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Nutrient Inactivation as a Lake Restoration Procedure–Laboratory Investigations

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NUTRIENT INACTIVATION AS A LAKE RESTORATION PROCEDURE

LABORATORY INVESTIGATIONS

by

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PREFACE

The Federal Water Pollution Control Act Amendments of 1972, PL 92-500, include the requirement that the Administrator of the United States Environmental Protection Agency issue such information on methods, processes, and procedures as may be appropriate to restore and enhance the quality of the nation's publicly owned lakes [Subsection 304(i)]. The concept of in-lake nutrient inactivation, wherein a critical nutrient is rendered unavailable through introduction of a complexing additive to the lake, is a promising restorative technique that has received limited attention in the United States and Europe. The present study is designed to more thoroughly evaluate this concept at the laboratory and pilot field scale levels, using a variety of potential inactivant materials over a range of simulated and actual operational conditions, to determine its value and potential as a practical tool in lake management.

Although the authors assume full responsibility for the investigations described in this report, the work could not have been carried out without the able assistance of a number of other members of this laboratory. In particular, the contributions of William Lauer in the work on efficiency of inactivant materials; Terry Smith in the toxicity studies; and Thomas Hamlin in the development of the sediment-water column systems, are gratefully acknowledged.

Dr. Alan V. Nebeker of the EPA Western Fish Toxicology Laboratory in Corvallis rendered valuable assistance in the design of the toxicity studies, and provided us with laboratory populations of invertebrate test organisms. Salmonid fish were kindly furnished by the U.S. Bureau of Sports Fisheries Eagle Creek Fish Hatchery and the State of Oregon Fall Creek and Roaring River Fish hatcheries.

We also wish to thank the Wah Chang Teledyne Company, Albany, Oregon, for supplying experimental quantities of zirconium refinery waste, crude zirconium tetrachloride, and nuclear grade zirconyl chloride, the latter for standards.

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I. INTRODUCTION

The Ecosystems Analysis and Methods Development Section of the Eutrophication and Lake Restoration Branch (Program Element 1BA031) is charged with the conduct of laboratory and field research on aquatic ecosystems to develop information relative to effective prevention and control of eutrophication processes. The concept of nutrient inactivation in eutrophication control and lake restoration appears to be relatively new, although it is essentially an extension of existing wastewater and water supply treatment methodology. Inactivants which have received the most attention relative to treatment technology are Al(III), Fe(III), and Ca(II) all of which react in water at various pH's to form a floc which may chemically bond or physically adsorb soluble phosphorus while also entrapping organic phosphorus to some degree. Of the three, Al (III) appears to be the only one applicable to lakes, since Ca(II) is ineffective in removing phosphorus at pH values less than 9 and Fe(III) is undesirable because of its tendency to be reduced to the soluble Fe(II) state (thereby resolubilizing phosphorus) under anaerobic conditions. The latterr conditions are found frequently in the hypolimnions of eutrophic lakes.

The first attempt to inactivate nutrients in an entire lake with aluminum appears to have been an experiment in phosphate precipitation at Lake Langsjon, Sweden, in 1968[1,2,3]. Results of alum application to the lake surface were favorable, with lowered phosphorus concentration, increased oxygen concentration in bottom waters, and decreased phosphate release from bottom sediments.

In 1970 Horseshoe Lake, Wisconsin, was treated experimentally with alum[4]. Results for this small, eutrophic lake were likewise encouraging. Total phosphorus concentrations decreased, transparency increased, nuisance algal blooms failed to appear, and dissolved oxygen conditions improved. A few other small Wisconsin Lakes, including Long, Snake, and Pickerel, have subsequently been treated with aluminum compounds and preliminary results appear to be similar to those for Horseshoe Lake[5].

In 1971 a one acre (0.40 ha) pond near Corvallis, Oregon was treated with sodium aluminate[6]. Although overall phosphorus concentrations were not greatly depressed, improvement in the pond during the following summer and fall, as compared with previous years, was clearly evident. There was a definite decrease in nuisance algae production and associated symptoms.

The encouraging results obtained in this earlier work, and the need for practical methods of controlling eutrophication in lakes where high water and sediment nutrient levels are encountered, made it appear worthwhile to investigate phosphorus inactivation in greater detail. Laboratory and field studies were designed to evaluate the suitability of a number of candidate materials for use in natural waters. The objectives were to determine (1) efficiency of each as a phosphorus inactivant, (2) possible toxicological or other adverse environmental effects related to the inactivant material, (3) stability of the initial result effected by treatment and (4) beneficial limnological effects resulting from phosphorus inactivation. Iron and calcium were not considered because of the unsuitable characteristics previously mentioned. In all, nine materials have been screened, consisting of eight metal salts and a crude waste product from a zirconium refinery. The metal salts are zirconium tetrachloride, zirconyl chloride, sodium aluminate, aluminum sulfate (alum), lanthanum rare earth chloride, lanthanum rare earth carbonate, sodium tungstate, and titanium sulfate.

Work to date has concentrated on laboratory investigations relating to the first three objectives. Information developed on the efficiency, toxicity and environmental consequences, and stability of the inactivation effects has been applied to the design of a follow-up pilot scale field evaluation, which was initiated in March 1974 at our Cline's Pond test site. Results of that phase of the study will be presented in a subsequent report.

II. SUMMARY

Compounds of certain metals are known to be capable of complexing phosphate ions, thereby removing them from solution. The application of this principle to the control of phosphorus levels in eutrophic lakes has been subjected to laboratory investigation in the present study. Salts of lanthanum, zirconium, and aluminum were found to effectively remove phosphorus from laboratory growth medium and natural pond water, with resulting depression of algal production. Toxicity to fishes and aquatic invertebrates was minimal, but the tests demonstrated that some components of metals salts may have adverse effects. The stability and duration of phosphorus inactivation is being studied in laboratory-scale water-sediment systems, under aerobic and anaerobic conditions. These experiments are expected to elucidate the effect of inactivant-phosphate precipitates on sediment-water phosphorus interchange. Preliminary results indicate that zirconium precipitates phosphorus from the water and holds it at low levels.

III. CONCLUSIONS

Compounds of lanthanum, zirconium, aluminum, tungsten, and titanium were capable of removing phosphorus from pond water and algal growth medium in the laboratory.

Based on molar ratios, the lanthanum rare earth mixtures were the most efficient phosphorus inactivants, followed by zirconium and aluminum, in that order.

The tungsten and titanium compounds tested did not remove phosphorus in sufficient quantity to merit further consideration as practical nutrient inactivants.

Zirconium and lanthanum rare earth mixtures exhibited optimum performance within pH ranges commonly encountered in eutrophic lakes, whereas optimum phosphorus removal with aluminum was at a lower and corsiderably narrower pH range rendering it less appropriate for application to field situations.

Algal assays demonstrated that lanthanum rare earth chloride, zirconium, and aluminum depressed algal growth in both AAP medium and pond water.

Reconstitution of phosphate to the AAP culture medium generally resulted in increased algal biomass approaching theoretical yields.

Available information indicates that zirconium and rare earths supplies and reserves are sufficient to make feasible their use in selected lake restoration activities, and that costs would not be prohibitive.

Toxicity studies using salmonid fish and cladocerans revealed severe detrimental effects only with lanthanum rare earth chloride, and in that case it is believed that the observed mortalities resulted from a component of the compound other than lanthanum.

Results of preliminary tests with zirconium tetrachloride to determine the extent and stability of phosphorus inactivation in laboratory microcosms warrant further investigation.

IV. RECOMMENDATIONS

Additional potential inactivants such as fly ash, volcanic ash, clay materials, and other metal salts alone and in combination should be tested for efficiency, toxic effects and treatment stability.

In view of its high efficiency in removing phosphorus, lanthanum should be subjected to further testing, particularly with respect to toxic effects.

Laboratory studies on permanency of inactivation and effect on sediment-water phosphorus interchange should be continued to produce estimates of long-term effects.

Additional pilot scale field testing should be pursued as quickly as possible, both to enhance and verify information developed in the laboratory.

Experiments should be designed and initiated to determine the availability of inactivated phosphorus to macrophytes as well as to algae.

V. EFFICIENCY OF INACTIVANT MATERIALS

OBJECTIVES AND APPROACH

Initial laboratory studies involved "jar tests" in which candidate inactivant materials were screened to determine their phosphorus complexing capacities. These tests included determination of the phosphorus removal efficiency of the inactivants at various pH levels.

In the jar tests, precipitated inactivant-phosphate phosphorus (PO_4-P) compounds were separated from the water by filtration, and phosphorus analyses performed on the filtrate to determine the phosphorus residual. The biological availability of the residual phosphorus was assessed by the algal assay procedure (AAP). Algal assays were also conducted to determine, through reconstituting phosphorus to inactivated media, whether other growth-limiting nutrients, in addition to phosphorus, had been removed.

METHODS, MATERIALS, AND EQUIPMENT

General

All chemicals were of reagent grade quality or better unless otherwise stated. Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were used for all pH adjustments and glass double distilled water was used to prepare all solutions.

Measurements of pH were made with Beckman Electromate and Beckman Zeromatic meters.* Phosphorus analyses were performed both manually using the single reagent ascorbic acid technique and by automated procedures using a Technicon AutoAnalyzer.

Sulfuric acid-persulfate digestion was used in the total phosphorus analyses. All determinations followed standard EPA methodology.[8]

A Coulter electronic particle counter was used for total cell counts and average cell volume determinations in the algal assay tests.

Phosphorus Removal Experiments ("Jar Tests")

Batch-type "jar tests" were used as the first step in screening inactivant materials. Intital tests were carried out on AAP culture medium, the chemical composition of which is given in Table 1. Potential inactivants which performed well in AAP medium were tested further in water from Cline's Pond #1 (henceforth referred to as pond water). Chemical composition of the pond water at the time of the jar tests is given in Table 2.

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*Mention of commercial products by EPA does not constitute an endorsement or recommendation for their use.

Table 1

CHEMICAL COMPOSITION OF
AAP CULTURE MEDIUM

Compound	Concentration (mg/l)	Compound	Concentration (µg/l)
$NaNO_3$	25.5	H_3BO_3	185.5
K_2HPO_4	1.0	$MnCl_2$	264.2
$MgCl_2$	5.7	$ZnCl_2$	32.7
$MgSO_4 \cdot 7H_2O$	14.7	$CoCl_2$	0.7
$CaCl_2 \cdot 2H_2O$	4.4	$CuCl_2$	0.0
$NaHCO_3$	15.0	$Na_2MoO_4 \cdot 2H_2O$	7.2
		$FeCl_3$	96.0
		$Na_2EDTA \cdot 2H_2O$	300.0

Table 2

Chemical Composition of Cline's Pond #1 Water (mg/l) at the Time of Jar Tests

Total - P	1.36
Orthophosphate - P	0.43
Ammonia - N	0.21
Nitrite - N	0.01
Nitrate - N	0.18
Total Kjeldahl - N	0.82
Chloride	5.00
Total Inorganic Carbon	3.30
Alkalinity (eq. $CaCO_3$)	31.0
Dissolved Iron	0.28
Total Hardness	36.3
Conductivity	96.0 μmho/cm

Known chemical characteristics of the inactivants permitted calculation of the theoretical quantity necessary to achieve 100 percent phosphorus (PO_4-P) removal. Following calculation, six 500 ml samples, with various concentrations of inactivant, bracketing the estimated 100 percent phosphorus removal concentrations, were stirred at 100 rpm on a Phipps and Bird 6-place mixer. A solution of inactivant (always less than 0.5 percent of the volume of the test solution) was added when stirring began and the pH was adjusted to 7.0. The mixture was stirred for 5 minutes more, and the stirring rate slowed to 20 rpm for an additional 30 minutes. Mixing was then terminated to allow a 30-minute settling period, after which the supernate was filtered through a prewashed 0.45 μ filter. The filtrate was analyzed for residual orthophosphate-phosphorus. (Note: Dissolved inactivant concentrations were not measured because sufficiently sensitive analytical methodology was not available. Therefore, inactivant concentrations as expressed in this report refer only to quantities of materials added).

The above procedure was varied slightly in the tests on sodium aluminate. With this material efficiency of phosphorus removal was variable when pH was adjusted downward to 7.0, and was less than expected when compared to alum. Initial downward adjustment of the pH to 5.0, followed by readjustment back to 7.0, resulted in less variation and increased phosphorus removal.

The results of these broad-spectrum tests were examined to determine the range of inactivant concentration over which the desired amount of phosphorus removal occurred; a second series was then run over a narrower concentration range. Results of these tests were plotted to show that the percent phosphorus removal was a function of the inactivant-to-phosphate molar ratio. This ratio was calculated by dividing molar quantity per liter of the inactivant added by the initial molar concentration of phosphorus. Expressed in this manner the phosphorus removal efficiencies of different inactivants or different concentrations of the same inactivant are directly comparable.

Algal Assay Experiments

The second step in the screening process involved the use of the standard Algal Assay Procedure[7]. Assays were conducted only on phosphorus-inactivated AAP medium.

Two hundred milliliters of filtrate from the inactivated AAP medium were placed in a sterilized 500 ml Erlenmeyer flask and inoculated with 1 ml of a Selenastrum capricornutum culture. The inoculum was prepared by centrifuging a 7-day-old stock culture of S. capricornutum, decanting the supernate, washing the cells in a 15 mg/l sodium bicarbonate solution, centrifuging again, and decanting the supernate. This was repeated twice, after which the inoculum was counted and diluted to approximately 200,000 cells/ml. The inoculated samples were incubated 14 days at 24°C ± 1°C and approximately 4300 lux (1300 $\mu W/cm^2$)[a] on a rotary shaker at 100 oscillations/min. One ml of sample was collected for total cell count and average cell volume determination on days 3, 4, 5, 6, 7, 10, 12 and 14 of the incubation period.

Effect of pH on Phosphorus Removal

Tests were conducted with sodium aluminate, zirconium tetrachloride and lanthanum rare earth chloride,, using both AAP medium and pond water, to determine the effect of pH on the phosphorus removal efficiency of the inactivant. A 4-liter container of test medium (either AAP medium or pond water) was adjusted to pH 2.0 with HCl. This was used as a stock solution for

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a The energy level output of a bank of six 48 inch "cool white" fluorescent lamps (GE 40 watt, @ 60 Hz) was approximately 1300 $\mu w/cm^2$ (range, 380-760 nm) at a distance of 26 3/4 inches, as measured with an ISCO Model SRC spectroradiometer. Using the same measurement geometry, a Weston Model 756 Illumination Meter read 400 footcandles. All reflecting surfaces were matte white. Therefore, utilizing a calibrated illumination meter with a footcandle readout, one may, by adjusting the height of the lights, achieve a known energy level output of 1300 $\mu w/cm^2$.

tests on a single inactivant material. One hundred ml of medium was pipetted into a 250 ml beaker, a quantity of inactivant material estimated to achieve about 90 percent phosphate removal added, and the mixture stirred. The remaining stock test solution was then adjusted to pH 3.0 with NaOH, and a second 100 ml aliquot pipetted into a beaker, inactivant added, and the mixture stirred. This procedure was repeated for each whole pH unit through 11.0. The supernatant portion of each 100 ml aliquot was then filtered through a 0.45 μ membrane filter, and analyzed for residual phosphorus.

EXPERIMENTAL RESULTS

Zirconium Refinery Waste

Jar Tests -

The zirconium refinery waste used in these experiments was a heterogeneous mixture containing a number of metals and other elements in addition to zirconium. An analysis provided by the refinery is presented in Table 3. It should be noted that the waste material analyzed was kiln dried at 300°C prior to analysis and therefore the oxides of the elements appear in the table. The non-kiln dried waste used in the experiments contained halogens in addition to the oxidized species. Because of this heterogeneity the relationship of refinery waste to phosphorus removed is expressed on a weight basis rather than as molar ratio as has been done for other inactivants tested. It was decided to conduct inactivation tests using both wet waste (65% water) and wet waste dried at 105°C for 24 hours. Drying the waste did not greatly affect the amount of phosphorus removed, although in both media the wet waste did remove more phosphorus than the dried waste (Figure 1). The differences in removal were much less significant in pond water than in AAP medium.

Algal Assays -

Results of algal assay tests on AAP medium treated with seven different concentrations of zirconium refinery waste are given in Table 4. The algal dry weight yields were higher than expected for the measured quantities of residual phosphorus. Although the excessive yields cannot be explained by the available data, it seems evident that all the residual phosphorus was in a form available for uptake by the test organisms. The observed growth further implies that toxic or other inhibitory effects were not associated with the inactivant.

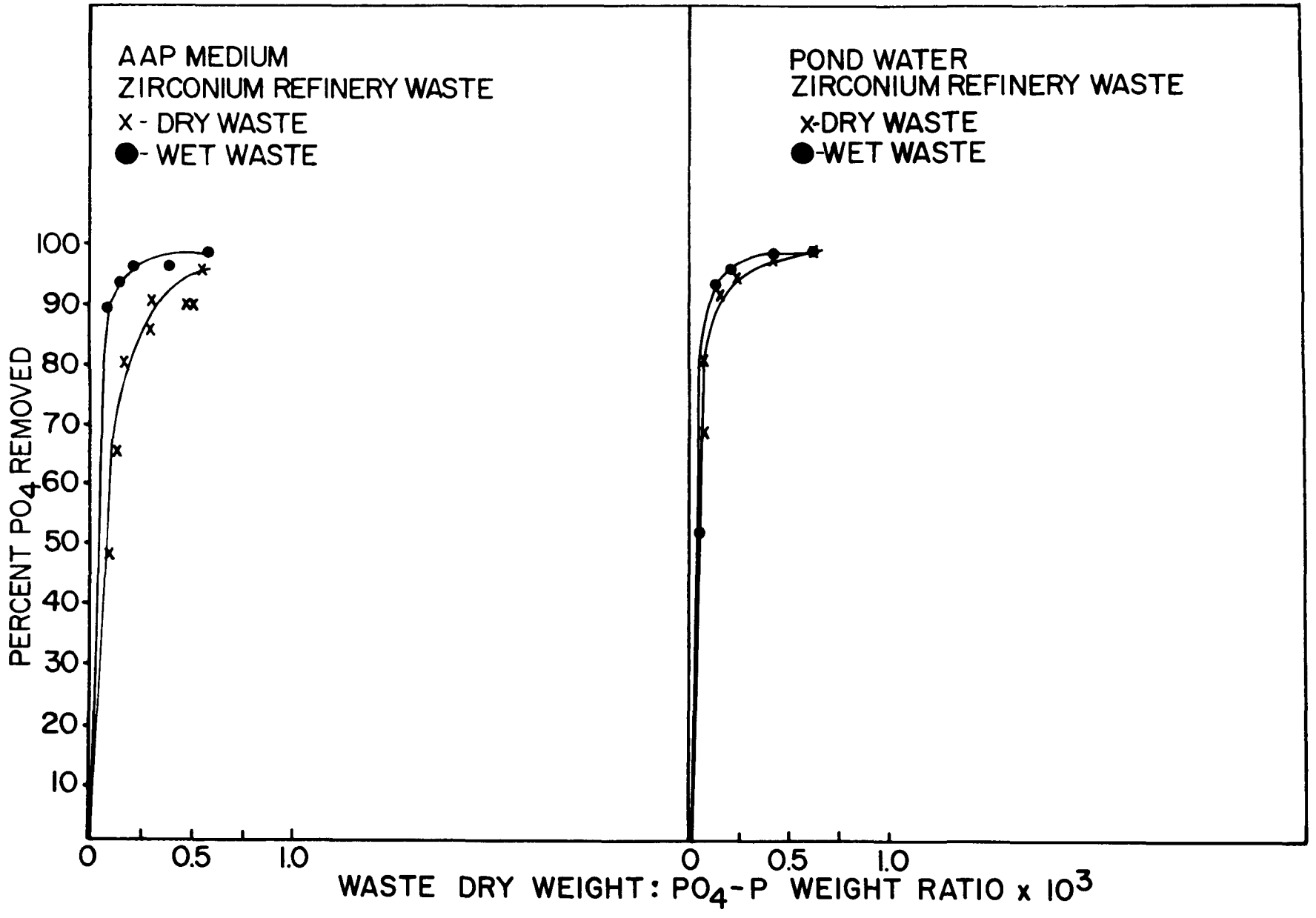

Figure 1. Phosphorus removal efficiency of zirconium refinery waste in AAP medium and pond water.

Table 3

Chemical Analysis of Zirconium Refinery Waste*

Compound	Percent
$(Zr+Hf)O_2$	55.30
$(Cb+Ta)O_2$	0.01
Al_2O_3	1.63
SiO_4	21.97
MgO	10.50
CaO	11.94
Fe_2O_3	3.34
	104.69

*Kiln dried at 300°C.

Table 4

Results of Algal Assay Tests on AAP Medium
Treated with Zirconium Refinery Waste

Waste (g/l)	Residual PO_4-P (mg P/l)	Waste:PO_4-P Weight Ratio (x 10^3)	Cell count Cells/mlx10^4	Cell Dry Wt. (mg/l)
0.0	0.215	0.0	686.1	94.7
0.2	0.075	0.9	231.4	48.0
0.6	0.018	2.8	59.0	12.7
1.0	0.008	4.6	18.9	4.7
4.0	0.001	18.6	0.8	0.2
10.0	0.001	46.5	0.6	0.2
15.0	0.001	69.8	1.6	0.4

Tungsten and Titanium

Jar Tests -

The maximum amount of phosphorus removed from AAP medium with sodium tungstate was 53 percent at a tungsten to phosphorus molar ratio of 8.4. As can be seen from the curve, no significant increase in phosphorus removal occurred at higher molar ratios (Figure 2).

Titanium sulfate (basic) was less efficient than sodium tungstate at the lower molar ratios, but resulted in much higher maximum phosphorus removal. Maximum removal was 80 percent at a titanium to phosphorus molar ratio of 28.6. Molar ratios greater than 28.6 did not appear to significantly increase the amount of phosphorus removed (Figure 2).

Aluminum Sulfate (Alum)

Jar Tests -

Aluminum sulfate was slightly more efficient in removing phosphorus from AAP medium than from Cline's Pond water (Figure 3). Maximum phosphorus removal in both media was 95 percent at an Al(III): phosphorus molar ratio of 3 in AAP medium and 4 in pond water. Molar ratios greater than these did not result in a significant decrease in residual phosphorus.

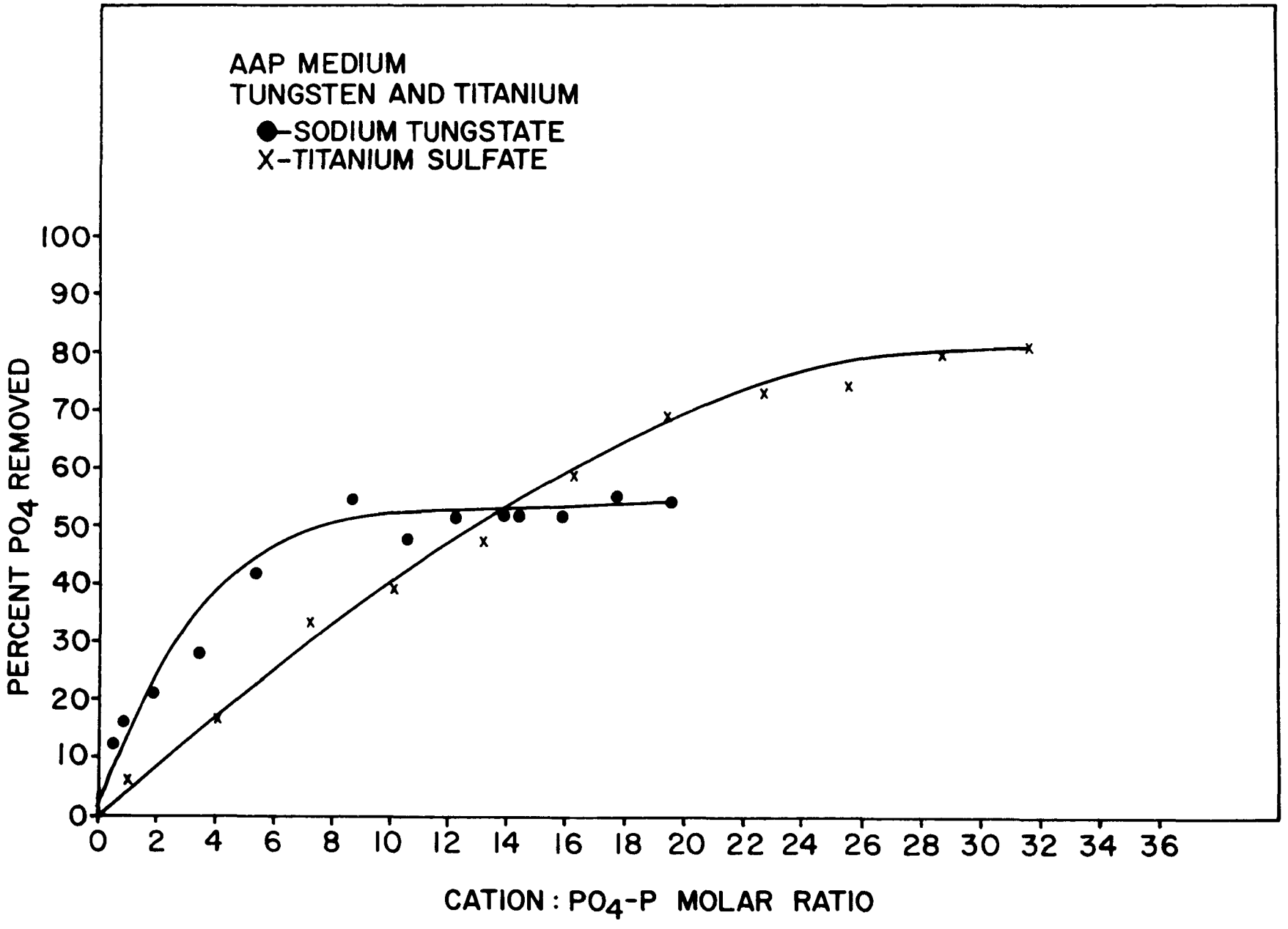

Figure 2. Phosphorus removal efficiency of tungsten and titanium in AAP medium.

