THE TIME-CONCENTRATION RELATIONSHIP OF TRACE METALS IN THE GROWTH OF ALGAE

by

DEAN STUART JEFFRIES

A Thesis

Submitted to the Department of Geology

in Partial Fulfilment of the Requirements

for the Degree

Bachelor of Science

GEOLOGY 4K6

McMaster University

April 1971

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. J. R. Kramer for his generous assistance in the preparation of this thesis. The help of Mr. H. B. McDonald (Canada Centre for Inland Waters) in obtaining a lake water sample is also acknowledged.

Abstract

The time relationship of metabolically important trace metals (Fe, Cu, Zn, Co, Cd, Cr) in Lake Erie plankton were analyzed from samples collected during an algal (Aphanizomenon) bloom. Iron and cobalt showed a rapid increase in concentration during the initial period of the bloom, followed by a decrease and levelling off to an equilibrium value. Copper and zinc were found to behave similarily undergoing a slight decrease at the beginning and then steadily increasing in concentration with time. Different depths of sampling were correlated to the life-death cycle of the organism and specific metal concentration trends. Algae from the lowest level of sampling (9.7 m; dead organism level) exhibited an increasing nutrient concentration (Fe, Zn, Co, and PO_4) with time.

Factor analysis suggested a Fe, Co, depth, 1/Biomass relationship; a Cu, Zn relationship; and a PO_4, time relationship. The first two were verified from the experimental results; the last was not.

LIST OF TABLES AND FIGURES

Introduction

The biochemical dependence of certain trace elements on almost all plant-life forms has been a well established fact for a number of years. (Lewin, 1962; Lamb et al, 1958; O'Kelley, 1968). In light of this, the relationship between the uptake of these elements and growth is of interest. An ideal plant-form for use in the study of this relationship is algae, both from the standpoint of the experimental ease of handling and from its very rapid growth rate. The algae samples used were collected during a continuous time study of Lake Erie (LETS) in August, 1969. (Kramer et al, 1969) They were collected at intervals prior to and during an algal bloom and thus represent a typical growth pattern. The trace metal concentration of the algae was correlated to the time of collection (and thus position in the growth pattern) in an attempt to determine an uptake rate relationship.

At least a dozen trace metals are essential to plant growth. These micro-nutrients are involved in a number of metabolic reactions, some being more important than others. Iron, copper, zinc, and manganese all fall into the category of "most important". (Lamb et al, 1958) All are directly related to growth, often through photosynthetic processes. For example, iron is known

to be an essential constitute of many enzymes and cytochromes, while Zn concentrations are known to vary directly with chlorophyll concentrations. (Lewin, 1962) Other less important but still "essential" trace metals include cobalt (a vitamin B_{12} constituent), vanadium (photosynthesis), and molybdenum (nitrogen metabolism). In the opposite sense, elements such as Cd, Pb, and Hg are known for their inherent toxicity.

The actual choice of metals to be analyzed was primarily influenced by the availability of suitable analytical equipment . Excellent selectivity and sensitivity made atomic absorption (A.A.) the most obvious analytical method. An air/acetylene apparatus was readily available with suitable hollow cathode lamps, which made analysis of Fe, Zn, Cu, Co, and Cd immediately possible. Mn, Mo, and U require the higher temperature nitrous-oxide/air system which was not available, and thus, analysis of these metals was not attempted. In the end, Cr was added to the list, not so much for its metabolic relationship (no relationship is known as yet), but measurable quantities of this element were detected in the water from which the algae samples were taken. In addition, as a further means of correlation from sample to sample, an analysis of total phosphate was made.

In an effort to determine the concentration

levels to be expected, I made preliminary studies on algae samples grown in aquaria under the controlled condition. A modification of two standard procedures was used in sample analysis.

Experimental

(A) Growth Studies:

The preliminary study involved growth of algae for sampling, the samples being analyzed in the same way as the Lake Eire samples. Two growth studies were carried out. The first was grown in a concentrated nutrient solution (Allen's Media, see Figure 1) at room temperature (20-25°C) and under constant light exposure. A variety of autotrophic blue-green species could be grown quickly and in sufficient quantity to provide the necessary organic matter for testing of the analytical method. The second algae were grown in a sample of lake water (Lake Ontario; October, 1970) with no added nutrients but under otherwise identical conditions as those above. It was necessary to maintain a growth period of three months in order to obtain a suitably representative algal sample.

(B) Analysis:

The metal concentration levels expected in these samples made it necessary to employ a chelation/extraction method, the organic extract then being aspirated in the A.A. spectrophotometer during the actual analysis. The main analytical problem was the development of a chelation system which would simultaneously chelate Fe, Cu, Zn, Cd, Cr, and Co,

FIGURE 1

ALLEN'S MEDIA (Allen, M.M., J. Phycol, 4, p 1-4, (1968))

(A) Macronutrients (weight dissolved in 10 l. lake water)

1.500	g.	$NaNO_3$ *
0.039	g.	K_2HPO_4 *
0.75	g.	$MgSO_4.7H_2O$
0.27	g.	$CaCl_2$
0.58	g.	$Na_2SiO_3.9H_2O$
0.06	g.	citric acid

* the weights suggested are only one-tenth that given by Allen; also not included in the macronutrients were 0.20 g. Na_2CO_3, 0.01 g. EDTA, and 0.060 g. iron citrate

(B) Micronutrients**(weight dissolved in 1 l. deionized water and 10 ml. of this solution added to the media as prepared above)

2.86	g.	H_3BO_4
0.39	g.	$Na_2MoO_4.2H_2O$

**not included but suggested by Allen were

1.8	g.	$MnCl_2.4H_2O$
0.222	g.	$ZnSO_4.7H_2O$
0.079	g.	$CuSO_4.5H_2O$
0.0494	g.	$Co(NO_3)_2.6H_2O$

