Carbon Sources in Algal Populations
and Algal Community Structure

Submitted to

The Oklahoma Water Resources Research Institute
Oklahoma State University
Stillwater, Oklahoma

by

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INTRODUCTION

Project B-005-OKLA, "Carbon Sources in Algal Populations and Algal Community Structure," was supported during the period of time July 1, 1966, to June 30, 1967, as a matching grant agreement. Matching money was provided by the Oklahoma Refiners Waste Control Council and the Oklahoma State University. Project B-005-OKLA, though supported for only one year by the Office of Water Resources Research, was the first year of a three year study. Year 1 was supported as indicated above. Year 2 was supported by the Oklahoma Refiners Waste Control Council and the Oklahoma State University and Year 3 was supported as Project B-008-OKLA, "Carbon Sources and Algae Community Structure and Metabolism in a Reservoir Undergoing Eutrophication by Domestic and Industrial Effluents," again by the Office of Water Resources Research, the Oklahoma Refiner's Waste Control Council and the Oklahoma State University. This abbreviated report is the technical completion report for the period July 1, 1966, through June 30, 1967. All of the material included was further developed during Year 2 and Year 3. Therefore, for full understanding of the three-year integrated study, one should refer to the technical completion report of Project B-008-OKLA.

Objectives

Objectives of this study were to determine:

(1) the source and distribution of organic compounds in the reservoir; and

(2) development of phytoplankton populations in response to eutrophication processes resulting from the decomposition
of organic compounds in effluents entering the Cimarron arm of the reservoir.

Organic Compounds from Industrial and Municipal Sewage Effluents

Organic compounds from industrial and municipal sewage effluents were sampled by adsorption on activated carbon filters at six stations (Fig. 1) as follows:

A. Bixby, Oklahoma, on the Arkansas River below Tulsa. Samples were taken twice a month to monitor water quality of effluents from the Tulsa area.

B. Dam site. A few samples were made to provide a base line for comparison with the Bixby station. However, sampling was not successful because of lack of suitable locations for the equipment.

C. Lower end of Cimarron arm. Samples were taken about every two weeks from the upper layer and from the lower layer alternately.

D. Cimarron River. Samples were taken about every two weeks from the top and bottom alternately.

E. Lower end of Arkansas arm.

F. Arkansas River. Samples were taken from stations E and F as in the Cimarron arm.

A year of field sampling was completed in December, 1966. The sampling program was designed to develop information on the following.

a. Seasonal changes in the quantity and nature of organic compounds.

b. Effect of stratification on movement and changes of compounds.

c. Pollution of the Arkansas River by effluents from Tulsa.
Extraction of adsorbed compounds from the carbon filters by successive reflux distillation with chloroform and alcohol has been accomplished on several samples as shown in Table 1.

The data are too incomplete to form any generalizations at this point, but we note that in a few instances chloroform extract was larger at the lower end of the reservoir (Cimarron arm) than at the river inlet. We know that a few leaking oil wells are submerged in the reservoir and it is possible that they contribute hydrocarbons to our samples.

We expect generally to find that alcohol extracts will decrease through the reservoir. Preliminary Biochemical Oxygen Demand tests substantiate this hypothesis with BOD declining from about 8 mg/l in the river to about 2 mg/l at the lower end of the reservoir. During fiscal year 1968 we will complete extraction and analysis of the carbon adsorption filter samples.

After extraction by reflux distillation, the samples will be fractionated by gas chromatography, and the fractions thus obtained will be further analyzed by infra-red spectroscopy and mass spectrometry. It is expected that the chloroform-extracted fractions, which generally contain compounds originating from oil refinery effluents, will be most readily analyzed by these procedures. We hope to be able to identify particular compounds, trace their progress through the reservoir and determine the extent to which they are degraded by passage through the reservoir.
Carbon 14 Analysis of Organic Compounds

The chloroform and alcohol extractions separate organic compounds originating from oil refinery effluents and domestic sewage effluents respectively. Industrial wastes generally are concentrated in the chloroform extract, whereas the more polar compounds of domestic wastes are more abundant in the ethanol extract. The weight ratio of the two extracts is in itself an indication of the type of pollution source.

The described premise is a simplification. Domestic wastes may contain a portion of fossil compounds, such as detergents and antifreeze. To a limited and unknown degree, natural biological cycles of oxidation and photosynthesis may rearrange a portion of both the contemporary and fossil carbon (organic and inorganic) in the stream into new organic compounds resembling those of domestic waste.

Nevertheless, experimental data appear to justify disregarding these factors in a first approximation, for the results of several isotope assays agreed with the actual known pollution sources.

