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PRELIMINARY REPORT ON THE INFLUENCE OF IRON, ORGANIC MATTER AND OTHER FACTORS UPON PRIMARY PRODUCTION OF A MARL LAKE*

By

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A seasonal study of nutrients limiting primary production in Blind Lake, a marl lake in Washtenaw County, has been in progress for the past 2 years. It is typical of the many hard-water lakes in southern Michigan which are characterized by a highly inorganic sediment composed mainly of calcium carbonate and a low production of organic matter. The broad objective is to learn how lakes of this type use and conserve their nutrient resources, so that methods of artificial enrichment can be worked out. Results presented in this paper are a portion of this study.

Blind Lake has a surface area of 68 acres, a maximum depth of 80 feet and a mean depth of 27.3 feet. Surface waters have a methyl-orange alkalinity of 150 ppm and a pH of 8.5. Dissolved oxygen is present typically throughout the lake in all seasons of the year. During the 1959 spring overturn, the lake became stratified before the bottom water was saturated with oxygen; consequently, less than one ppm was present below 50 feet in September, 1959.

Since ferric or oxidized iron is practically insoluble, very little iron is found in solution when oxygen is abundant in a lake. Thus, it might be

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possible for iron to be a factor limiting primary production when oxygen is present in all strata throughout the year. However, iron may be present in oxidized waters in colloidal suspension or as an organic complex.

Iron and other metals are complexed by chelating agents. A chelating agent may be defined as an organic molecule which reacts reversibly with a polyvalent metal ion to form a soluble stable ring complex. In other words, it maintains metal ions in solution. Chelating agents and their relation to primary production will be discussed further.

To determine the possible effects of chelating agents on the iron cycle in a lake, the mud-water experiments shown in Table 1 were performed. In these experiments, as well as others presented in this paper, chelate refers to the trisodium salt of N-hydroxyethylethylenediaminetriacetic acid (HEDTA) or its iron salt if the term chelated iron is mentioned. The results represent the chemical characteristics of distilled water which had been in contact with hypolimnetic mud from Blind Lake for 22 days. These parameters would not have been changed materially if Blind Lake water had been used instead of distilled water. The effect of the chelating agent was to increase the iron content of the aerobic

Table 1.--Some chemical characteristics of distilled water modified by 22 days of contact (in the laboratory) with hypolimnetic mud from Blind Lake

Experiment	Dissolved oxygen (ppm)	pH	Iron (ppm)
Aerobic control	5.4	8.63	0.02
Aerobic with chelate	5.2	8.32	0.45
Anaerobic control	0.15	7.30	0.55
Anaerobic with chelate	0.15	7.32	0.93

bottle approximately 20 times over the control and nearly double the iron content of the anaerobic bottle with chelate as compared to the anaerobic control. Thus it can be concluded that chelating agents could play an important part in the iron cycle of a lake by maintaining in solution iron which would normally be precipitated on the bottom as insoluble ferric salts.

Various workers have given ecological significance to chelation in determining the fertility of waters, although fertility or the nutritional value of water for plants has sometimes been referred to only in terms of its mineral content--mainly on the basis of nitrogen and phosphorus. The latter viewpoint neglects the possible implications of dissolved organic substances. Data will be presented later to show the importance of a synthetic chelating agent to primary production in marl lakes.

Early workers in plant nutrition were able to obtain maximum growth by using known quantities of potassium, nitrogen, phosphorus, magnesium, calcium and sulfur. These quantities were quite low initially, but as chemicals of greater purity became available the needed amounts increased. It soon became apparent that the need for greater quantities of purified chemicals was caused by a decrease in trace element contaminations.

Today it is known that some plants cannot be grown in purely inorganic or mineral media. This was indicated by Pringsheim (1924) and others working with algal cultures who found that either soil extracts or defined organic substances were required in culture media to grow some species of algae. Harvey (1939) reported that sea water collected in late summer, enriched only with inorganic nutrients, would not support diatom growth; however, water collected at other times of the year would support growth when enriched only with inorganic nutrients. In order to grow diatoms in water collected during the late summer, organic substances had to be added to the water. The function of the organic matter, in part if not wholly, is chelation.