Clearly, the components left out correspond to the metals which were to be analyzed.

which could be effectively extracted for analysis. For example, Sachdev and West (1970) employ a diphenylthiocarbazone/8-hydroxyquinoline (oxine)/acetyl acetone chelate mixture and extract into ethyl propinate (tartrate buffer). Effective determination of eight different elements using this method was reported. This method was carefully tested because of its obvious applicability. Unfortunately, the method did not work well with the apparatus available (a Perkin-Elmer 290 AA. spectrophotometer). The gas flow dynamics of the burner were such that the dithizonate chelates tended to coat the inside of the chamber rather than passing on to the burner head. The aspiration rate was very low; this phenomenon has been noted in the literature on previous occasions and usually occurs with low boiling point extractive solvents and the cheaper spectrophotometer models (both applicable in this case).

A second possible method involved the use of the ammonium pyrrolidine dithiocarbonate (APDC)/ methyl isobutyl keotine (MIBK) system. The usefulness of this system arises from the large number (16) of metals which APDC is known to chelate. Cobalt, however, is not included in this list; therefore, I formulated a hybrid variety of the above methods. The method involved an APDC-oxine chelation followed by a MIBK extraction

(See Appendix 1). Since APDC is a low pH chelating agent, while oxine chelates at a higher pH, intermediate buffering was necessary. A tartrate buffer system (pH 5.0-6.0) was found to be effective. Solution from the low parts per billion (ppb) to 0.5 parts per million (ppm) were easily handled by this method.

The phosphate (PO_4) analysis was carried out using the modified colorimetric method of Proctor and Hood (Sutherland et al, 1966; see Appendix 2). I found it necessary to dilute the samples by a factor of forty in order to obtain isobutanol extracts which fell in the straight line portion of the calibration curve. Although the concentration range present meant less sensitive phosphate methods were feasible, effective control of several interferences by the isobutanol procedure made its use necessary.

The natural samples were treated with a small amount of formaldehyde and stored in mason jars. I prepared them for analysis by filtering and by wet oxidation of the organic material (See Appendix 3).

Results

(A) Growth Studies:

The purpose of the preliminary studies was primarily to develop and test the analytical method to be used on the natural samples. Three additional conclusions, however, can be made about the second algal sample grown (i.e. in lake water with no additional nutrients):

(1) Analysis of the lake water used showed detectable Zn, Cu, and Fe in the range 5-15 ppb.

(2) The algae analysis, itself, showed detectable Zn, Cu, Fe, and Co and Cd as well.

(3) Finally, analysis of the water in which the algae were grown showed detectable Cu only; no other element was detected.

(B) Natural Samples:

The natural samples were collected during the period August 11 to August 18, 1969, during which the algal bloom commenced and proceeded to essential completion. The specific time of commencement of the bloom may be set at approximately noon on August 11 (between samples E7 and E17) when a sudden change in particulate carbon results and dissolved oxygen content was noted by the LETS study. The results of the natural sample analysis

are in Table 1. The concentrations of metals are given in units of milligrams (mg) per kilogram (kg) biomass (ppm). The biomass of each sample was calculated using the LETS particulate carbon analyses and assuming the stoichiometry of the biomass is $C_{106}H_{263}O_{110}N_{16}P_1$ (Stum and Morgan, 1970); it is also given in the results expressed in mg per litre of sample taken. The time of each sample is expressed in the decimal equivalent of days (i.e. 11.50 corresponds to 12:00 noon, Aug. 11, 1969). In addition, it is noted that each sample was taken at four different depths (1.0m, 3.6m, 5.7m, 9.4m), and finally, when no metal was detected, it is indicated by the presence of a horizontal bar in the appropriate position.

Other facts noted from the LETS programme are given below:

(1) Cd was undetectable in the water throughout the period of study.

(2) Cr concentrations decreased from 3-6 ppb at the beginning of the bloom to undetected quantities at the end.

(3) Cu and Zn concentrations decreased marginally during the study period.

(C) Factor Analysis:

The data in Table 1 was subjected to factor

TABLE I

EXPERIMENTAL RESULTS

ALGAL TRACE METAL CONCENTRATION (PPM) *

		Fe	Zn	Cu	Cd	Cr	Co
SAMPLE E7	1	2670	687	436	44	109	22
	2	5108	3133	450	27	136	27
	3	3663	241	174	19	96	29
	4	3426	301	251	33	167	33
SAMPLE E17	1	29418	411	364	-	-	239
	2	7372	1180	929	-	-	369
	3	9847	1217	680	-	-	448
	4	35939	793	940	-	-	461
SAMPLE G9	1	7520	269	163	-	125	157
	2	12533	279	9	-	186	232
	3	7206	63	163	-	251	313
	4	39608	709	307	-	473	591
SAMPLE G22	1	3264	346	72	104	131	209
	2	9905	243	57	24	162	194
	3	10930	146	102	29	291	291
	4	48476	355	166	71	473	1135
SAMPLE Q6	1	23493	1823	168	70	281	421
	2	14622	738	111	14	279	223
	3	17227	588	118	101	336	437
	4	16901	440	126	31	314	346
SAMPLE Q7	1	5610	603	182	70	281	252
	2	4735	599	70	84	279	348
	3	4790	924	118	134	336	420
	4	5346	472	110	63	314	393
SAMPLE T1	1	4324	1258	896	110	314	283
	2	4382	769	197	72	358	233
	3	4919	322	72	36	358	322
	4	21323	1340	171	195	487	609

TABLE I (con'd)

