THE EFFECTS OF COPPER, ZINC AND CADMIUM ON *SELENASTRUM CAPRICORNUTUM*

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ABSTRACT

The algicidal and algistatic effects of copper, zinc and cadmium on *Selenastrum capricornutum*, a unicellular green alga were analyzed by using a modification of the Algal Assay Procedures Bottle Test.

Algicidal concentrations of copper, zinc and cadmium were 0.30, 0.70, and 0.65 mg/l. Treatment of *Selenastrum* with various concentrations of the metals resulted in similar growth rates characterized by extended lag growth phases.

Combinations of copper, zinc and cadmium were similar in toxicity to equal concentrations of zinc. Combinations of copper and cadmium resulted in a greater growth rate than equal concentrations of copper suggesting that cadmium may inhibit copper toxicity.

Selenastrum was able to exist in waters from the upper South Fork and North Fork of the Coeur d'Alene River where zinc and other metals were in low concentration. However, *Selenastrum* was not able to grow in water from other parts of the drainage where zinc concentrations were greater than 0.041 mg/l. These observations were consistent with laboratory findings where 0.7 mg/l zinc was algicidal and 0.12 mg/l inhibited the growth of *Selenastrum*.

INTRODUCTION

The Coeur d'Alene River and lake drainage has received metallic sulfide, mineral wastes and mine tailings for the past 80 years. Ellis (1932) working at the mouth of the Coeur d'Alene River made a general survey of the toxic effects of the water on plankton and fish. A number of more recent Coeur d'Alene River system studies, supported by the Office of Water Resources Institute, were initiated in 1969 at the University of Idaho with participation from the Environmental Engineering Section at Washington State University.

Sappington (1970) reported 96 hr. T_{lm} values of 0.09 mg Zn/l for cutthroat trout after adding zinc to the unpolluted North Fork Coeur d'Alene River water. Savage and Rabe (1973) observed that macrobenthic diversity of riffle areas in the Coeur d'Alene River was much less than in adjacent North Fork waters. They attributed this in part to the effects of mine wastes in the waters. Wissmar (1972) reported that zinc, copper and cadmium were acutely and synergistically toxic to carbon uptake by phytoplankton in Lake Coeur d'Alene.

In 1969 a joint industry/government task force on eutrophication (EPA, 1971) proposed that standardized algal growth tests be adopted. Ideally, these tests would provide a means of comparison between cooperating laboratories and geographical areas. In this study, these tests were extended to compare the growth rates of algae cultured in synthetic medium to those cultured in waters from the Coeur d'Alene River system. In this way the toxic effects of a natural water supply contaminated by mineral wastes was compared to that of a synthetic

growth medium containing metals of known concentration.

The principle objective was to determine what algicidal and algistatic effects copper, zinc and cadmium had on the growth rate of *Selenastrum capricornutum*, a unicelluar green algae of the family Oocystaceae. This would provide a reference to compare the toxicity of Coeur d'Alene River water or other river systems polluted by mineral wastes, to that of a synt[illegible]dium.

The physical and c[illegible]ical characteristics of the river and lake system are presented in Table 1 (Wissmar, 1972). The average total alkalinity of the water, expressed as methyl orange alkalinity, is 17 mg/l. As a result, the toxic effect of heavy metals on the biota can be expected to be relatively great because of negligible interference or antagonism by [illegible]rbonates.

Table 1 — Physical and chemical characteristics of the Coeur d'Alene River and Lake, May, 1969 to November, 1970 (Wissmar, 1972)

Factor	N[c]	Coeur d'Alene River			Coeur d'Alene Lake		
		Mean	S.E.[b]	Range	Mean	S.E.	Range
Sodium (mg Na/l)	26	3.40	±0.20	1.4 - 5.1	1.7	±0.1	0.1 - 2.5
Potassium (mg K/l)	26	0.90	±0.05	0.5 - 1.4	0.7	±0.03	0.5 - 1.1
Magnesium (mg Mg/l)	32	3.20	±0.30	0.4 - 6.4	1.5	±0.1	0.2 - 2.5
Calcium (mg Ca/l)	32	7.80	±0.70	0.6 - 13.1	4.4	±0.3	0.5 - 7.1
Copper (mg Cu/l)[a]	28	0.10	±0.02	0.0 - 0.6	0.10	±0.02	0.0 - 0.4
Iron (mg Fe/l)	32	0.30	±0.09	0.0 - 2.0	0.3	±0.09	0.3 - 2.0
Manganese (mg Mn/l)[a]	32	0.60	±0.09	0.01 - 1.90	0.10	±0.01	0.0 - 0.3
Zinc (mg Zn/l)[a]	32	2.70	±0.40	0.1 - 11.2	0.40	±0.05	0.01 - 1.00
Cadmium (mg Cd/l)[a]	18	0.02	±0.005	0.01 - 0.05	0.010	±0.001	0.01 - 0.02
Lead (mg Pb/l)[a]	32	0.20	±0.07	0.0 - 1.9	0.20	±0.07	0.0 - 1.7
Nitrate (mg NO_3/l)	30	0.50	±0.10	0.0 - 3.1	0.20	±0.06	0.0 - 1.1
Phosphate (mg PO_4/l)	30	0.30	±0.10	0.0 - 2.8	0.10	±0.03	0.0 - 1.7
Bicarbonate (mg HCO_3/l)	35	17.10	±0.40	13.0 - 25.0	17.2	±0.3	13.8 - 20.0
Total CO_2 (mg CO_2/l)	35	5.50	±0.20	3.9 - 11.1	5.0	±0.1	3.6 - 8.2
Dissolved oxygen (mg/l)	29	8.70	±0.50	4.8 - 11.6	8.0	±0.4	5.2 - 13.6
B.O.D. (mg/l)	26	1.50	±0.20	0.3 - 3.8	1.6	±0.2	0.6 - 4.9
pH	35	——	——	6.4 - 7.3	——	——	6.6 - 8.2
Conductance (umho/cm at 25 C)	35	111	±6	<50 - 315	46	±3	<50 - 75
Temperature (Centigrade)	35	13.7	±1	2.0 - 21.7	12.7	±1.0	2.6 - 19.7
Secche disc (Meters)	35	2.3	±0.9	1.5 - 3.1	2.8	±0.1	0.8 - 4.9
Extinction coefficient ($\bar{\varepsilon}_\lambda$)	34	0.744	±0.046	0.437- 1.537	0.717	±0.049	0.408- 1.857

[a]Trace values of < 0.01 mg/l treated as 0.01 mg/l

[b]Standard error

[c]Number of observations

MATERIALS AND METHODS

Growth and maintenance of algal cultures follow procedures outlined in the Algal Assay Procedures Bottle Test (EPA, 1971). The bottle test is intended primarily for assessment of eutrophic conditions and is based on Liebig's law of the minimum which states that growth is limited by the substance that is present in minimal quantities with respect to the needs of the organism. My research deals with heavy metals, that when present in excess limit the growth of the organism. Consequently, the procedures were modified to fit the objectives of the research.

