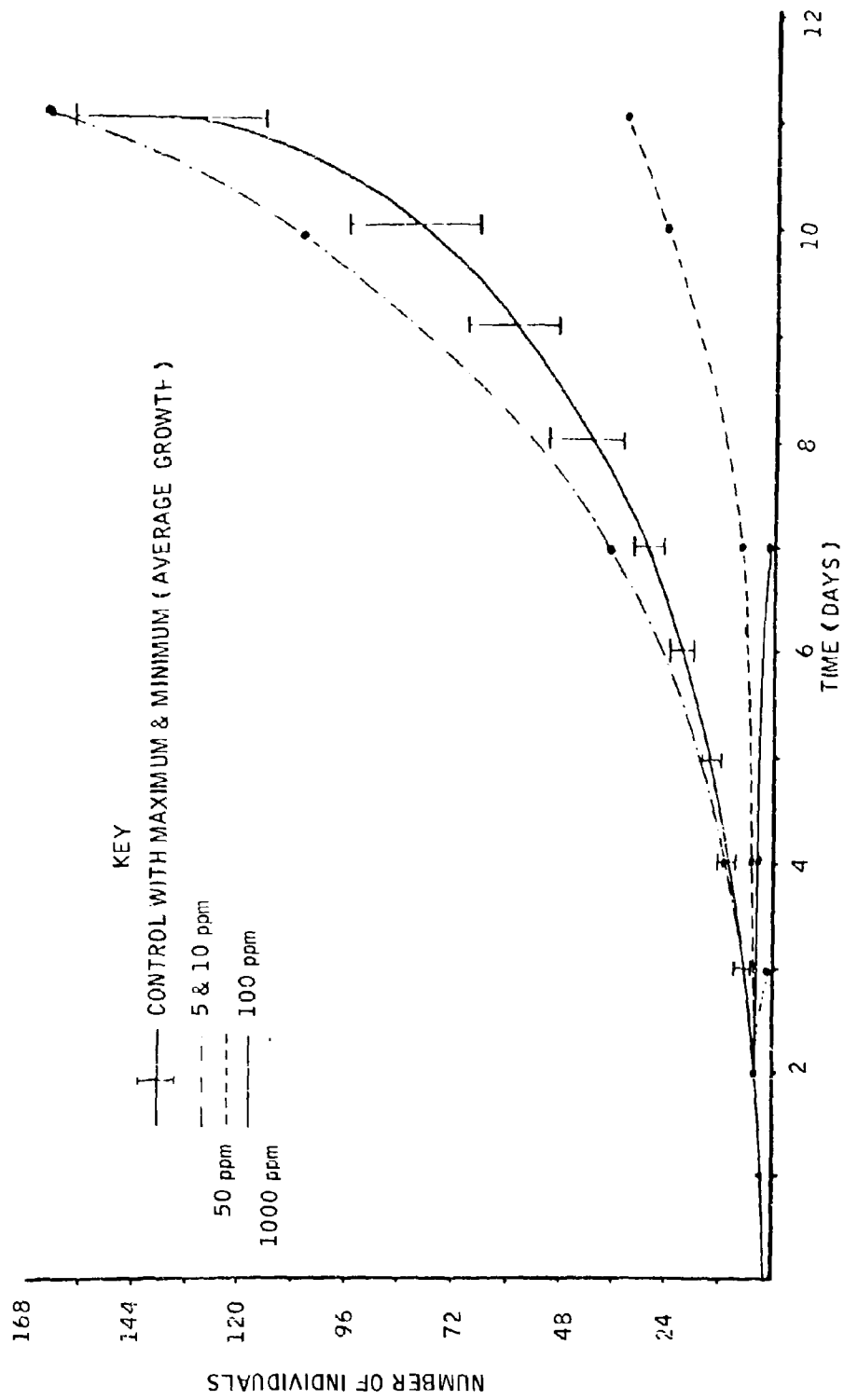


Figure 9. Growth Profile of *Wolffia pepulifera* When Exposed to Varying Concentrations of Diazinon