EXPERIMENTAL RESULTS					
		Time (days)	Depth (m)	Biomass (mg/l)	PO_4 (ppm)
SAMPLE E7	1	11.48	1.0 m.	3.28	4141
	2		3.6 m.	2.62	7084
	3		5.7 m.	3.70	5399
	4		9.4 m.	2.14	5347
SAMPLE E17	1	11.62	1.0 m.	3.73	12437
	2		3.6 m.	2.42	13639
	3		5.7 m.	1.99	12980
	4		9.4 m.	1.94	6911
SAMPLE G9	1	12.47	1.0 m.	5.70	5953
	2		3.6 m.	3.85	12533
	3		5.7 m.	2.85	13535
	4		9.4 m.	1.51	15962
SAMPLE G22	1	12.66	1.0 m.	5.47	6136
	2		3.6 m.	4.42	11037
	3		5.7 m.	2.45	12183
	4		9.4 m.	1.51	10499
SAMPLE Q6	1	14.47	1.0 m.	3.96	17812
	2		3.6 m.	3.99	9720
	3		5.7 m.	3.31	13865
	4		9.4 m.	3.53	5503
SAMPLE Q7	1	14.49	1.0 m.	3.96	15372
	2		3.6 m.	3.99	15206
	3		5.7 m.	3.31	15630
	4		9.4 m.	3.53	7295
SAMPLE T1	1	18.36	1.0 m.	3.53	10377
	2		3.6 m.	3.11	9658
	3		5.7 m.	3.11	11161
	4		9.4 m.	2.28	20226

* metal and PO_4 concentrations are given in parts per million (ppm), ie. mg. per kg. of biomass

analysis in an effort to establish relationships among the variables. This computerized analysis allows an objective empirical grouping of variables which show common trends through the data. Both principal axis and varimax factoring were employed. The results of this factor analysis are given in Table 2. There are three significant factors (eigenvalues greater than one) from the principal component analysis. Varimax analysis of the three factors shows similar results.

The differences between the two factor analyses arise primarily from the different mathematical natures of the two. In the principal axis analysis, the basic data matrix is handled in such a way that the first factor is weighed most heavily in the determination of the relationships among the variables. In the varimax (orthogonally rotated) matrix, however, the three factors are weighed much more evenly and thus slightly different results may occur.

The principal component factor matrix indicates the following possible relationships between the variables tested: (note that Cd and Cr have been omitted due to the generalized nature of the Cr results and the lack of completeness of the results for both elements)

(1) 35% of the data can be explained by a direct relationship between Fe, Co, and depth with

TABLE 2

FACTOR ANALYSIS RESULTS

	MEAN	STANDARD DEVIATION	UNITS	EIGENVALUES
Fe	13020	12180	ppm.	2.815
Zn	723	630	ppm.	1.589
Cu	272	270	ppm.	1.448
Co	323	222	ppm.	0.842
PO_4	10990	4240	ppm.	0.545
Biomass	3.26	1.03	mg/l.	0.456
Time	13.65	2.27	days	0.173
Depth	4.93	3.13	m.	0.131

	FACTOR MATRIX			ORTHOGONALLY ROTATED FACTOR MATRIX		
				FACTOR		
				#1	#2	#3
Fe	0.800	-0.039	-0.168	0.814	0.028	0.075
Zn	0.113	0.549	0.670	-0.105	0.833	0.243
Cu	0.217	0.820	0.210	0.125	0.828	0.243
Co	0.865	-0.286	0.053	0.817	-0.044	0.405
PO_4	0.446	-0.382	0.620	0.250	0.102	0.810
Biomass	-0.813	-0.350	0.051	-0.733	-0.413	0.050
Time	0.055	-0.509	0.598	-0.113	-0.087	0.775
Depth	0.710	-0.066	-0.424	0.805	-0.149	-0.132
Cumulative % Eigenvalues	35.2	55.1	73.2			

an inverse relationship to biomass (i.e. Fe $\propto$ Co $\propto$ depth $\propto$ 1/Biomass)

(2) 20% of the data might be explained by a Cu, Zn, 1/Time relationship, although the factor analysis shows this correlation to be weak

(3) 18% of the data may be explained by a Zn, PO_4, Time correlation.

The orthogonally rotated matrix verifies many of the relationships indicated in the principal component matrix. Factor #1 produces the same relationship as above (i.e. Fe $\propto$ Co $\propto$ depth $\propto$ 1/Biomass), while factor #2 supports the possibility of a relation between Cu and Zn, but excludes the 1/Time correlation. Similarily, factor #3 shows a proportional relationship between PO_4 and Time, but excludes the Zn correlation noted above.

Exclusion of some variables and substitution of other nutrient variables such as nitrate or dissolved oxygen from the LETS study into further factor analysis might prove very worth while, although time was not available for this at present.

The method devised for the analyses proved to work well. In general, Fe, Zn, and Cu were of high enough concentration that the results may be considered to be accurate to within 10%. Similarily, the high

sensitivity of the spectrophotometer to cadmium analysis made determination of this element reasonably exact (± 15 %). Cobalt and chromium analyses must be considered as approximate figures only, due to fairly high reading fluctations in the case of cobalt, and very low sensitivity, in the case of Cr. A problem encountered during the analysis arose from the reaction between formaldehyde and APDC to give a white to grey precipitate in samples with an unexpectedly high formaldehyde content. This was observed in samples E17 and G9, and to a lesser extent in G22 and Q6. Analyses of the precipitate indicated that it was purely organic, containing no appreciable quantities of metals; however, in order to remove all possible interference, these samples were suitably diluted until no precipitate formed on the addition of APDC. As a result of this dilution, certain metals, (particularily Cd and Cr) were reduced to a concentration below the detection limit of the spectrophotometer and are noted as "not-detected" in the chart of experimental results. Despite the precautions taken by dilution, it is evident that the formaldehyde present, did have some effect on the analytical results. Samples Q6 and Q7 should have reasonably comparable values, and for all the metals except iron, this is true. Higher formaldehyde concentrations (as in Q6 but not in Q7) apparently have

a significant effect on the iron determination; despite this, the iron results are felt to be reasonably accurate since sample Q6 was the least diluted of any of the samples.