Chemical constituents of the synthetic medium are listed in the bottle test procedures. Chemical characteristics of the medium resembles a soft water lake with adequate nutrients to support algal growth. The medium is divided into two groups of nutrients, macronutrients and trace elements.

Macronutrient stock solutions were made up individually to 100 times the final concentration and stored in polyethlene bottles. Trace elements, $FeCl_3$ and Na_2EDTA were combined in a single solution 100 times the final concentration. A new trace element solution was prepared periodically as a precautionary measure against possible chemical changes over time. A 10 ml aliquot of each solution was added to glass-distilled water to give a final volume of one liter.

Concentrations of stock solutions as well as the order of mixing were found to be critical. Preparation of stock solutions to 1000 times the final concentration results in precipitation of salts after a short

period of time and seriously affects the shelf life of the solutions. Stock solutions made to 100 times the final concentration provides a solution with a longer shelf life. The order in which the macronutrient solution is mixed must be closely adhered to. Stock solutions containing $CO_3^{=}$ and $PO_4^{=}$ must be added last and only after the others have been sufficiently diluted to prevent precipitation in the final solution.

Stock solutions of copper, zinc and cadmium were made up in 100 mg/l concentrations and stored in polyethylene bottles. Analytical grade $CuCl_3$, $ZnCl_3$ and $CdCl_3 \cdot 2\ 1/2\ H_2O$ were used in these solutions.

Alkalinity and pH were periodically monitored. The pH of day-old medium ranged from 7.1 to 7.2. Heavy metal additions to 0.7 mg/l did not lower the pH below 6.8. Alkalinity of the medium was 8.2 mg/l and the total hardness was 14.9 mg/l as $CaCO_3$.

All glassware was washed in a detergent solution and rinsed in deionized water before a final rinse in 10% HCL to remove nutrient traces which may have adhered to the surface of the glass. Glass distilled water was used for the final rinse. Clean glassware was prerinsed with the type solution before use.

A stock culture of *S. capricornutum* was obtained from the National Environmental Research Center, Corvallis, Oregon. Cells from the stock culture were transferred to liquid medium and subcultures were incubated four to five days at 24 ± 2 C. Cultures used in the tests were inoculated with cells from these subcultures. The subcultures were centrifuged in Goetz centrifuge tubes for ten minutes at 1000 g., the supernatant discarded and the cells resuspended in fresh medium. The overall volumes of the subcultures were adjusted so that 1 ml contained approximately

0.001 ml packed cell volume. Each "test" culture was inoculated with 1 ml of subculture.

Ideally, the test cultures for all three metals should be inoculated from the same subculture and run simultaneously. This would reduce the change of growing each group of cultures with a different bacterial flora that may affect the outcome of the experiment. In this study, space and facilities did not allow all test groups to be run at the same time. Therefore, a new subculture was prepared for each test group.

Test cultures were incubated on a shaker platform at 24 ± 2 C. with 380 ft-c light intensity at the liquid level. Five hundred ml Kimax flasks contained 100 ml of culture medium. Polyethylene foam stoppers were used to facilitate gas exchange. Agitation rate of the shaker was 70 to 80 rpm. The light source was six continually illuminated 48 inch Ken Rad "Pland Aid" flourescent tubes.

The source and intensity of light was determined to be an important factor concerning the reproduccability between cultures of the same test group. A New Brunswick incubation chamber equipped with a shaker platform was found to be inadequate due to wide variations in light intensity at the platform surface. An overhead lighting system was constructed to insure that the light intensity was uniform at the liquid level of the culture flasks. The 48 inch flourescent tubes were long enough so that the shaker platform could be positioned under the center portion of the tubes where the light intensity was more uniform.

The method of biomass monitoring was selected from sources outside the Algal Assay Procedures. This method provides means of monitor-

ing a culture's growth without sacrificing any portion of it.

Cultures were assayed on a daily basis by the packed cell volume method of Oswald and Gaonkar (1969). Test cultures were centrifuged at 1000 g for 10 minutes in Goetz centrifuge tubes with a stem volume of 0.2 ml in 0.01 ml graduations. These were cultured in triplicate and the packed cell volume averaged.

Waters of the Coeur d'Alene River system were filtered through HA 0.45 micron Milipore® filters and stored at 4 C in polyethylene bottles. The samples were analyzed for copper, zinc and cadmium with a Perkin-Elmer Model 303 atomic absorption spectrophotometer. Zinc and cadmium concentrations above 0.01 mg/l were determined by direct aspiration. Copper and cadmium concentrations below 0.01 mg/l were determined by ammonium-pyrrolidine-di-phiocarbamate-methyl-isobutyl-ketone extraction (FWPCA, 1969).

Macronutrient additions to the river and lake waters were made up to 1000 times the final concentration. $MgSO_4 \cdot 7H_2O$ and $CaCl_2 \cdot 2\ 1/2\ H_2O$ were prepared as individual solutions. The remaining salts were combined into a single stock solution. Immediately after preparation, one ml of each solution was added to one liter of water from each collecting point to insure nutrients were available and to minimize dilution of the sample.

Methods for determination of algicidal concentrations of toxicants are not provided for in the procedures. The method chosen is a simple positive/negative test that indicates, within 50 ug, the concentration at which a metal is algicidal in AAPBT medium.

Determination of algicidal concentrations follow the method

proposed by Fitzgerald and Faust (1963). Two hundred and fifty ml flasks containing 50 ml of spiked medium were inoculated with 1 ml of inoculum culture. Triplicate flasks were incubated seven days and the cells centrifuged in Goetz centrifuge tubes, washed with stock medium and reinoculated into 50 ml of stock medium. Visible growth at the end of a second seven day period indicated a positive test. Concentrations of copper, zinc and cadmium, initially of 100 ug/l, were increased in 50 ug/l increments until negative results were observed. A control consisting of noninoculated medium was incubated simultaneously to check for contaminated stock medium.

RESULTS AND DISCUSSION

The concentrations of copper, zinc and cadmium toxic to *S. capricornutum*, in AAPBF medium, are listed in Table 2. Copper was found to inhibit growth completely at 90 ug/l and was algicidal at 300 ug/l. Growth inhibition for zinc began at 30 ug/l and zinc was found to be algistatic at 120 ug/l while 700 ug/l was algicidal. Cadmium began to supress growth of *Selenastrum* at 50 ug/l, was algistatic at 80 ug/l and algicidal at 650 ug/l.

Comparison between the growth rates of cultures treated with copper, zinc and cadmium are presented in Figures I through III. The cultures were assayed from day 2 through day 7 of the incubation period. The smallest amount of biomass that can be measured by this method is 0.01 ml packed cell volume. Therefore, packed cell volume less than 0.01 ml is estimated and reported in Figures I through VI and Figure VIII between dashed lines. The maximum standing crops of the control cultures were reached in 4 to 5 days and those maintained beyond 8 days degenerated rapidly necessitating a test period of 7 days.

The most noticeable effect on the growth rate is a extension of the lag growth phase as metal concentrations are increased. For copper, 50 and 60 ug/l results in a 24-hour extension of the lag growth phase, 70 ug/l results in a 48-hour extension and 80 ug/l in a 144-hour extension. Zinc and cadmium cultures result in similar extensions (Figures II through III).