In the experimental procedure at Blind Lake, a known volume of lake water from a given depth was placed in a 5-gallon carboy. Control sample bottles of 250 cc volume were then filled from the carboy. Measured quantities of the various nutrients were added to the water remaining in the carboy to provide the necessary enrichments for experimental bottles. The nutrient medium used was Chu No. 10 (Chu, 1942), modified by the omission of ferric chloride, soil extract and minor elements. Chelating agents were added to the experimental bottles after they had been filled with the nutrient-enriched lake water.

The experimental bottles were placed horizontally in racks and suspended from a buoy at a depth of 1.5 meters for periods of five to nine days. Radioactive carbon was added to the bottles four to six hours before the termination of the experiments. After this time, the bottles were taken from the lake and aliquots were filtered for radioactivity determinations. The activity of the filtered material gave a measure of the photosynthetic fixation of carbon by the algal populations in the bottles.

The first experiments used in studying the effects of nutrient enrichment were designed so that responses of populations with the passage of time could be followed. One of these experiments (Fig. 1) shows the responses of treatments with nutrient, and nutrient plus chelated iron, in relation to a control. Nutrient in this experiment, as in all others from Blind Lake, refers to Chu No. 10 nutrients which are calcium nitrate, dibasic potassium phosphate, magnesium sulfate, sodium carbonate and sodium silicate. Eight bottles for each experimental series were filled at the beginning of the experiment, and on each day of the experiment carbon-14 was added to two bottles from each series to yield duplicate determinations of the carbon-14 uptake rate for that day. It can be seen that there is initially a lag phase in the response to the treatment of nutrient plus chelate and that this treatment increased the rate of carbon-14 uptake over the control about 8 times at the termination of the experiment.

Figure 1.--Carbon-14 uptake (cpm/hr) in nutrient experiments, August 4-8, 1958, Blind Lake, Washtenaw County, Michigan.