As was noted earlier, the biomass of each sample was calculated using the assumed agal protoplasm stoichiometry of $C_{106}H_{263}O_{110}N_{16}P_1$. This assumption was tested by calculating the carbon to phosphorus ratio for the data. This is given below:

Mean of C/P ratio = 116

Standard Deviation = 53

This mean value corresponds well with the assumed ratio (C:P = 106:1) considering the experimental errors involved. No large error has been introduced by assuming the stoichiometry above.

Discussion

As an aid to the interpretation of the results, graphs of metal concentrations vs time were plotted for Fe, PO_4, Zn, Cu, and Co at each of the four depths of sample collection, (see Figures #2-5). Superimposed on the uppermost graph (Fe) is a plot of biomass concentration vs time for each depth. Definite trends are quickly noted from these graphs and are commonly found to correlate with the factor analysis results.

Iron: It is noted that in all cases, the algal concentration increases sharply at the beginning of the bloom period followed by a decrease and levelling off to a concentration slightly greater than the pre-bloom equilibrium value. The 9.7m samples show increasing iron content with time towards the end of the bloom period. The increased iron uptake is followed by the peak in the biomass concentration, although the iron peak, itself, commonly corresponds closely to a low biomass value. It is thus evident that the bloom was initiated by the growth of a small quantity of biomass of extremely high iron content. After the initial surge, the iron concentration tends to level off, except in the samples taken at the 9.7m depth. If a model is adopted, which assumes that the major growth of the

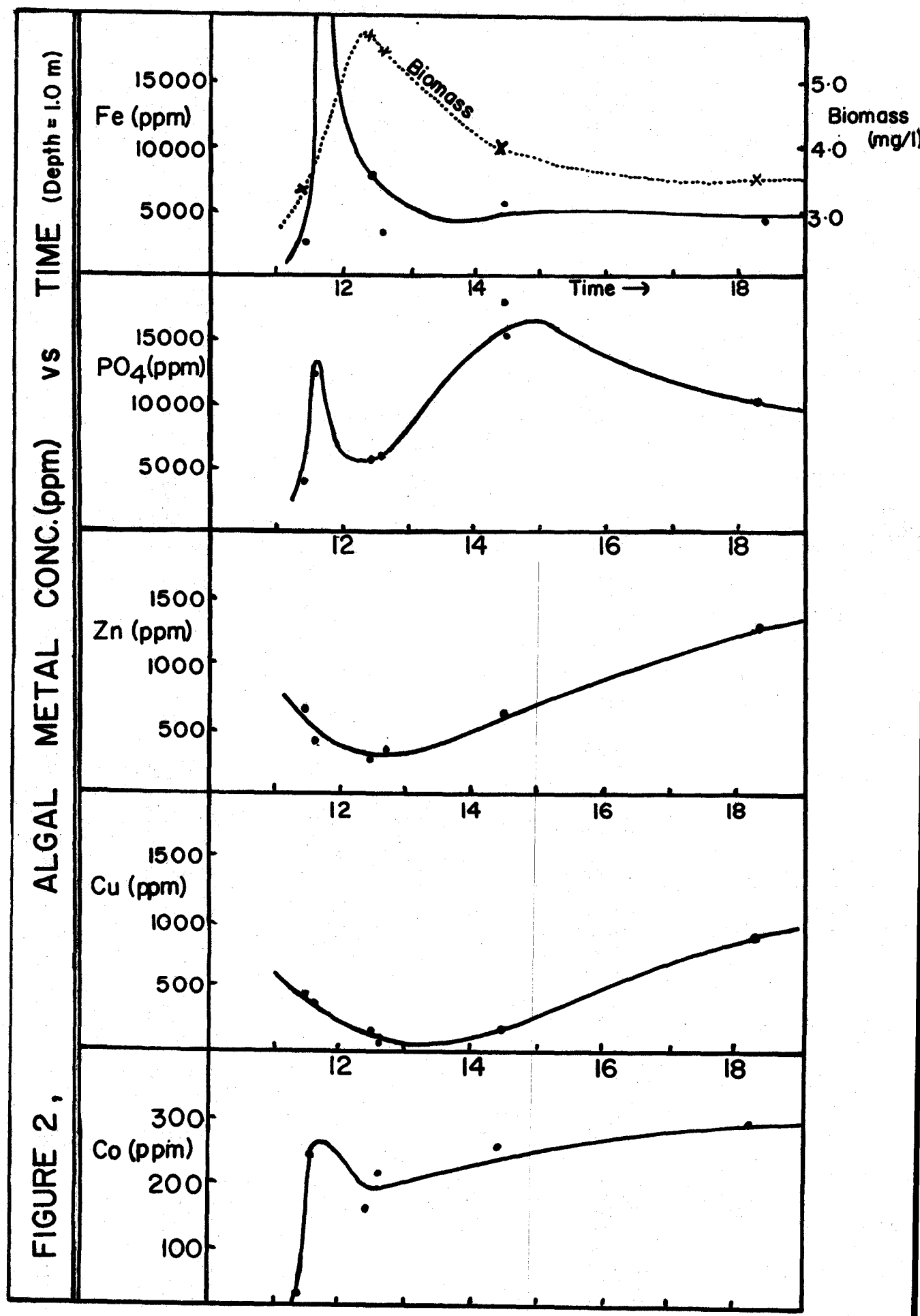
FIGURE 2, ALGAL METAL CONC.(ppm) vs TIME (Depth = 1.0 m)
15000
Fe (ppm)
10000
5000
Biomass
5·0
4·0
3·0
Biomass (mg/l)
12
14
Time →
18
15000
PO4(ppm)
10000
5000
12
14
16
18
1500
Zn (ppm)
1000
500
12
14
16
18
1500
Cu (ppm)
1000
500
12
14
16
18
300
Co (ppm)
200
100