Copper and cadmium treated cultures produce "near-normal" growth rates upon resumption of growth (Figures I and III). Zinc treated

Table 2

Toxic levels (ug/l) of copper, zinc and cadmium for *S. capricornutum* in AAPBT medium

Element	Incipient Growth Inhibition	Complete Growth Inhibition	Algicidal
Copper	50	90	300
Zinc	30	120	700
Cadmium	50	80	650

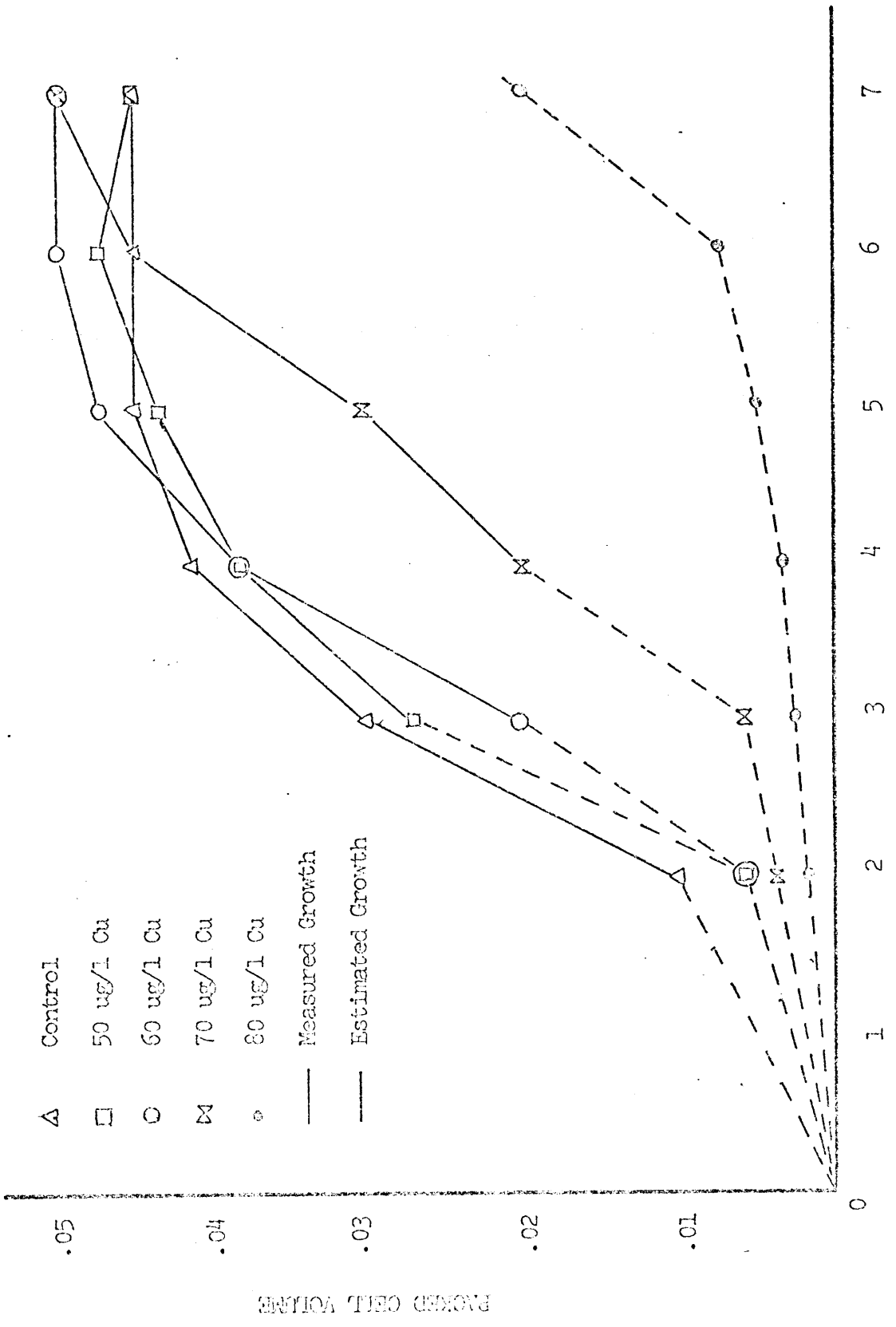

FIGURE I EFFECT OF COPPER ON THE GROWTH OF *S. capricornutum* in AAPST medium

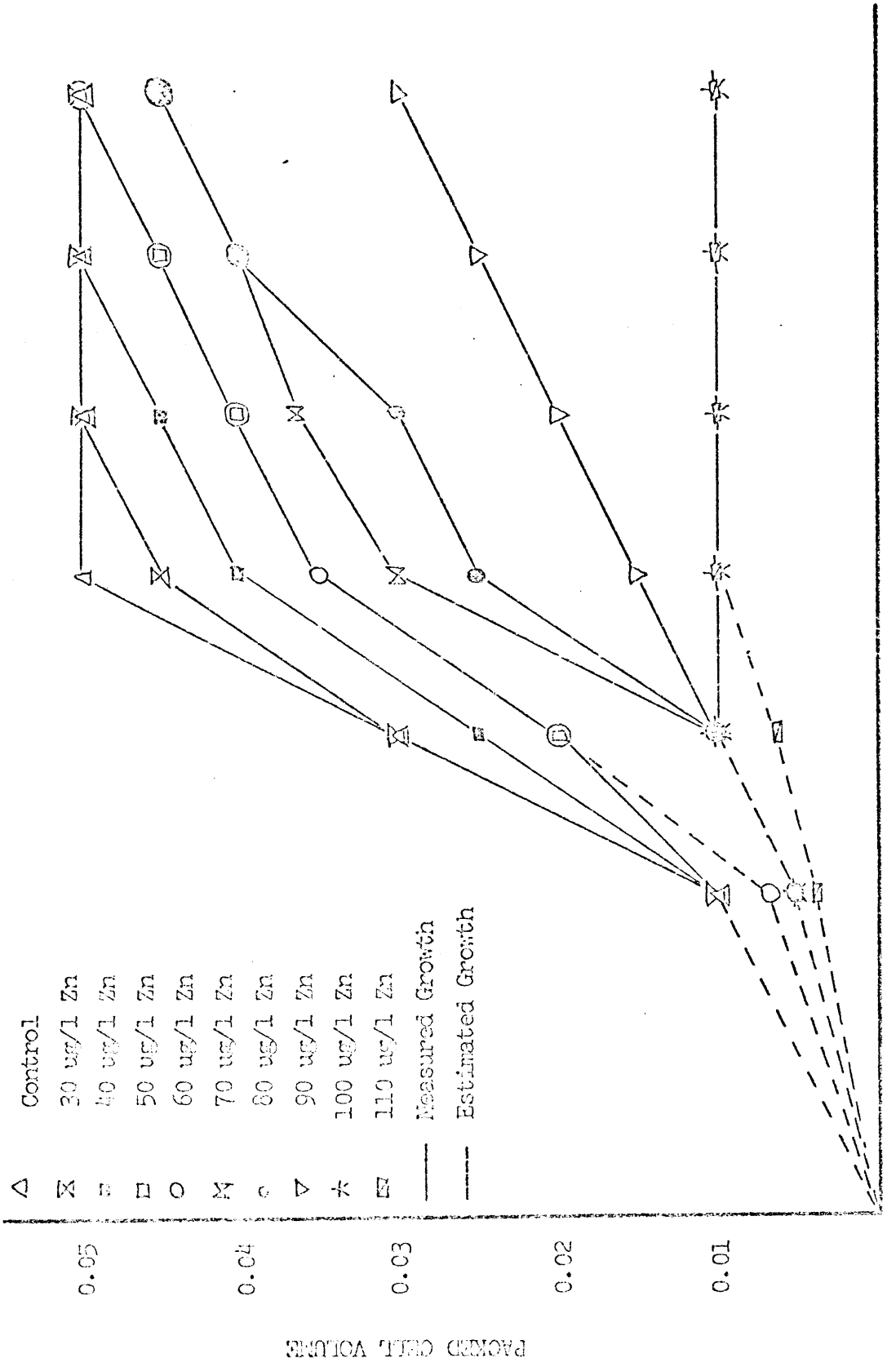

FIGURE II EFFECT OF ZINC ON THE GROWTH OF *S. capricornutum* in AAPBT medium

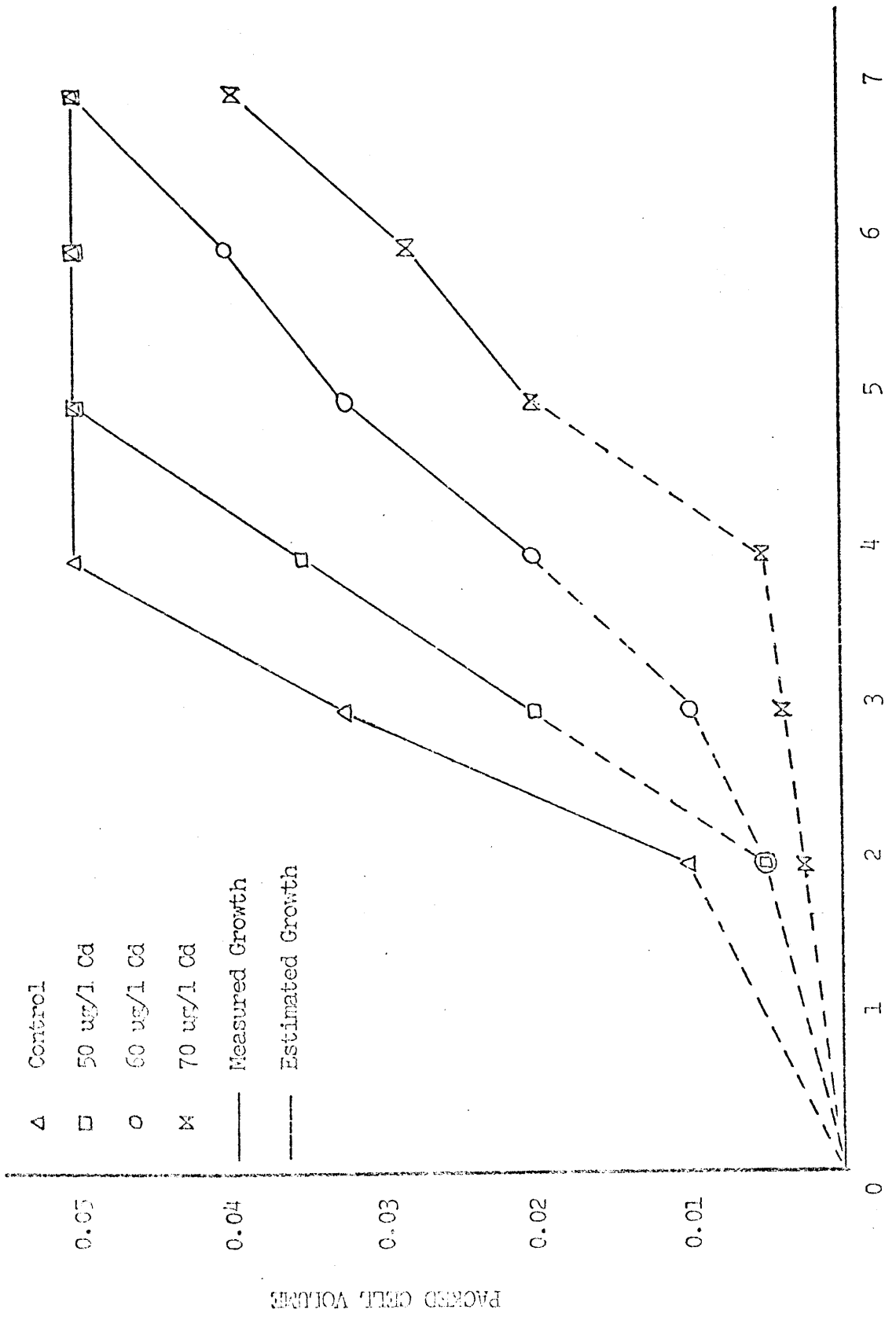

FIGURE III EFFECT OF CADMIUM ON THE GROWTH OF *S. capricornutum* in AAPBT MEDIUM

cultures did not resume "near-normal" growth rates but were depressed with increasing zinc concentrations. For example, cultures treated with 30 ug/l are similar to the control cultures while cultures treated with 110 ug/l stabilized at approximately 0.01 ml packed cell volume throughout the 7 day test period (Figure II).

Of the three elements tested, copper is the most toxic. However, in relation to copper, lower concentrations of zinc will cause growth rate inhibition (Table 2) and lower concentrations of cadmium will completely inhibit growth. Algicidal concentrations of zinc and cadmium both exceed twice that of copper (Table 2). This suggests that the toxicity of these elements may be governed by their metabolic roles. Morgan and Lackey (1958) and Steeman-Nielsen, *et al.* (1969) discuss the effects of copper on proteins and photosynthesis. Ting kai Li (1966) discusses the role of zinc in metalloenzymes.

The variation in the toxicity of copper, zinc and cadmium may be due to the tightness in which copper and other heavy metals bond with protein and enzymes. Morgan and Lackley (1958) report that in living organisms copper and other heavy metals combine with -SH groups to form a complex and deprive the organism of the -SH groups necessary for metabolism. This is effectual in precipitating and coagulating protein structures, many of which are denatured. They believe another denaturing mechanism is brought about by the presence of a peptide bond -CONH-, where a copper ring is formed by the coordination of the copper ion with the peptide bond. The copper in this ring is joined to the oxygen by a coalesing, covalent bond and the other valence requirement for the copper is satisfied by an -OH- radical. Denaturation is sometimes reversible

for most protein after exposure to low concentrations of metals but enzymes are reported to be almost always permanently denatured (Morgan and Lackey, 1958). This may be an explanation for the differences observed in the recovery ability of *Selenastrum* after exposure to the various concentrations of copper, zinc and cadmium.