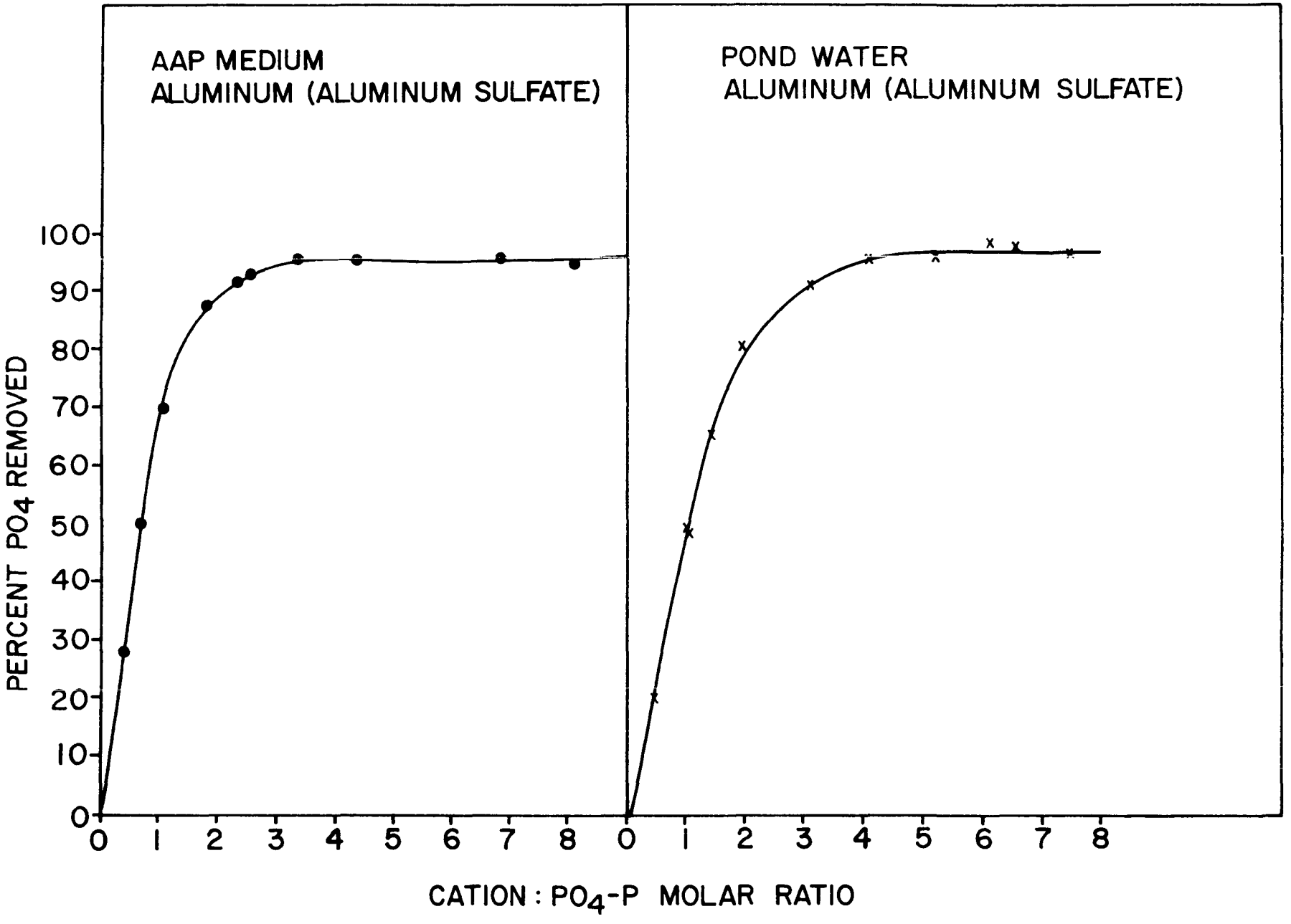

Figure 3. Phosphorus removal efficiency of aluminum sulfate in AAP medium and pond water.

Sodium Aluminate

Jar Tests -

Two different experiments were conducted with sodium aluminate. In one the pH of the treated solution was adjusted down to 5.0 and then readjusted to 7.0. In the other the pH was simply adjusted down to 7.0. Results are summarized in Figure 4, where it will be noted that lowering the pH to 5.0 before neutralizing resulted in increased phosphorus removal. Results differed, however, between AAP medium and pond water: the relationship between phosphorus removal and inactivant concentration was linear in AAP medium, but non-linear in pond water. When the pH was simply adjusted down to 7.0, the non-linear relationship was found in both AAP medium and pond water. Not only was efficiency of phosphorus removal lower, but results were less consistent than when pH was first lowered to 5.0.

Algal Assays -

Algal assay growth response to the filtrates from the sodium aluminate experiments indicated that the residual phosphorus existed in an available form, that is, the test organism was able to assimilate it (Table 5).

Additional filtrates were reconstituted with phosphorus to concentrations approximately the same as in control samples. These would have been expected to produce about the same biomass as the controls, assuming (1) no toxic effects and (2) that phosphorus was the only growth-limiting nutrient removed by the sodium aluminate. In the case of treatment with 0.99 mg Al/l, algal biomass produced was equal to the calculated theoretical yield (Table 6). However, where 1.32 mg Al/l was added to the growth medium, reconstitution of the phosphorus content failed to produce the expected growth. These disparate results will be the subject of future investigation, particularly with regard to assumptions (1) and (2), above.

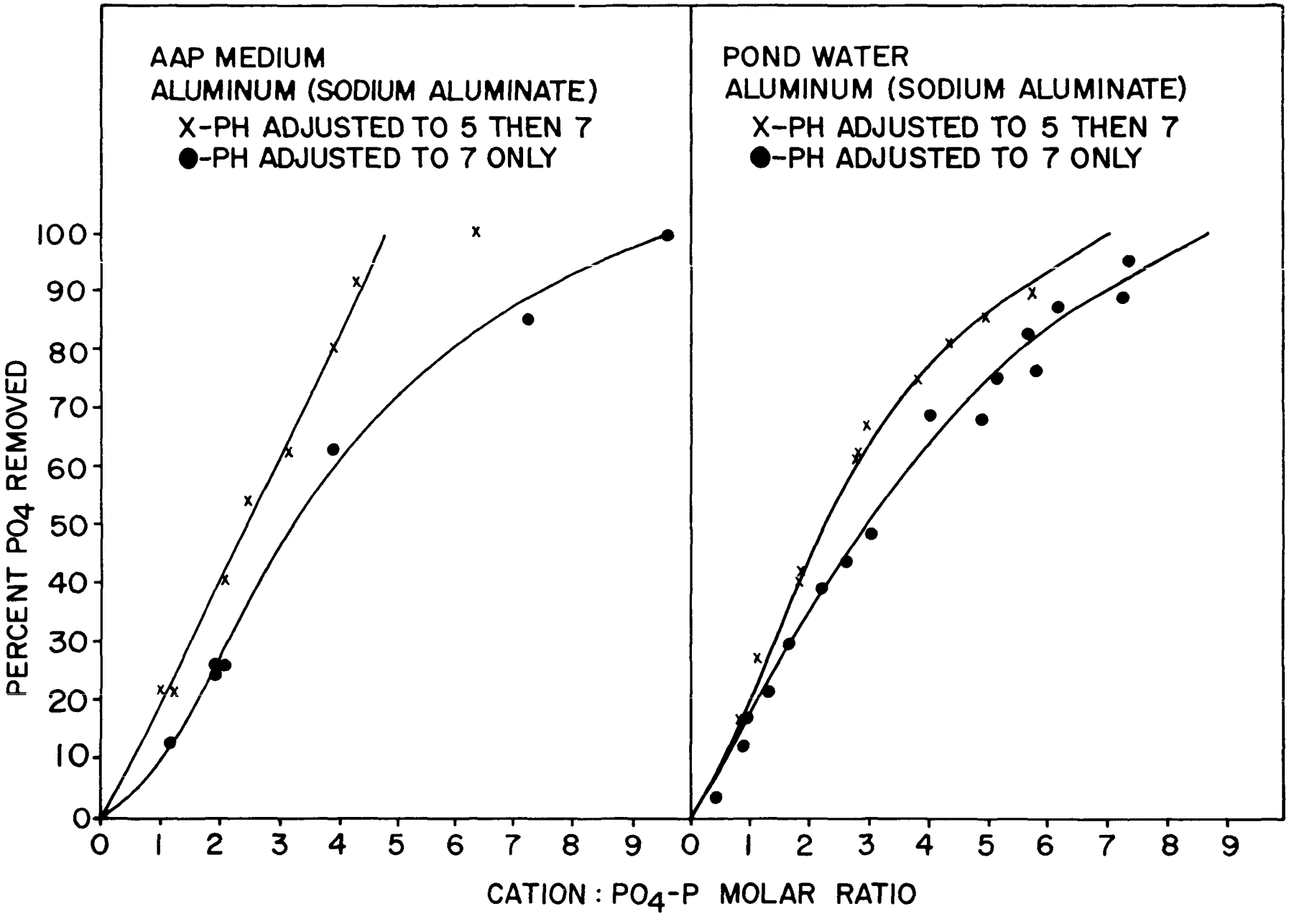

Figure 4. Phosphorus removal efficiency of sodium aluminate in AAP medium and pond water.

Zirconium

Jar Tests -

Testing was carried out on two forms of zirconium: zirconium tetrachloride ($ZrCl_4$) and zirconyl chloride ($ZrOCl_2 \cdot 8H_2O$). Reagent grade zirconium tetrachloride and zirconyl chloride were used in the experiment with AAP medium. Both zirconium forms used in the experiments on pond water were of a less refined quality; the zirconium tetrachloride contained from 0.1 to 2.0 percent impurities. The zirconyl chloride was precipitated from a saturated water solution of the less pure zirconium tetrachloride and thus contained some impurities.

Zirconium tetrachloride (reagent grade) removed 100 percent of the phosphorus in AAP medium at a Zr(IV)/P molar ratio of 4.3 (Figure 5). Results with zirconyl chloride (reagent grade) were very similar, but zirconium tetrachloride appeared to be somewhat more efficient.

Both zirconium inactivants were more effective in pond water than in AAP medium. Zirconyl chloride (crude) appeared to be a slightly more efficient phosphorus inactivant than zirconium tetrachloride (crude) in pond water.

Algal Assays -

Algal assay growth yields on the filtrates from zirconium tetrachloride and zirconyl chloride jar tests (Tables 7 and 8) were proportional to the amount of residual phosphorus present in the filtrate, indicating that the residual phosphorus was in a form usable by the test organisms. The single exception was a marked decrease in the dry weight yield in samples treated with 0.25 mg Zr/l zirconyl chloride.

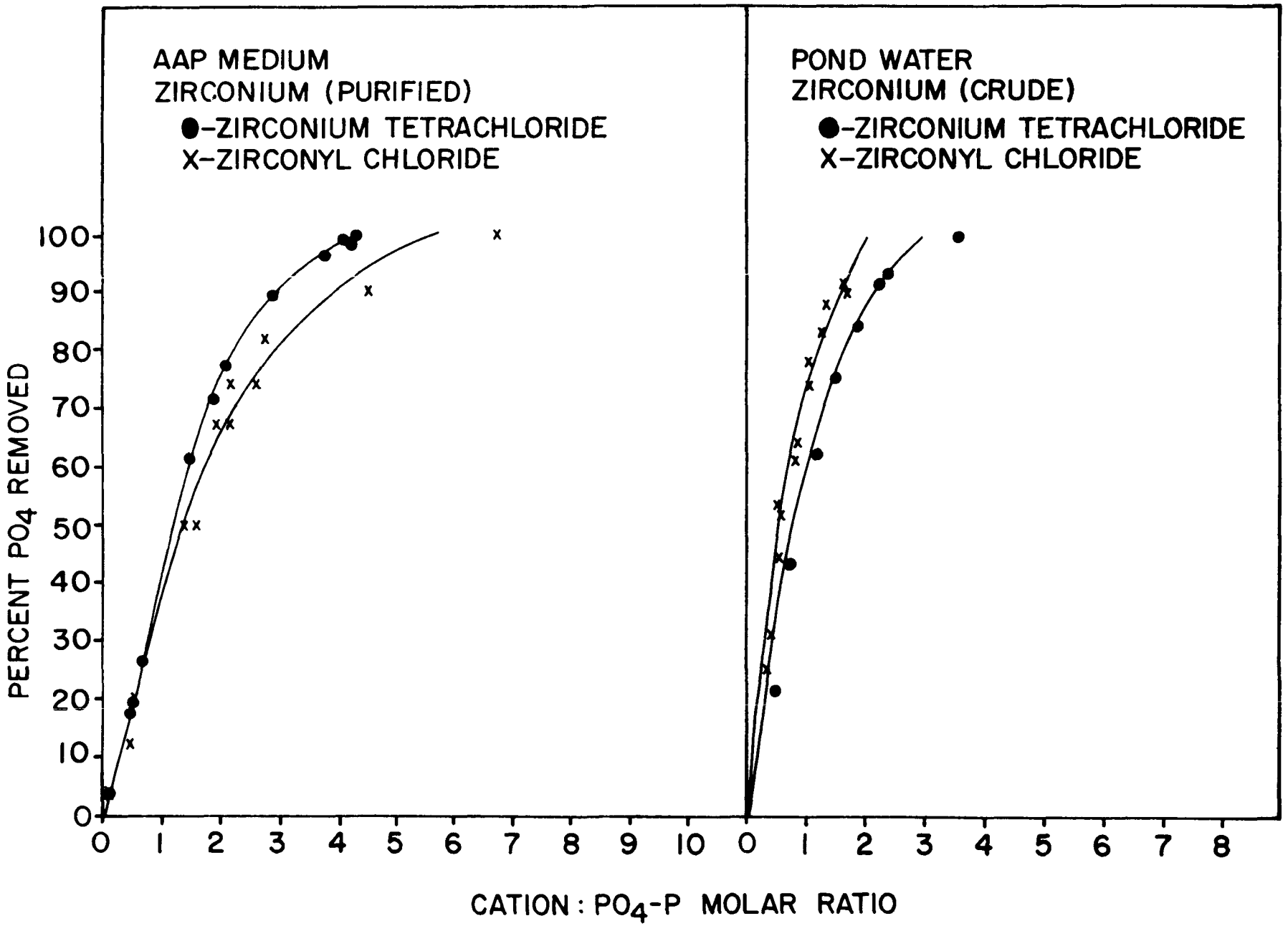

Figure 5. Phosphorus removal efficiency of zirconium tetrachloride and zirconyl chloride in AAP medium and pond water.

This decrease in yield did not appear to be the result of a toxic effect since samples treated at higher concentrations (1 mg Zr/l) yielded greater dry weights. A like decrease did not occur in samples treated with zirconium tetrachloride at the same Zr concentration.

Table 5

Results of Algal Assay Tests on AAP Medium Treated with Sodium Aluminate

$NaAlO_2$ (mg Al/l)	Residual PO_4-P (mg P/l)	Cation: PO_4-P Molar Ratio	Cell Count (Cells/ml x 10^4)	Cell Dry Wt. (mg/l)
0.00	0.184	0.0	285.34	32.73
0.66	0.014	4.1	34.55	8.75
0.99	0.008	6.2	0.32	0.06
1.15	0.002	7.2	0.12	0.03
1.32	0.003	8.3	0.13	0.02
1.65	0.001	10.3	0.03	0.00

Table 6

Results of Algal Assay Tests on Sodium Aluminate-Treated AAP Medium in Which Phosphorus was Reconstituted

$NaAlO_2$ (mg Al/l)	Residual $PO_4$4-P (mg P/l)	Cation: PO_4-P Molar Ratio	Cell Count (Cells/mlx10^4)	Cell Dry Wt. (mg/l)
0.00	0.180	0.0	285.34	32.72
0.99	0.190	6.2	265.14	39.83
1.32	0.180	8.3	5.96	1.90

Table 7

Results of Algal Assay Tests on AAP Medium Treated with Zirconium Tetrachloride

$ZrCl_4$ (mg Zr/l)	Residual PO_4-P (mg P/l)	Cation: PO_4-P Molar Ratio	Cell Count (Cells/mlx10^4)	Cell Dry Wt. (mg/l)
0.00	0.163	0.0	403.5	54.35
0.25	0.135	0.5	328.3	45.48
1.00	0.036	2.1	118.1	21.73
2.00	0.004	4.2	0.3	0.07
3.00	0.0	6.3	0.2	0.03
4.00	0.0	8.4	0.2	0.03
5.00	0.0	10.5	0.2	0.03

Table 8

Results of Algal Assay Tests on AAP Medium Treated with Zirconyl Chloride

$ZrOCl_2$ (mg Zr/l)	Residual PO_4-P (mg P/l)	Cation: PO_4-P Molar Ratio	Cell Count (cells/ml x 10^4)	Cell Dry Wt. (mg/l)
0.00	0.155	0.0	482.04	54.32
0.25	0.120	0.5	7.20	1.13
0.25	0.155	0.5	1.67	0.22
1.00	0.049	2.2	134.23	16.61
1.00	0.039	2.2	105.42	12.70
3.0	0.0	6.6	0.08	0.01
4.0	0.0	8.8	0.08	0.01
5.0	0.0	11.0	0.08	0.01
7.0	0.0	15.3	0.09	0.01

Table 9

Chemical Composition of Lanthanum Rare Earth Carbonate.
Ratio of Rare Earth Oxide/Total Rare Earth Oxides (REO)*

La_2O_3	65%	Na_2O	.10%
Nd_2O_3	27%	CaO	.05%
Pr_6O_{11}	7%	Cl	.25%
CeO_2	1%	Fe_2O_3	.03%
Other REO	1%	Loss on Ignition	33.00%

*Total REO = 65% of solids

Table 10

Chemical Composition of Lanthanum Rare Earth Chloride.

Ratio of Rare Earth Oxide/Total Rare Earth Oxides (REO)*

La_2O_3	60.0%	Fe_2O_3	0.02 %
CeO_2	15.0%	CaO+SrO	1.00%
Nd_2O_3	17.5%	Na_2O	0.50%
Pr_6O_{11}	7.0%	(Pb+V+Ni+Cu)	0.01%
Other REO	0.5%	MgO	0.25%
		Cl	25.00%

*Total REO = 46% of solids

Table 11

Results of Algal Assay Tests on AAP

Medium Treated with Lanthanum Rare Earth Chloride

LaRECl (mg/l)	Residual PO_4-P (mg P/l)	Cation: PO_4-P Molar Ratio	Cell Count (Cells/mlx10^4)	Cell Dry Wt. (mg/ml)
0.00	0.185	0.00	285.34	32.72
0.25	0.150	0.12	271.42	33.77
1.00	0.100	0.47	146.78	18.69
1.50	0.103	0.71	138.82	20.89
2.00	0.015	0.94	41.19	6.70

Lanthanum

Jar Tests-

Testing was carried out on lanthanum rare earth carbonate and lanthanum rare earth chloride, the chemical compositions of which are given in Tables 9 and 10. Because the two compounds are mixtures of rare earths, the molar ratios as presented represent the ratio of the sum of the individual rare earth molar concentrations to the phosphate-phosphorus molar concentration of the test medium.

Lanthanum rare earth carbonate is only slightly soluble in water, and that used in this experiment was dissolved in dilute HCl (pH 2.0) before addition to the test medium. Both the carbonate treated with HCl and chloride salts removed 100 percent of the phosphorus in AAP medium and pond water, at a cation: phosphate-phosphorus molar ratio of approximately 0.9 - 1.0 (Figure 6).

Algal Assays -

Assays were conducted only on lanthanum rare earth chloride. Results are summarized in Table 11. Assays included tests on filtrates in which the phosphorus concentrations had been readjusted to approximately that of the control (Table 12).

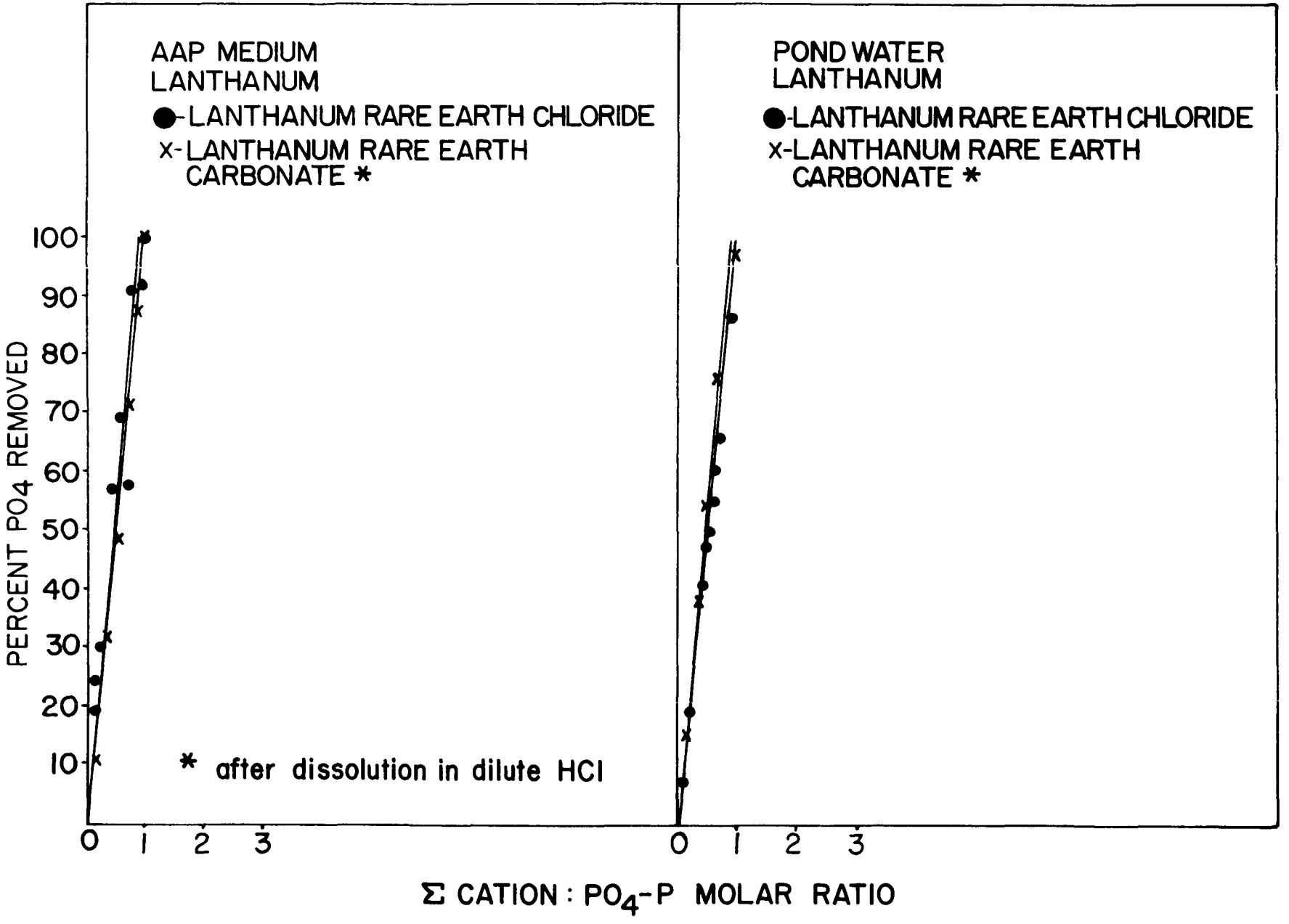

Figure 6. Phosphorus removal efficiency of lanthanum rare earth chloride and lanthanum rare earth carbonate in AAP medium and pond water.

Table 12

Results of Algal Assay Tests on Lanthanum Rare Earth Chloride-Treated AAP Medium in Which Phosphorus was Reconstituted

LaRECl (mg/l)	PO_4-P (mg P/l)	Cation: PO_4-P Molar Ratio	Cell Count (Cells/ml x 10^4)	Cell Dry Wt. (mg/l)
0.00	0.185	0.0	285.34	32.72
1.0	0.180	0.47	418.98	52.23
2.0	0.195	0.94	263.03	27.29

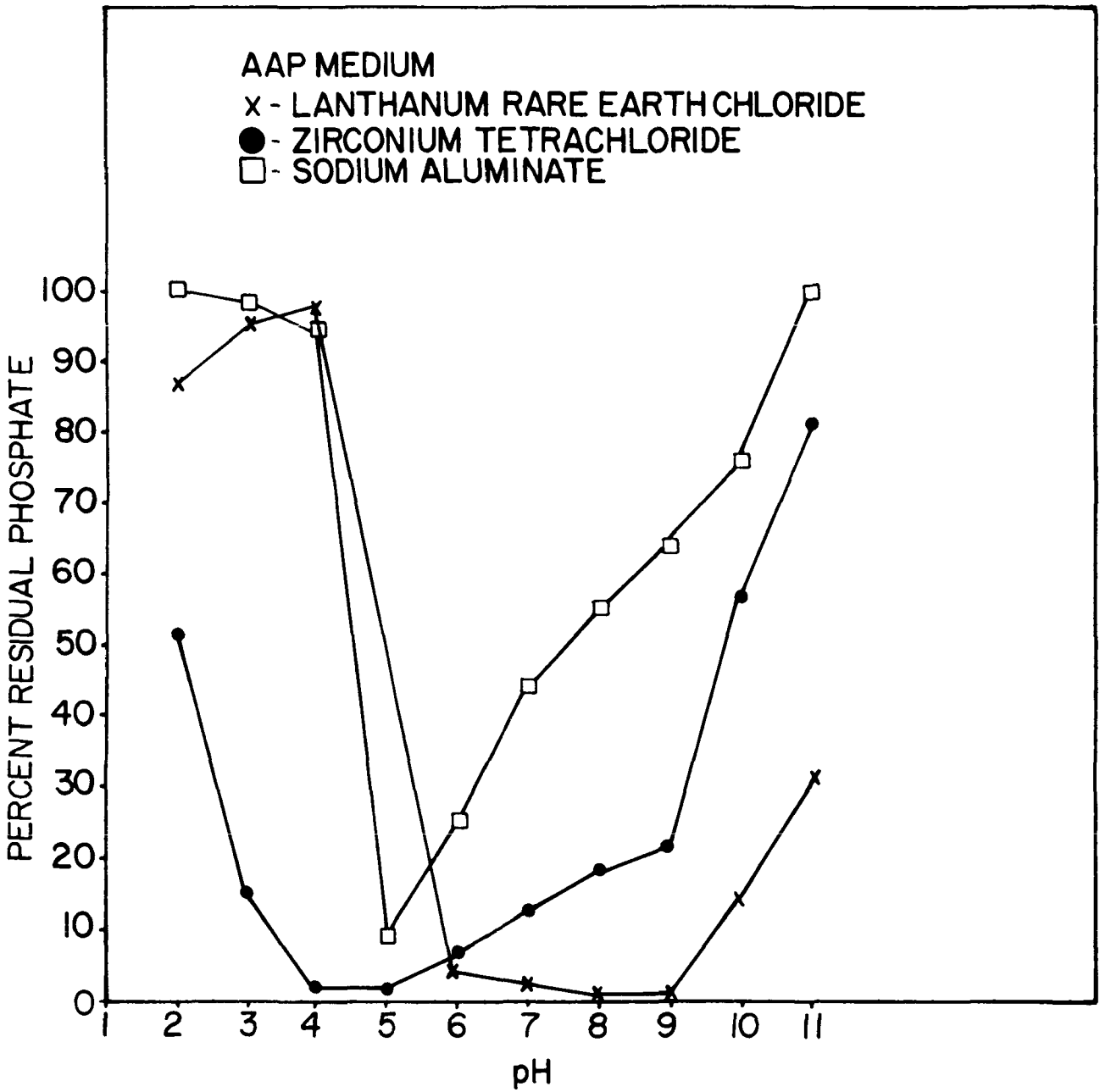

Figure 7. Effect of pH on phosphorus removal by lanthanum, zirconium, and aluminum in AAP medium.