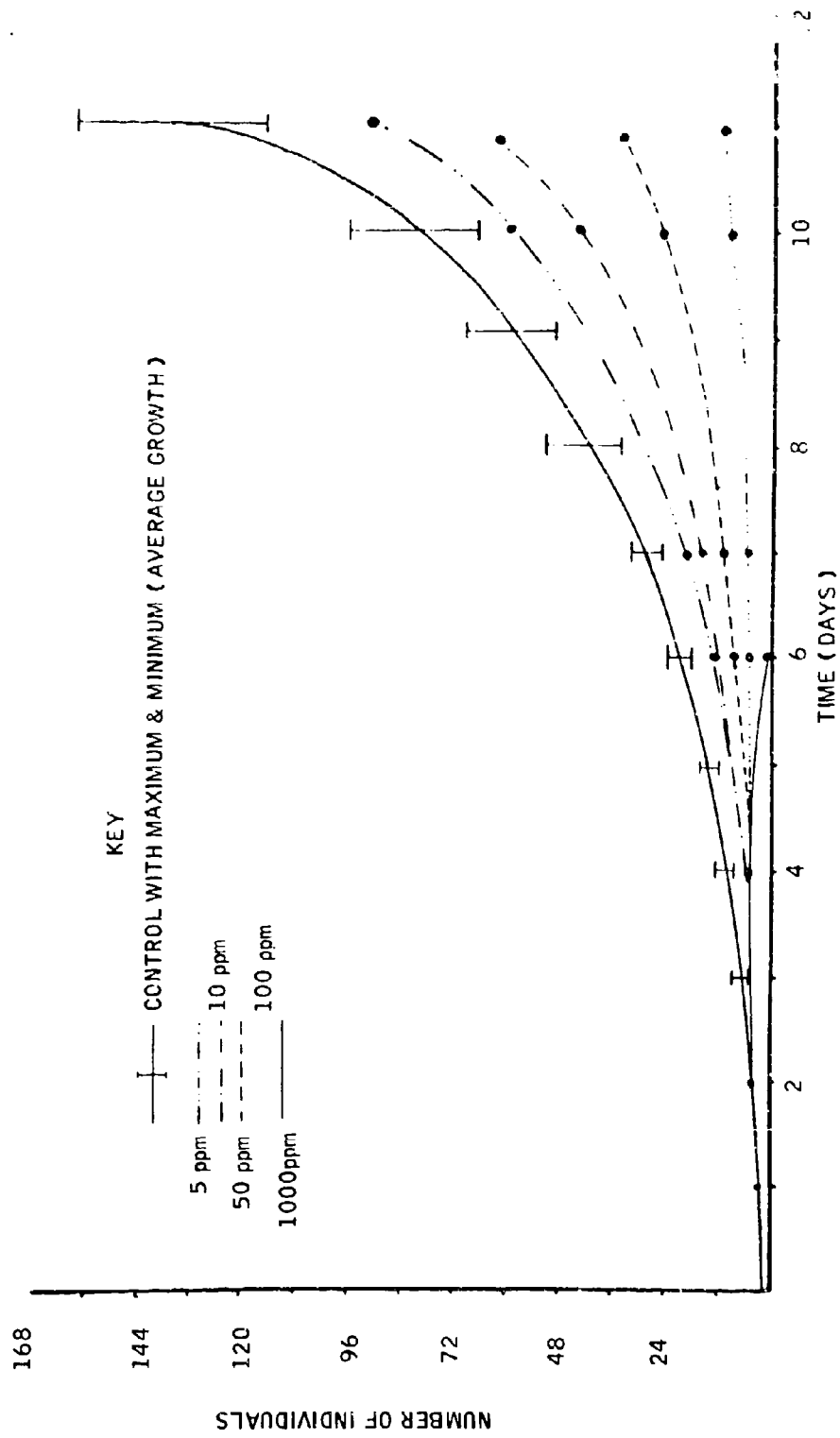


Figure 10. Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of Sewin