Steeman-Nielsen *et al.* (1969) reports that in *Chlorella pyrenoidosa*, copper does not at first penatrate into the cell but influences it by blocking mechanisms in the cell membrane in such a way that no division takes place. In addition, they observed a decline in the rate of photosynthesis after several hours which they attributed to the accumulation of photosynthethetic products which secondarily blocks photosynthesis. These observations may explain some of the observed effects of copper, zinc and cadmium on *Selenastrum*. Specifically, reduction of biomass over time as metal concentrations are increased. "Yellowing" of the algal cells were also observed after prolonged exposure to low concentrations of copper, zinc and cadmium.

Wissmar (1972) defines metal synergism as "resulting from simultaneous cooperation of separate elements which produce an effect greater than that of any metal taken alone." He observed that copper, zinc and cadmium were synergistic in combination when introduced to phytoplankton communities endemic to Coeur d'Alene Lake. His tests indicate that copper has an over-riding effect on zinc and cadmium and appears to be the principal toxic component.

The growth rates of *Selenastrum* treated with combinations of zinc and copper, zinc and cadmium and copper and cadmium are presented in Figures IV through VI. A 5:1, 5:2, 5:3 ratio of zinc and copper

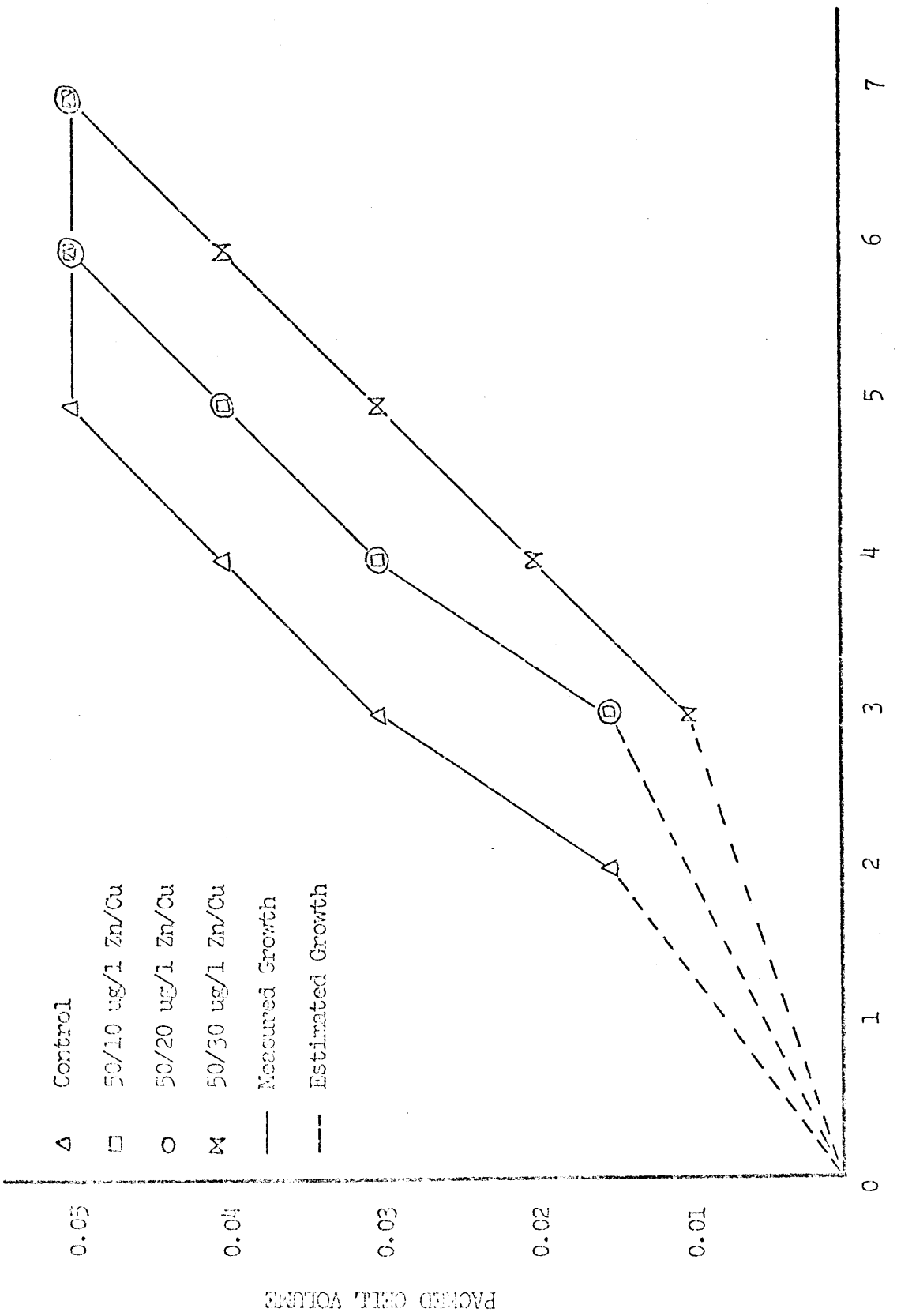

FIGURE IV EFFECT OF ZINC AND COPPER ON THE GROWTH OF *S. capricornutum* in AAPBT MEDIUM