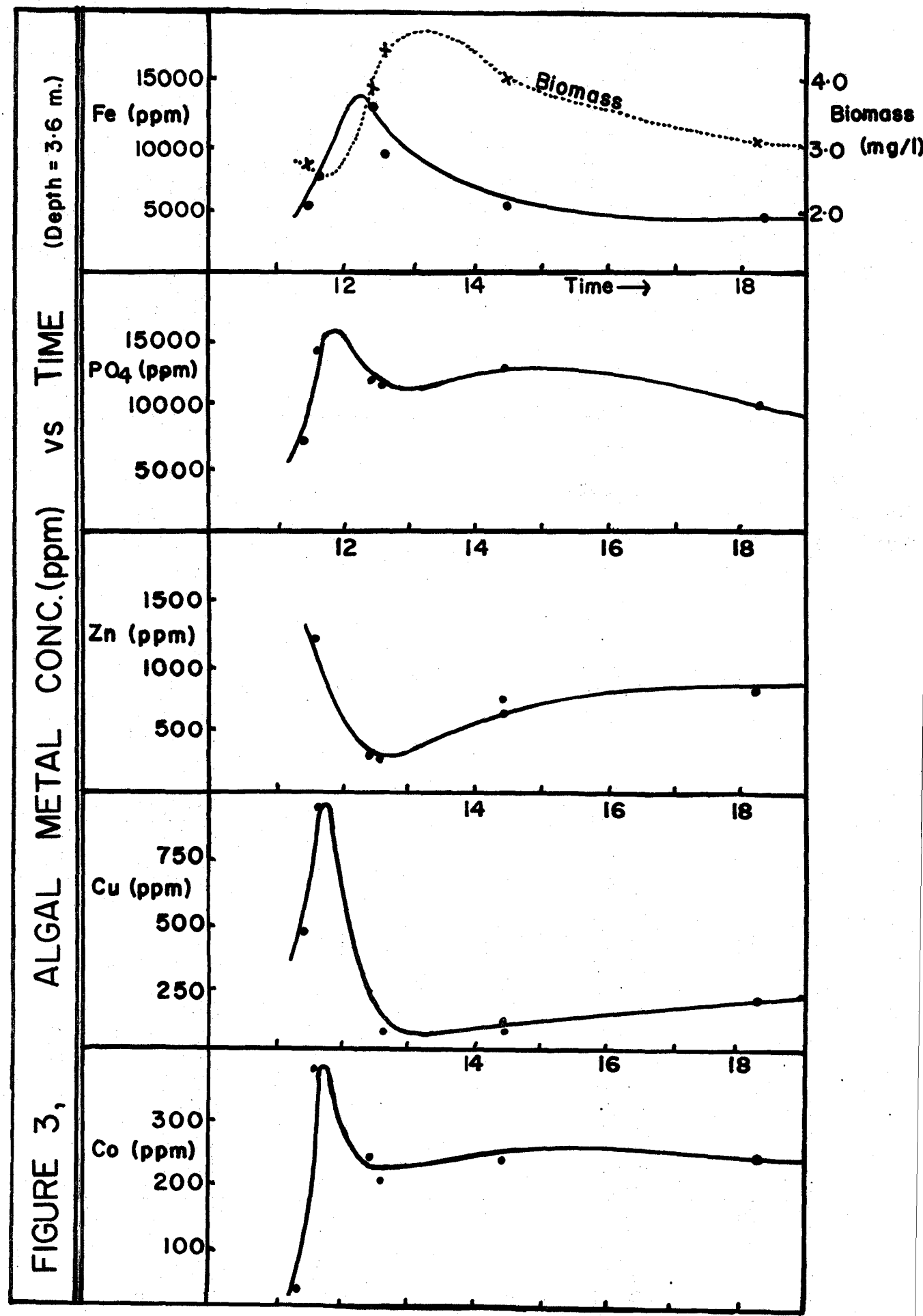
FIGURE 3, ALGAL METAL CONC.(ppm) VS TIME (Depth = 3·6 m.)
15000
Fe (ppm)
10000
5000
Biomass
4·0
Biomass
3·0 (mg/l)
2·0
12
14
Time →
18
15000
PO4 (ppm)
10000
5000
12
14
16
18
1500
Zn (ppm)
1000
500
14
16
18
750
Cu (ppm)
500
250
14
16
18
300
Co (ppm)
200
100