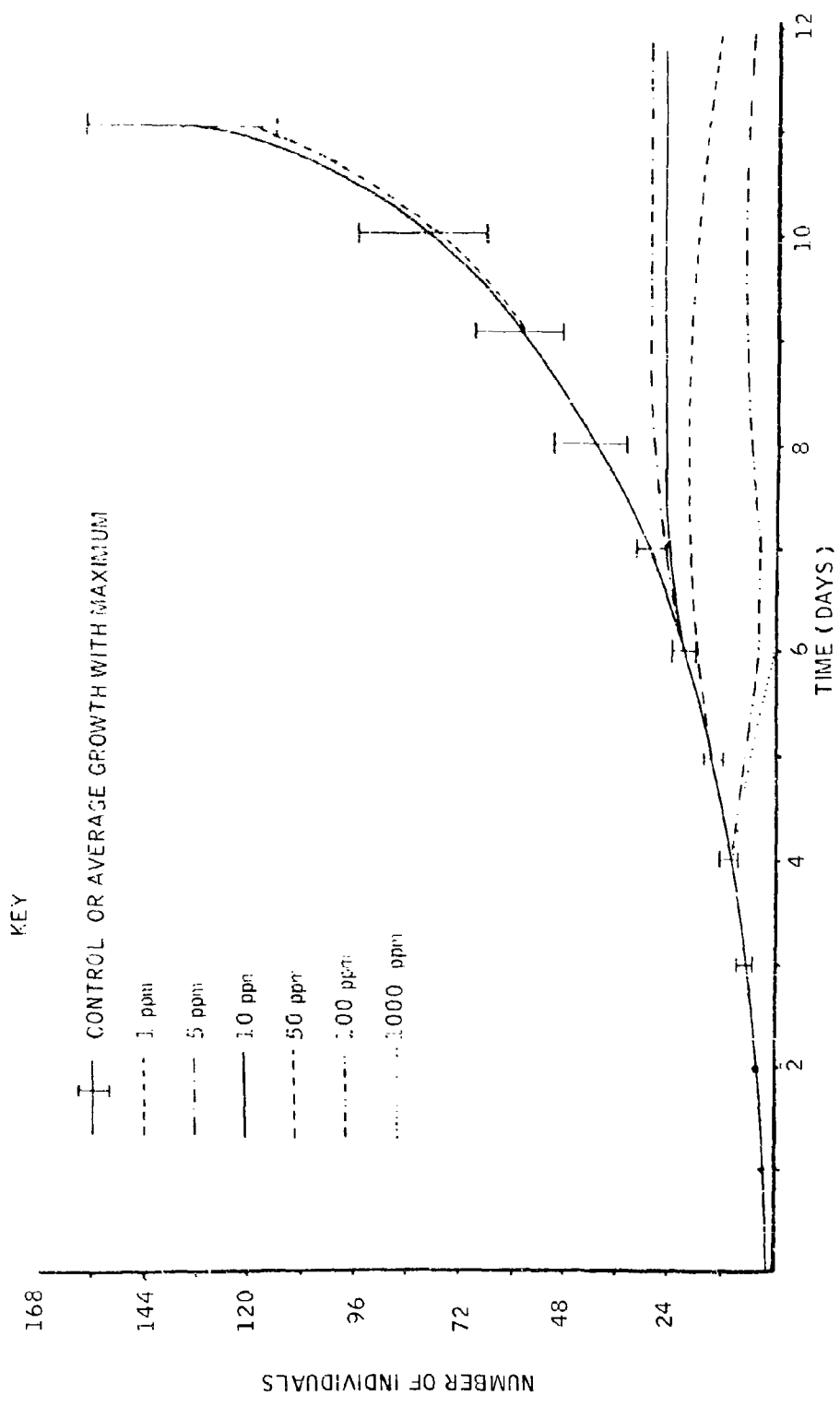


Figure 11. Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of 2,4-D