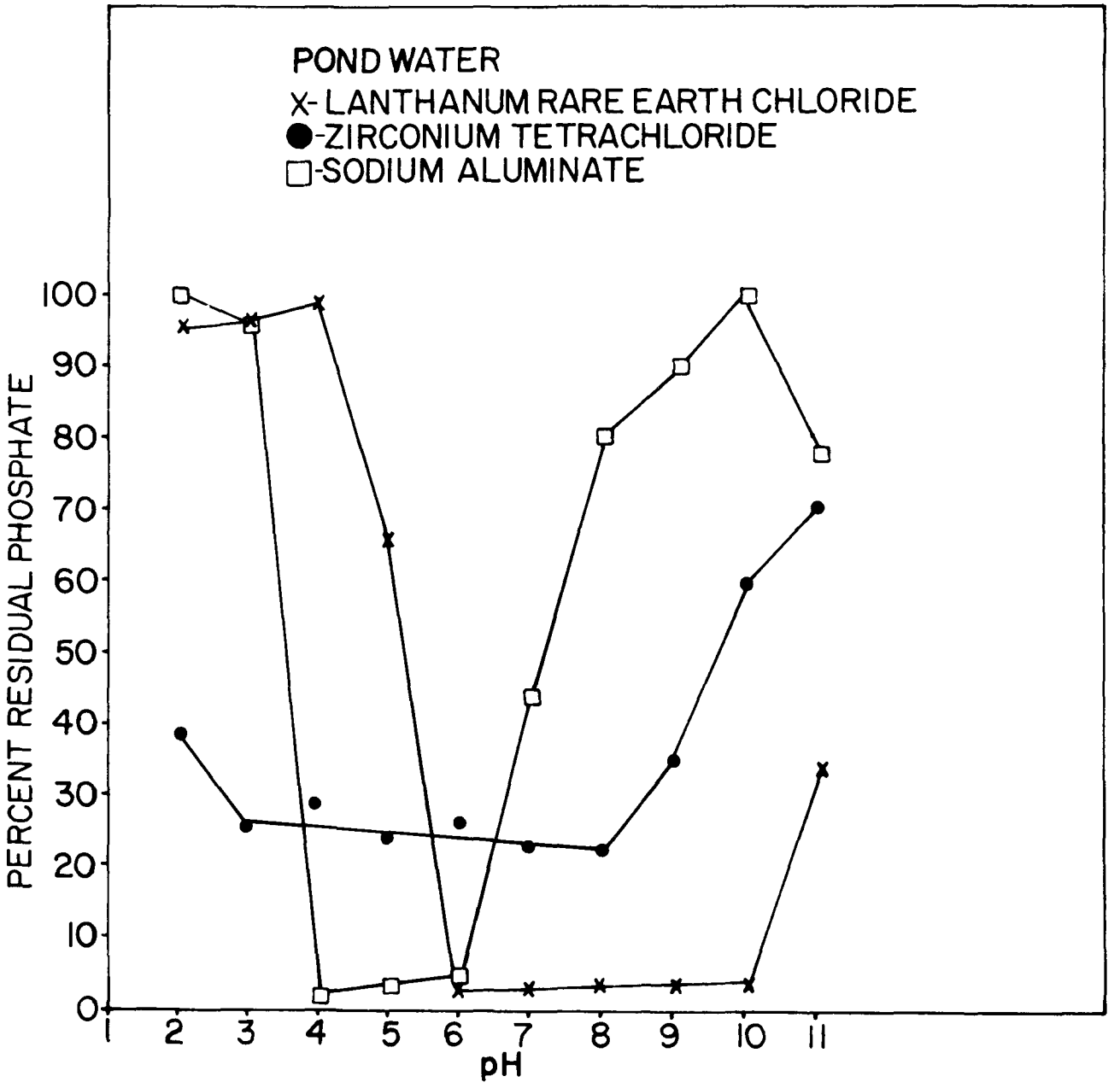

Figure 8. Effect of pH on phosphorus removal by lanthanum, zirconium and aluminum in pond water.

With the exception of the sample which received 0.25 mg/l LaRECl, the dry weight yields of the inactivated samples were less than those of the control (Table 11). The biomass yields from filtrates in which phosphorus was reconstituted showed mixed results (Table 12). The sample which was treated with 1 mg/l lanthanum rare earth chloride (LREC) produced an unexpectedly high dry weight yield after reconstitution which has not been explained. The results do show, however, that any residual inactivant was non-toxic to the test organism. The sample treated at 2 mg LREC and reconstituted with phosphorus did produce a somewhat lower dry weight yield than the control, however, it was well within the ±20% accuracy of the algal assay and thus within the expected range. Again, this indicates the absence of a toxicant.

Effect of pH on Inactivant Efficiency

The data obtained from the experiments to determine the optimum pH for phosphorus removal by Al (III), Zr (IV), and La-rare earths are presented in figures 7 and 8.

The concentrations of inactivants used in AAP medium (Figure 7) were 2.4×10^{-5} M Al, 1.2×10^{-5} M Zr, and 1.0×10^{-5} M La-rare earths. Concentrations used in pond water (Figure 8) were 12.2×10^{-5} M Al, 3.2×10^{-5} M Zr, and 1.9×10^{-5} M La-rare earths.

The pH range for optimum phosphorus removal by aluminum was quite narrow. Minimum residual phosphorus concentrations occurred at pH 5 in AAP medium and between pH 4 and 6 in pond water. Residual phosphorus concentrations increased sharply above and below these pH values.

The most effective pH range for phosphorus removal by zirconium was 4 to 6 in AAP medium and 3 to 8 in pond water. In AAP medium residual phosphorus rose gradually between pH 6 and 9 and increased sharply above pH 9. In pond water a large increase occurred above pH 8.

The optimum pH range for phosphorus removal by lanthanum was between 6 and 9 in AAP medium and between 6 and 10 in pond water. At pH values above and below these ranges the amount of residual phosphorus increased sharply.

SUMMARY AND DISCUSSION OF PRELIMINARY SCREENING RESULTS

Aluminum sulfate (alum), sodium aluminate, zirconium tetrachloride, zirconyl chloride, lanthanum rare earth chloride, and lanthanum rare earth carbonate have all proven to be efficient phosphorus inactivants. All were capable of removing 100 percent of the phosphorus contained in the test media, if added in sufficient quantity, except aluminum sulfate which removed 96 percent.

Relationships between inactivant added and phosphorus removed were sometimes linear and sometimes curvelinear. It appears likely that a linear relationship is indicative of an actual chemical reaction involving the inactivant and phosphorus while a curvelinear relationship would be associated with phosphorus removal by a sorption process. The rationale for this is as follows.

During a chemical reaction such as

$$AB \leftrightarrow A + B$$

where A = inactivant species,
B = phosphorus species, and
AB = inactivant-phosphorus compound,

a constant relationship,

$$K_{sp} = [A][B]$$

where K_{sp} = solubility product, and
$[\ ]$ = molar concentration,

would exist, according to the law of mass action. The amount of phosphorus, [B], in solution would be equal to

$$[B] = K_{sp} \cdot \frac{1}{[A]}$$

which results in an inverse <u>linear</u> relationship between [B] and [A].

During an adsorption reaction the relationship can be expressed by the Freudnlich isotherm equation

$$\frac{x}{m} = kC^{\frac{1}{n}}, \text{ or } C = \left(\frac{x}{mk}\right)^{n}$$

where $\frac{x}{m}$ = amount of phosphorus removed per unit weight of adsorbent,
C = concentration of phosphorus in solution after adsorption, and
k and n = constants ($n > 1$).

Expressed graphically, this equation results in a curvelinear relationship.

Of the inactivants tested, the two lanthanum rare earth mixtures removed phosphorus from solution at the lowest inactivant to phosphorus molar ratio, with 100 percent removal in both AAP medium and pond water occurring at a ratio of approximately 0.9 to 1.0. The relationship was linear and has been observed by others at much higher phosphorus concentrations.[10,11] In those studies 100 percent phosphorus removal was obtained at a cation: phosphate-phosphorus ratio of about 0.9, as was the case in our experiments. A chemical reaction between La^{+++} and a combination of $HPO_4^{--} + PO_4^{---}$ is strongly suggested.[10,11] Further, the similarity of our results and those of others on media of very different chemical compositions implies that phosphorus removal by lanthanum is not greatly affected by the presence of contaminants.

Zirconyl chloride and zirconium tetrachloride were next in efficiency, with 100 percent removal at molar ratios of 2.1 and 3.1, respectively. A curvelinear relationship existed between these two compounds and phosphorus removed, suggesting that phosphorus removal was by some process other than a chemical reaction, such as sorption or occlusion.[12]

Zirconium refinery wastes were required in much larger quantities than the refined products to achieve comparable phosphorus removal (Note that in Figure 1 the ratios are expressed in terms of weight x 10^3). It was further noted that the wet waste product removed more phosphorus than an equivalent amount of dried product. This may have been conversion of a small fraction of the Zr (IV) species to zirconium oxides during the drying process. Zirconium dioxide is nearly insoluble in water and probably is not an effective phosphorus inactivant.[12]

Greater quantities of aluminum sulfate (alum) and sodium aluminate were required than zirconium tetrachloride and zirconyl chloride. A molar ratio of 7.0 was necessary for 100 percent phosphorus removal with sodium aluminate, while aluminum sulfate removed 95 percent of the phosphorus at a molar ratio of 3.0. Although the 95 percent removal figure could not be exceeded with alum its removal efficiency on a molar basis was better than for sodium aluminate. The slightly greater effectiveness of both aluminum compounds in AAP medium than in pond water probably resulted from the difference in chemical composition of the two media and the different concentration of inactivant used in each experiment.[9,13] With the exception of sodium aluminate in AAP medium, a curvelinear relationship resulted between aluminum added and phosphorus removed, suggesting that at pH 7.0 phosphorus removal was by some process other than chemical reaction. The linear response by sodium aluminate in AAP medium cannot be explained from these data.

Algal assay experiments showed that when less than 100 percent of the ortho-phosphate phosphorus was removed from solution by inactivation, the residual was available for uptake by the test alga. It is not clear, however, whether the phosphorus passing through the 0.45 μ filter was not bonded to the inactivant or whether it was usable by the algae in spite of being bonded.

VI. TOXICITY AND ENVIRONMENTAL EFFECTS

OBJECTIVES AND APPROACH

Candidate materials which satisfactorily passed the initial screening tests were next required to undergo testing for the determination of possible adverse environmental effects, particularly toxicity to aquatic fauna. The primary objectives were to examine organisms which would be representative of the natural system and various levels in the food web. If toxicity was demonstrated by an inactivant during the tests this would have to be taken into consideration before any subsequent application.

The toxicity tests were designed to evaluate the effects of the inactivants under the most extreme environmental conditions. Static rather than continuous flow systems were used, on the premise that static systems would provide environmental conditions more stringent than the organisms would be expected to encounter. The fish in particular were under increased stress because of the absence of flowing water and the confined conditions of the testing chambers. All the test organisms were constantly exposed to quantities of inactivant-phosphorus precipitant greater than those which they would likely encounter under natural conditions. As noted for the jar tests, dissolved inactivant concentrations could not be satisfactorily measured, and, unless noted, concentrations refer only to quantities added.

Tests consisted of 96-hour, one generation bioassays using salmonid fish and cladocerans (Daphnia magna), and 9-week, three generation bioassays on D. magna only. Additional tests are planned for the larva of the benthic midge Paratanytarsus sp; therefore, when all tests are completed, organisms from three critical portions of the aquatic ecosystem will have been subjected to inactivant stress. The midge larvae fish and cladocerans will represent the benthos nekton, and zooplankton communities, respectively.

METHODS

96-Hour Tests

Fish -

Fish bioassays were conducted in accordance with the Static Bioassay described in Standard Methods, 13th edition[14]. Test fish were obtained from local fish hatcheries at Eagle Creek, Fall Creek, and Roaring River. The tests were conducted with specimens of Oncorhynchus tshawytscha (chinook salmon), Oncorhynchus kisutch (coho or silver salmon), and Salmo gairdneri (rainbow trout). Species were not mixed in a given test.

Fish were transported from the hatcheries to our laboratory in aerated hatchery water, then placed in a 10°C environmental room to acclimate for 24 hours before transfer to a holding tank supplied with carbon-filtered tap water. Dissolved oxygen levels were maintained at 8-9 mg/l. The fish were provided with commercial fish food daily in amounts such that a minimal residue remained the following day. All fish were allowed to acclimate for a period of 14 days and were not fed for two days prior to the initiation of testing. The toxicity tests were conducted at 10°C. Twenty-liter wide-mouth soft-glass containers were filled with 15 liters of carbon-filtered tap water, and the inactivant added at concentrations 4 to 10 times expected operational levels.

Because of the basic nature of the aluminum compound and the extreme acidity of the zirconium compounds, it was necessary to neutralize the mixtures to pH 7.0 ± 0.2 with reagent grade sodium hydroxide

or hydrochloric acid. The tests consisted of duplicates of the controls and the four different additions of the material being examined. Five fish were used in each of two containers at each concentration, and for each control. Daily observations were made on the general behavior and mortality of the fish.

TL_m (mean tolerance limits) values were determined from the percent survival of the test organisms over the 96-hour test period. The TL_m is defined as the concentration of toxicant at which 50 percent of the test organisms survive a preselected time period. For example, the 96-hour TL_m is the concentration at which 50% of the organisms are alive after 96 hours. The values are determined graphically by extrapolation between any two successive concentrations in which one value is above the 50% survival value and one is below. This is illustrated in Figure 12.

Cladocera -

The test used is one developed by the EPA National Water Quality Laboratory, Duluth, Minnesota, for pesticide testing. A clone of Daphnia magna (Straus) was kept in well water at room temperature (22° ± 3°C), with 16-hour light and 8-hour dark photoperiods. Well water from the EPA Western Fish Toxicology Station, Corvallis, Oregon, was used as a test medium because of the extreme sensitivity of these organisms to chlorine and its derivatives. Stock cultures were maintained in one gallon soft-glass jars and fed a mixture of ground nettle powder, water, and commercial fish pellets twice weekly.

Tests were conducted in a manner similar to the 96-hour fish bioassays. Four hundred ml of well water were added to 600 ml acid-washed Griffin beakers. The inactivant was added to each beaker from a fresh stock solution, and the mixture neutralized with either hydrochloric acid or sodium hydroxide to pH 7.0 ± 0.2. Reagent grade sodium aluminate was used in the toxicity tests on aluminum. The zirconium compounds were the "crude" forms, containing less than two percent impurities, as used in the "jar tests." Additions as presented in this text were not corrected for these impurities, thus, the actual quantity of zirconium could be up to 2 percent less than that listed. A considerable deposit of insoluble material remained when the zirconium tetrachloride was mixed with the water; therefore, the supernatant was carefully pipetted from the stock solution to avoid contamination with the undissolved solids. Lanthanum rare-earth chloride (LaRECl) was added as the complete compound and data represent mg/l of the entire compound. Actual lanthanum content of the LaRECl was about 23.5 percent.

One day after neutralization five 24-hour-old (± 12 hours) Daphnia magna were added to each beaker, and the beakers placed in environmental chambers at 18° ± 2°C. Light, provided by cool white fluorescent bulbs at an intensity of approximately 2150 lux, was cycled on a 16-hour light and 8-hour dark photoperiod. Surviving organisms were enumerated every 24 hours until the 96-hour test was completed, and the results used in the calculation of the 96-hour TL_m. Three separate tests were conducted on each inactivant. During each test, four replicates were run for each inactivant addition, making a total of twelve replicates for each concentration of a given inactivant material.

9-Week Three-generation Tests

Cladocera -

The rearing methodology and general experimental procedure were the same as for the 96-hour tests, but the counting procedure differed. Counts were made at weekly intervals, at which time the surviving adults (parental generation) were transferred to new water-inactivant mixtures. The young were counted and discarded. At the end of week 3 the adults were discarded and five of the youngest offspring (F. generation) were transferred to fresh water-inactivant mixtures. Counts and transfers were conducted for the following 3 weeks in the manner described for the original test organisms. At the end of week 6, the adults were again discarded and five of the youngest offspring (F_2 generation) transferred to fresh water-inactivant mixtures. Counts and transfers were made as previously described. Three different generations were thereby examined: the original (parental) generation which was never exposed to the inactivant before birth, one generation (F_1) whose parents were exposed after birth and who themselves were exposed to the inactivant for all phases of their life cycle, and one generation (F_2) whose parents as well as themselves were exposed to the inactivant throughout all phases of their life cycle. Replication was the same as in the 96-hour tests.

Graphic determinations of 1-week TL_m values were made and reproductive rates per individual were calculated. The reproductive rate per individual was estimated by dividing the total number of young surviving at the end of the one-week interval by the mean of the adults existing at the beginning and at the end of the week.

Tests With Benthic Organisms

The benthic test organism is a midge, originally classified as Tanytarsus dissimilus (Johannsen). Its exact taxonomic status is presently in doubt. Professor J. E. Sublette, Eastern New Mexico University, (personal communication) considers it a form of Paratanytarsus. The organism is ideal for stress studies because of its extreme sensitivity to toxicants (Nebeker and Puglisi[15], Nebeker[16], Bell[17]), and its parthenogenetic mode of reproduction. We have maintained cultures in one-gallon soft-glass jars for over six months and the organisms have continued to reproduce at the expected frequency. They are fed, twice weekly, with the same food used for the Daphnia. Water levels are maintained by addition of glass-double-distilled water.

Tests using this organism have not yet been conducted, but they will be designed to measure survival through all stages of the aquatic life cycle. The egg masses will be collected from the adults and placed in petri dishes to hatch. Five first instar larvae will be removed, placed in beakers, and incubated for approximately 18 days at 22°C, the approximate time necessary for the midge to complete its aquatic life cycle. The number of cast pupal skins found on the water surface after adult emergence will be used to calculate the TL_m values.

Chemical Data

Alkalinity, hardness and dissolved solids were determined at the beginning of each study. Average values for each pair of duplicated containers appear in Table 13, which lists all the tests that were carried

Table 13. Chemical Composition of Water Used in Toxicity Tests

Inactivant	Organism	Inactivant Concentration mg/l	Alkalinity mg/l	Hardness mg/l	Dissolved Solids mg/l
Sodium Aluminate	Chinook Salmon	0.0 (Control)	25.0	28.0	---
Sodium Aluminate	Daphnia	0.0 (Control)	27.0	27.0	69.0
Zirconium Refinery Waste	Coho Salmon	0.0 (Control)	28.0	29.0	67.8
Zirconium Refinery Waste	Daphnia	1.0	45.3	69.8	139.5
		5.0	104.8	165.5	268.5
		10.0	220.5	279.3	426.3
		15.0	398.0	420.0	536.3
Zirconium Tetrachloride	Coho Salmon	0.0 (Control)	34.0	38.0	92.0
		0.5	28.0	37.5	98.0
		1.0	27.0	37.0	89.0
		6.7	28.0	38.5	100.0
		10.0	27.5	36.0	81.0
Zirconium Tetrachloride	Daphnia	0.0 (Control)	25.7	28.0	77.0
		0.5	22.7	27.7	81.3
		1.0	23.0	28.0	76.7
		5.0	20.0	29.7	121.0
		10.0	26.3	26.7	99.7
Zirconium Tetrachloride (Week 1)	Daphnia	0.0 (Control)	30.0	28.0	70.0
		1.0	28.0	30.0	73.0
		5.0	26.0	28.0	77.0
		10.0	24.0	27.0	84.0
		20.0	21.0	25.0	118.0
Zirconium Tetrachloride (Week 2)	Daphnia	0.0 (Control)	25.0	34.0	73.0
		1.0	23.0	24.0	74.0
		5.0	18.0	30.0	81.0
		10.0	6.0	32.0	91.0
		20.0	2.0	26.0	110.0
Zirconium Tetrachloride (Week 3)	Daphnia	0.0 (Control)	25.0	----	78.0
		1.0	23.0	29.0	77.0
		5.0	19.0	27.0	72.0
		10.0	10.0	30.0	96.0
		20.0	4.0	27.0	103.0
Zirconium Tetrachloride (Week 4)	Daphnia	0.0 (Control)	27.0	28.0	81.0
		1.0	31.0	32.0	70.0
		5.0	23.0	28.0	80.0
		10.0	2.0	32.0	99.0
		20.0	2.0	30.0	124.0

Table 13. (Continued)

Inactivant	Organism	Inactivant Concentration mg/l	Alkalinity mg/l	Hardness mg/l	Dissolved Solids mg/l
Zirconium Tetrachloride (Week 5)	Daphnia	0.0 (Control)	27.0	28.0	67.0
		1.0	27.0	29.0	67.0
		5.0	19.0	28.0	70.0
		10.0	11.0	28.0	74.0
		20.0	4.0	25.0	98.0
Zirconium Tetrachloride (Week 6)	Daphnia	0.0 (Control)	30.0	26.0	79.0
		1.0	29.0	28.0	76.0
		5.0	20.0	24.0	83.0
		10.0	12.0	25.0	92.0
		20.0	7.5	26.0	103.0
Zirconium Tetrachloride (Week 7)	Daphnia	0.0 (Control)	32.0	30.0	78.0
		1.0	22.0	32.0	76.0
		5.0	18.0	29.0	80.0
		10.0	2.0	25.0	85.0
		20.0	2.0	26.0	108.0
Zirconium Tetrachloride (Week 8)	Daphnia	0.0 (Control)	28.0	28.0	71.0
		1.0	26.0	30.0	73.0
		5.0	22.0	28.0	79.0
		10.0	20.0	28.0	88.0
		20.0	16.0	28.0	118.0
Zirconium Tetrachloride (Week 9)	Daphnia	0.0 (Control)	30.0	33.0	63.0
		1.0	----	36.0	90.0
		5.0	24.0	34.0	70.0
		10.0	21.0	35.0	77.0
		20.0	20.0	42.0	103.0
Lanthanum Rare Earth Chloride	Coho Salmon	0.0 (Control)	32.0	25.0	89.0
		1.0	29.5	35.5	93.0
		5.0	27.5	35.5	96.0
		10.0	28.0	36.0	96.0
		20.0	27.0	36.0	110.0
Lanthanum Rare Earth Chloride	Daphnia	0.0 (Control)	28.0	29.0	79.7
		0.5	28.0	29.3	84.3
		1.0	28.0	29.3	86.0
		2.0	27.7	28.7	89.3
		5.0	28.3	29.7	107.0
Zirconyl Chloride	Daphnia	0.0 (Control)	30.0	32.7	77.3
		1.0	31.7	32.7	73.3
		5.0	31.3	32.0	105.3
		10.0	32.0	31.7	98.7
		20.0	34.0	30.7	114.3

out. In tests using sodium aluminate the soluble aluminum fraction was measured at the beginning of the tests; those results are given in the sections dealing with the aluminum bioassays.

Experimental Difficulties

The floc, in some instances, acted as a physical barrier to movement for the Daphnia. In higher concentrations, particularly with sodium aluminate, they could be seen trailing strings of floc from their caudal spine. It was necessary to feed the organisms because of the test duration, which may have resulted in reduced toxicity.

RESULTS

Sodium Aluminate

Fish -

Chinook salmon (12-15 cm) were used to test sodium aluminate in concentrations of 0.0 (Control), 5.0, 10.0, 20.0, and 40.0 mg Al/l. These values represent the amount added (as aluminum), not the amount of particulate or soluble aluminum remaining in solution after the precipitation reaction. Table 14 gives actual soluble aluminum concentrations. The erratic distribution of values can not be explained adequately, but probably lies within the limits of error of the analysis.

Table 14
Dissolved Aluminum Concentrations, Sodium Aluminate
Fish Bioassay

Added Aluminum Concentration	Aluminum Concentration at 0 hours (mg/l)	Dissolved Aluminum Concentration at 96 hours (mg/l)
0.0 (Control)	<0.02	<0.02
5.0	0.03	<0.02
10.0	0.07	0.05
20.0	0.04	<0.02
40.0	<0.02	<0.02

All fish survived the 96-hour test period. Only five fish were tested at the 40 mg/l concentration because of a shortage of fish, and only nine at 10 mg/l because of an initial miscount. The test was allowed to continue for 216 hours. One fish whose caudal fin had been chewed by the others died in the 40 mg/l concentration at 120 hours; all the others survived.

Cladocera: 96 Hours -

Sodium aluminate was relatively non-toxic to D. magna. Somewhat higher mortalities were observed in the higher concentration levels but at no time was a 96-hour TL_m reached. Experimental concentrations of 0.0 (Control), 5.0, 10.0, 20.0, and 40.0 mg Al/l were used in the bioassay. The average soluble aluminum values for these tests are given in Table 15. A graphic presentation of the percentage survival is shown in Figure 9.

Table 15

Aluminum Concentrations, Sodium Aluminate Daphnia magna Bioassay

Added Aluminum Concentration (mg/l)	Dissolved Aluminum Concentration at 96 hours (mg/l)
0.0 (Control)	0.045
5.0	0.055
10.0	0.045
20.0	0.040
40.0	0.080

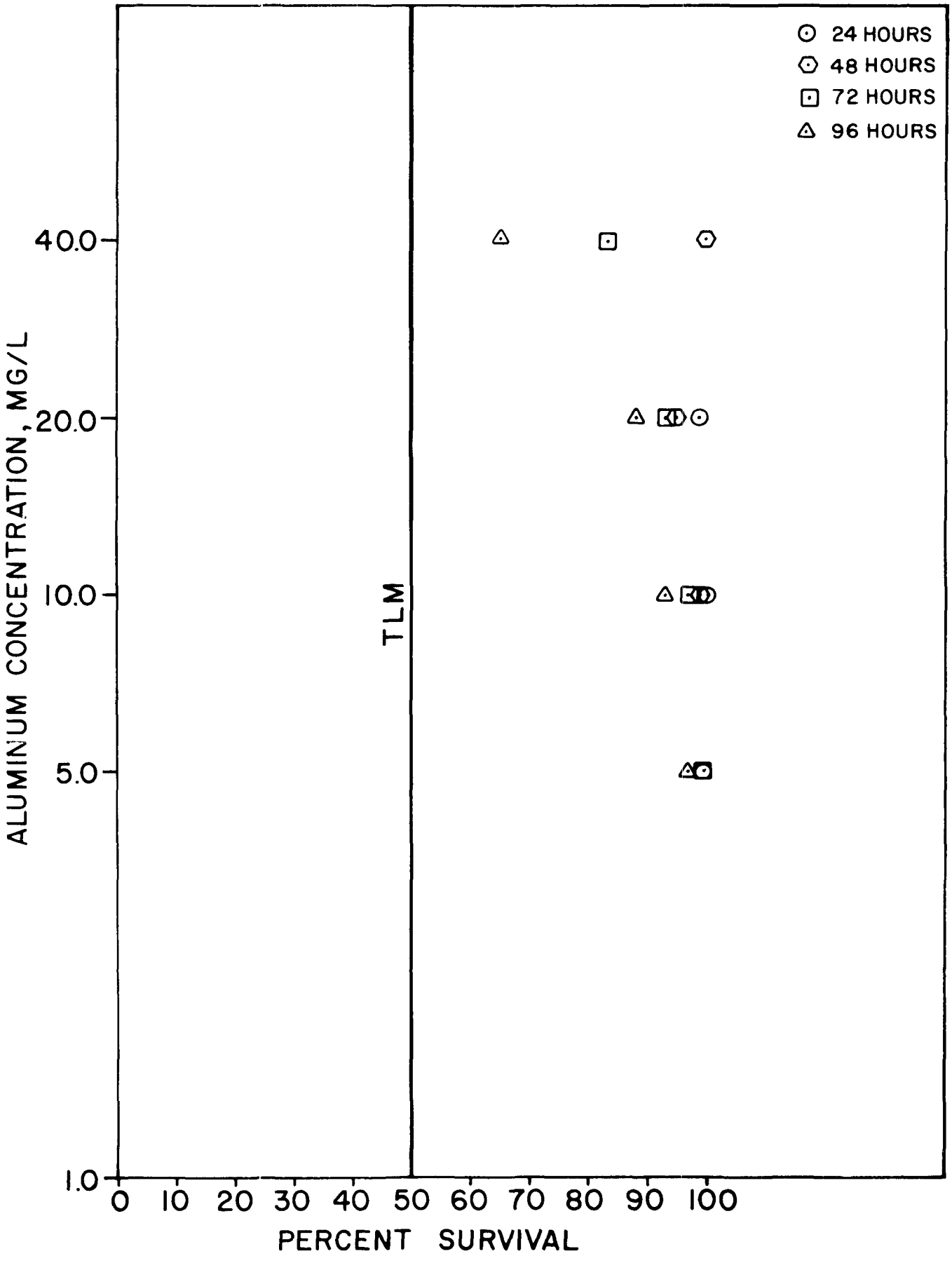

Figure 9. Percent survival of Daphnia magna in four concentrations of sodium aluminate over 96 hours

Zirconium Refinery Waste

Fish -

Coho salmon of similar size were used in the zirconium refinery waste toxicity tests (Table 16). Quantities added were 0.0 (Control), 1.0, 5.0, 10.0, and 15.0 grams of waste per liter. A highly turbid condition resulted upon addition of the waste material, sufficient to prevent visual observation of the fish during the early stages of the test. At the end of 96 hours most of the material had settled. All fish survived the 96-hour test and a continuation to 192 hours.