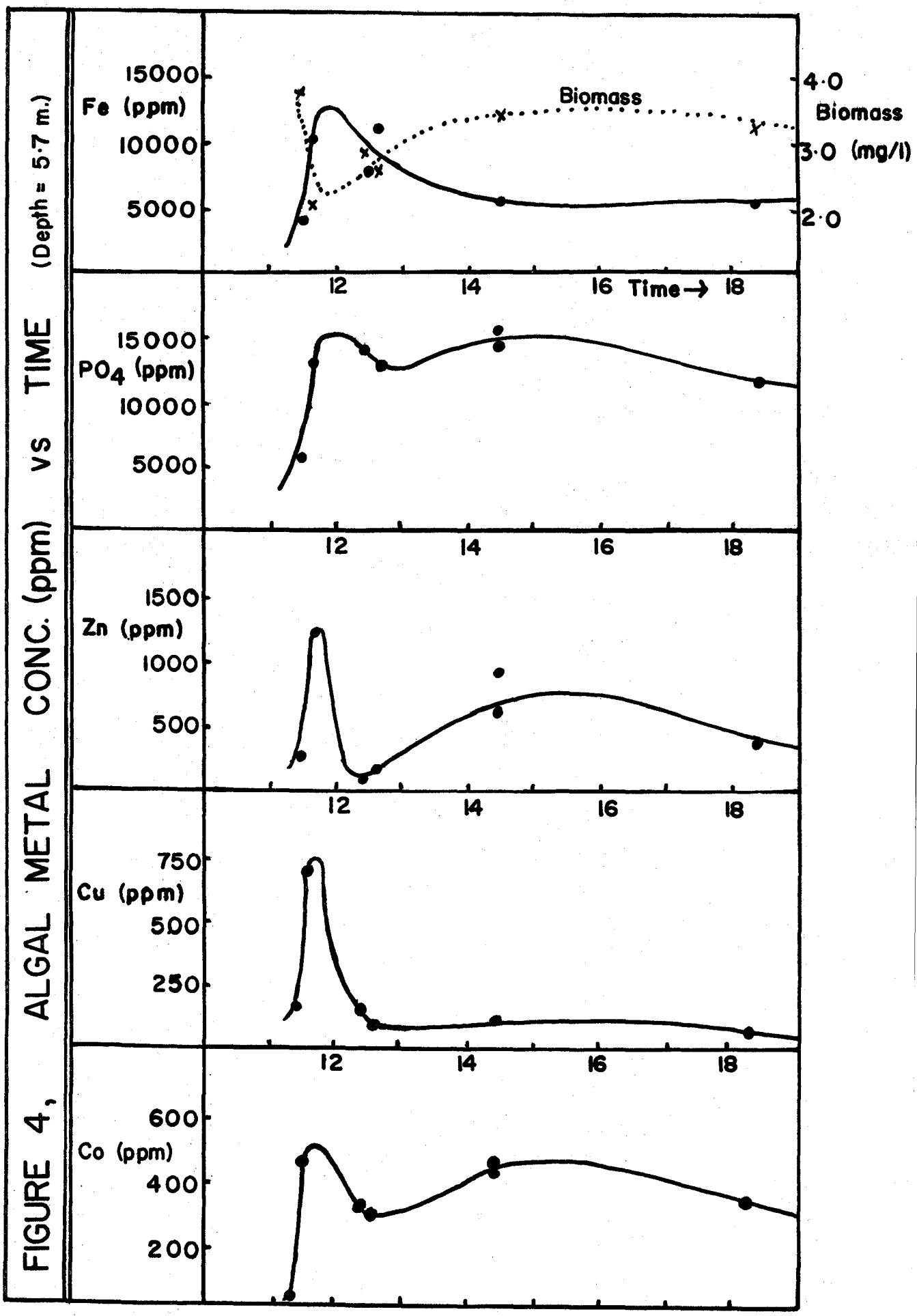
FIGURE 4, ALGAL METAL CONC. (ppm) vs TIME (Depth = 5·7 m.)
Fe (ppm)
15000
10000
5000
Biomass
4·0
3·0
2·0
Biomass (mg/l)
12
14
16
Time→
18
PO4 (ppm)
15000
10000
5000
Zn (ppm)
1500
1000
500
Cu (ppm)
750
500
250
Co (ppm)
600
400
200

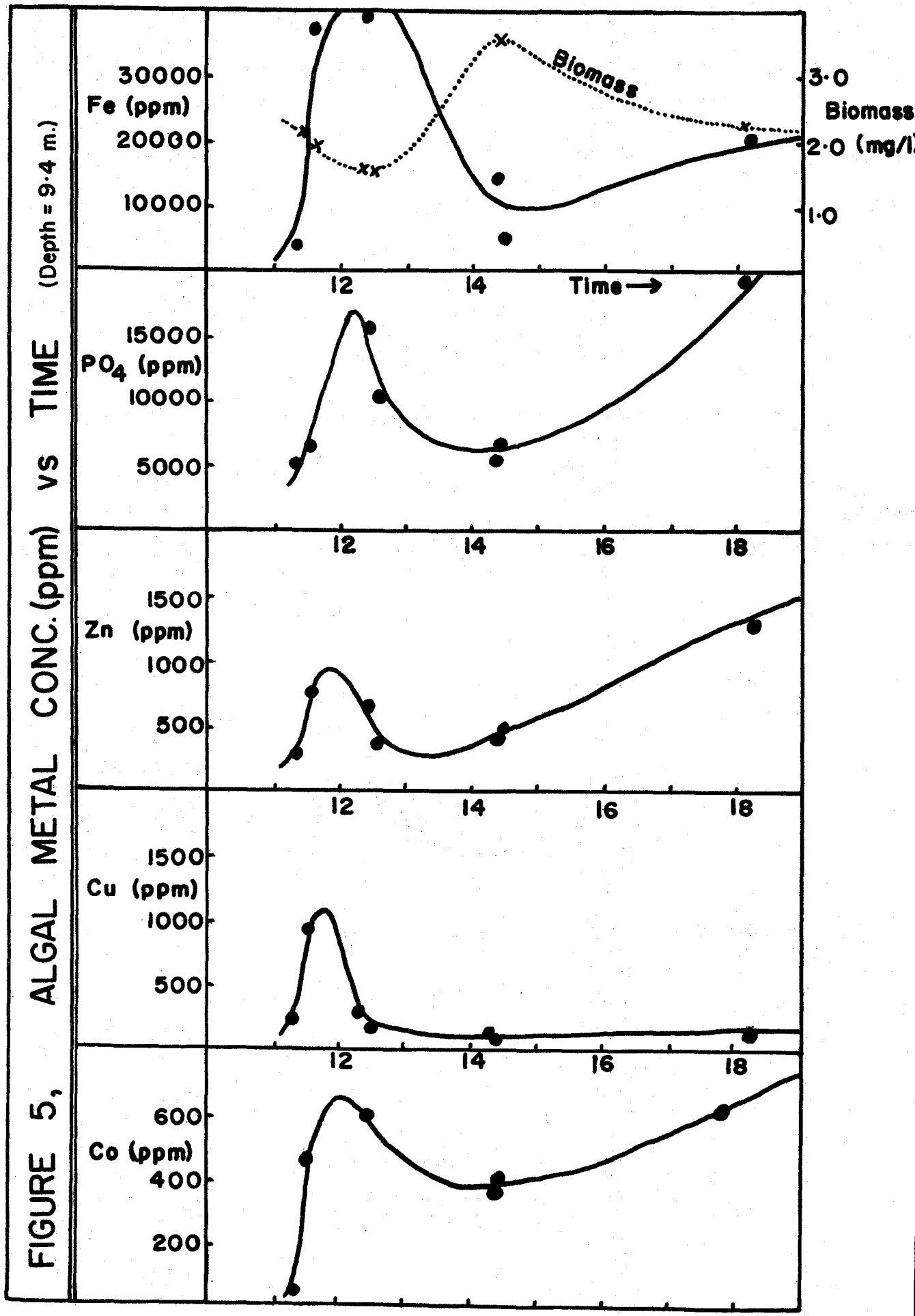
FIGURE 5, ALGAL METAL CONC. (ppm) vs TIME (Depth = 9·4 m.)
30000
Fe (ppm)
20000
10000
Biomass
3·0
Biomass
2·0 (mg/l)
1·0
12
14
Time →
15000
PO4 (ppm)
10000
5000
12
14
16
18
1500
Zn (ppm)
1000
500
12
14
16
18
1500
Cu (ppm)
1000
500
12
14
16
18
600
Co (ppm)
400
200