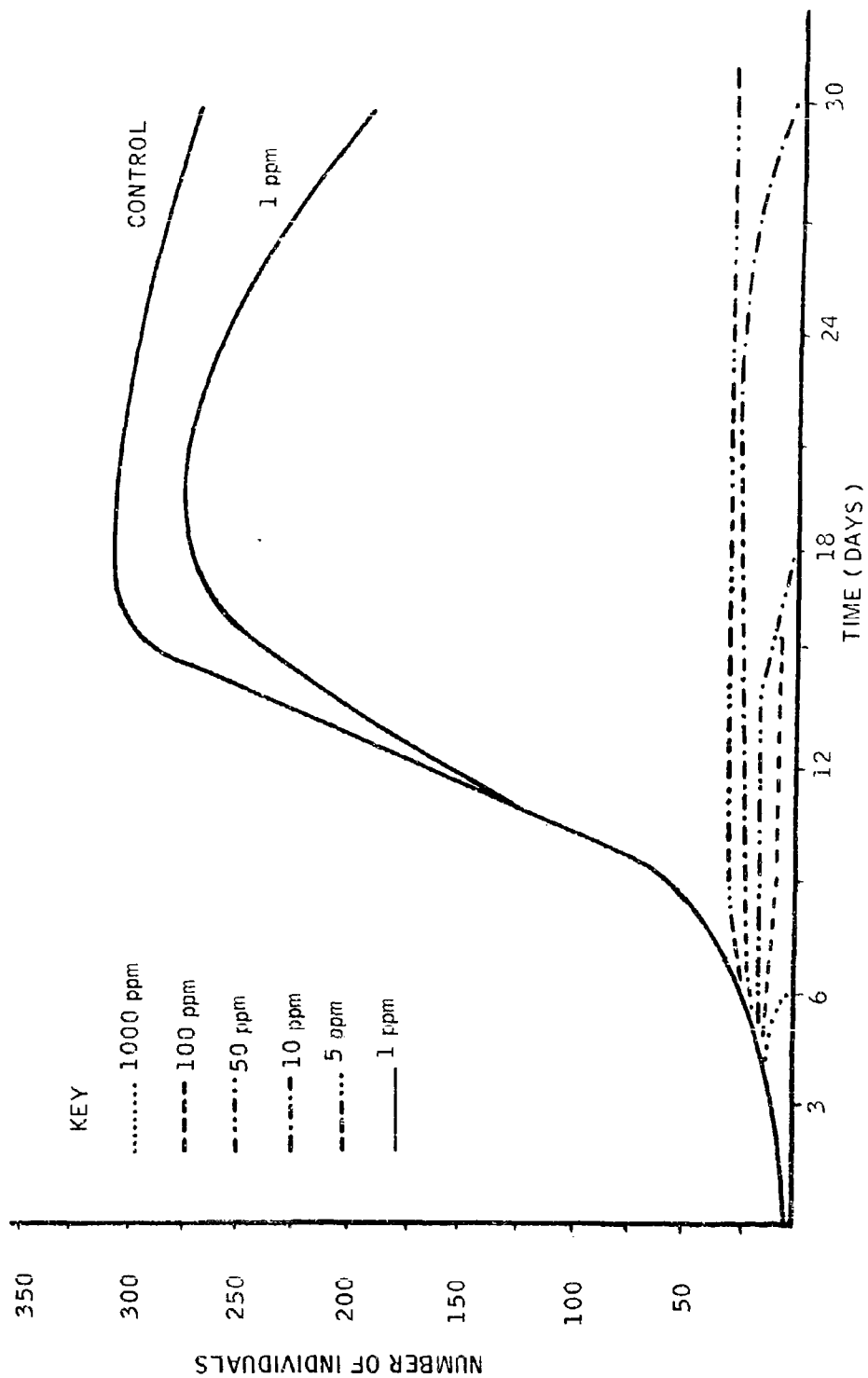


Figure 12. Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of 2,4-D for a Period of 30 Days