Table 16

Average Length/Weight Measurements of Coho Salmon at Termination of 96-Hour Zirconium Refinery Waste Bioassay

Refinery Waste Added g/l	Avg. Length (cm)	Avg. Wt. (g)
0.0 (Control A)	14.2	24.4
0.0 (Control B)	14.1	21.1
1.0 A	14.0	21.9
1.0 B	14.0	21.9
5.0 A	13.9	21.9
5.0 B	14.3	23.6
10.0 A	14.0	22.1
10.0 B	14.4	23.1
15.0 A	14.3	21.8
15.0 B	13.7	21.0

Cladocera -

The zirconium refinery waste was also tested with D. magna. The dry weight of material added was 0.0 (Control), 1.0, 5.0, 10.0, and 15.0 g/l. As with the fish, the experimental solutions were very turbid after the material was added; however, after 24 hours clarity was sufficient to permit enumeration. Observed mortalities reached 15% of the total population (Figure 10).

Zirconium Tetrachloride

Fish -

Zirconium tetrachloride was tested with coho salmon from the same group used for zirconium refinery waste. The concentrations tested were 0.0 (Control), 0.5, 1.0, 6.7, and 10.0 mg Zr/l.

All fish survived the 96-hour test. The tests were then continued to 240 hours during which time two fish died, a control at 120 hours and one in the 1.0 mg Zr/l solution at 192 hours. The remaining fish still survived when the test ended at 240 hours. Table 17 lists the average length and weight measurements at the termination.

Table 17

Average Length/Weight Measurements of Coho Salmon at Termination of 96-Hour Zirconium Tetrachloride Bioassay

Zirconium Concentration (mg/l)	Avg. Length (cm)	Avg. Wgt. (g)
0.0	12.5	16.5
0.0	12.7	20.0
0.5	13.4	22.4
1.0	13.0	21.0
1.0	12.3	17.6
1.0	12.4	19.3
6.7	14.2	27.7
6.7	12.2	17.4
10.0	13.4	22.9
10.0	13.7	22.3

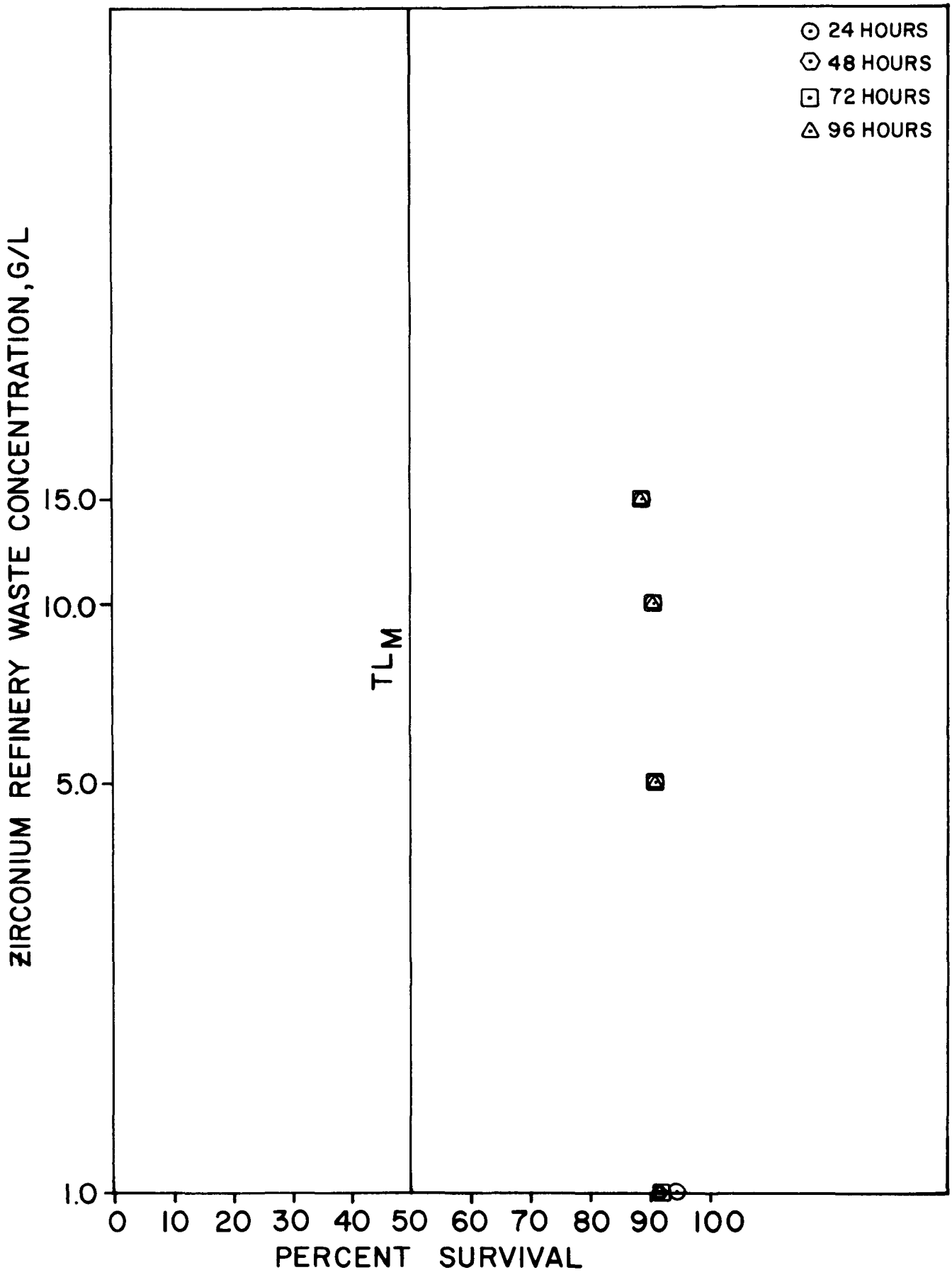

Figure 10. Percent survival of Daphnia magna in four concentrations of zirconium refinery waste over 96 hours.

Cladrocera: 96 Hours -

Crude zirconium tetrachloride was relatively non-toxic to D. magna at the concentrations tested: 0.0 (Control), 1.0, 5.0, 10.0, and 20.0 mg Zr/l. A 96-hour TL_m was not reached within this range. Higher mortalities were noted in the 10.0 and 20.0 mg/l concentrations; the maximum number of deaths represented 22% of the original population (Figure 11).

Cladocera: 9 Weeks -

Crude zirconium tetrachloride was also subjected to the 9-week test. The concentrations were: 0.0 (Control), 5.0, 10.0, and 20.0 mg Zr/l. A 7-day TL_m was not found during either the first or second week. At the end of week 3, a TL_m of 2.0 mg Zr/l had been reached (Figure 12). Following the first three-week test period, five of the smallest (or the total surviving if less than five) offspring in each beaker were transferred to another beaker containing the same concentration of zirconium, and the test carried out for another three weeks. The week 4 animals (comparable in age structure to the week 1 individuals of the initial three-week run) exhibited a TL_m of 20.0 mg Zr/l. The week 5 organisms (comparable in age to the second-week organisms) had a TL_m of 11.5 mg Zr/l and the week 6 (comparable in age to week 3), 1.1 mg Zr/l (Figure 13).

The transfer procedure was repeated at the end of week 6. The TL_m for week 7 organisms, of a comparable age to those in weeks 1 and 4, decreased to 18.5 mg Zr/l. A week 8 TL_m was observed at two points--16.0 and 4.4 mg Zr/l. The week 9 TL_m was 1.8 mg Zr/l (Figure 14).

Reproductive rate per individual per week was also calculated for the nine-week test. Figure 15 indicates that, in general, the higher the concentration the fewer young produced.

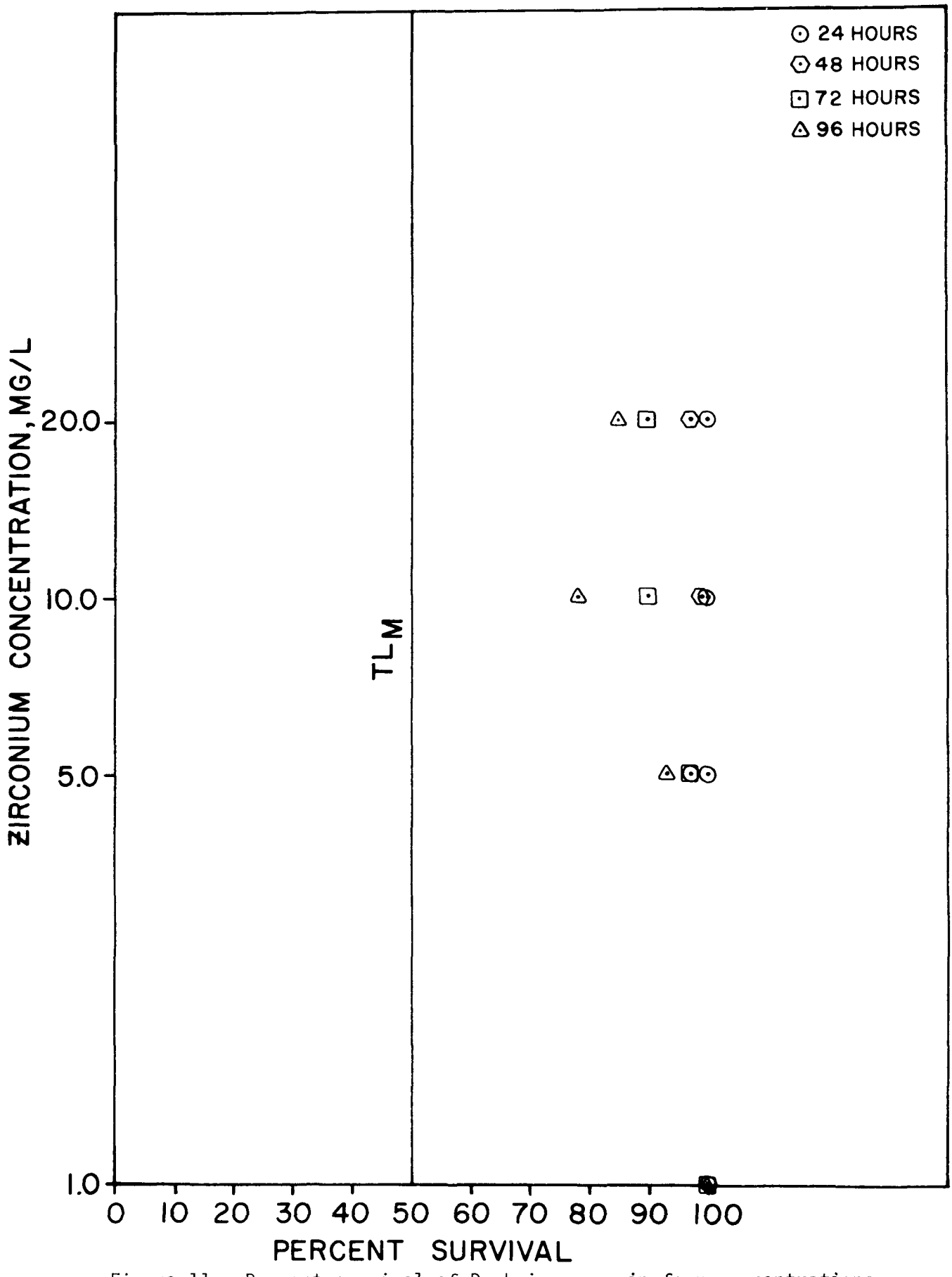

Figure 11. Percent survival of Daphnia magna in four concentrations of zirconium tetrachloride over 96 hours.

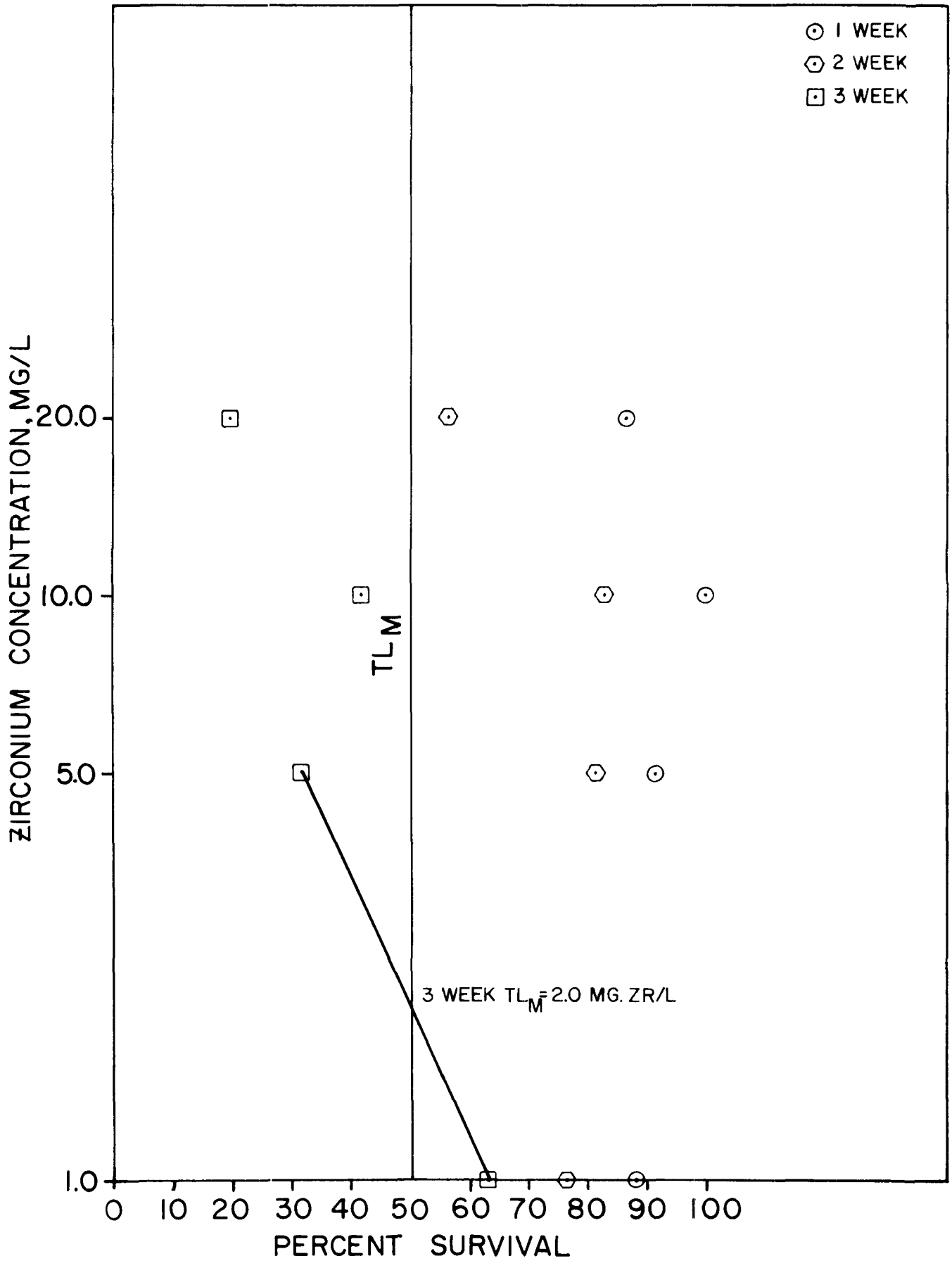

Figure 12. Percent survival of Daphnia magna in three concentrations of zirconium tetrachloride during weeks 1-3 of 9-week toxicity test.

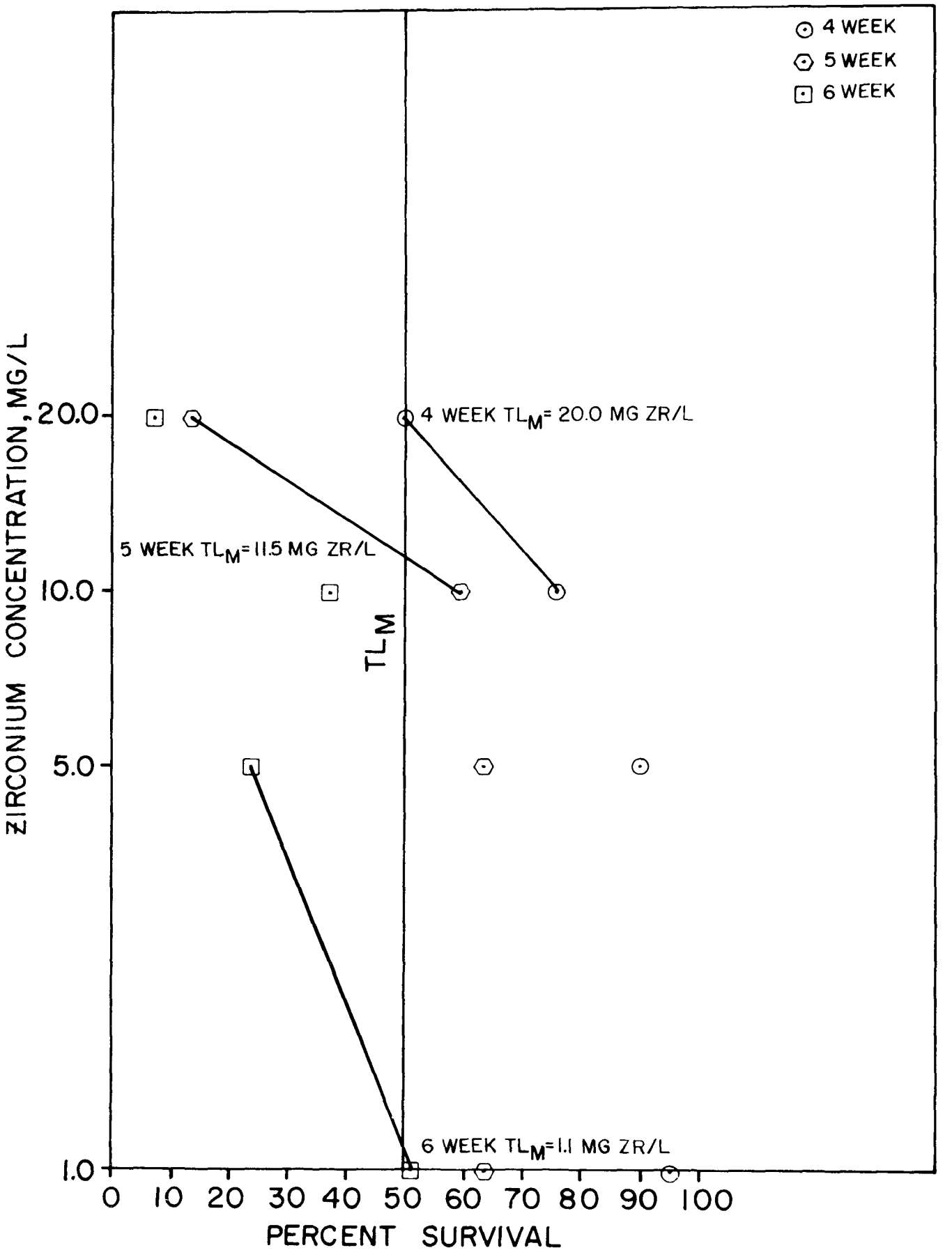

Figure 13. Percent survival of Daphnia magna in three concentrations of zirconium tetrachloride during weeks 4-6 of 9-week toxicity test.

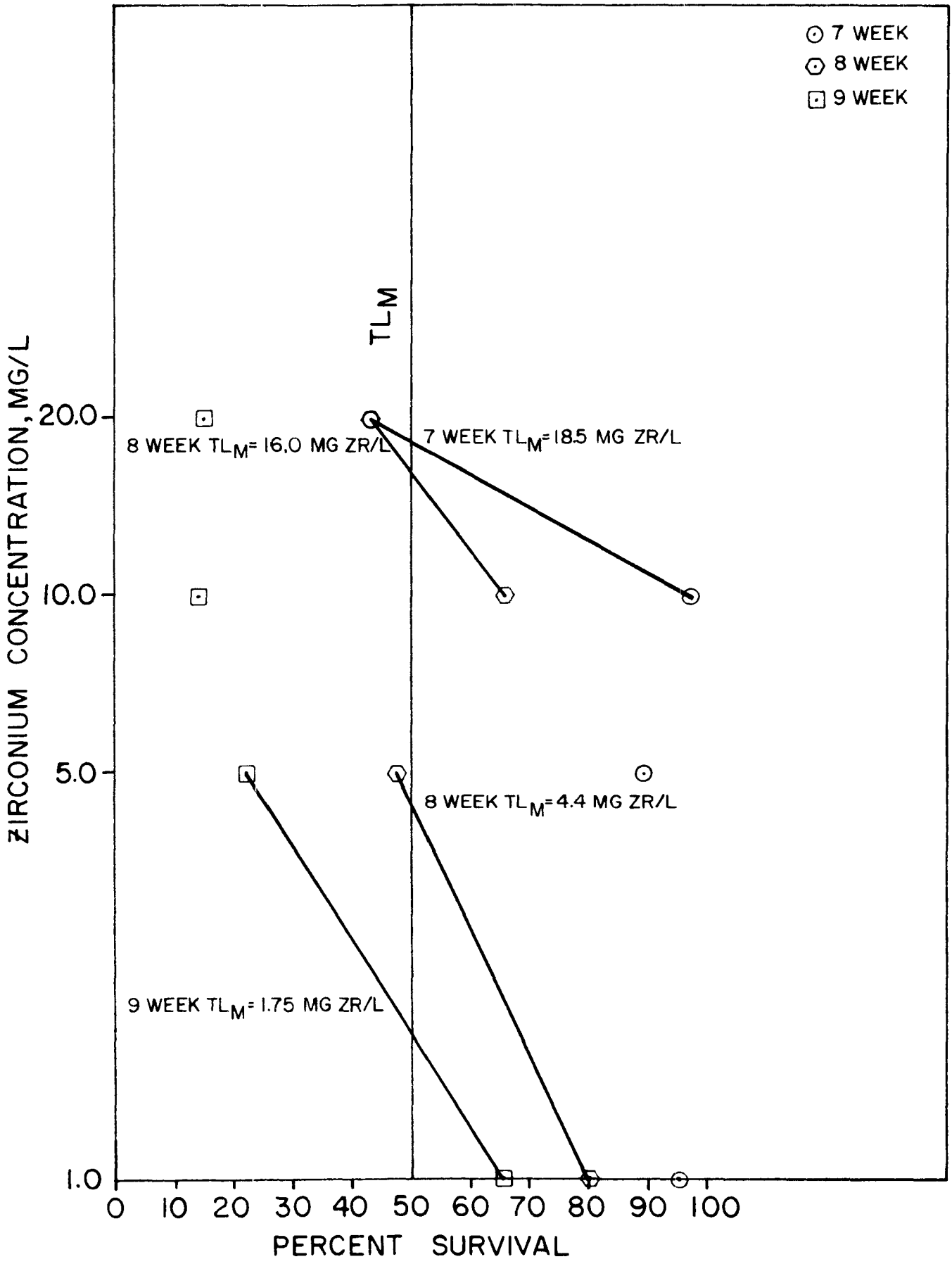

Figure 14. Percent survival of Daphnia magna in three concentrations of zirconium tetrachloride during weeks 7-9 of 9-week toxicity test.