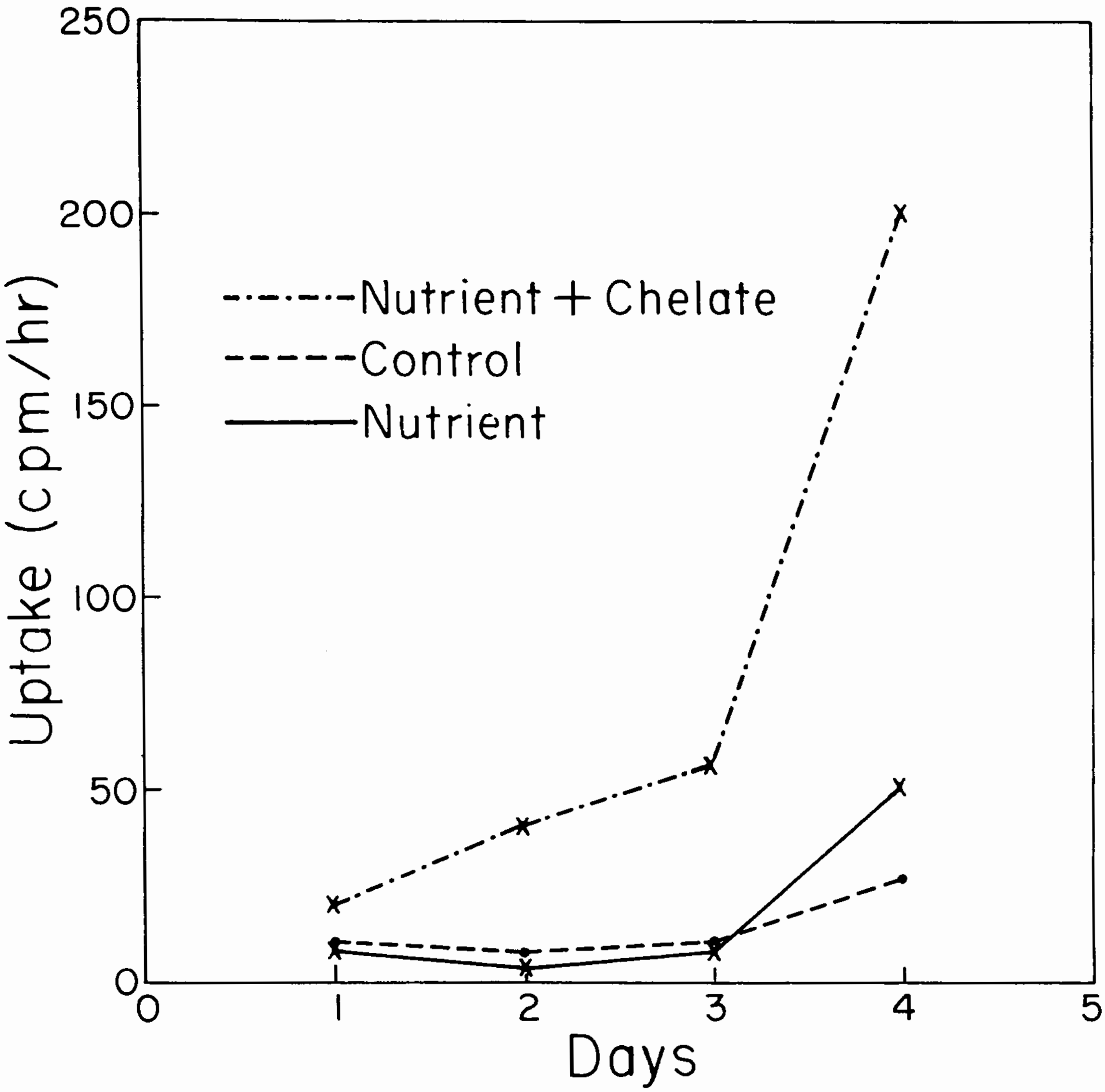

Nutrient + Chelate
Control
Nutrient
Uptake (cpm/hr)
250
200
150
100
50
0
0
1
2
3
4
5
Days

Figure 1

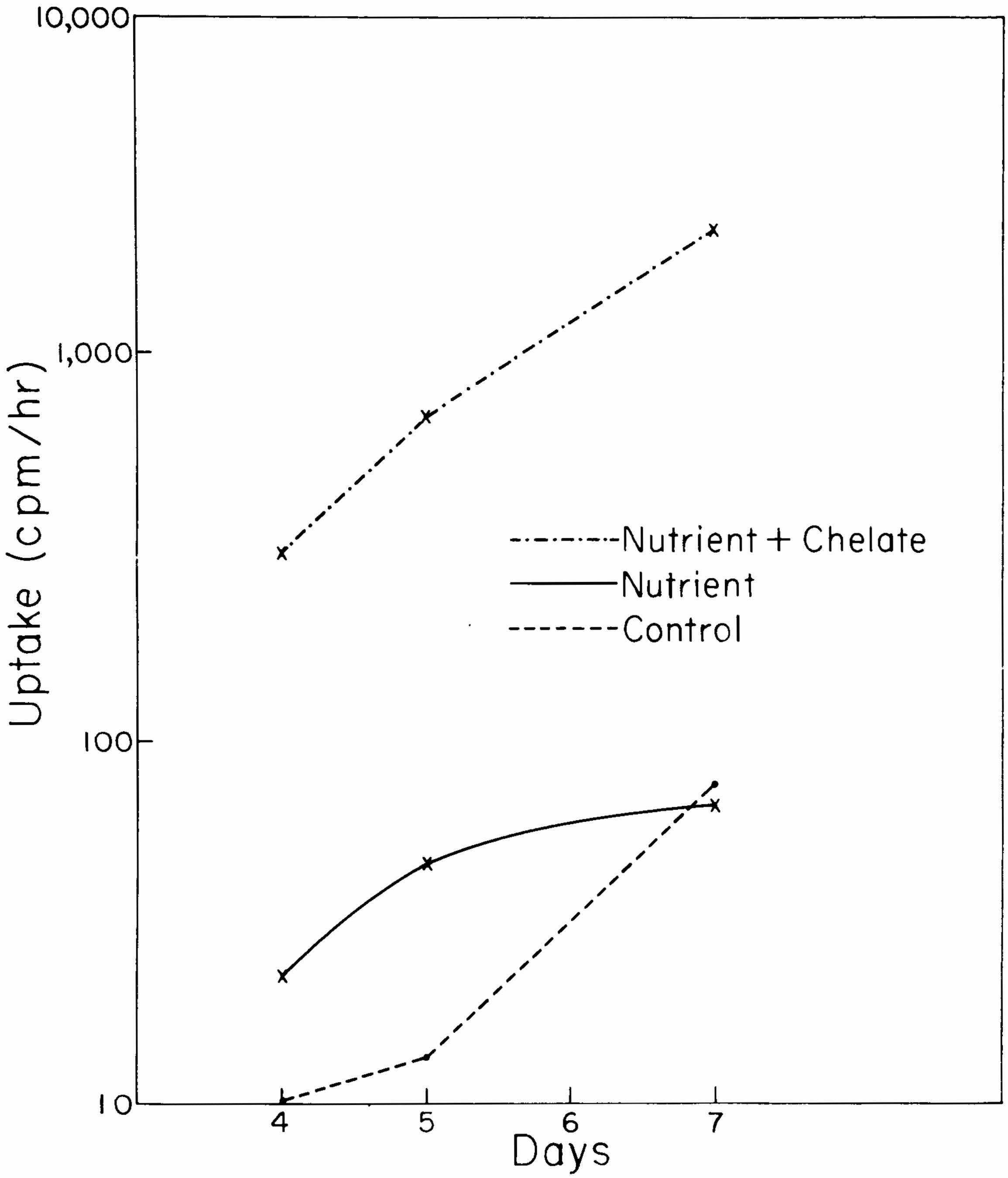
Figure 2 shows a semi-log plot of the same type of experiment, except that C-14 uptake was not determined until the fourth day. In this experiment, the ratio of the nutrient plus chelated iron/control carbon-14 uptakes is 33 on the 7th day. It can also be seen that the treated population, on the basis of carbon-14 uptake rates, is in the log phase of growth. The importance of chelated iron in these experiments cannot be overlooked since it, in combination with Chu 10 nutrients, has a powerful effect on the photosynthetic rate.