algae is a near surface phenomenon as is implied by the data and would be expected from a nutrient and light availability standpoint (i.e. nitrate, dissolved O_2, CO_2, etc.), and the lower levels constitute different stages of decay, then some inference regarding the 9.7m iron content can be drawn. The rising iron concentration with time indicates that the release of iron from the dead algae is restricted, and thus a certain fraction of the iron is removed from the system as a whole, until enough time has elapsed for the complete breakdown of the dead organisms.

This increasing concentration with the time relationship is not restricted to iron at the 9.7m (dead organism) level. Phosphate, zinc, and cobalt also show the same trend. Several nutrients are thus concentrated in the dead algae and removed from the system for a period of time. This nutrient removal followed by recycling on eventual complete breakdown of the organic material may be an important factor controlling the advent and periodicity of algal blooms.

A noticeable feature of the graphs is the obvious similarity between the behaviour of iron and cobalt, and zinc and copper. Both iron and cobalt show the "sudden increase" relationship at all depths, followed by a levelling off or gradual increase (in the case of cobalt).

If this relationship is an important factor in the initiation mechanism of the bloom, then the importance of Fe and Co concentrations relative to Zn and Cu concentrations is obvious. Supporting this idea is the fact that at the 1m depth (growth level), the Zn and Cu concentrations actually decrease at the beginning of the bloom and only later show a steadily increasing relationship. At lower levels, Zn and Cu show the "sudden increase" relationship similar to iron. The parallel behaviour of Zn and Cu is very apparent at all depths.

Phosphate shows an extremely interesting time relationship. At each of the four depths a sudden increase at the initiation of the bloom is followed by a decrease until a minimum PO_4 concentration is reached, after which a second maxima is obtained (except for the 9.7m depth). In three of the four cases, the PO_4 minima corresponds reasonably close to the greatest biomass concentration, This PO_4-biomass relationship may be coincidental, but appears worth further study. The irregular phosphate relationship is born out by the LETS study where a maximum reactive PO_4 content was noted about noon, August 12, 1969, (compare with Figure #2) and then showed a decrease, presumably as it was incorporated into the algae.

The above interpretations show a good correlation

with the factor analysis results. Both the iron-cobalt and zinc-copper relations were noted. Also correlated with iron and cobalt, were depth and 1/Biomass. The depth correlation is immediately obvious on inspection of Figures#2-5. An increasing Fe and Co content with depth but decreasing biomass weight is compatible with the model proposed earlier. The decreasing biomass with depth indicated that the organic material quickly degrades on the death of the organism, but Fe and Co remain tied up for a longer period of time. This phenomenon was noted during the aquaria algae studies; dead algae were found to retain high concentrations of iron with little being returned to the aqueous environment. Finally, the PO_4-time correlation suggested by the factor analysis is also evident on inspection of Figures #2-5. Disregarding the initial sharp increase and decrease in PO_4 content, the concentrations showed a general increasing trend with time at all depth levels.

The "sudden increase" relationship of nutrient metals and phosphate would seem reasonable from a supply standpoint. At the beginning of the bloom, the water concentration of these nutrients would be at a maximum and thus readily available for incorporation by the algae; however, on the resultant fast depletion of the metals, the concentration within the biomass must similarily

undergo a decrease until a reasonable equilibrium can be attained between algal uptake, supply from outside the system, and nutrients released from dying plant life.

Two further features apparent from the concentration vs time relationships are as follows: first, Fe, PO_4, and Co consistently level off to concentrations greater than their respective "pre-bloom" values, while Zn and Cu do not. This fact is probably the result of the increased necessity for these essential nutrients to support rapid algal growth as experienced during the bloom. Secondly, the relative proportion of metals remains fairly constant throughout the study period (i.e. Fe, highest concentration, Zn next, then Cu and Co).