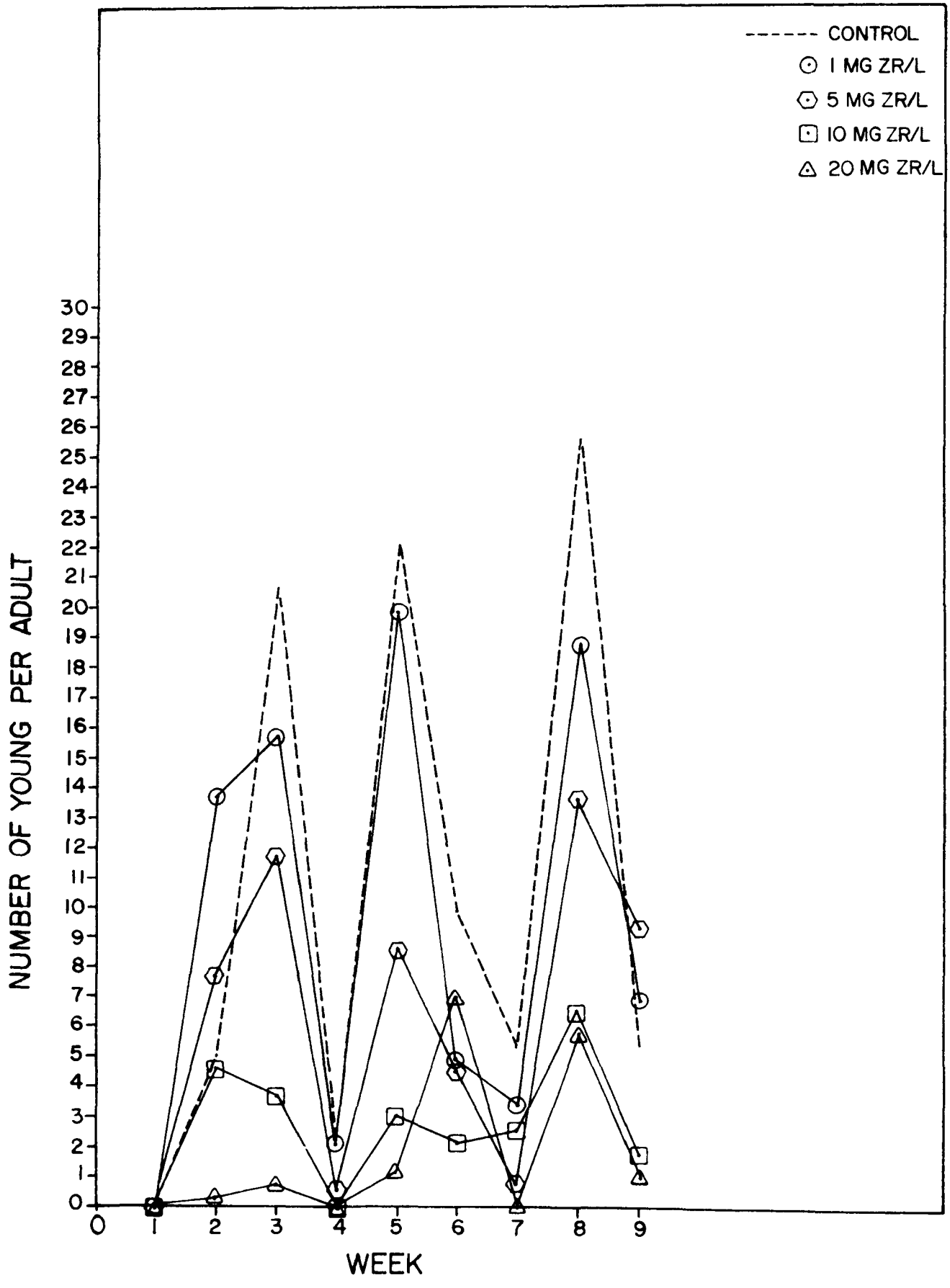

Figure 15. Daphnia magna reproductive rate per individual per week during 9-week zirconium tetrachloride toxicity test.

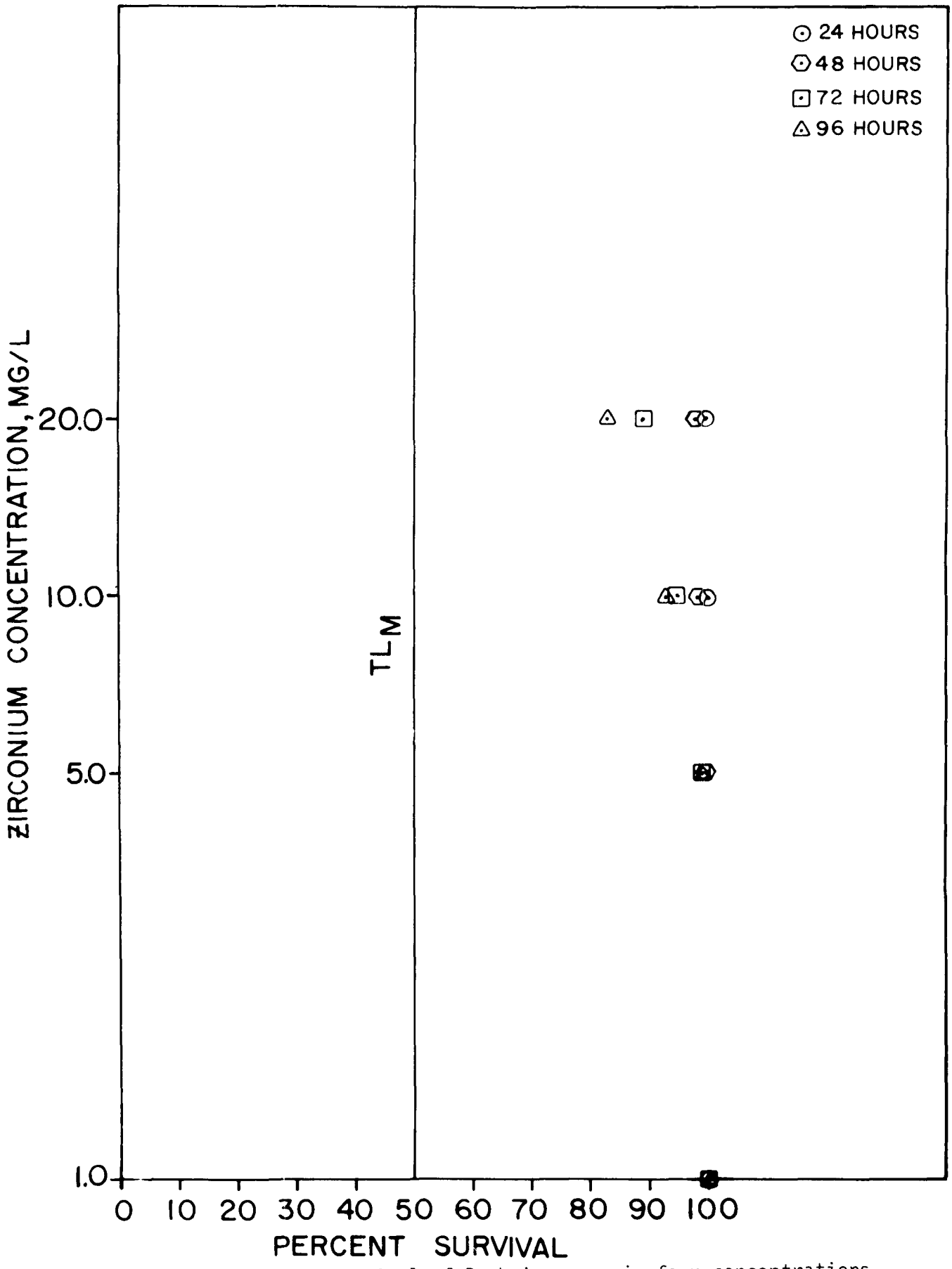

Figure 16. Percent survival of Daphnia magna in four concentrations of zirconyl chloride over 96 hours.

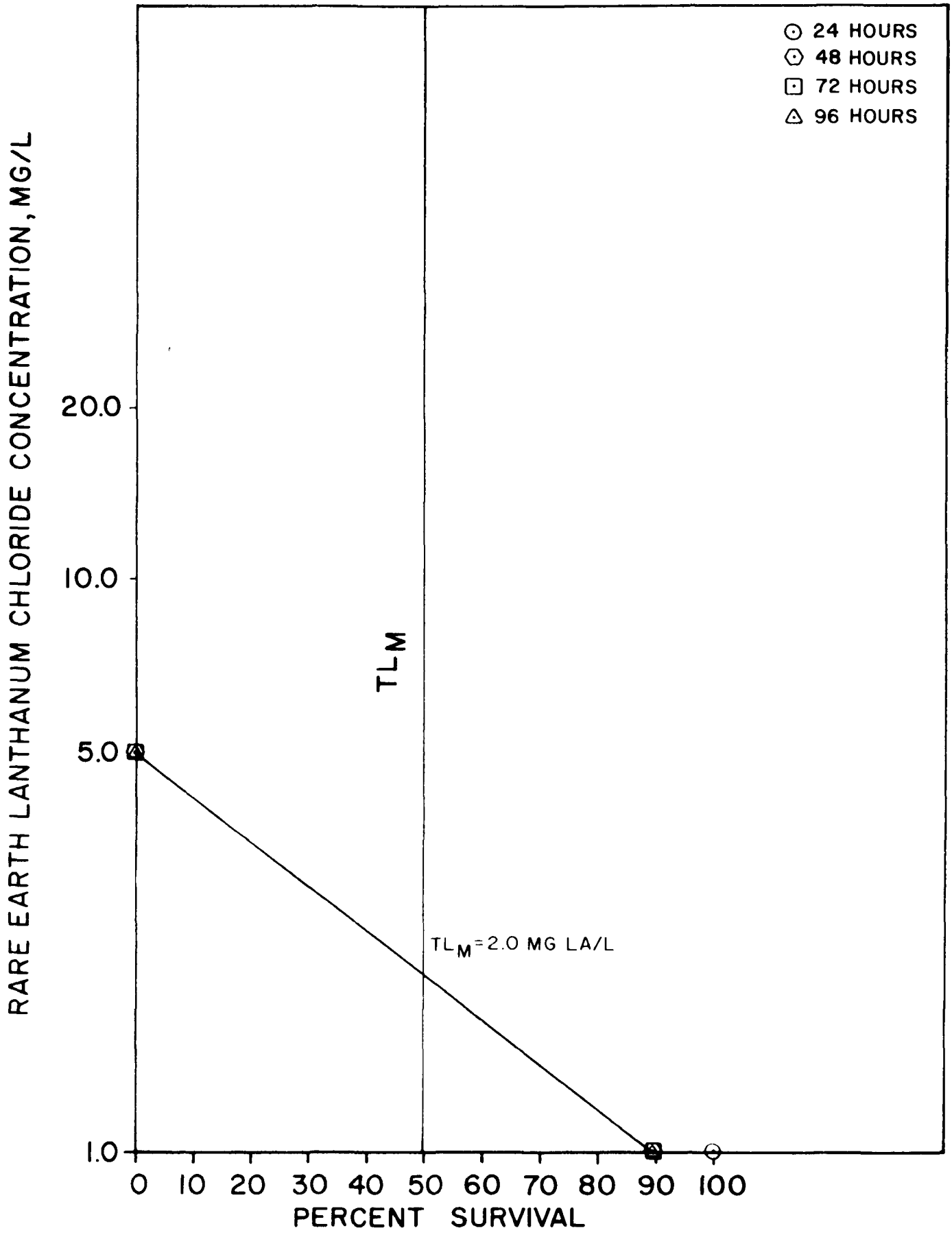

Figure 17. Percent survival of coho salmon in four concentrations of lanthanum rare earth chloride over 96 hours.

Zirconyl Chloride

Fish -

No tests were carried out on fish.

Cladocera -

Zirconyl chloride ($ZrOCl_2$) was tested at 0.0, 1.0, 5.0, 10.0, and 20.0 mg Zr/l. The results were similar to those obtained with zirconium tetrachloride, with excellent survival at all concentrations. Maximum mortality was 17%, occurring at the 96 hours, 20 mg Zr/l level (Figure 16).

Lanthanum Rare-Earth Chloride

Fish -

A lanthanum rare earth chloride was tested in the same manner as the previous substances, using coho salmon. The concentrations used were: 0.0 (Control), 1.0, 5.0, 10.0, and 20.0 mg La rare earth chloride mixture/l. All fish exposed to concentrations greater than 1.0 mg/l died within the first 24 hours (Figure 17). The TL_m for 24 hours was approximately 2.0 mg/l. The control and 1 mg/l organisms survived to 240 hours with the loss of only one fish. Average length and weight measurements at the termination of the test are listed in Table 18.

Table 18

Average Length/Weight Measurements of Coho Salmon at Termination of 96-Hour Lanthanum Rare Earth Chloride Bioassay

Lanthanum Rare Earth Chloride Concentration (mg/l)	Avg. Length (cm)	Avg. Weight (g)
0.0	13.7	20.1
0.0	13.2	16.1
1.0	13.4	17.4
1.0	12.9	15.1
5.0	14.3	24.5
5.0	14.5	26.1
10.0	13.7	23.1
10.0	14.5	23.9
20.0	14.0	24.8
20.0	14.0	23.4

Cladocera -

Rare earth lanthanum chloride proved to be more toxic than the other substances. Because of the high mortality of fish, Daphnia were examined at decreased inactivant concentrations to include 0.0 (Control), 0.5, 1.0, 2.0, and 5.0 mg La rare earth chloride mixture/l. A 72-hour TL_m of 2.81 mg/l and a 96-hour TL_m of 1.61 mg rare earth lanthanum chloride were obtained (Figure 18).

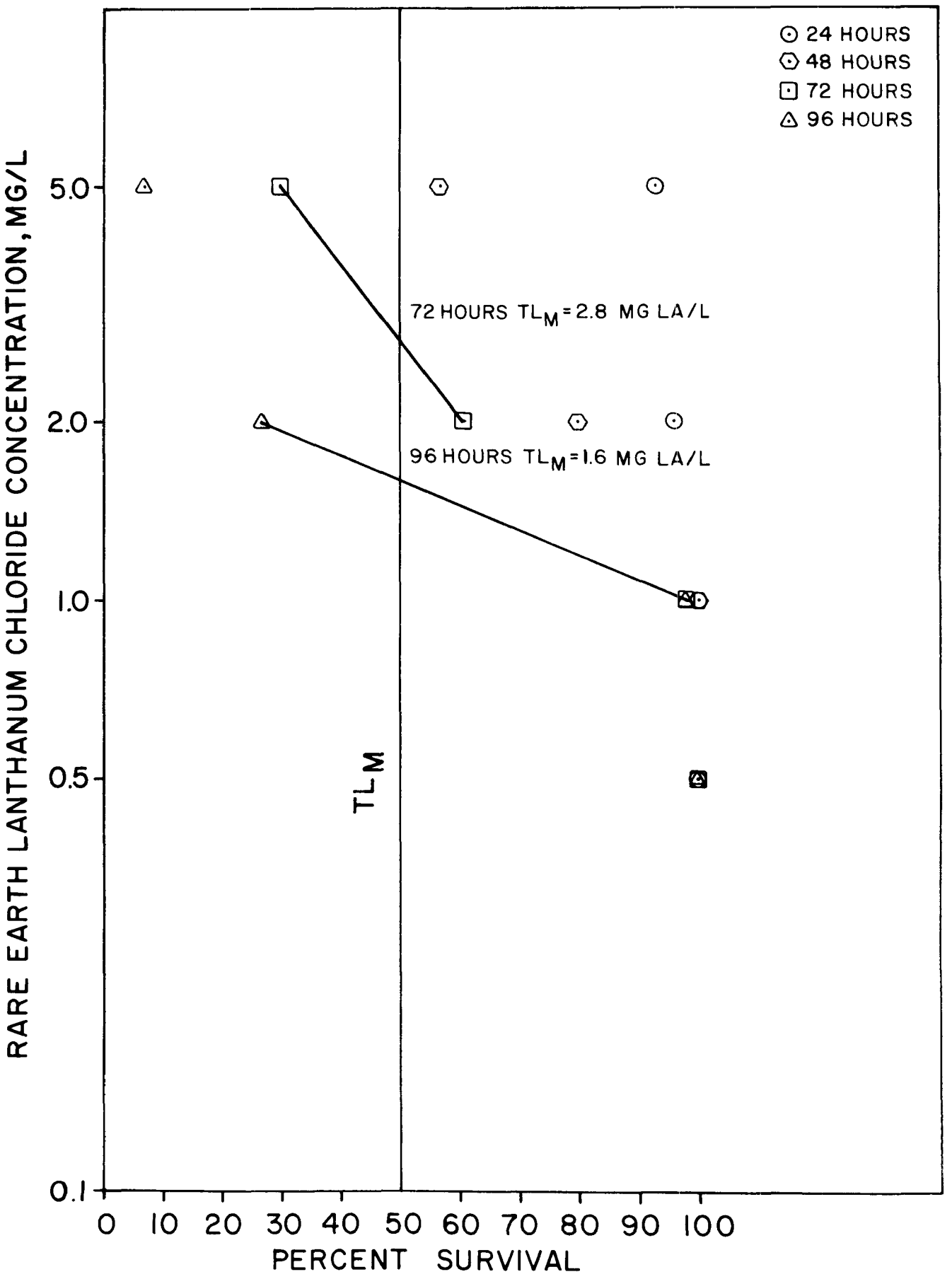

Figure 18. Percent survival of Daphnia magna in four concentrations of lanthanum rare earth chloride for over 96 hours.

DISCUSSION

The foregoing tests were designed to determine whether substances being screened as possible nutrient inactivants could be expected to be harmful to aquatic organisms over anticipated ranges of operational concentrations. Results should not be interpreted as criteria for discharge levels or water quality standards.

The results of the toxicity tests should be interpreted in a general manner. As previously stated, they were designed specifically to test the inactivant under laboratory conditions and the relation of these results to actual field conditions is unknown. In those cases where a gradual increase in toxicity occurs with increasing inactivant concentration, as demonstrated by zirconium and aluminum, a workable concentration range could probably be established. However, the results with highly toxic materials, as seen with the lanthanum rare earth chloride, suggests the use of extreme caution even though loss of biota might be considered non-critical to the project. Potable water supplies for humans and animals could be involved.

The importance of toxicity in relation to field conditions is likely to be variable. Under some conditions a massive reduction in the native biota may be tolerable if the toxicity is of short duration. Heavy application of an inactivant could in some cases result in a blanketing of the sediment water interfaces. The composition of this substance might prevent recolonization by benthic populations and thus lead to a significant reduction in the consumer population with an overall shift in the structure of the ecosystem. The use to

which a particular aquatic environment is to be put becomes an important factor in determining if, and what type of nutrient inactivation will be used. The loss of a critical nutrient (the primary focus of the inactivation treatment) will in itself cause a reduction in the consumer population through reduction of the available food supply. Thus, if it is sought to achieve conditions where nuisance algal growth is limited but a productive fishery is maintained, toxicity of an inactivant will likely become a critical consideration. If the desire is to create a quasi-oligotrophic system, however, a short term toxicity may be tolerable providing the treatment does not prevent recolonization by aquatic organisms for an extended period of time.

The introduction of materials with cumulative effects must be avoided. Such materials would damage not only the aquatic population but might eventually be cycled to humans. Therefore, the utmost precaution, involving thorough testing of inactivants, must preceed their general use.

Following is a brief resume of the data available on the toxicity of various compounds tested during the present study. Our results are compared to those from the literature where possible to determine if the operational inactivant levels established by our experiments are compatible with non-toxic levels of the material as reported by others.

Aluminum

Everhart and Freeman[18] found (1) debilitation of rainbow trout after one week's exposure to the addition of 5.2 mg/l aluminum at any pH examined; (2) acute mortalities at 5.2 mg/l dissolved aluminum, while mortality rates were more chronic with equivalent

suspended amounts (chronic being defined as a long-termed debilitation without lethality); (3) toxic effects were lower for suspended aluminum than dissolved aluminum, and (4) physiological damage incurred by young fish appeared to be reversible in the survivors. They also found the fish were able to tolerate 0.05 mg/l dissolved aluminum, with normal-appearing growth and behavior. This concentration represents the saturation point at pH 7.0 and will remain in solution at all higher pH levels.

Anderson[19] found aluminum chloride toxic to Daphnia magna at 6.7 mg/l in Lake Erie water. Pulley[20], on the other hand, found no toxicity to small marine invertebrates and adult fish from 44 mg/l of aluminum chloride at a pH range of approximately 5.2 - 6.8. Water Quality Criteria[21] lists various aluminum salts which have been found to be toxic to fish and other aquatic life. Aluminum nitrate at 0.10 mg/l was lethal to sticklebacks exposed for one week; 0.30 mg/l was lethal after an exposure of only one day. Aluminum chloride at 0.27 mg/l was lethal to eels by 50 hours, and 2.7 mg/l was lethal by 3.6 hours. Biesinger and Christensen[22] established an LD_{50} of 1.40 mg Al/l for Daphnia magna in Lake Superior water over a three-week period (pH range 6.5 - 7.5) at a concentration of 5 mg Al/l.

Our tests indicated that sodium aluminate adjusted to pH 7.0 ± 0.2 at the beginning of the test was not lethal to fifty percent of the organisms tested, either chinook salmon or cladocera. A maximum of 35% of the Daphnia died after 96 hours at approximately 40 mg Al/l. The actual concentration of dissolved aluminum ranged from 0.06 to 0.02 mg/l. This appears to be within the range of Everhart and Freeman[18] who found 0.05 mg Al/l to be relatively non-toxic to

rainbow trout. The results of our chinook salmon test were also within that range, varying from 0.02 to 0.07 mg Al/l. Gahler and Sanville (unpublished), in a pilot field study, found no deleterious effects to resident rainbow trout when a neutralized aluminum hydroxide slurry was applied to a pond at 10 mg Al/l. Aluminum has been applied to several lakes by the Inland Lake Renewal and Management Demonstration Project, Upper Great Lakes Regional Commission[23] and they, too, have reported no serious disruptions of the food chain or evidence of direct toxicity.

Zirconium

Zirconium has been reported as relatively inert with respect to biological systems. Cochran, et. al.,[24] found that 800 to 1600 mg of zirconium per kilogram of body weight was necessary to demonstrate acute toxicity when administered orally to rats. This included the acetate, chloride, nitrate and sulfate salts. Palange, et. al.,[25] reported 48-hour TL_m values of 14.4 and 17.8 mg/l, respectively, for zirconium sulfate and zirconyl chloride to fathead minnows in soft water. Tarzwell and Henderson[26] found a 96-hour TL_m for the fathead minnow of 14 mg Zr/l in soft water and 115 mg/l in hard water. Zirconyl chloride exhibited 96-hour TL_m values of 18 and 240 mg Zr/l, for soft and hard water, respectively. The actual dissolved concentrations were not reported. Collier[27] has reported zirconium concentrations of 0.34 - 3.4 mg Zr/l during a bloom of Gymnodinium off the coast of Florida.

The results of our study indicate that for a 96-hour test the zirconium was not extremely toxic to either coho salmon or Daphnia. In general, the fish were affected less. All fish survived the 96-hour test period and a 96-hour TL_m was not observed in the Daphnia study. As pointed out, the concentrations of soluble zirconium were not determined and the material used was known to contain some impurities. Observed mortalities could have been the result of contaminants.

In general, heavy metal toxicity is greater in soft water than hard. The water used in our tests had relatively low hardness levels of 30-40 mg/l which might amplify the toxic effects of elements in our tests. Harder water environments could possibly reduce toxicity.

The 9-week-three generation test was designed to explore chronic effects of exposing D. magna to zirconium tetrachloride. Two facets were examined, (1) effect on adult survival, and (2) effect on reproduction. The test was designed so that all young used after the initial introduction were exposed to the zirconium for their entire life cycle. One might expect a natural selection in which more resistant Daphnia are used as new assay animals. The expected result of this would be lower mortality and higher reproductive rates during the second and third phase of the study. However, mortalities and reproductive rates did not indicate occurrence of selective processes during the bioassay.

The test results generally showed a gradual increase in sensitivity to crude zirconium tetrachloride with time. TL_m values for weeks 1 through 9 were shown in Figures 12, 13, and 14. In interpreting the graphs, it must be remembered that weeks 4 and 7 represent young transferred from previous tests. Week 1 individuals had never been exposed previously to the inactivant, week 4 individuals were offspring of adults which had been exposed for three weeks, and week 7 individuals were offspring of adults whose parents had been exposed for three weeks as adults and up to three weeks as immature organisms. Week 2, 5, and 8 individuals were approximately one week old and by the end of the week had been exposed to the inactivant as adults for two weeks. Week 3, 6, and 9 individuals were all of the same age structure and by the end of the week had been exposed to the inactivant as adults for 3 weeks. Therefore, in assessing the

week to week effects of exposure, one must compare weeks 1, 4, and 7; 2, 5, and 8; and 3, 6, and 9. The TL_m value for week 1 (Figure 4) was above the concentrations tested, i.e., survival at all concentrations was >50%. The TL_m was 20.0 mg/l for week 4, and for week 7 it was 18.5. The same trend was seen in weeks 2-5-8. There was no indicated TL_m for week 2, but for week 5 a TL_m of 11.5 was found. Two TL_m values were indicated for week 8; the higher (16.0) is probably in error, since the lower value of 4.4 is nearer the expected level. The lowest TL_m values were found in weeks 3, 6, and 9, and probably represent the maximum stress the animals can tolerate independent of previous exposure to the test material.

Data on effects of exposure on reproduction (Figure 7) indicate a decreased number of young produced with each succeeding increase in concentration until a maximum level of reproductive impairment is reached. It appears that this level is reached between 5.0 and 10.0 mg Zr/l after which higher concentrations have little effect. At 10.0 and 20.0 mg Zr/l reproductive impairment appeared to be comparable after the fourth week, with the exception of an erratic peak for 20.0 mg/l at week 6. It must be borne in mind, however, that at the higher inactivant concentrations the number of surviving adults is so low that a single tolerant adult which produced a large number of young could sufficiently increase the reproductive rate per individual to signficantly bias the result.

Lanthanum

Lanthanum has been reported as relatively non-toxic. Cochran et al[24] found LD_{50} values of 4,200 mg $LaCl_3$ per kilogram body weight for rats to which lanthanum chloride had been administered orally. He concluded that the LD_{50} value was directly related to absorption

of the ingested compounds and that lanthanum ammonium nitrate was the most soluble and the most toxic of the administered lanthanum compounds. Interperitoneal LD_{50} levels were much lower. Kyker and Cress[28] reported LD_{50} values for interperitoneal injections of lanthanum considerably lower than those of Cochran, but did not experiment with oral dosage. Bringmann and Kuhn[29] found a median threshold effect for Daphnia of 160 mg La/l after 48 hours and 0.15 mg La/l for Scenedesmus after four days.

Our results with lanthanum rare earth chloride showed an extreme toxicity to coho salmon and a relatively high toxicity to D. magna tested at lower inactivant concentrations. All fish exposed to concentrations greater than 1 mg lanthanum rare earth chloride/l died within the first 24 hours and a 96-hour TL_m of 1.6 was observed with Daphnia.

VII. STABILITY AND DURATION OF EFFECTIVENESS

OBJECTIVES AND APPROACH

One of the most important aspects of the nutrient inactivation concept is the stability of the effect. Whereas this problem is not critical in the treatment of municipal water supplies or wastes, where conditions can be carefully manipulated, reaction times are short, and preciptants are removed, in natural waters it may be affected by a number of uncontrollable variables such as temperature, pH, oxygen level, etc. It is particularly important to determine the behavior of inactivants in aerobic and anaerobic systems when considering the interaction between inactivant-phosphorus complexes and sediment and whether such complexes will affect the release of phosphorus from the sediment.

The problem has been approached here by developing experimental laboratory setups in which natural, undisturbed sediment-water systems are approximated. Using these systems as laboratory microcosms, inactivant materials are added to the water following the addition of ^{32}P. A second isotope tracer, ^{33}P, is then added to the sediment. Through use of the dual tracer system, the effect of precipitated inactivant-phosphorus complexes on release of exchangable phosphorus from the sediment, and the rate of release of phosphorus from the inactivant complex are expected to be determined.

DEVELOPMENT OF QUANTITATIVE METHODS

Sampling

A Jenkin coring apparatus[30] was used to obtain sediment-water samples from Cline's Pond for the laboratory studies. Experience

with this device has shown that essentially undisturbed samples can be obtained, to the extent that midge larvae tubes are maintained intact on the surface of the sediment. Initially the plastic coring tubes were fitted with glass liners to minimize phosphorus adsorption. All of our studies to date have used the glass-lined coring tubes; however, we have since found that there is no signficant difference in phosphorus adsorption on the glass liners and the acrylic plastic coring tubes themselves. Plastic tubes will be used in future studies. A series of sampling ports were bored in each column and filled with silicone rubber (Figure 19), allowing the sample collector to serve as the laboratory experimental vessel, and precluding the need for transferring and disturbing the sample.