In Figure 3 the ratios of carbon-14 uptakes of the experimental treatment/control have been graphed for various experiments conducted in 1957. The experimental conditions were: nutrient plus chelated iron; nitrogen and phosphorus plus chelated iron; chelated iron only; nitrogen and phosphorus only; and nutrient only. With the exception of the experiment involving nitrogen and phosphorus plus chelated iron (September 2) and that with chelated iron only (July 5), all points are readily separable into two groups--one group of treatments containing chelated iron and the other group without chelated iron.

Two possible explanations for the increasing effectiveness of nitrogen and phosphorus plus chelated iron are (1) that some limiting nutrient was being circulated into the epilimnion preceding the fall overturn or (2) that different algal populations developed, and their nutrient requirements were favored by this enrichment. These conditions might also explain the greater effectiveness of the chelated iron only on October 21 and November 7.

In all experiments discussed thus far, the concentration of chelated iron was 2.0 ppm. Since this greatly exceeds the amount of iron found in most lakes, experiments were performed to determine the amount of chelated iron necessary to give the effects already noted. This amount seems to vary seasonally, but in no instance was a concentration of more than 0.2 ppm needed to give a significant increase in the rate of carbon fixation (Table 2).

Figure 2.--Carbon-14 uptake (cpm/hr) in nutrient experiments, August 18-25, 1958, Blind Lake, Washtenaw County, Michigan.



10,000
1,000
100
10
Uptake (cpm/hr)
4
5
6
7
Days
Nutrient + Chelate
Nutrient
Control

Figure 2

Figure 3.--Ratio of $\frac{\text{experimental}}{\text{control}}$ carbon-14 uptake in nutrient experiments, July-October, 1958, Blind Lake, Washtenaw County, Michigan.