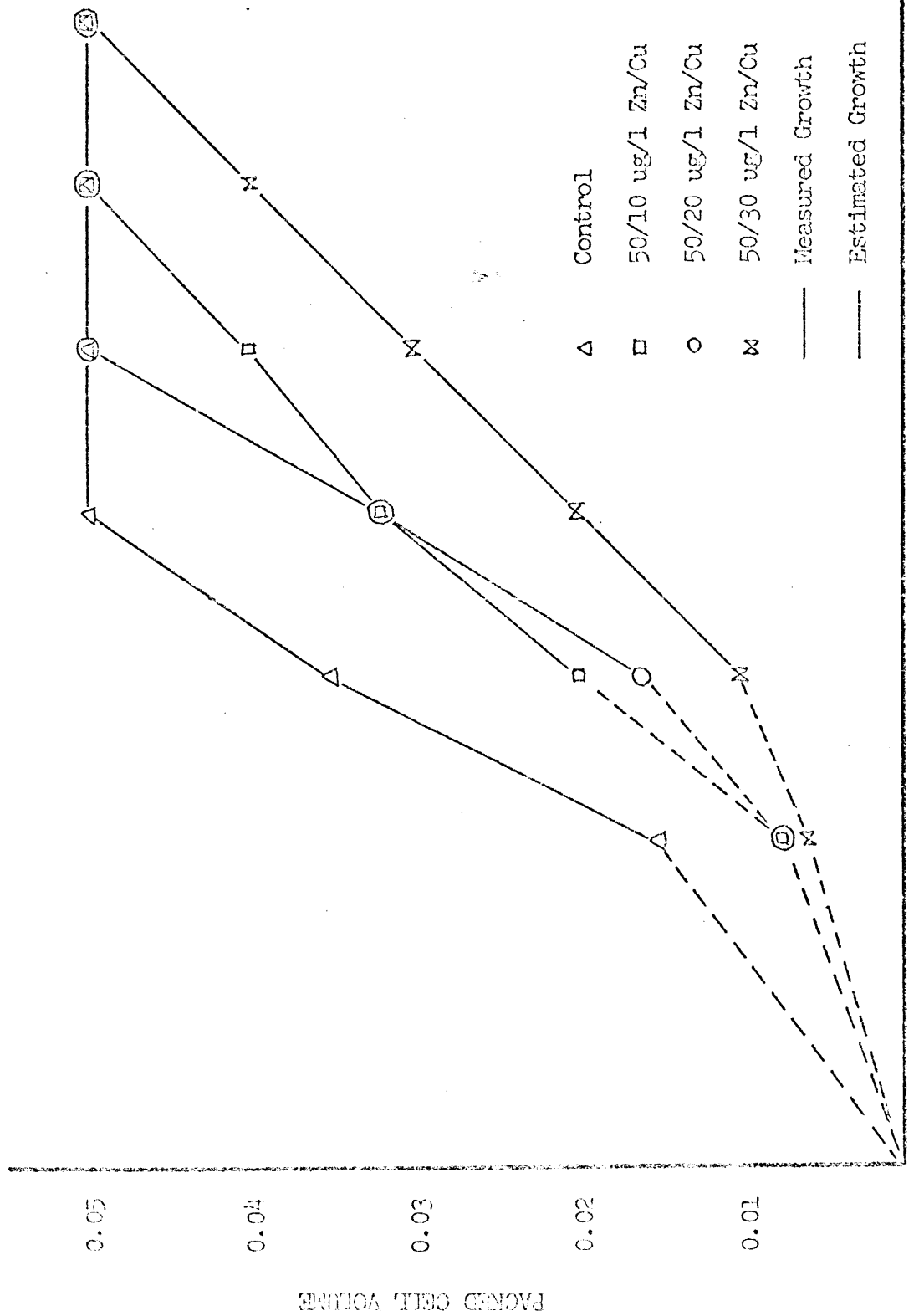

FIGURE V EFFECT OF ZINC AND CADMIUM ON THE GROWTH OF *S. capricornutum* in AAPBT MEDIUM

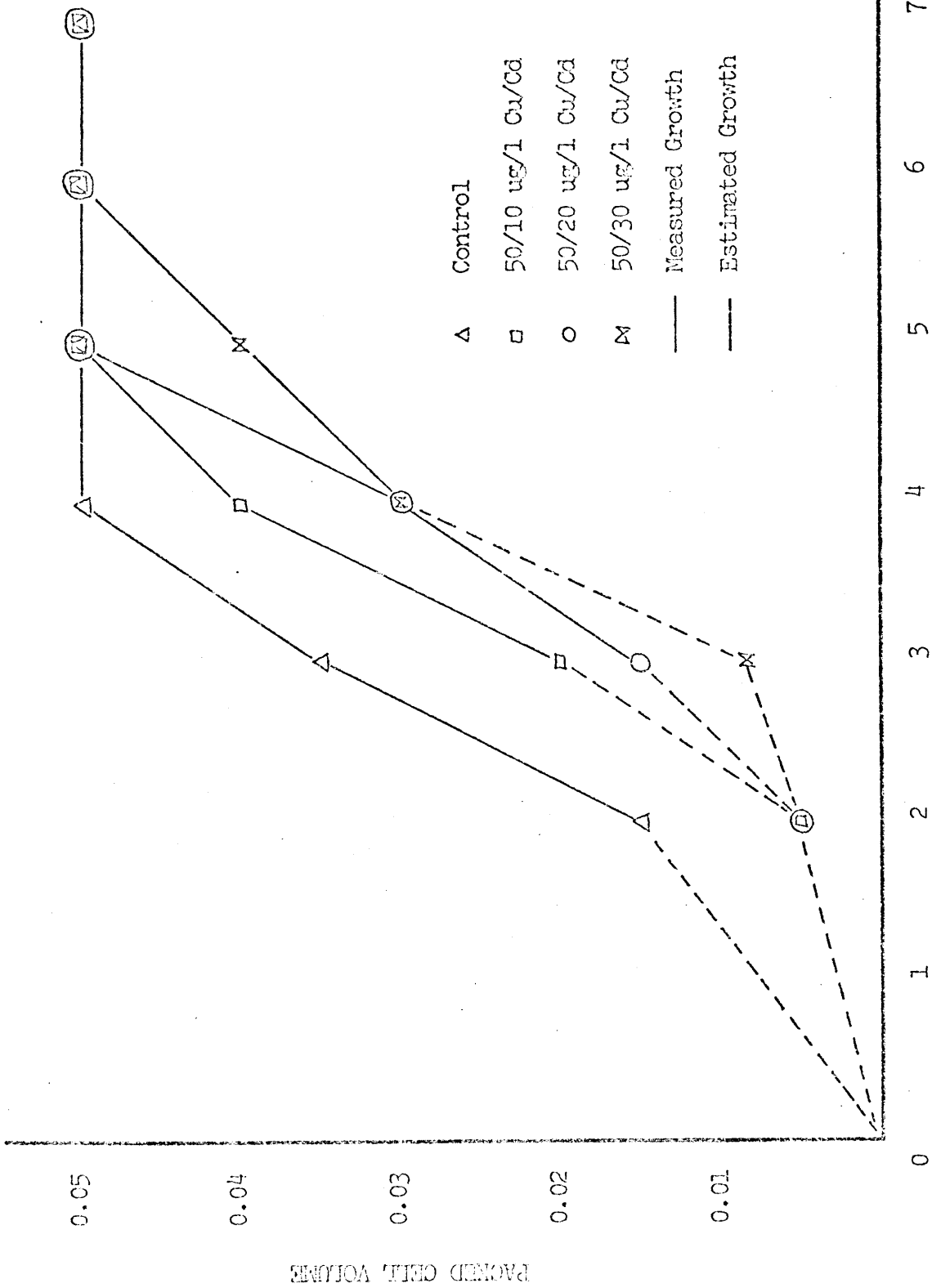

FIGURE VI EFFECT OF COPPER AND CADMIUM ON THE GROWTH OF *S. capricornutum* in AAPBT MEDIUM

(50 ug/l zinc + 10,20 and 30 ug/l copper) resulted in growth rates similar to 60,70 and 80 ug/l zinc. The same combinations of zinc and cadmium also resemble zinc. This indicates that copper and cadmium when in combination with zinc may not exhibit an over-riding effect on zinc. Combinations of copper and cadmium resulted in slightly more growth per equal time than the same concentration of copper. This observation is in opposition to that of Wissmar and suggests that in some cases cadmium in combination with copper may reduce the toxicity of copper.

Compareing the growth rates of algae grown in synthetic medium to those grown in natural waters is a relative comparison at best. The flow level and the presence of complexing agents in the Coeur d'Alene River system could effect the toxicity of metals found there. This is suggested because several genera of algae flourish in the South Fork and main rivers.