Meyer and Rubin (1964) found that by recovering organic substances from a stream and determining carbon-14 activity, it is possible to estimate more closely the relative contributions of both domestic sewage and oil refinery effluents. Domestic wastes consist primarily of animal and vegetable matter in garbage and sewage, which are contemporary in their content of the radioactive isotope, carbon-14. Conversely, oil refinery effluents contain nonradioactive fossil carbon materials.

Low-level carbon-14 assays can be done by several methods, including gas counting, solid counting, and liquid scintillation. Meyer and Rubin used a gas counter by which organic extracts were converted to acetylene
and counts were made on one liter samples. Shielding of the sample from background radiation was a serious problem, being accomplished by a ring of cosmic ray counters in anticoincidence with the sample counter.

As suitable gas counter was not available for our use, investigations were made to determine the feasibility of using solid or liquid scintillator counters available on the University campus.

Sample preparation for solid counting is simple and fast. However, even with a large sample area, the thickness of samples of the required volume resulted in quenching of the radiation. After considerable study, we were forced to conclude that with solid samples it was impossible to obtain a count with natural carbon-14 sufficiently large enough to give the desired precision.

Liquid scintillation counting can be used to count samples of carbon-14 at the natural abundance provided that the background radiation is sufficiently reduced. This may be accomplished by the use of noise-free photomultiplier tubes, good shielding, and small volumes of sample solution. This method was particularly appealing to us because liquid scintillation spectrometers are available on this campus.

We found that the dark color of the extracted samples adversely affected the liquid scintillator counter, so that the samples could not be directly dissolved in a carrier, such as xylene.

The sample can be converted to a chemical form which has a high counting efficiency in the liquid scintillator. Other workers have converted the carbonaceous material to benzene and achieved high efficiency of counting. However, our approach to this technique uncovered several problems. The benzene conversion is accomplished by a series of processes, which by ordinary laboratory procedures
available to us resulted in so much loss from an already small sample that useable samples could not be prepared.

We attempted a simpler solution by converting the sample to carbon dioxide which was dissolved in xylene and other carriers. Conversion of the sample to carbon dioxide is an efficient process yielding a high return. However, we failed in this because we could not get enough carbon dioxide dissolved in the carrier to produce meaningful counts.

In another approach, we attempted to count carbon dioxide samples with a vibrating reed counter with ion chamber. However, we could not use a volume of carbon dioxide large enough to provide counts significantly different between sample and control.

We have been forced to abandon our attempts to apply the carbon-14 analysis to our carbon filter samples.

**Phytoplankton**

Phytoplankton studies on the Cimarron arm of the reservoir have been designed to study the effects of eutrophication of the water by domestic and industrial effluents. Evaluation of algal populations is difficult and tedious by the usual procedures, which require careful counting of the microscopic algae. Table 2 shows the results of seven weeks sampling.

Field studies are still being made on algal populations and will continue until summer, 1967. We have been measuring the full spectrum of photosynthetic and other pigments as each sample was taken. The spectra require complex analysis and this work lags behind the field and laboratory work.

Pigment composition of the algal populations will be correlated with phylogenetic composition. This work will require identification
and counting of the species of algae composing each population. Preliminary efforts have been made in the species identification. This work, along with spectral analysis, will be undertaken after field work is terminated. We will finish this study during fiscal year 1968.

We feel that good progress has been made on this study. The carbon filter studies and the algal pigment studies each required at least a full year of good samples to be meaningful. Both studies have been attended by considerable difficulty in the setting up and continuation of the field sampling program. However, the carbon filter field sampling program was completed in the first 18 months, and the algal field studies will be completed before the end of the second year. Each of the studies requires very extensive laboratory analysis. This is particularly true of the carbon filter program. Continued research through the third year is essential to successful completion of the studies.
**TABLE 1 Organic Compounds Adsorbed on Carbon Filters - Keystone Reservoir**

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<th>C</th>
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<th>R</th>
<th>C</th>
<th>A</th>
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*Deep samples - 35'*

F Arkansas River
E lower end of Arkansas R. arm of reservoir
D Cimarron River
C lower end of Cimarron R. arm
B below dam
A below Tulsa at Bixby
Table 2. Algal pigments, Keystone Reservoir, 7-week period, 25 July - 23 September, 1966.

Quantities per unit area (mg/m²). Average of seven weekly samples for the total water column at each station.

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FIG. 1  KEYSSTONE RESERVOIR.
Sampling stations
1-4 PHYSICO-CHEMICAL AND ALGAL PIGMENTS
A-E CARBON FILTER

SCALE OF MILES
1 0 1 2 3

ARKANSAS RIVER

CIMARRON RIVER

D AM SITE
A BIXBY