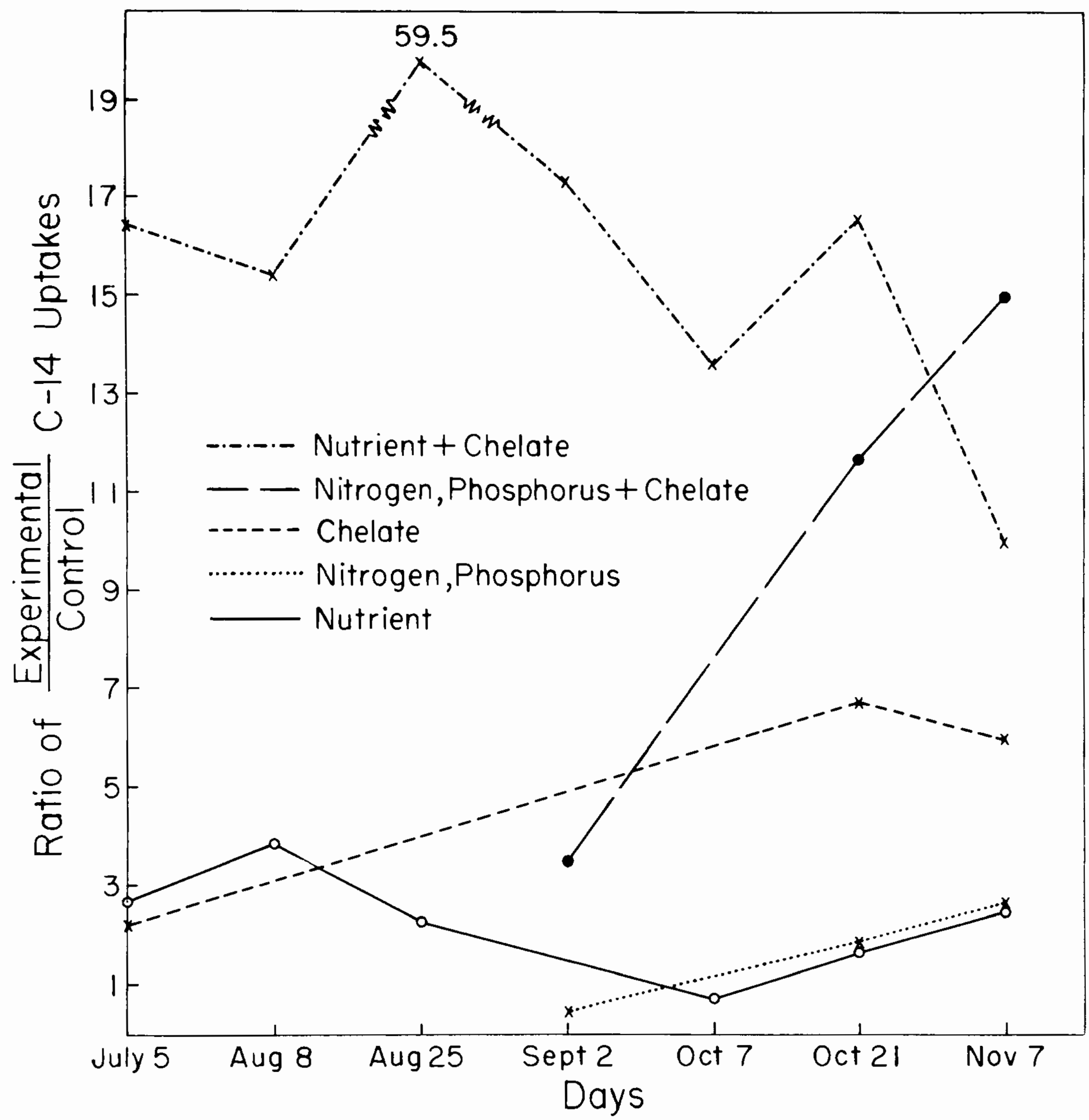

59.5
19
17
15
13
11
9
7
5
3
1
Ratio of $\frac{\text{Experimental}}{\text{Control}}$ C-14 Uptakes
Nutrient + Chelate
Nitrogen, Phosphorus + Chelate
Chelate
Nitrogen, Phosphorus
Nutrient
July 5
Aug 8
Aug 25
Sept 2
Oct 7
Oct 21
Nov 7
Days

Figure 3

Table 2.--Ratio of $\frac{\text{experimental}}{\text{control}}$ carbon-14 uptakes with various concentrations of chelated iron, Blind Lake, Washtenaw County, Michigan

Chelated iron (ppm)	Date			
	April 14, 1959	May 8, 1959	June 2, 1959	Sept. 8, 1958
5.0	.....	...	......	13.6
2.0	24.4	7.4	128	12.3
1.0	22.0	...	......	9.1
0.2	.....	6.0	40.5	.....
0.1	17.5	...	......	12.0
0.02	.....	2.5	3.39	.....
0.01	19.5	...	......	0.68
0.002	.....	2.0	......	.....
0.0	0.59	1.1	1.00	0.67

The natural phytoplankton populations, instead of pure or unialgal cultures, were used in the experiments discussed because the problem was concerned mainly with the factors limiting primary production and not with algal physiology. Natural populations are better suited for such investigations, since the physiological state of algae is determined by many factors, some of which are unknown. In a natural system the algal population has a physiological state which has been determined by the dynamics of the system itself and can not be duplicated experimentally. Therefore, only the algal populations of the lake have a physiological state that is dependent upon temporal occurrences limiting productivity within the lake system.