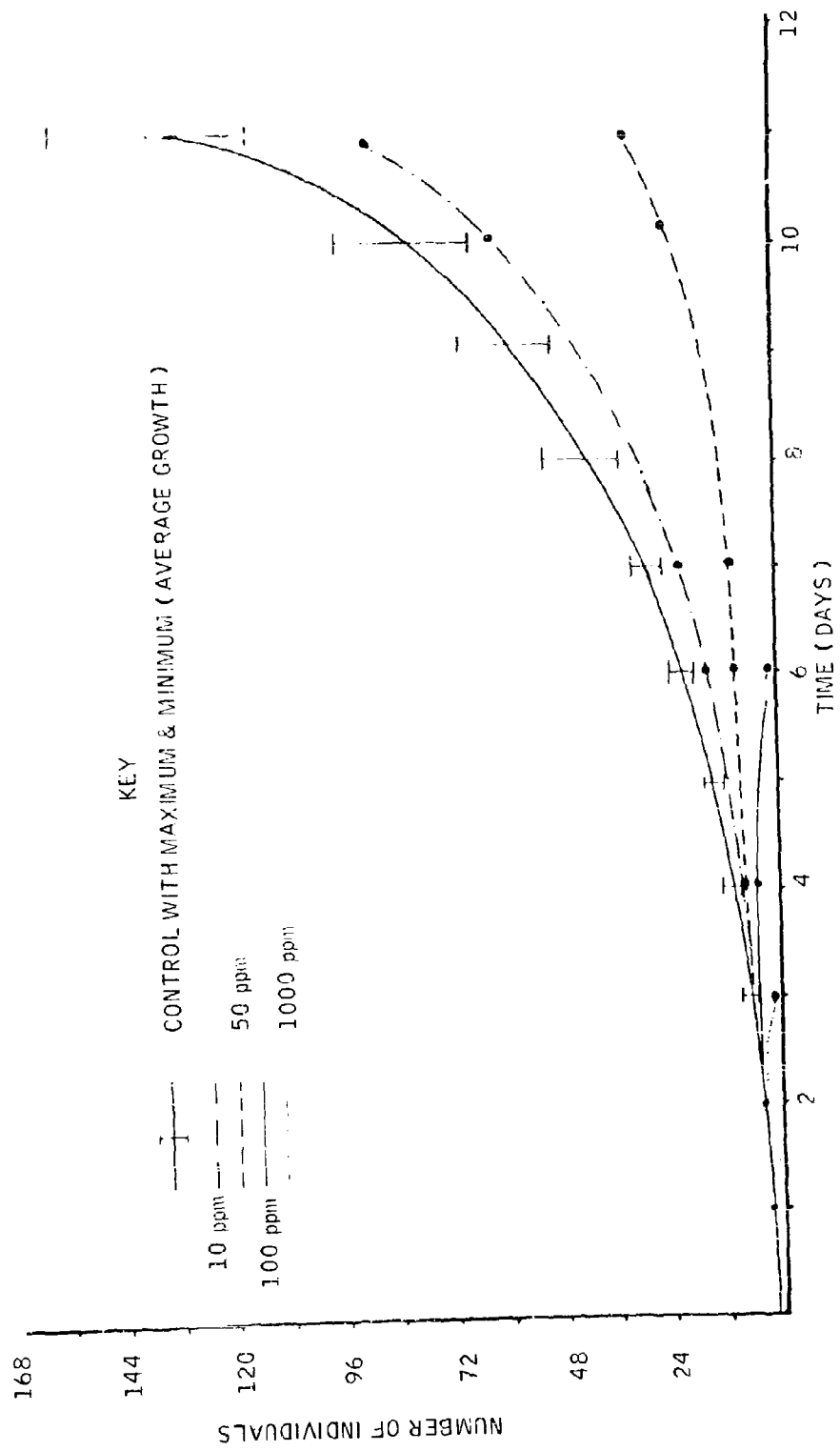


Figure 13. Growth Profile of *Wolffia papulifera* When Exposed to Varying Concentrations of Indole Acetic Acid