Algae collected at Station 3 include species of *Cladophora, Stigeoclonium, Ulothrix, Scenedesmus, Chaetophora* and *Euglena*. *Cladophora* appeared to be the dominant form. Palmer (1959) lists *Cladophora* as susceptible to copper and Whitton (1970) lists it as being sensitive to zinc and suggests that it be used as a pollution indicator. The highest dissolved zinc level recorded by Whitton from a stream containing *Cladophora* was 0.17 mg/l zinc. The highest dissolved zinc level recorded at Station 3 by Stokes and Ralston (1972) was 23.7 mg/l indicating that *Cladophora* can be tolerant to zinc. This study indicates *Selenastrum* is sensitive to zinc but its potential to acclimate to high zinc concentrations is unknown.

Zinc was chosen as the dominant metal in the combination tests because of high concentrations existing in the South Fork and main branch of the Coeur d'Alene River at low flow. Collecting sites on the river system are listed on Figure VII. Zinc concentrations at Stations 3 through 6 range from 0.55 mg/l in the lake to 19.1 mg/l in the South Fork of the river (Table 3). The South Fork above the source of pollution (Station 1) and the North Fork (Station 2) both have zinc concentrations less than [illegible].

The growth rate of *S. capricornutum* in Coeur d'Alene River and Lake waters is represented by Figure VIII. Of the Stations listed in Table 3, *Selenastrum* will grow only in water from Stations 1 and 2. Zinc concentrations at Stations 3 through 6 exceed those listed in Table 2 as completely inhibiting growth in synthetic medium.

Stokes and Ralston (1972) report that zinc is the only heavy metal found in Coeur d'Alene Lake in significant amounts. Transects of lake surface waters above and below the mouth of the river averaged 0.68 and 0.27 mg/l respectively. They reported the lake to be thermally stratified and zinc concentrations were at a seasonal low.

The concentration of zinc in surface waters of the lake at Station 6 compares favorably with the findings of Stokes and Ralston. The lake was thermally stratified at 10 meters and the reported zinc concentration assumed to be the seasonal low. Laboratory test using synthetic medium (Table 2) indicate that *Selenastrum* will not grow in water exceeding 0.12 mg/l zinc. Lake water from Station 6 contained 0.55 mg/l zinc and attempts to grow *Selenastrum* failed.

Table 3

Heavy Metal Concentrations in the
Coeur d'Alene River System
October 3, 1971

Station	Location	Concentration (mg/l)		
		Cu	Zn	Cd
1	Shoshone Park, Mullan, Idaho	0.0004	0.041	0.0014
2	N.F. Coeur d'Alene River 0.5 mi. above confluence	0.0004	0.016	0.0027
3	S.F. Coeur d'Alene River 0.5 mi. above confluence	0.0021	19.1	0.17
4	Cataldo, Idaho	0.0015	5.5	0.044
5	Coeur d'Alene River Delta	0.0004	1.13	0.010
6	Spokane Point, Lake Coeur d'Alene	0.0015	0.55	0.005

Table 4

Packed Cell Volume, *S. capricornutum*

		DAY					
	1	2	3	4	5	6	7
	Control	0.01	0.03	0.05	0.05	0.05	0.05
	30	0.01	0.03	0.045	0.05	0.05	0.05
	40	0.01	0.025	0.040	0.045	0.05	0.05
	50	0.01	0.02	0.040	0.040	0.045	0.05
	60	< 0.01	0.02	0.035	0.040	0.045	0.05
ug/l Zn	70	< 0.01	0.01	0.03	0.035	0.04	0.045
	80	< 0.01	0.01	0.025	0.03	0.04	0.045
	90	< 0.01	0.01	0.015	0.02	0.025	0.03
	100	< 0.01	0.01	0.01	0.01	0.01	0.01
	110	< 0.01	<0.01	0.01	0.01	0.01	0.01
	120	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01

	1	2	3	4	5	6	7
	Control	0.01	0.032	0.05	0.05	0.05	0.05
	50	< 0.01	0.02	0.035	0.05	0.05	0.05
ug/l Cd	60	< 0.01	0.01	0.02	0.032	0.04	0.05
	70	< 0.01	<0.01	<0.01	0.02	0.028	0.038
	80	< 0.01	<0.01	<0.01	<0.01	<0.01	0.02

	1	2	3	4	5	6	7
	Control	0.01	0.03	0.04	0.045	0.045	0.045
	50	< 0.01	0.027	0.037	0.042	0.047	0.045
	60	< 0.01	0.02	0.037	0.047	0.05	0.05
ug/l Cu	70	< 0.01	0.01	0.02	0.03	0.045	0.05
	80	< 0.01	<0.01	<0.01	<0.01	<0.01	0.02
	90	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 4 (Con't.)

		DAY					
	1	2	3	4	5	6	7
	Control	0.015	0.035	0.05	0.05	0.05	0.05
ug/l	50/10	< 0.01	0.02	0.032	0.04	0.05	0.05
Zn + Cd	50/20	< 0.01	0.016	0.032	0.05	0.05	0.05
	50/30	< 0.01	0.01	0.02	0.03	0.04	0.05

	1	2	3	4	5	6	7
	Control	0.015	0.035	0.05	0.05	0.05	0.05
ug/l	50/10	< 0.01	0.02	0.04	0.05	0.05	0.05
Cu + Cd	50/20	< 0.01	0.015	0.03	0.05	0.05	0.05
	50/30	< 0.01	< 0.01	0.03	0.04	0.05	0.05

	1	2	3	4	5	6	7
	Control	0.015	0.03	0.04	0.05	0.05	0.05
ug/l	50/10	< 0.01	0.015	0.03	0.04	0.05	0.05
Zn + Cu	50/20	< 0.01	0.015	0.03	0.04	0.05	0.05
	50/30	< 0.01	0.01	0.02	0.03	0.04	0.05

Coeur d'	1	2	3	4	5	6	7
Alene	Station I	0.02	0.03	0.04	0.05	0.05	0.05
River	Station II	0.017	0.03	0.04	0.045	0.062	0.072