In the use of pure or unialgal cultures, two problems related to physiological state are: (1) optimum requirements and (2) biological dilution.

Theoretically, at least, each species has optimum conditions under which it grows best. If all these conditions were known exactly, it is possible that the use of cultures would prove valuable. At present, however, our knowledge of algal nutrition, algal physiology and algal succession (ecology) is not sufficient to use this approach. Even if this information were available, experiments with more than one species would be necessary in a seasonal study, since great changes occur in the natural system and in optimum conditions throughout the year.

Since certain trace elements are concentrated in algal cells in quantities many times in excess of amounts the cells utilize, the excess nutrients must be "diluted" to limiting quantities by cell division (biological dilution). In the lake system, because of biological dilution, nutrient concentrations in algal cells are reduced over a period of time to limiting quantities if the rate of utilization and loss exceeds the rate of absorption (uptake from water). In this case a limiting nutrient is a reflection of not only present, but also preceding environmental conditions within the lake. Consequently, the study of a limiting nutrient with cultures must be carried through many generations involving several successive transfers to media deficient in the nutrient being studied to determine whether the nutrient is limiting. Rodhe (1948) found that algae, which had previously been cultured in a medium containing iron, could be grown in an iron-deficient medium for six months. The advantages and importance of using natural populations in the study of iron as a limiting factor cannot be overemphasized.

The effects of bacterial activity and surface area to volume relationships have been questioned in long-term bottle experiments. Data presented in this paper might also be questioned on that basis. However, Figure 4 shows the

Figure 4.--Carbon-14 uptake profiles (cpm/6 hrs), July 21-August 15, 1959, in Titus Lake, Grand Traverse County, Michigan.

Iron chelate added on July 27 and nitrogen, phosphorus and potassium added on August 6.