Height of sediment in the coring tubes is dependent on the texture and compactness of the substrate being sampled and thus varies somewhat from tube to tube. Following collection, therefore, sediment levels are adjusted to approximately 1 cm above the lowest sampling port. Excess sediment is "drained off" by removing the seal on top of the core and gently rocking the bottom seal from side to side, allowing sediment to seep out the bottom. This manipulation does not significantly disturb the mud-water interface. The water level is then restored by adding water from a reserve supply obtained from just above the lake bottom at the time of sample collection.

Establishment of Aerobic and Anaerobic Systems

Aerobic and anaerobic columns are subjected to the same standard conditions except for the type of gas (air or nitrogen) supplied.

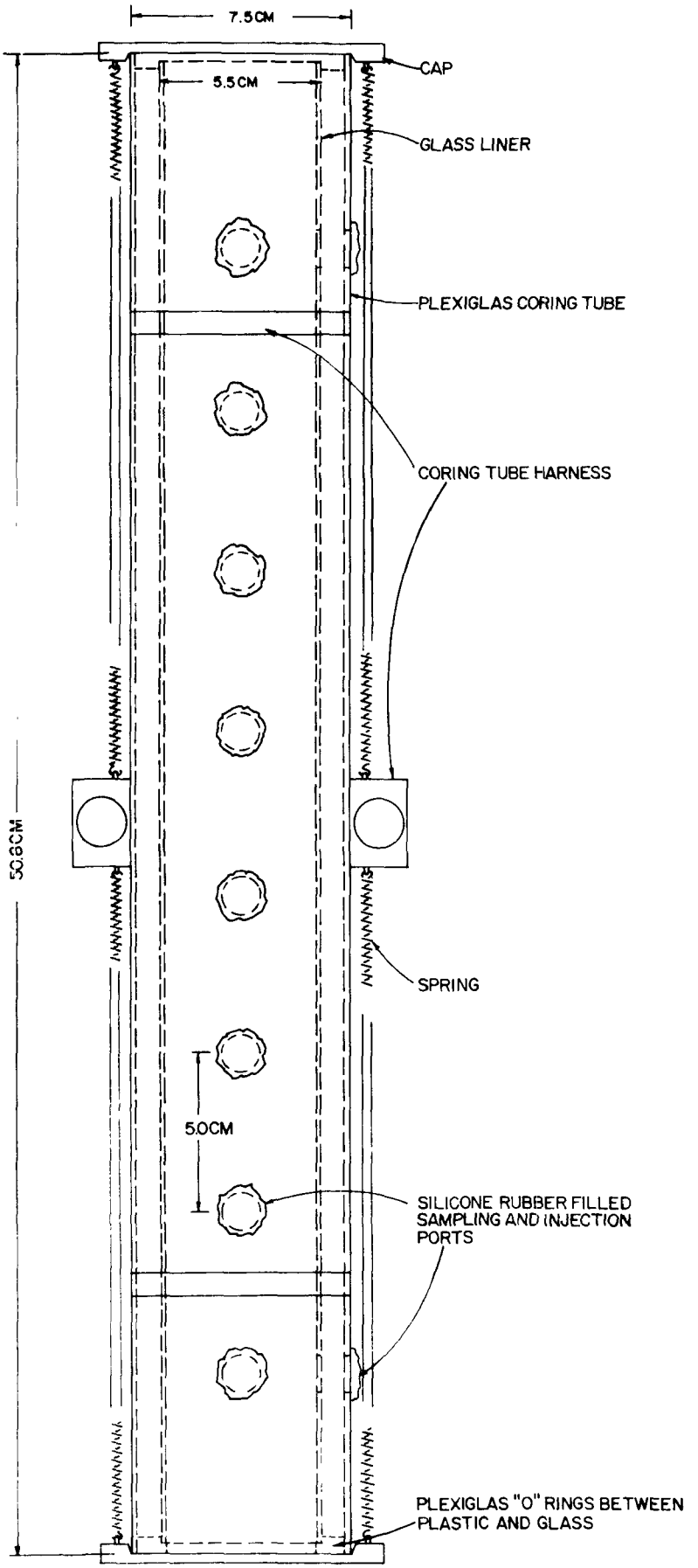

Figure 19. Jenkin corer tubes modified for laboratory experiments.

It has been necessary to establish the minimum identical air and nitrogen inputs to the columns required to ensure that one set remains aerobic and the other anaerobic, while at the same time water circulation is held to a minimum. A distribution manifold with gas flow regulators proved unsatisfactory and was replaced with a 15-channel Technicon peristaltic pump which meters equal gas flows to duplicate pairs of aerobic and anaerobic sealed columns. An underwater bleed-off prevents air from leaking back into the columns (Figure 20) and maintains equal pressure on all columns. Gas pumping rates of 0.10, 0.25, 0.80, and 1.60 cc/min were tested to determine the minimum acceptable gas flow. It was determined that a rate of 0.25 cc/min produced the desired aerobic and anaerobic conditions with a minimum of turbulence (Table 19).

Low concentrations of oxygen were initially present in the nitrogen purged columns (0.4 - 0.8 mg/l). Our nitrogen source was a pre-purified gas containing a claimed concentration of < 5mg/l oxygen. An "oxy-trap" (Attech Associates) was installed in the line between the nitrogen cyclinder and the Technicon pump, reducing oxygen levels in the columns to a range of 0 - 0.2 mg/l. This was considered operationally anaerobic.

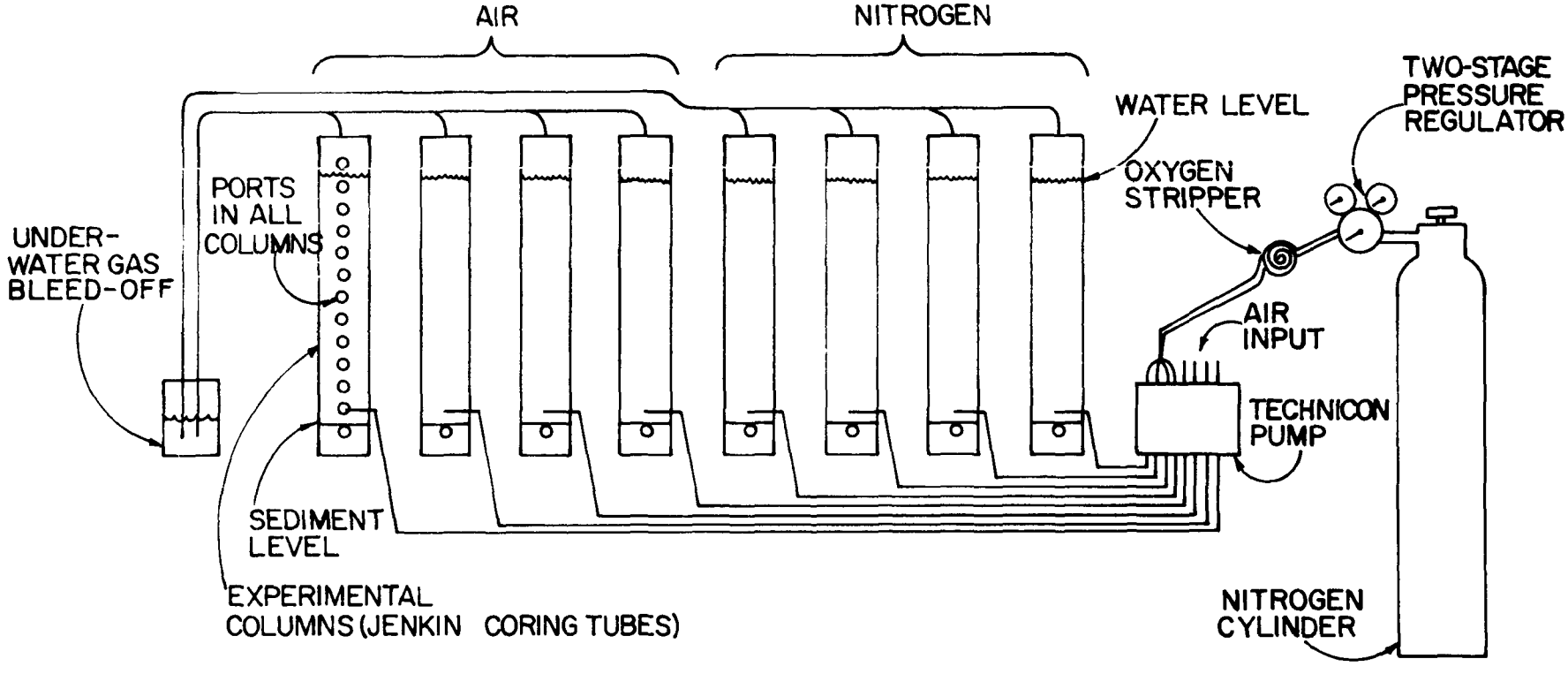

Figure 20. Aerobic-anerobic experimental set-up with modified Jenkin tubes.

Table 19

Nitrogen-Air Pumping Rates to Experimental Columns as Related to Dissolved Oxygen Levels

Pumping Rate cc/min	Gas	Dissolved Oxygen mg/l Day 1	Day 2
1.60	N_2	0	0
1.60	Air	10+	9.4
0.80	N_2	0	0
0.80	Air	10+	8.0
0.25	N_2	0	0.2
0.25	Air	10+	8.0
0.10	N_2	1.5	1.6
0.10	Air	9.2	7.2

Subsampling and Analytical Techniques

The glass lined Jenkin coring tubes have an approximate volume of 1300 ml and a cross-sectional area of 23.8 cm^2. The relatively small water volume and the frequent analyses required by these experiments necessitates the use of very small sample volumes and micro-analytical techniques. Micro-techniques have been used by other investigators for some of the parameters we wished to measure, but for most, our own techniques had to be developed.

Phosphorus -

The method usually employed for phosphorus determination in our laboratory is that given in "Methods for Chemical Analysis for Water and Wastes, 1971"[8]. This method, which is a modification of the Murphy and Riley[31] single solution method, requires a 50 ml sample size. Our plan was to proportionately scale-down all of the reagent volumes to suit our 2.5 ml sample size. Results using this procedure were poor: replicates gave 25 to 100 percent error. Although contamination of the samples was magnified because of their small volume, lack of precise acidity and temperature control appeared to produce the greatest variation. This method was ultimately abandoned in favor of an EPA automated method[8] where acidity and temperature variation could be eliminated.

The automated method, however, also produced poor replicates, until it was discovered that acid-washed plastic syringes used to load samples into the specially adapted 5 ml containers used by the automated system were an apparent source of contamination.

Initially the syringes were acid-washed, rinsed in distilled water, dried in a circulating air oven, and stored under cover until the next use. Acceptable replication was not obtained until the syringes were allowed to soak in 1:1 HCl during storage and rinsed with distilled water immediately before use. Following this modification, the automated procedure utilizing 2.5 ml sample volumes and acid-soaked, distilled water-washed plastic syringes was consistently reliable and reproducible (see Figure 21 and Table 20). Differences between 20 sets of replicates with phosphorus concentrations ranging from 0.005 to 1.61 mg/l was 0.01 - 0.02 mg/l. The mean variation between 20 sets of replicate samples (both standards and pond water) was 0.008 mg/l phosphorus.

Oxygen -

Several micro and semimicro oxygen techniques were considered for use in our experiments and discarded for various reasons. A method described by Carpenter[32] required a 125 ml sample, much larger than we could use. A polarographic technique described by Koch and Kruuv[33] was rejected for the same reason. A microprobe used for the determination of oxygen levels in microbial slime films was considered (Bungay et. al.[34], Sanders et. al.[35], and Whalen et. al.[36]), but it was decided that sulfides in our experimental columns might rapidly poison the 1.5 μ diameter microprobe.

An apparatus similar to that used by Whitney[37] was then assembled and tested. The technique was essentially a micro-Winkler system requiring a 10 ml sample. The sample was collected in a syringe which also served as the titration vessel, thereby eliminating prolonged exposure to air. However, the short exposure time when the syringe needle was removed and the titration burette needle reinserted was apparently sufficient to allow atmospheric contamination.

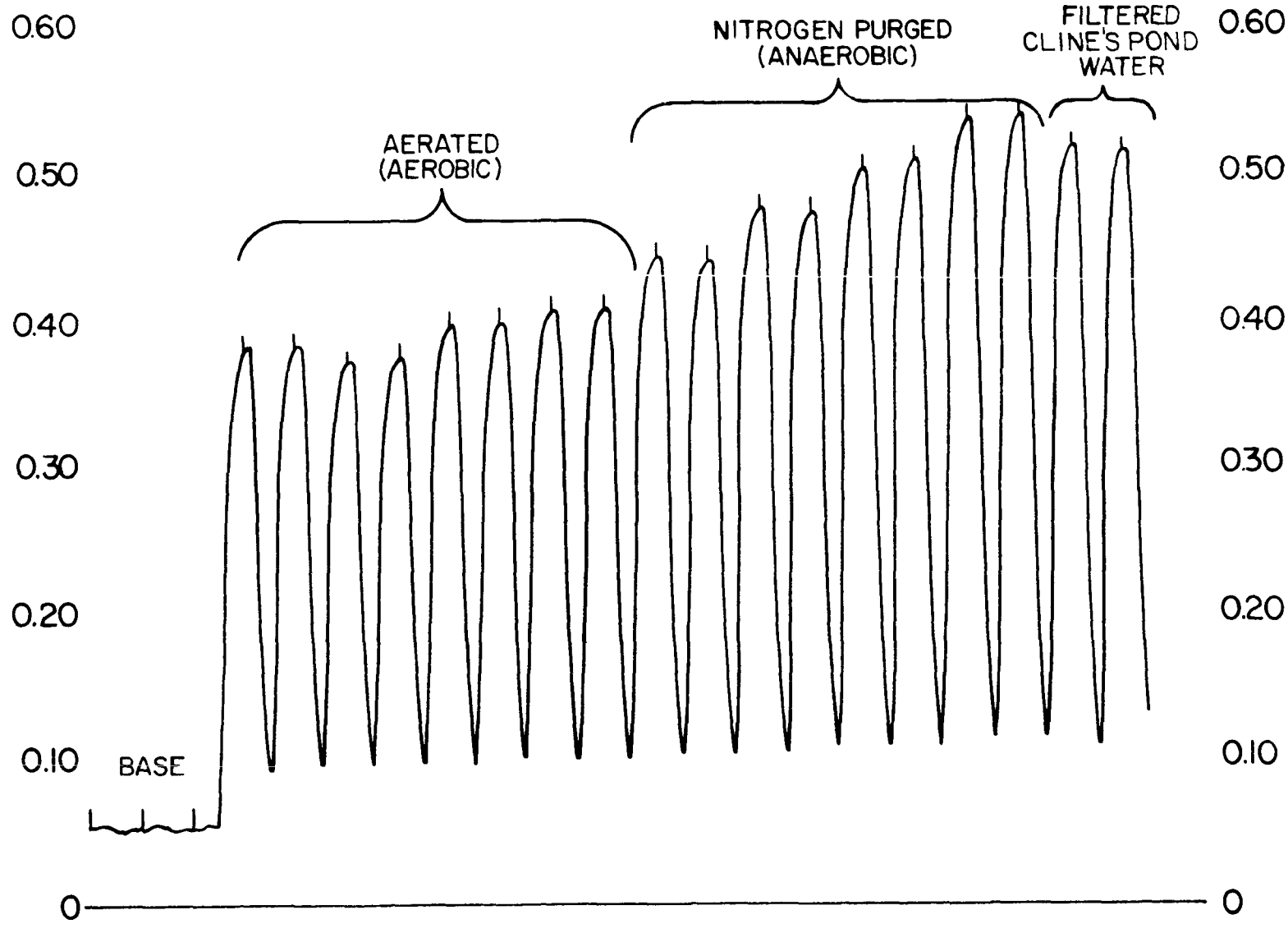

Figure 21. Autoanalyzer record demonstrating reproducibility of duplicates for phosphorus analysis in small volume (2.5 ml) samples.

Table 20. Results of Replicate Sample Tests for Total Phosphorus Using the Autoanalyzer with 2.5 ml Samples (Base = 0.50, C/A = 0.333).

Replicate Sample Number	Peak Height	Percent Variation in Peak Height Between Replicates	Calculated Phosphorus Concentration mg/l	Measured Phosphorus Concentration* mg/l	Difference Between Measured Phosphorus Concentrations of Replicates mg/l
STANDARDS					
1	0.52	0.0	0.000	0.007	0.000
	0.52			0.007	
2	0.53	0.0	0.000	0.009	0.000
	0.53			0.009	
3	0.50	2.0	0.000	0.000	0.010
	0.51			0.006	
4	0.50	4.0	0.000	0.000	0.020
	----			-----	
5	0.54	0.0	0.005	0.010	0.000
	0.54			0.010	
6	0.52	0.0	0.005	0.006	0.000
	0.52			0.006	
7	0.57	0.0	0.020	0.020	0.000
	0.57			0.020	
8	0.61	6.6	0.020	0.040	0.020
	0.57			0.020	
9	0.83	2.7	0.100	0.110	0.000
	0.82			0.110	
10	1.70	2.3	0.400	0.400	0.010
	1.74			0.410	
11	1.60	3.6	0.400	0.370	0.020
	1.66			0.390	
CLINE'S POND WATER					
12	3.74	0.7		1.08	0.01
	3.77			1.09	
13	3.66	0.8		1.05	0.01
	3.69			1.06	
14	3.91	0.2		1.14	0.00
	3.92			1.14	
15	4.01	0.7		1.17	0.01
	4.04			1.18	
16	4.37	0.7		1.29	0.01
	4.34			1.28	
17	4.69	0.4		1.40	0.01
	4.67			1.39	
18	4.96	1.4		1.49	0.02
	5.03			1.51	
19	5.29	0.6		1.60	0.01
	5.32			1.61	
20	5.11	0.4		1.54	0.01
	5.09			1.53	
	Mean Variation =	1.4		Mean Difference =	0.008

*Concentration = (Peak Height - Base Height) (C/A)

C/A = 0.3333 = Slope of Calibration Curve = $\frac{\text{Concentration}}{\text{Absorbance}}$

This introduced error was greatest at low oxygen concentrations, when increased sensitivity was most desired. This technique, therefore, was also discarded. The problem was finally resolved by a return to polarographic methodology.

In polarographic oxygen analysis oxygen diffused through a membrane is reduced electrochemically, inducing a current which is proportional to the concentration of oxygen in the sample. Most of the sensors available for field and laboratory use, however, have relatively large surface areas which consume excessive amounts of oxygen when tensions are low. International Biophysics Corporation supplied an IBP bedside blood oxygen analyzer probe (a hospital unit) and a modified Model 300 compact oxygen analyzer which proved to be satisfactory. The small sensor from the hospital unit was coupled to the Model 300 meter, the circuitry of which was modified to increase sensitivity to low oxygen tensions at low temperatures. The sensor has a diameter of approximately 2.5 mm and is mounted in a plexiglas flow-through sample chamber which accomodates a 0.25 ml sample. Since oxygenated water from the previous sample tends to adhere to the plastic walls it is necessary to flush the chamber with approximately three to four volumes of each succeeding sample. One problem encountered was drying of the membrane. This resulted from rapid evaporation and capillary movement of electrolyte up the vent tube of the small volume (2 ml of 1M KCl) electrolyte reservoir. This was overcome by disconnecting the sensor from the read-out unit at the end of each day, immersing it in electrolyte, and subjecting it to a strong vacuum. This forces electrolyte under the membrane and prolongs the life of the sensor.

Oxygen meter readings are plotted as a function of Winkler oxygen concentrations to establish a standard curve on any given day the

meter is used. Calibrations are made by filling BOD bottles with various mixtures of aerated and deoxygenated water to create a graduated series of oxygen concentrations. After zeroing the meter with nitrogen gas, subsamples from each mixture are injected into the meter and readings made. Dissolved oxygen content of the mixtures is then determined by the alkaline azide modification of the standard Winkler method. The standard curve is plotted with the meter reading as a function of Winkler oxygen concentration. The meter-probe combination is stable for 2 to 3 hours, after which it must be recalibrated.

Typical calibration curves are shown in Figure 22. A distinct departure from linearity at higher dissolved oxygen concentrations was observed for those made on September 17 and 24. This condition improved following development by IBP of an improved sensor. Calibration curves made after September 24 demonstrated good linearity over a range of 0-10 mg/l. The slope of the latter curves was nearly the same, although there was a shift downward with time. This probably indicates decreased sensitivity of the sensor with use. Gain on the instrument was not readjusted during this period.

Hydrogen Ion Concentration -

A plexiglas semi-micro combination type pH electrode adapter (Figure 23) was fabricated to accommodate a 2.5 ml sample volume without atmospheric contamination. The probe is calibrated while in the adapter using standard non-phosphate buffers of pH 4.6 (0.2 M KH phthalate and 0.2 M NaOH) and pH 8.0 (0.2 M H_3BO_3 + 0.2 M KCl and 0.2 M NaOH)[38].

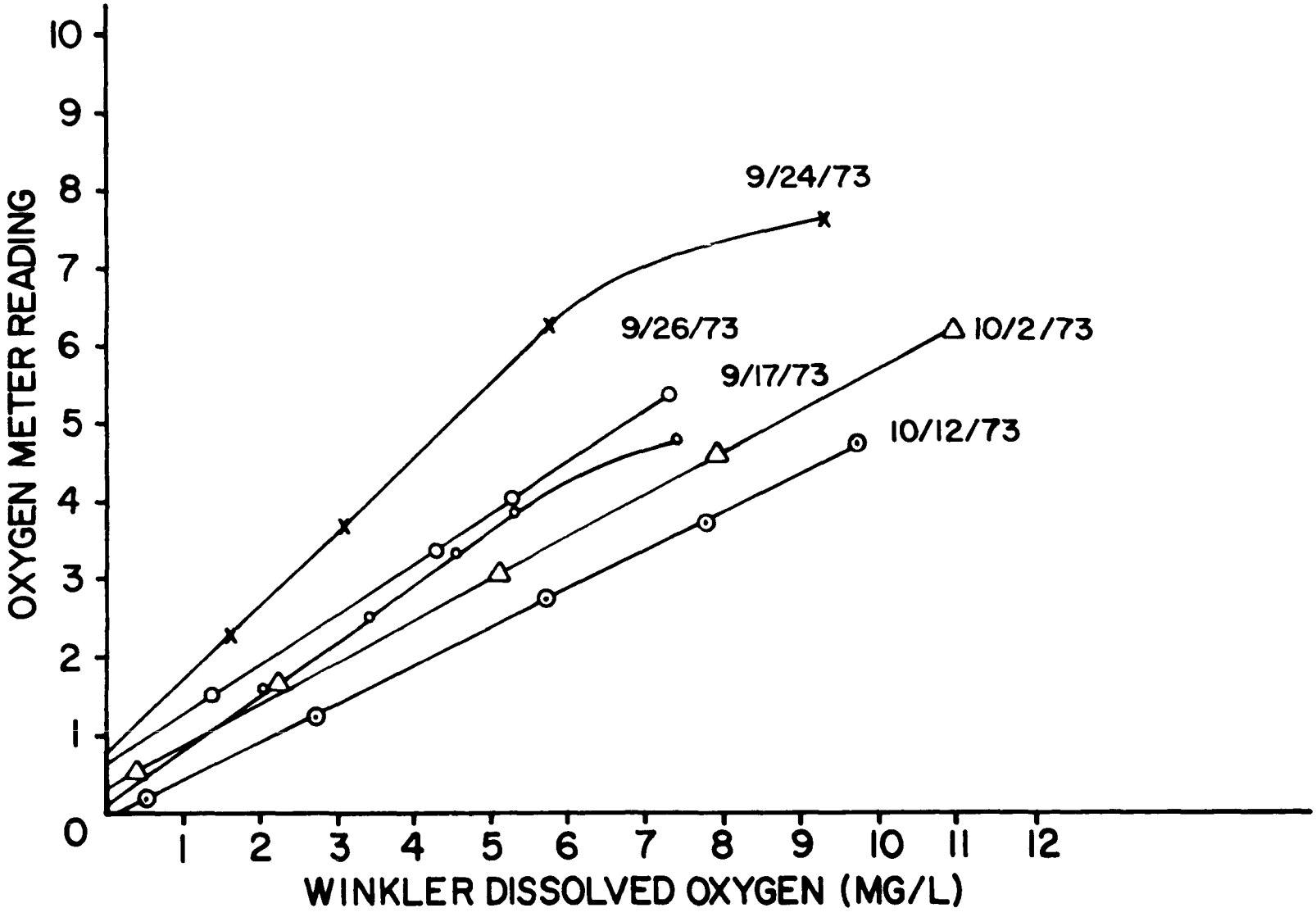

Figure 22. Typical calibration curves for micro oxygen meter.

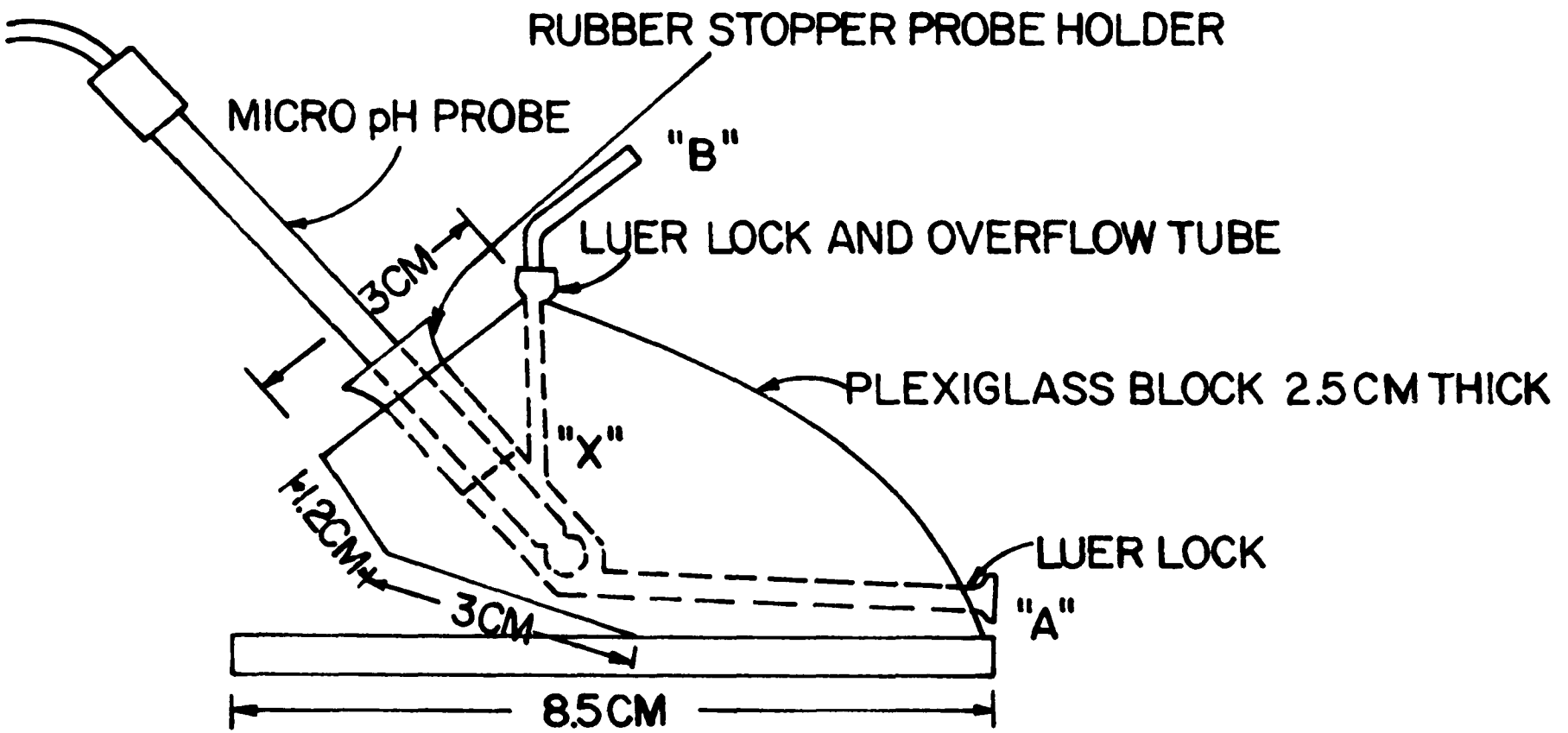

Figure 23. Semi-micro combination pH probe adapter.