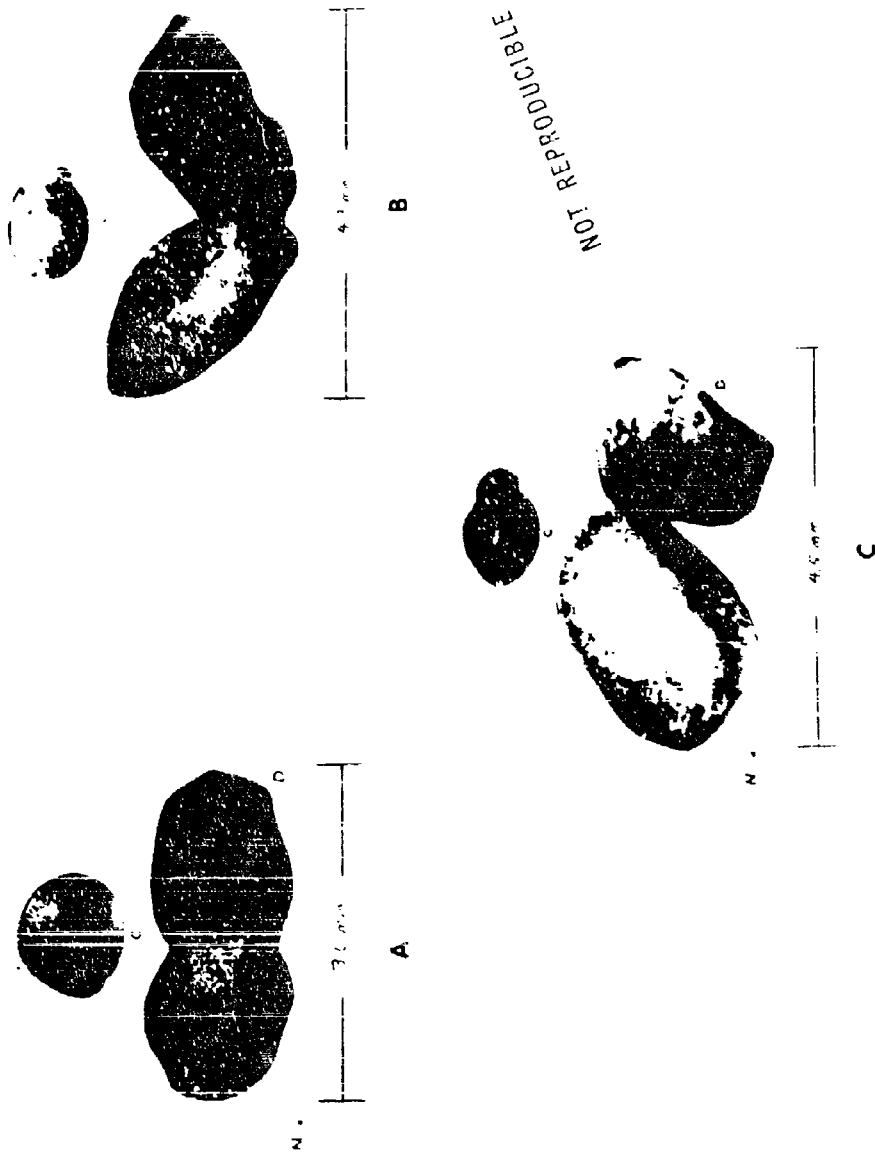


Figure 14. Dorsal (A and B) and Lateral (C) Views of Individual *Wolffia* 14 Days After Exposure to 0.1 ppm 2,4-D
 c - Control at similar stages; c - growth; N indicates the actual size of one individual *Wolffia*.

Table III. Comparison of Pollutant Susceptibility in Selected Plant and Animal Species

Compound	LD100 ^a				ED100	Photosynthesis ^f inhibition
	Mosquito ^b	Shrimp ^c	Fish ^d	Wolffia ^e	Wolffia ^e	
	<i>ppm</i>					
CS	-	-	-	100	10	-
DDT	0.01	0.04	0.02	100	10	0.1
Aldrin	0.03	-	0.04	1000	1	-
Dieldrin	0.01	0.02	0.01	1000	5	0.1
Malathion	0.03	-	12, 1.0, 0.01	100	1	-
Diazinon	0.01	-	0.5	100	5	-
Sevin	-	0.02	-	1000	5	-
2,4-D	-	-	5.0	1000	0.1	-
IAA	-	-	-	100	5	-

^aThis is the 100% lethal dose or LD100.

^bAll tests utilized *Anopheles quadrimaculatus* Thom., 4th instar, for test organism. Data from Negherbon, W. O. *Op. cit.*³

^cShrimp. Test organisms were white and brown shrimp. Data from Wilber, C. G. The Biological Aspects of Water Pollution. C. C. Thomas, Springfield, Illinois. 1970.¹⁵

^dTest organisms were the fathead minnow (*Pimephales promelas* Raf.), the mosquito fish (*Gambusia affinis* B. & G.), and the bluegill (*Lepomis macrochirus* Raf.). Results for all three species are the same when only one figure is listed except that only the bluegill was used as the test organism for Diazinon and 2,4-D. Data were obtained from: Wilber, C. G. *Op. cit.*¹⁵ Calley, D. D., Jr., and Ferguson, D. E. Patterns of Insecticide Resistance of the Mosquito Fish, *Gambusia affinis*. J. Fish. Bull. Canada 26, 2395-2401 (1969).¹⁶ Cope, O. B., Wood, E. M., and Wallen, G. H. Some Chronic Effects of 2,4-D on the Bluegill (*Lepomis macrochirus*). Trans. Amer. Fish Soc. 99, 1-12 (1970).¹⁷

^e*Wolffia* LD100 and ED100 values obtained from table II.

^fFour species of algae were used: *Skeletonema costatum* (Grev.) Cl., *Dunaliella tertiolecta* Butcher, *Coccolithus huxleyi* (Lohm.) Camptner, and *Cyclotella nana*. Data from: Menzel, D. W., Anderson, J., and Radtke, A. *Op. cit.*¹²

This suggests that there may be more sensitive plants that can be used in assessment of pollutant contamination.

V. CONCLUSIONS.

Varying concentrations of nine potential pollutants were tested for effects in vitro against colonies of *Wolffia papulifera*. Death was observed in colonies of *Wolffia* exposed to 100 ppm or above of CS, DDT, Malathion, Diazinon, and IAA; and to 1000 ppm of Aldrin, Dieldrin, Sevin, and 2,4-D. No effects were seen in *Wolffia* colonies exposed to 5 ppm or below of CS, DDT, and IAA; 1 ppm of Dieldrin, Diazinon, and Sevin; 0.1 ppm of Aldrin and Malathion; and 0.01 ppm of 2,4-D. Teratogenic effects were observed in *Wolffia* colonies exposed to Malathion at 1 ppm, 2,4-D at 0.1 ppm and above, and Diazinon at 10 ppm.

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