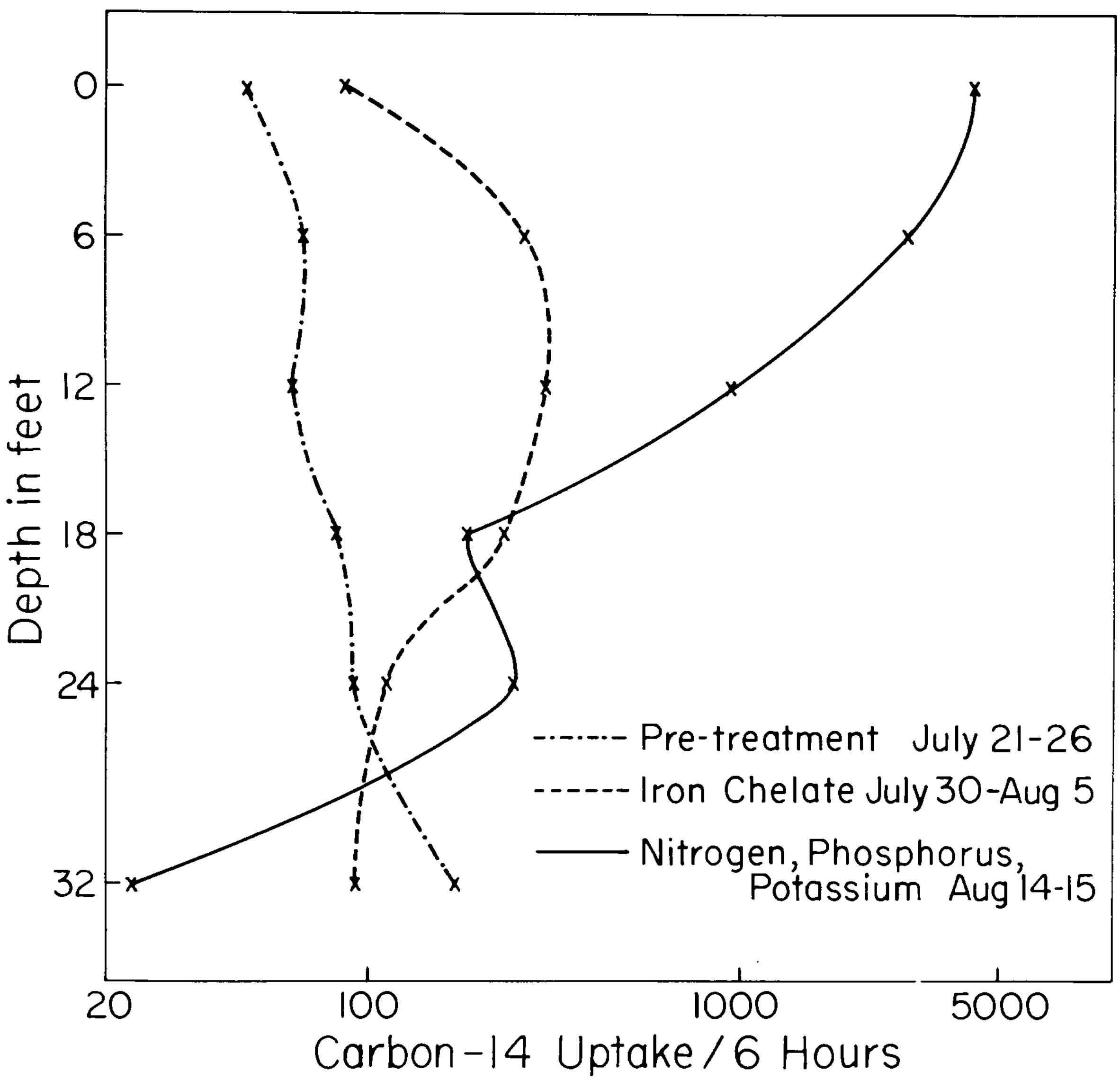

0
6
12
18
24
32
Depth in feet
20
100
1000
5000
Carbon-14 Uptake/6 Hours
Pre-treatment July 21-26
Iron Chelate July 30-Aug 5
Nitrogen, Phosphorus, Potassium Aug 14-15

Figure 4

results of an experiment in which nutrient enrichments were made in a 14-acre marl lake, Titus Lake, Grand Traverse County. The data presented are the average photosynthetic profiles determined by carbon-14 uptake in the lake at six depths. The pretreatment curve corresponds to the control series of bottle experiments and is the average of profiles on four dates from July 21-26. On July 27, iron chelate was added to the lake and the profile shown for iron chelate is the average of determinations made on four dates from July 30 to August 5. This enrichment with only chelated iron increased the area under the photosynthetic profile approximately four times in comparison with the pretreatment or control profile. This ratio is in close agreement with the ratios of 2, 5, and 6 found in Blind Lake bottle experiments (Fig. 3). On August 6, commercial fertilizer (nitrogen, phosphorus and potassium) was added to the lake. The average of two photosynthetic profiles on August 14 and 15 is shown. The area under this curve is approximately 60 times greater than the area under the pretreatment curve. This is in agreement with the maximum ratio of 59.5 from nutrient enrichments in the Blind Lake experimental bottles (Fig. 3). In Blind Lake experiments during 1959 this ratio has exceeded 100.

In addition to the great effect the chelated iron has on primary productivity, two conclusions may be made from this study concerning chelating agents in general: (1) that the use of synthetic organic compounds offers possibilities for evaluating one of the roles of dissolved organic material in natural systems and (2) that naturally occurring chelating agents have ecological significance in determining the fertility or productivity of waters.

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Literature cited

Chu, S. P.

1942. The influence of the mineral composition of the medium on the growth of planktonic algae. Part I. Methods and culture media. Jour. Ecol. 30: 284-325.

Harvey, H. W.

1939. Substances controlling the growth of a diatom. Jour. Mar. Biol. Assoc. U. K. 23: 499-520.

Pringsheim, E. G.

1924. Algenkultur. Aberhalden's Hand. biol. Arbeitsmethoden. Abt. XI, Teil 2: 377-406.

Rodhe, W.

1948. Environmental requirements of plankton algae. Symb. Bot. Ups. 10: 1-149.

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