Both standards and samples are injected from a syringe into part "A". Air is expelled from part "B" ahead of the sample and steady pH meter readings are obtained when the liquid level reaches point "X" in the adapter.

Oxidation-Reduction Potential (Redox) -

Some of the earlier work on the importance of oxidation-reduction relationships in lakes was carried out by Pearsall and Mortimer[39], who studied the effects of redox potential on several of the common ions found in lakes, and by Mortimer[30], who compiled an extensive treatise on the exchange of dissolved substances between mud and water in lakes. Many of Mortimer's findings emphasized the importance of redox changes. He showed that increased concentrations of ammonia, ferrous iron, and phosphorus at the mud water interface resulted from oxygen depletion and accompanying reducing conditions. Hayes, Reid, and Cameron[40] have pointed out several of the problems associated with redox measurements. These include the lack of reproducibility in non-poised samples (samples other than standards), differential readings depending on the area of platinum in the electrode, hydrogen sulphide alteration of readings, and air contamination of samples removed from a reducing environment.

We have minimized the problem of air contamination by making redox measurements on water from the experimental columns with an Orion Model 96-78 combination redox probe mounted in a specially constructed plexiglas adapter (Figure 24). The platinum electrode and fluid junction are seated tightly into the plastic block. Water samples (2.5 ml) are withdrawn from the columns by syringe and injected into the sample holding chamber, flushing through 3-4 volumes to expel air and residual sample.

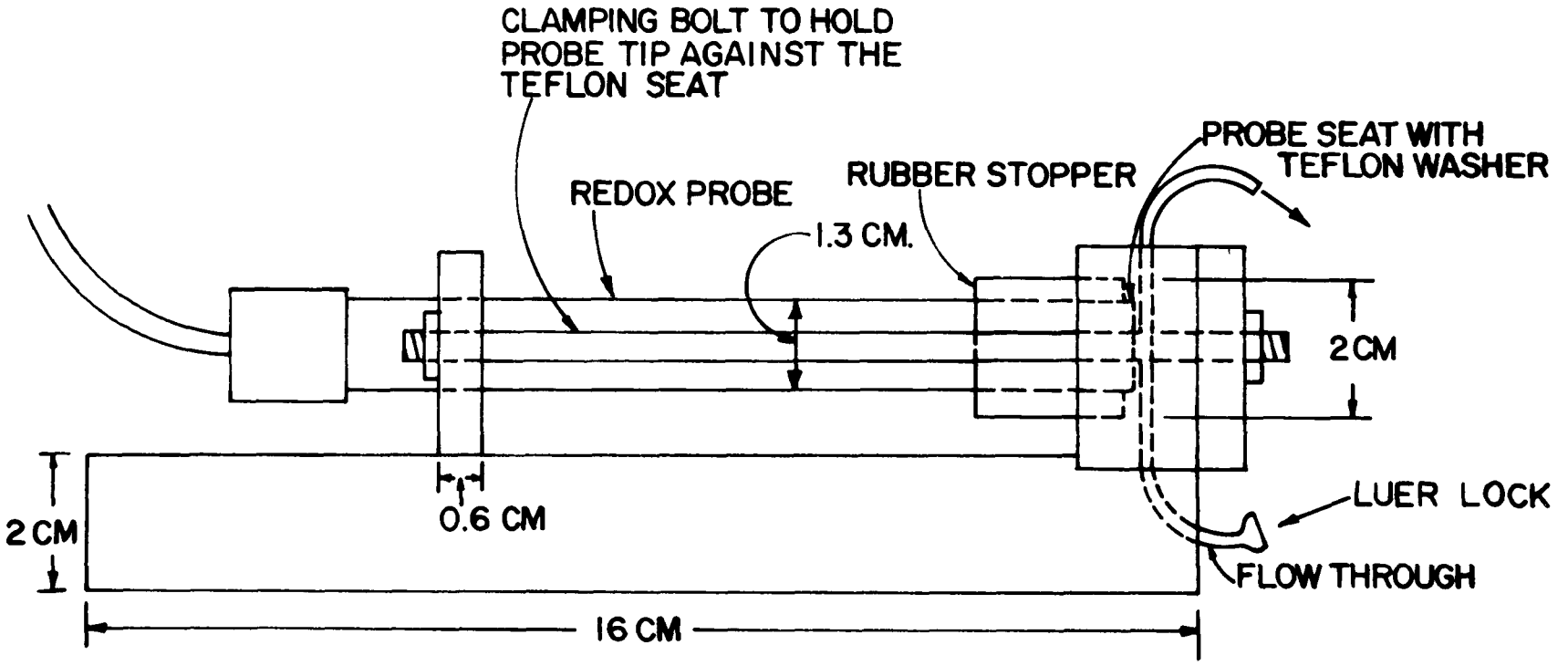

Figure 24. Plastic redox probe adapter for excluding air from anaerobic samples.

The redox probe is standardized using the potassium ferric and ferrous cyanide solutions descibed by Zobell[41]. Subsequent readings are corrected to the normal hydrogen electrode (NHE) according to the formula:

$$E_{NHE} = E_o + C$$

where E_{NHE} = oxidation reduction potential of the sample relative to the NHE

E_o = potential developed by the platinum redox electrode

C = potential developed by the reference electrode portion relative to the NHE.

TENTATIVE EXPERIMENTAL PROCEDURE

A tentative procedure for the experimental column studies has been established and partially verified. At this point, however, an entire experiment has not been completed. A preliminary test run without radioactive tracers has been conducted to determine the effect of one candidate inactivant on phosphorus concentrations, and to verify that the microcosm system functions as expected. This will be described later in this report.

The tentative experimental procedure is as follows:

DAY	EXPERIMENTAL PROCEDURE
1	A) Nutrient content of sediment. 1) Collect three core samples (see B). 2) Extrude the cores in 1 cm increments (Figure 25). 3) Composite the sequential increments from the three cores for chemical analysis. a) Centrifuge the sediment sample in N_2-purged containers for separation into interstitial water and solid phases. b) Analyze each phase for total phosphorus, total nitrogen, iron, and the inactivant being tested. B) At the same time collect cores for laboratory work and six liters of pond water from just above the sediment. Filter the latter through 0.45 μ membrane filter, divide the filtrate equally between two containers (replacement water).

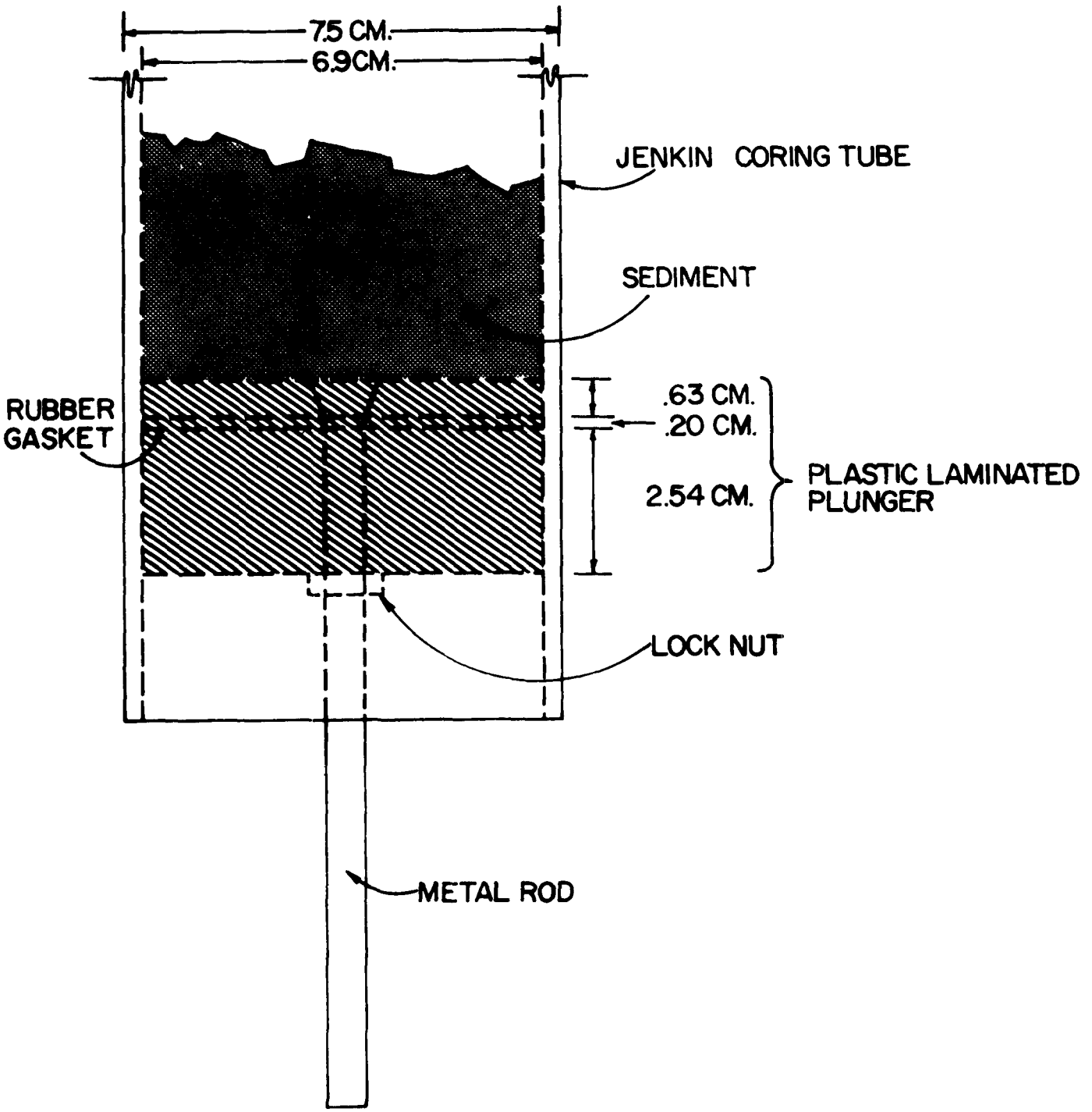

Figure 25. Device for extruding sediment samples from Jenkin coring tubes.

1) Place cores from (B) and replacement water in a dark environmental chamber at 18°C (approximate summer bottom temperature of Cline's Pond).

2) After two hours withdraw water samples from port number 4 with a syringe (ports are numbered from bottom to top) and analyze for the following parameters:

Analysis	Required Water Volume (ml)
Total soluble phosphorus	2.5
Soluble orthophosphorus	2.5
ATP	5.0 (Filtrate from above)
Total Phosphorus	2.5
Orthophosphorus	
Ammonia	2.5
Nitrite	
Nitrate	
	5.0
Kjeldahl nitrogen	25.0
Oxygen	2.0
pH	2.5
Redox	2.5
Inactivant material	5.0
	37.0
Total volume required for complete analysis	47.0

3) As water samples are removed from the columns, replace the volume with replacement water (See B).

4) Start air flow into half the columns and one replacement water container at 0.25 cc/min.

5) Start N_2 flow into half the columns and one replacement water container at 0.25 cc/min.

C) Allow the columns to equilibrate two weeks, rerunning all of the analyses under B-2 every 2 to 3 days.

15 D) Add one millicurie ^{32}P to the water phase of each aerobic and anaerobic column.

1) Remeasure the phosphorus concentration (total-P and soluble ortho-P) in each column after one hour, during which time air and N_2 flow has continued.
2) Measure ^{32}P in the water.
3) Add nutrient inactivant by syringe through port number 8 (amount will be dependent on phosphorus concentration) and continue aeration and N_2 purge for 30 minutes.
4) Terminate air and N_2 flow for 24 hours while the inactivant floc settles to the bottom.

16 5) Inject 0.50 millicuries ^{33}P (contained in 200 micro-liters of water) into the sediment 1.0 cm below the surface (Figure 26).

6) Resume air and N_2.

E) One hour after air-N_2 has been resumed, withdraw samples from port number 4 for complete analysis series (B-2), excluding Kjeldahl-N. Also analyze for ^{32}P, and ^{33}P in the water phase:

Analysis	Required Water Volume (ml)
^{32}P	2.5 ml water redox measurement
^{33}P	

F) Analyze for all parameters in B-2 (excluding Kjeldahl-N) and the parameters in F.

G) Tentatively, analysis frequency will be two-day intervals the first week, three-day intervals the second, and weekly thereafter.

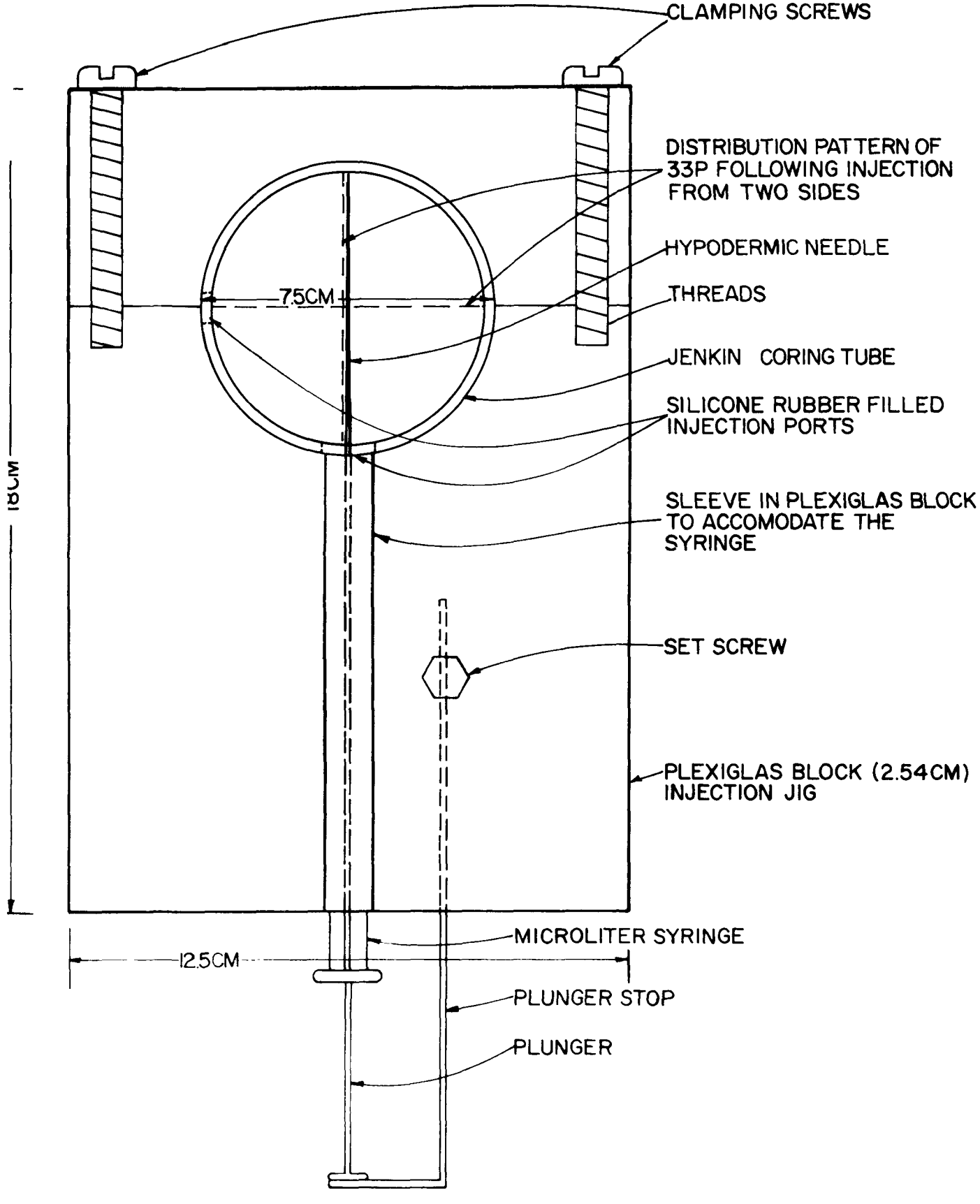

Figure 26. Jig for injecting ^{33}P into sediment.

PRELIMINARY DETERMINATION OF INACTIVANT EFFECT

Having resolved the technical problems associated with the chemical analysis and experimental apparatus, the first inactivant was selected for effect and stability experiments in the laboratory. The choice was made on the basis of phosphorus removal capacity, results of toxicity tests and other information available from the literature.

Laboratory experiments demonstrated clearly that lanthanum, zirconium, and aluminum compounds were more efficient phosphorus removers than any of the other materials tested. Based on phosphorus removal efficiency alone lanthanum would be the first choice with its 1:1 inactivant to phosphorus molar ratio. The lanthanum rare earth chloride used in the toxicity experiments, however, demonstrated a 100 percent mortality to the fish tested when applied at $\geq$1 mg/l lanthanum rare earth chloride. A relatively high mortality rate for D. magna also resulted. The lanthanum mixture was therefore, not used in this series of tests.

Zirconium removed phosphorus efficiently at an inactivant to phosphorus molar ratio of approximately 2:1 and was relatively non-toxic to fish and cladocerans. The optimum phosphorus removal capacity of zirconium extended over a broad pH range (see Figures 7 and 8). Optimum phosphorus removal with aluminum occurred at a molar ratio of approximately 8:1 and was confined to a rather narrow pH range between 4 and 6. Sorbing efficiency decreased rapidly outside this pH range. The pH of most eutrophic waters is greater than 6. Zirconium, therefore, possessed more of the characteristics desired of an inactivant than the other materials tested and was thus selected as the initial material to undergo more thorough examination with regard to stability of the inactivation treatment.

A preliminary experiment was then conducted to determine the effect of zirconium tetrachloride on phosphorus concentration in the test columns prior to beginning the major experiments with ^{32}P and ^{33}P. Mud-water interface samples were collected and treated according to the methods described with regard to sediment level adjustment and set-up of the columns (see Figure 20). The tentative test procedure as described in the foregoing section was not followed beyond that point with regard to time schedule or chemical analyses. Phosphorus, oxygen and pH were the only parameters measured. Four columns were aerated and four purged with nitrogen. These were arranged in four aerobic-anaerobic pairs. One pair served as controls and received no treatment other than the gas flow. The other three pairs received 10, 15, and 17 mg Zr/l (zirconium tetrachloride solution without neutralization), respectively, on the 15th day of the experiment (not the same as day 15 under "Experimental Procedure"). On the 20th day air and nitrogen flows were reversed, that is, those receiving air were switched to nitrogen and vice-versa. The experiment was terminated after 34 days.

Results

In columns initially aerated, the total phosphorus concentrations declined by an average of 44 percent prior to zirconium addition on day fifteen (Figure 27). On day 16 total phosphorus concentrations had declined 57, 94, and 91 percent from the previous day in the 10, 15, and 17 mg Zr/l columns, respectively. The control decreased only 5 percent. Total soluble phosphorus (Figure 28) decreased 89, 100, and 98 percent, respectively, while the control showed the same 5 percent decrease.

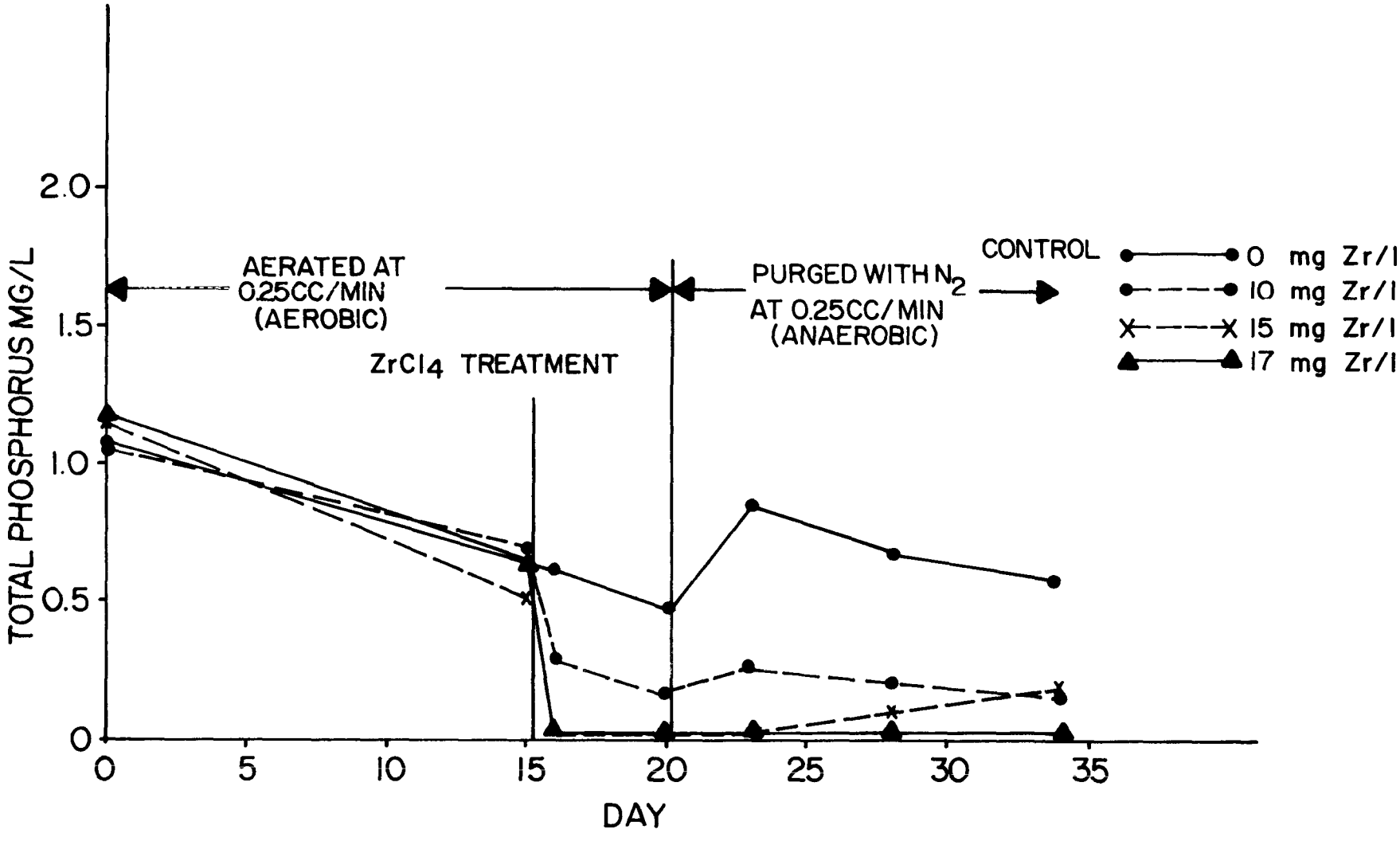

Figure 27. Total phosphorus concentration of an aerated system before and after zirconium tetrachloride addition with subsequent nitrogen purging.

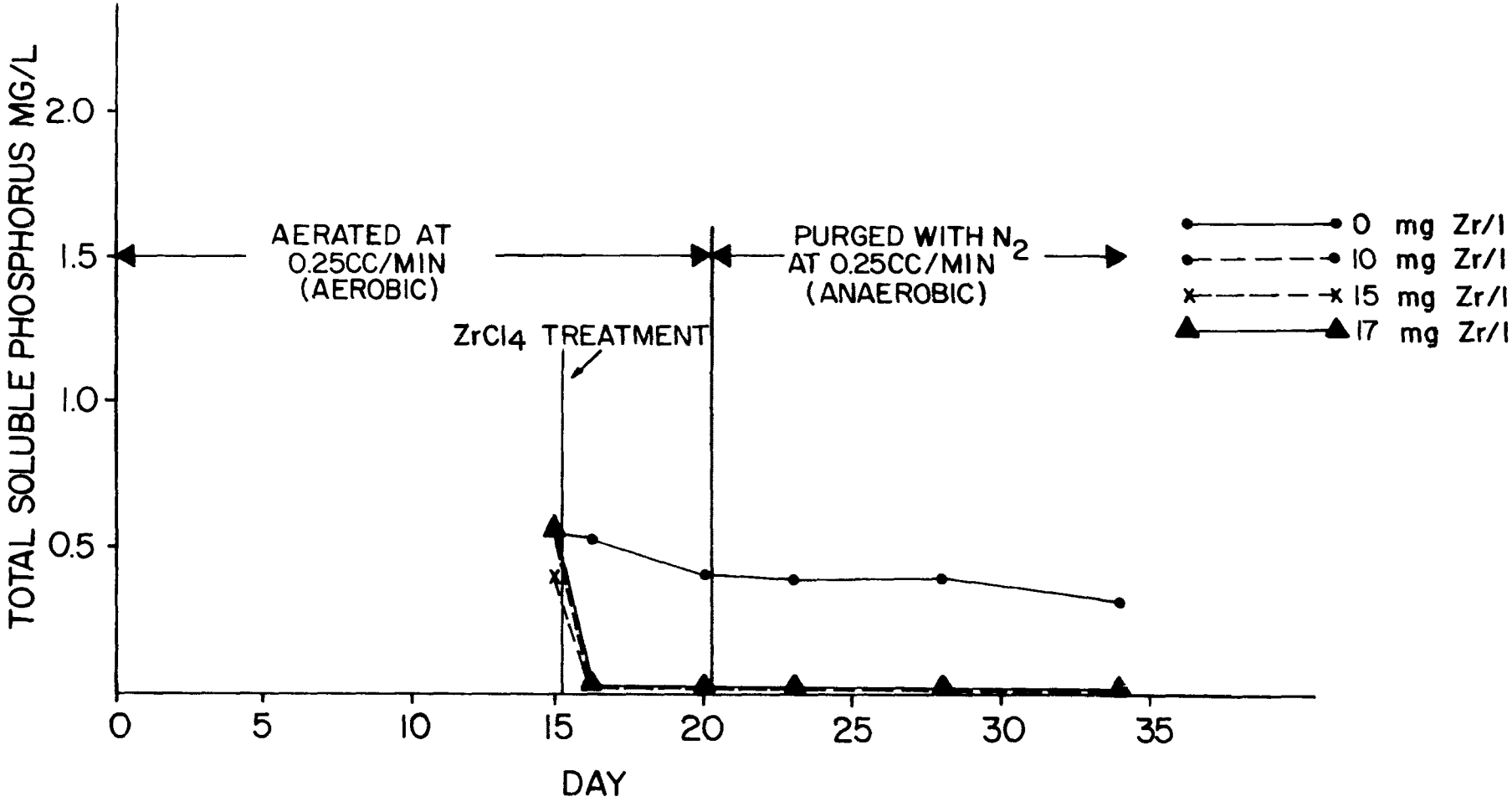

Figure 28. Total soluble phosphorus concentration of an aerated system before and after zirconium tetrachloride addition with subsequent nitrogen purging.