The toxic element Cd and Cr (as of yet unknown metabolic relationship) were also analyzed in this study. Clearly the algae have a high affinity for concentrating Cd, since it was easily detected in the analysis, but remained at an undetectable level in the water during the LETS study. Likewise, Cr was concentrated by the algae and a corresponding decrease of Cr in the water was noted from the LETS data. Some studies have been carried out on the metabolic effect of Cr on animals (Schroeder et al, 1963), but little information is available with respect to plant-forms. The obvious uptake of Cr by the algae indicates the presence of some

kind of relationship and further study is warranted.

APPENDIX 1

Chelation and Extraction of Metals for AA. Analysis

Chelation/extraction solutions:

(1) 1.0 g. APDC dissolved in 100 ml. deionized water and filtered through a forty-five micron filter.

(2) 1.0 g. oxine dissolved in 200 ml. MIBK.

One hundred millilitres of sample is treated with 10 ml. of buffer solution (1M. ammonium tartrate) and the pH adjusted to 5.5 using either NH_3OH or tartaric acid solution as required. This solution is then placed in a 250 ml. separation funnel and 10 ml. 1% APDC solution added followed by 10 ml. oxine/MIBK solution; mechanical shaking for five minutes is required.

After separation, the extract is centrifuged at 1500 rpm. for five minutes in order to remove all remnants of water from the organic phase. The sample is then ready for aspiration.

Determination of each metal involved calibration of the spectrophotometer using standard solutions and calculating the sample concentration directly from the readout.

Analytical grade reagents and deionized water were used in all stages of the analysis.

APPENDIX 2

Phosphate Analysis (Sutherland et al, 1966)

The following solutions were required:

(1) 15 g. ammonium molybdate in 500 ml. solution

(2) 0.34 g. potassium antimony tartrate in 500 ml. solution

(3) 5N H_2SO_4

(4) ethanol

"Mixed reagent" is prepared as follows: 20 ml. ammonium molybdate solution and 10 ml. potassium antimony tartrate solution are added to 50 ml. 5N H_2SO_4 along with 1.08 g. ascorbic acid. After dissolution, the mixture is made up to 100 ml. using deionized water.

One hundred millilitres of sample is treated with 10 ml. mixed reagent and the blue phosphomolybdate complex extracted into 20 ml. isobutanol; 0.8 ml. ethanol is added to the seperated extract to homogenize any water present. A calibration procedure using standard PO_4 solutions is employed. A Bausch-Lomb Spectronic 20 spectrophotometer was used; transmittance was read at 690 millimicrons.

The samples normally required dilution by a factor of forty to bring the resultant coloured extract into the straight line portion of the calibration graph.

APPENDIX 3

Sample Preparation

Each sample is prepared by filtering through a 45 micron millipore (previously well-washed) and the algae placed in a 125 Erlenmeyer flask; the filtrate is placed in a 250 volumetric flask. Three millilitres of concentrated nitric acid and one millilitre concentrated perchloric acid are added to the algae and the flasks set aside for a few hours. The solution is then heated on a hot-plate to complete the oxidation of the organic material and one millilitre each of concentrated nitric and perchloric acids added (at the end of this procedure a slight residue was commonly observed).

The solution is then added to the corresponding volumetric flask and made up to 250 ml. using deionized water. The sample is now ready for treatment in the chelation/extraction procedure.

REFERENCES

Burrel, D.C., "Determination of Co and Ni in Natural Waters by Atomic Absorption", Atomic Absorption Newsletter, No. 7, July-August,(1965).

David, D.J., Modern Methods of Plant Analysis, Vol.5, Paech and Tracy (eds), Springer Verlog, Berlin, (1962).

Kramer, J.R., Allen, H.E., Bauline, G.W., Burns, N.M., Lake Eire Time Study (LETS), Canada Centre for Inland Waters Publication, Burlington, Ontario, (1970).

Lamb, C.A., Bentley, O.G., Beattie, J.M. (eds), Trace Elements, Academic Press Inc., New York, (1958).

Lewin, R.A. (ed), Physiology and Biochemistry of Algae, Academic Press Inc., New York, (1962).

Mansell, R.E., Emmel, H.W., "Trace Metal Extraction from Brines with APDC and Oxine", Atomic Absorption Newsletter, Vol. 4, November-December, (1965).

Morrison, G.H., Freiser, H., Solvent Extraction in Analytical Chemistry, John Wiley & Sons, Inc., New York, (1966).

Mulford, C.E., "Solvent Extraction for Atomic Absorption", Atomic Absorption Newsletter, Vol. 5, July-August, (1966).

O'Kelley, J.C., "Mineral Nutrition of Algae", Annual Review of Plant Physiology, 19, (1968).

Pulido, Fuwa, Vallee, "Determination of Cd in Biological Materials by Atomic Absorption", Anal. Biochem., 14, (1966).

Ramirez-Munoz, J., Atomic Absorption Spectroscopy, Elsevier Publishing Company, New York, (1968).

Sachdev, S.L., West, P.W., "Concentration of Trace Metals by Solvent Extraction and their Determination by Absorption Spectrophotometry", Environmental Science and Technology, Vol. 4, September, (1970).

Schroeder, H.A., Venton, W.H., Balassa, J.J., "Effect of Cr, Cd, and Other Trace Metals on the Growth and Survival of Mice", Journal of Nutrition, 80(1), (1963).

Slavin, W., Atomic Absorption Spectroscopy, Interscience Publishers, New York, (1968).

Slavin, W., Spraque, Rieder, Cordova, "Determination of Certain Toxicological Trace Metals by Atomic Absorption", Atonic Absorption Newsletter, Vol. 17, January, (1964).

Srum, W., Morgan, J.J., Aquatic Chemistry, Wiley-Interscience, Toronto, (1970).

Sutherland, J.C., Kramer, J.R., Nichols, L., Kurtz, T.D., "Mineral-Water Equilibria, Great Lakes: Silica and Phosphorous", Pub. No. 15, Great Lakes Research Division, The University of Michigan, (1966).