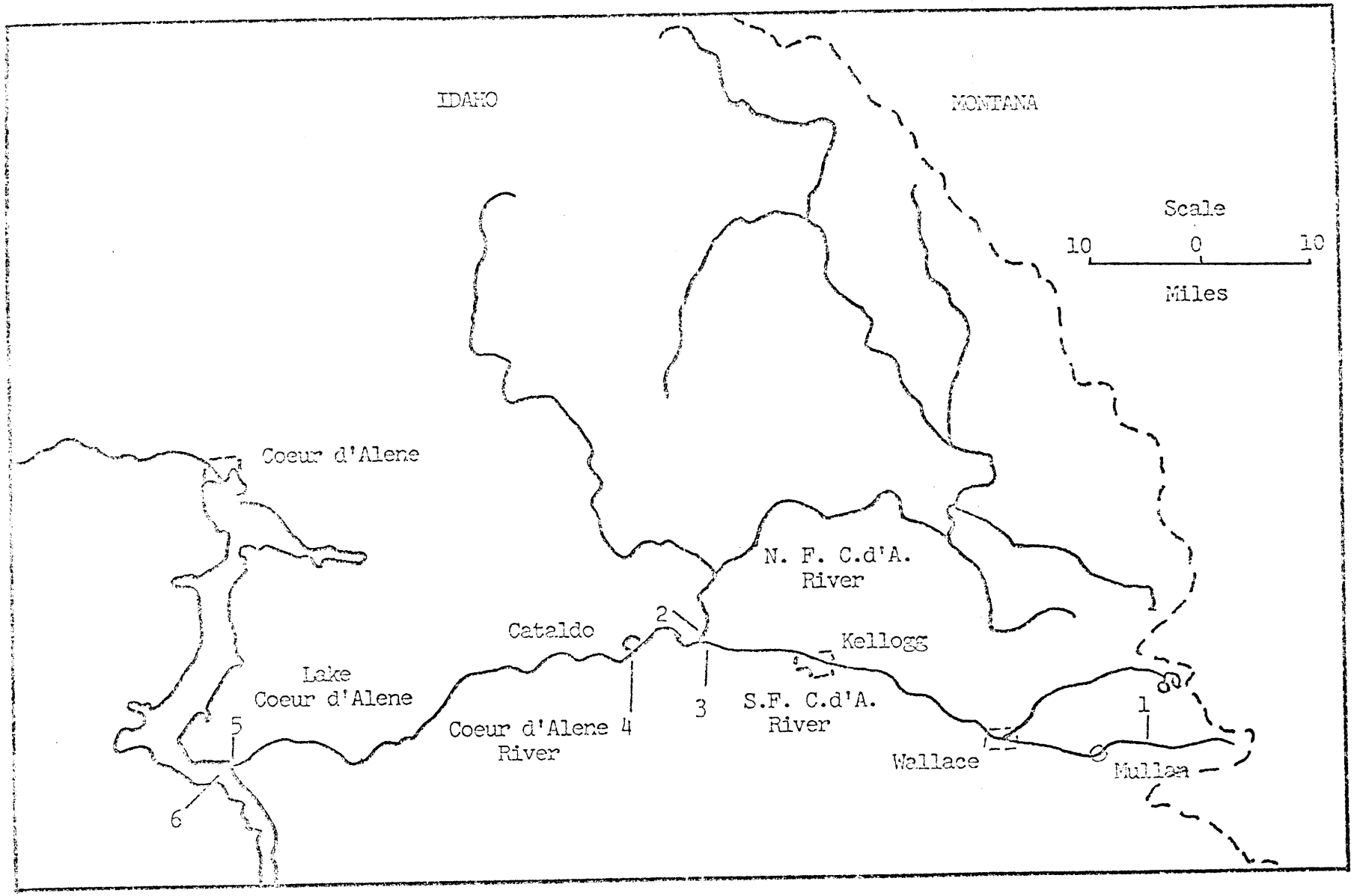

FIGURE VII COLLECTING STATIONS, COEUR D'ALENE RIVER SYSTEM, IDAHO

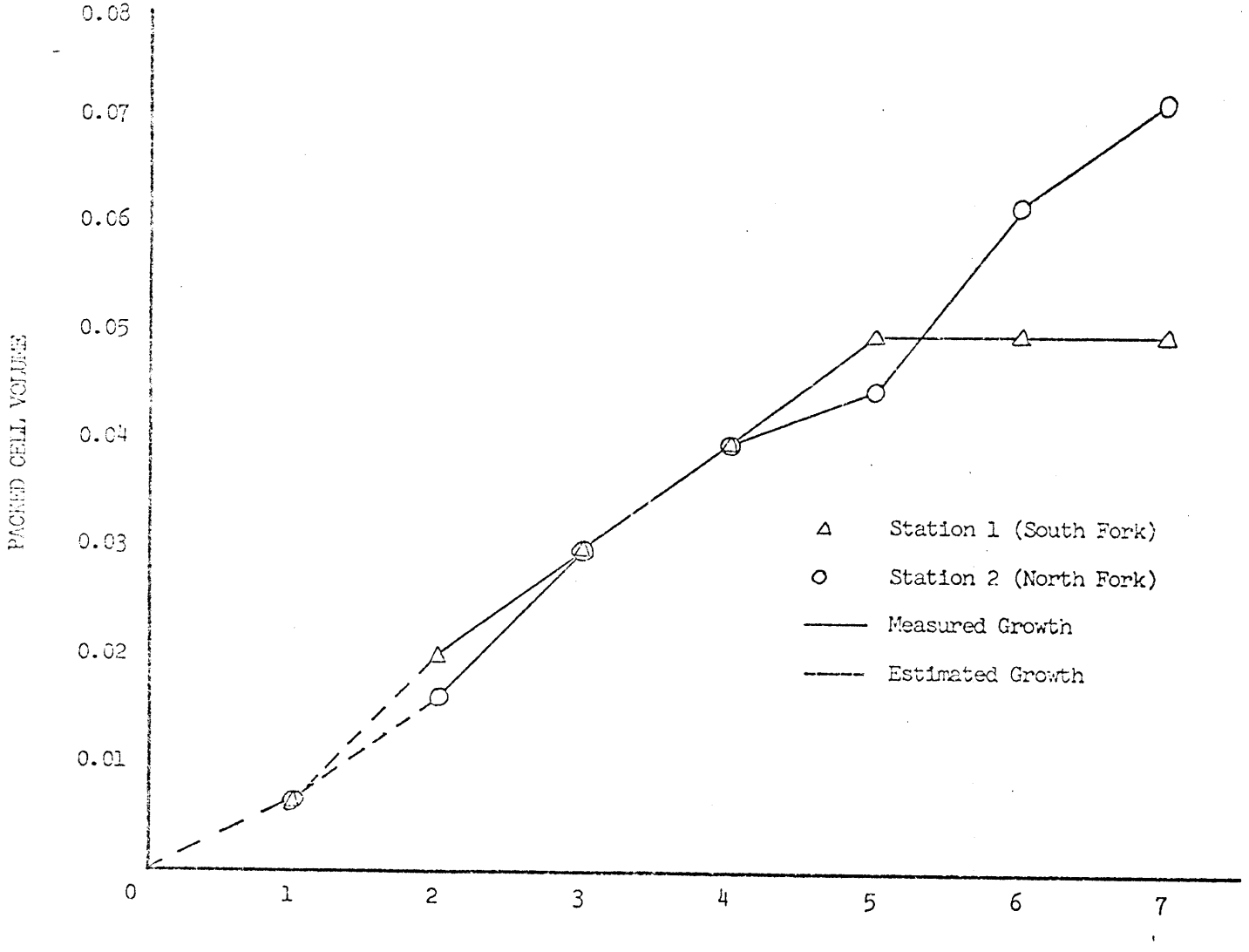

FIGURE VIII EFFECT OF COEUR D'ALENE RIVER SYSTEM WATER ON THE GROWTH OF *S. capricornutum*

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