No increase in phosphorus concentration was evident over the next five days, and the total phosphorus in the control and 10 mg Zr/l columns continued to decrease at the same rate as before inactivation. Following the change to anaerobic conditions after day twenty, an increase in total phosphorus was noted in all columns. In the inactivated columns this increase was small. Concentrations in the control and 10 mg Zr/l columns decreased after day 23, increased slightly in the 15 mg Zr/l column, and remained unchanged in the 17 mg Zr/l column. Total soluble phosphorus showed no increase until day 34 when a maximum increase of 0.03 mg P/l was noted in the 10 mg Zr/l column.

Decreasing phosphorus concentrations during the initial aeration period (days 1-5) were probably due to high oxygen concentration and associated moderate pH levels (Figure 29). On the third day of the experiment the pH of all four columns was about 6.8. On the day following zirconium addition pH values were 7.05, 6.75, 5.90, and 5.45 in the control and the 10, 15, and 17 mg Zr/l columns, respectively. The inactivant solution was not neutralized, thus the hydrochloric acid formed during the hydrolysis of $ZrCl_4$ was responsible for rapid pH reduction. Five days after treatment the lower pH levels had increased under the continued aeration. On the 20th day, five days after treatment, aeration was replaced by nitrogen purging. By the 22nd day pH levels of all columns were nearly identical, and close to the original values.

Figure 30 shows that total phosphorus concentrations in the initially anaerobic columns increased by an average of 30 percent over the 15 day period before zirconium addition. Increases for the four columns ranged from 24 to 41 percent.

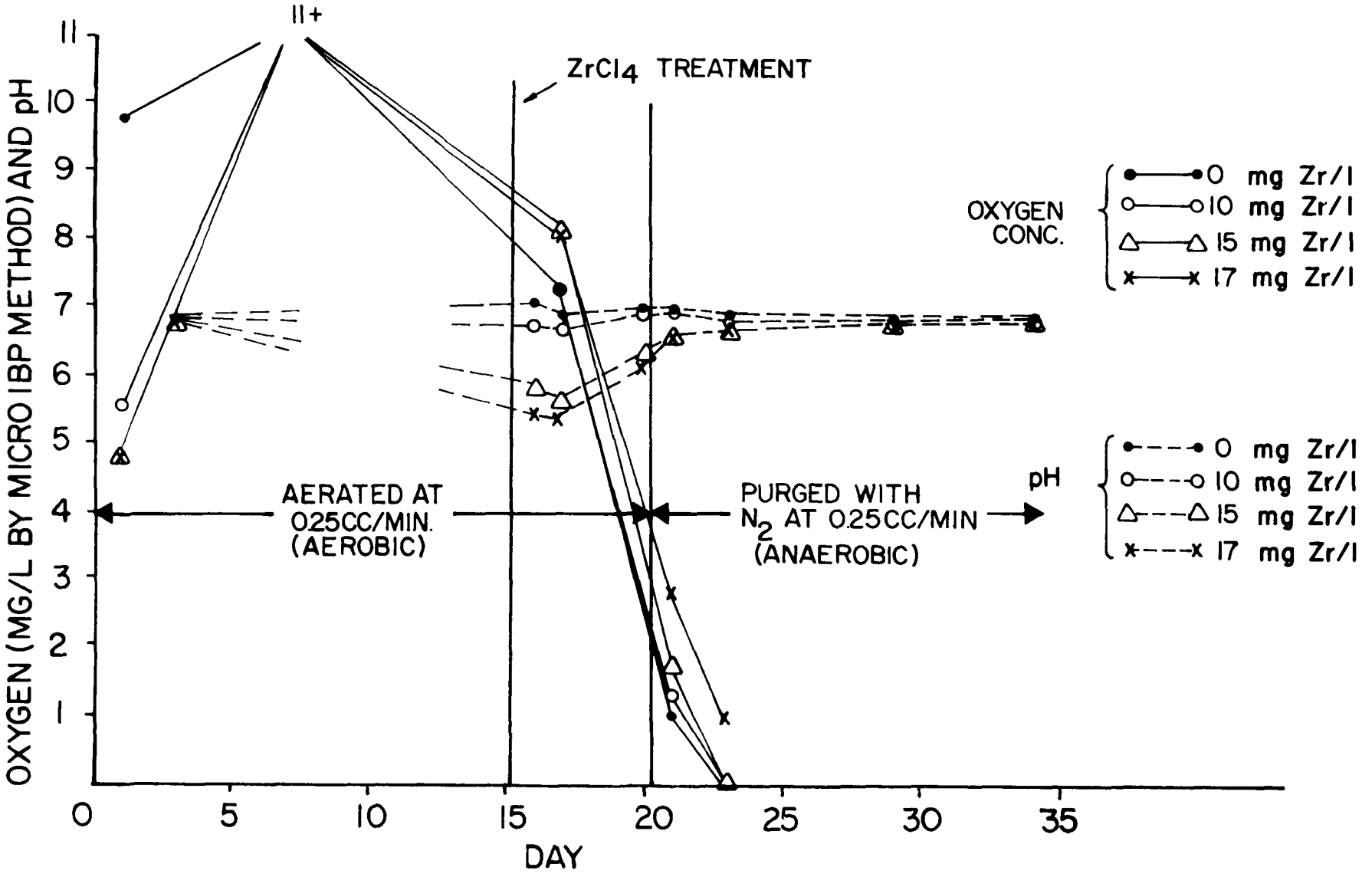

Figure 29. Dissolved oxygen and pH in an aerated system before and after zirconium tetrachloride addition with subsequent nitrogen purging.

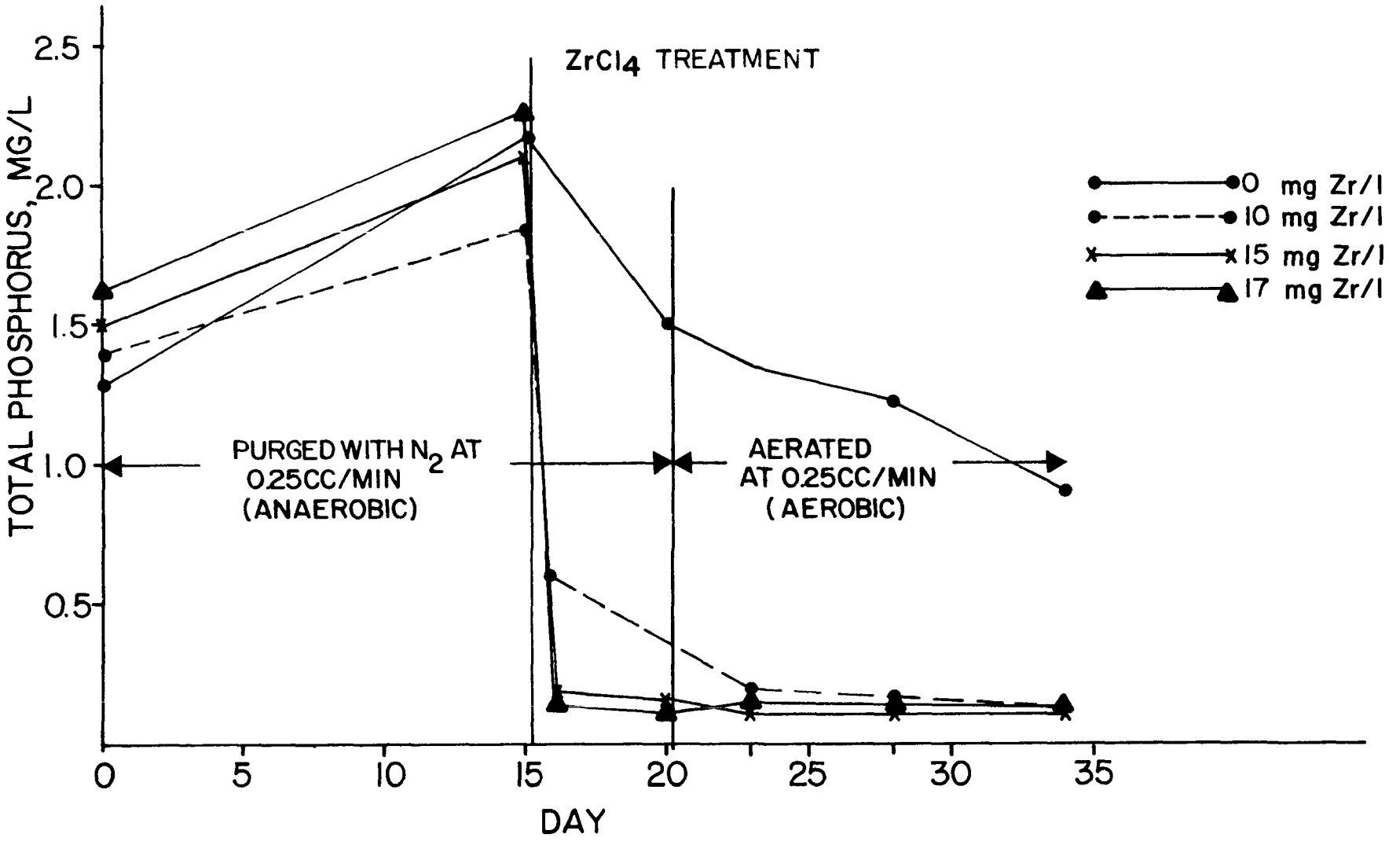

Figure 30. Total phosphorus concentration of an anaerobic system before and after zirconium tetrachloride addition with subsequent aeration.

On day 16, the day following zirconium treatment, total soluble phosphorus concentrations had declined from those of the previous day by 67, 95, and 93 percent for the columns treated with 10, 15, and 17 mg Zr/l, respectively. Total phosphorus decreased in the control by 28 percent. Total soluble phosphorus decreases for the same period were 94, 94, and 97 percent, respectively, with the control column showing no change (Figure 31).

In the nitrogen purged columns (Figure 32) pH was the same (approximately 7.0) as in the aerated columns on day 3 of the experiment. On day 16 (one day after zirconium tetrachloride treatment) pH values of the two systems were more varied with greater depression of pH in the aerobic than in the anaerobic columns. Under anaerobic conditions a minimum pH of 6.4 was noted in the 17 mg Zr/l column on day 16. In contrast to the aerated system, pH recovery in the anaerobic columns was quite rapid. Four days after zirconium inactivation (Day 19) the pH had risen in all columns to 7.2, and 2 days later had returned to approximately 7, remaining at that level until termination of the experiment. Recovery to the initial pH in the aerated columns required nearly twice as long. The difference in pH behavior between the nitrogen purged and aerated columns may have been the result of an ammonium carbonate buffering system operating under anaerobic conditions, while in the aerated system ammonia would have been rapidly oxidized. This possibility will be examined in future experiments when inorganic nitrogen fractions in the test columns will be monitored.

Total phosphorus remained low (it decreased even further for the 10 mg Zr/l treatment) for the remainder of the experiment. The change-over from nitrogen to air on day 20 may have contributed to the continued low levels. Total soluble phosphorus was at

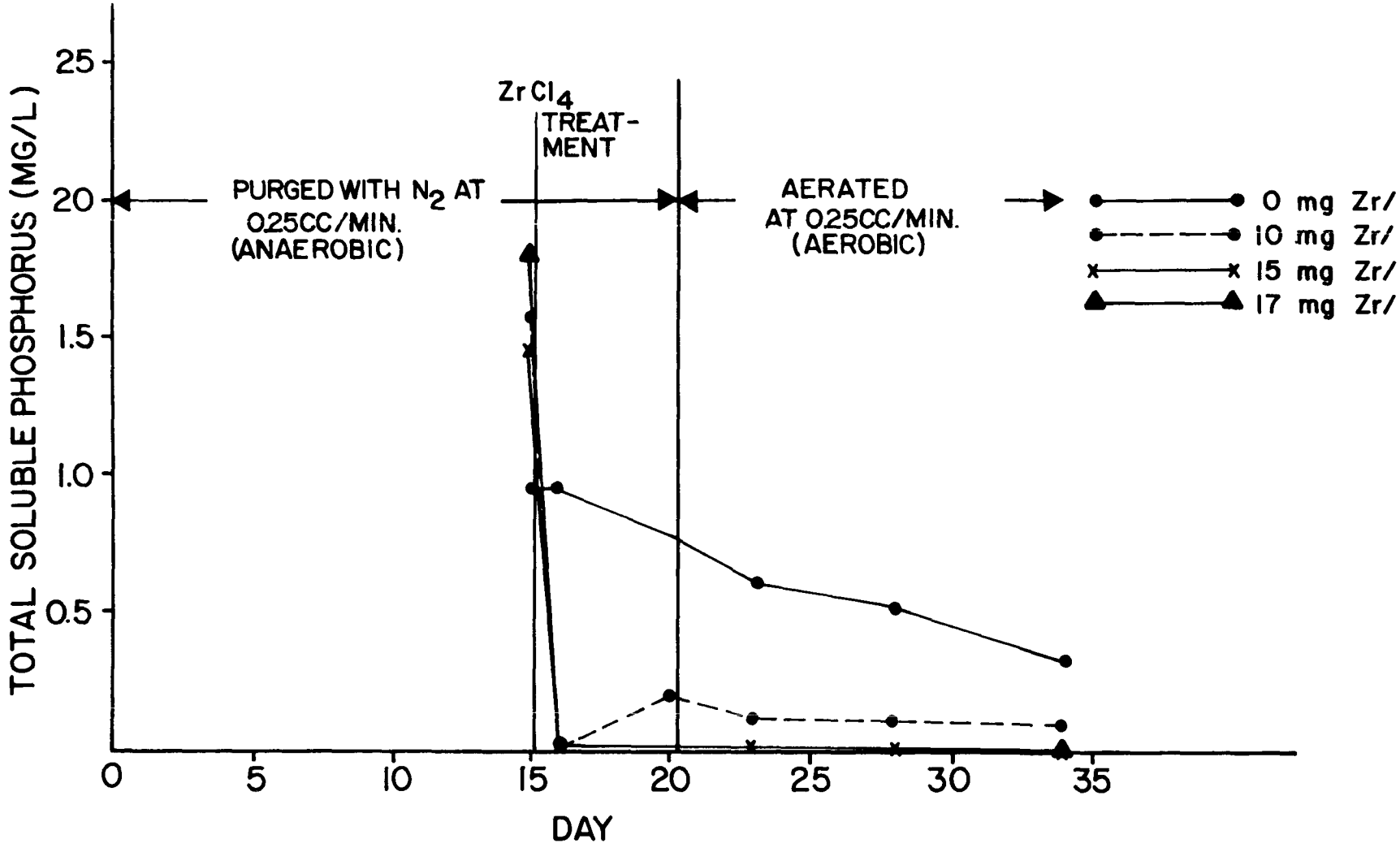

Figure 31. Total soluble phosphorus concentration of an anaerobic system before and after zirconium tetrachloride addition with subsequent aeration.

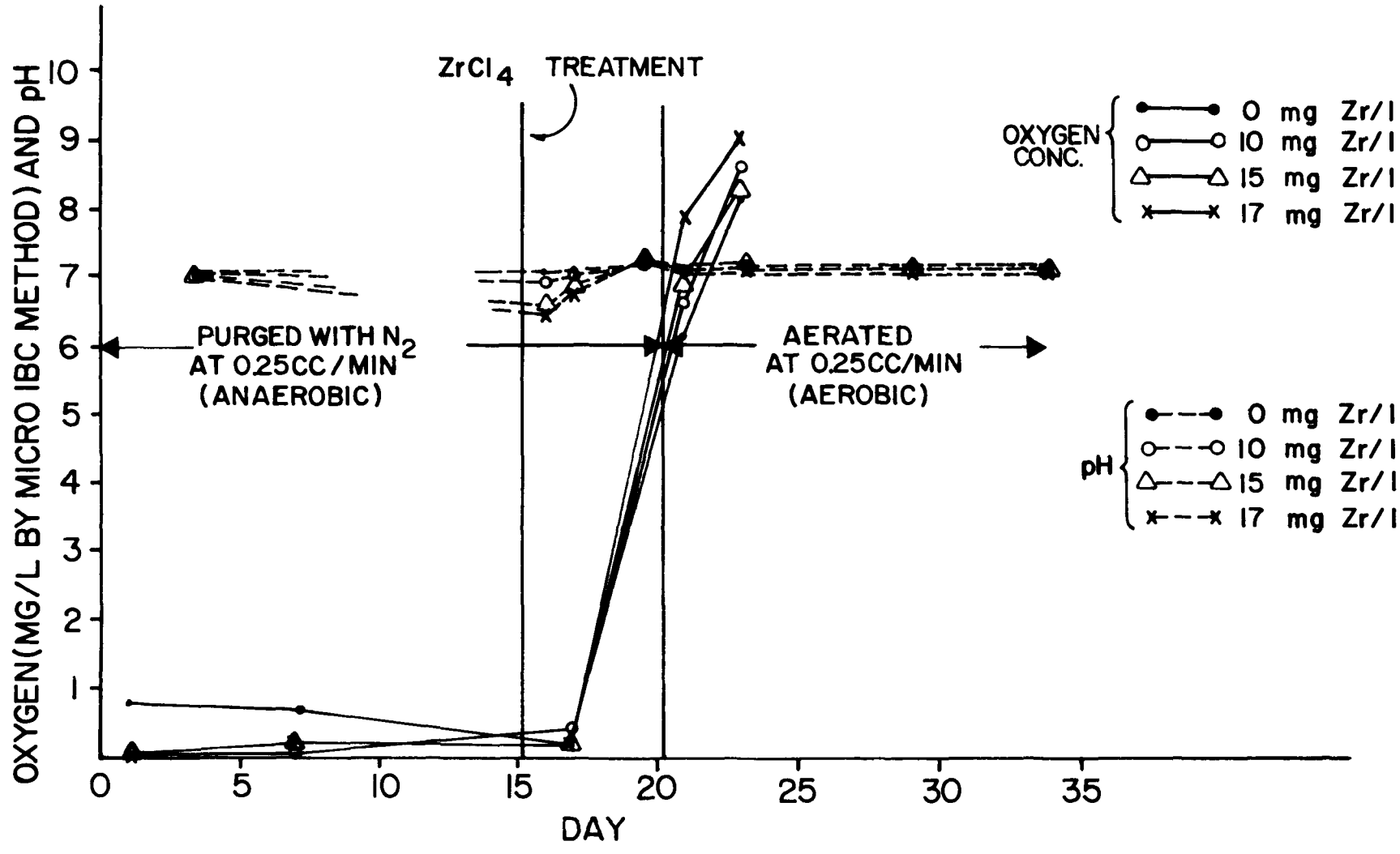

Figure 32. Dissolved oxygen and pH in an anaerobic system before and after zirconium tetrachloride addition with subsequent aeration.

a minimum on day 16 following inactivation, but the 10 mg Zr/l column showed an increase at day 20 just prior to switching from nitrogen to air. By day 23 total soluble phosphorus in that column was again reduced and remained constant until the experiment terminated. The other experimental columns showed no change in phosphorus concentration from date of inactivation onward.

Although the data are not conclusive, it appears that anaerobic conditions may contribute to the slow release of some soluble phosphorus fraction from the sediment or the zirconium-phosphorus complex. The fraction is extremely small, however, and will be examined more closely in the experiments using phosphorus isotopes.

Decreased phosphorus levels in the controls after the 15th day are not understood at this point. Development of an air leak is one possibility, but total phosphorus analyses for that day are also suspect. This phenomenon will be scrutinized closely in subsequent experiments.

Discussion

Results of the preliminary laboratory study confirm that the experimental procedure and sediment-water systems perform as anticipated. Under anaerobic conditions prior to inactivation there was apparently

substantial release of phosphorus from the sediment to the overlying water, while aerating resulted in a phosphorus concentration decline. Treatment of the "columns" with crude zirconium tetrachloride produced a precipitous drop in the phosphorus concentrations. When treated at the rate of 15 and 17 mg Zr/l the soluble phosphorus levels were reduced by 94 to 97 percent under both aerobic and anaerobic conditions.

In the toxicity experiments zirconium tetrachloride concentrations up to 20 mg Zr/l did not produce a 96 hour TL_m. The 9-week experiments produce TL_m values at < 20 mg Zr/l with D. magna; however, this represented the worst possible condition, in which the organisms were continually subjected to reconstituted solutions of the inactivant each week over a nine-week period. The column experiments and any field applications of zirconium probably would be more nearly represented by the 96 hour toxicity experiments, since the zirconium floc is dense and settles rapidly to the bottom. The concentration of zirconium remaining in solution is unknown at present but preserved samples will be analyzed shortly. An initial shock to aquatic organisms due to concentration of the inactivant at the surface is a possibility in field applications; however, it would normally be of short duration. In jar tests all visible zirconium floc settled to the bottom in less than 1 hour. Therefore, treatment of the columns with 15 to 17 mg Zr/l, as in the preliminary experiment, would remove approximately 94 to 97 percent of the soluble phosphorus from the water and would probably be relatively non-injurious to the majority of the pelagic aquatic organisms (Ostracods were observed in several of the columns following treatment).

The pH in treated columns dropped to a low of 5.45 with the addition of 17 mg Zr/l under aerated conditions. Although recovery to the original pH of approximately seven required only three days, a pH shock of that magnitude and duration could be extremely detrimental to many aquatic organisms. The column experiments demonstrated again that pH adjustment is critical when zirconium tetrachloride is used in poorly buffered waters. Adjustment of pH would be of far less concern in well buffered water systems.

The most encouraging information from the preliminary experiment was that the soluble phosphorus levels, once reduced by inactivation, tended to remain low for the duration of the experiment in both the aerobic and anaerobic systems. A slight increase in total phosphorus levels occurred 20 days after inactivation; it is necessary to know whether this phosphorus was resolubilized from the inactivant-phosphorus complex or originated from the sediment beneath the inactivant-phosphorus layer. The dual phosphorus tracer experiments are expected to shed light on this problem. If so, the techniques using sediment-water columns may be refined into a method for calculating the exchangeable phosphorus content of lake sediments, a problem which has plagued limnologists and systems ecologists for some time.

VIII. AVAILABILITY AND COSTS OF INACTIVANTS

An important criterion for determining the suitability of a material as a practical nutrient inactivant is its availability and cost. Depending on the phosphorus removal characteristics of the material, the size of the lake, its phosphorus concentration, and the proximity of the lake to sources of supply, both the quantity of inactivant needed and the total cost of treatment could vary over a wide range.

Very large lakes probably would not be subjected to nutrient inactivation; quantities, costs, and mechanics of application would become prohibitive. If field testing verifies the operational capability and reliability of the technique, however, it could conceivably be applied to many smaller, overly productive lakes throughout the country.

Information from the literature indicates that world supplies of zirconium and rare earths are sufficiently great to allow consideration of these materials for lake restoration. According to Kleber and Love,[42] total rare earths are about half as abundant as carbon or chlorine, in the same range of abundance as chromium, vanadium, or zinc, and more abundant than nickel or copper. The annual United States production capacity of rare earths of the cerium group (of which lanthanum constitutes about 25 percent) is estimated by Kleber and Love at 20,000-25,000 tons. The potential available supply, however, is in the range of hundreds of thousands of tons from undeveloped domestic sources alone. These minerals presumably would become available on demand.

The present United States demand for zirconium is about 72,000 tons of ore per year, most of which is imported from Australia. Reserves in that country are estimated at 3,250,000 tons.[43] Other sources would be expected to become available if demand increased.

As an illustration of the quantities of inactivants which might actually be required in the treatment of a eutrophic lake, we have computed the amounts of lanthanum, zirconium, and aluminum which would be needed to inactivate all the phosphorus in Diamond Lake, Oregon, a moderately eutrophic lake with a volume of 90×10^6 m^3 and a mean total phosphorus content of 2900 kg. Using values obtained with Cline's Pond water from the jar tests, the following calculations were made:

Lanthanum rare earth carbonate: 24,600 kg
Lanthanum rare earth chloride: 33,400 kg
Zirconyl chloride: 60,260 kg
Zirconium tetrachloride: 65,390 kg
Sodium aluminate: 53,600 kg

These numbers are qualitative estimates, being based only on the results of preliminary laboratory tests. They do, however, provide a reasonable idea of the order of magnitude of quantities needed in full scale operations. Diamond Lake is a relatively large lake, with a surface area of approximately 13,000 ha, but its phosphorus content can only be considered moderate. Phosphorus concentrations in many problem lakes could be a number of times greater. On the other hand, lakes selected for nutrient inactivation are likely to be small and require only moderate quantities of inactivant.

The costs of inactivation are also difficult to estimate because of variation in availability of materials from place to place, and the cost of transporting them to the lake. Some recent estimates of approximate costs per kilogram for various possible inactivants at their source are as follows:

lanthanum rare earth carbonate	$1.43
lanthanum rare earth chloride	0.64
zirconyl chloride	1.10
zirconium tetrachloride	1.10
sodium aluminate	0.22
aluminum sulfate	0.07

These are estimates only and actual prices will vary with location, purity of products and other factors. Further research should show whether performance characteristics of more expensive inactivants will warrant the higher initial cost. Stability of the treatment with the various inactivants will be an important consideration in determining their actual cost.

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16. ABSTRACT

Compounds of certain metals are known to be capable of complexing phosphate ions, thereby removing them from solution. The application of this principle to the control of phosphorus levels in eutrophic lakes has been subjected to laboratory investigation in the present study. Salts of lanthanum, zirconium, and aluminum were found to effectively remove phosphorus from laboratory growth medium and natural pond water, with resulting depression of algal production. Toxicity to fishes and aquatic invertebrates was minimal, but the tests demonstrated that some components of metals salts may have adverse effects. The stability and duration of phosphorus inactivation is being studied in laboratory-scale water-sediment systems, under aerobic and anaerobic conditions. These experiments are expected to elucidate the effect of inactivant-phosphate precipitates on sediment-water phosphorus interchange. Preliminary results indicate that zirconium precipitates phosphorus from the water and holds it at low levels.

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