ALGAL NUTRIENTS: Sources and Patterns of Flow in the Occoquan Watershed

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1. INTRODUCTION

The Occoquan Watershed lies on the southern periphery of the Washington, D. C., suburbs and includes portions of Fairfax, Prince William, Loudoun, and Fauquier Counties.

In recent years there has been a rapid expansion of population in this area. Concomitant with this population increase has been a large increase in construction, paving, sewage treatment, and drinking water requirements. At the present time some 600,000 people in the four localities depend on the Occoquan and its tributary streams as a means of removing sewage effluent and/or as a source of drinking water.

Arising with the rapid increases in population of this area has been an obvious eutrophication of the Occoquan reservoir. The drinking water obtained from the Occoquan, particularly in the summer months, is occasionally found by the general public to have a repulsive odor and a disagreeable taste. Even more typical is the fact that the Fairfax County Water Authority spends very large sums of money each year on algicide for direct application to the reservoir to attempt to inhibit the development of nuisance algae. In addition to taste and odor problems, serious questions come to mind concerning the public health aspects of drawing water from a reservoir that is downstream from sewage treatment plants.

In response to these public health questions and the concern for preservation of a major Northern Virginia water supply, the State Water Control Board of Virginia adopted a Policy of Waste Treatment and Water Quality Management in the Occoquan Watershed. The present research is part of the Water Quality Management program and seeks to document the particular causes of eutrophication encountered in the Occoquan Watershed.

Nature of the Problem

The Occoquan Reservoir is a long, narrow, relatively shallow impoundment that drains a total area of approximately 570 square miles. Within this drainage area are 11 sewage treatment plants that discharge directly into tributary streams of the reservoir. The treatment plant effluent contributes in excess of 5 million gallons per day to the drainage system. In addition, there are numerous non-point sources of potential pollutant such as urban drainage and agricultural drainage, and excessive levels of siltation are encountered due to the recent increase in construction. The apparent result of all these contributions is a reservoir that shows many of the characteristics of cultural eutrophication.

As may be expected, the reservoir has high productivity, with high concentrations of algae in the epilimnion and extreme depletion of dissolved oxygen in the hypolimnion during periods of thermal stratification. At varying times during the summer season, and particularly during fall turnover, water samples show excessive values for odor. These periods of excessive odor typically correspond with the appearance, in bloom proportions, of such bluegreen algae as

Anabaena circinalis, Microcystis aeruginosa, or Aphanizomenon flos-aquae.

The entire watershed has a relatively short detention time such that storms are a major influence on water quality. This has two main effects: (1) a scouring effect which tends to purge the reservoir of organic and nutrient accumulations during the winter and spring, and (2) a mixing effect which tends to upset thermal stratification during the summer releasing algal nutrients from the hypolimnion and making them available to rapidly growing algae at the surface.

The present study is concerned with locating the sources and patterns of flow of algal nutrient through the watershed, and in demonstrating the relative effectiveness of several chemical parameters in predicting growth responses of the most troublesome algal types.

Description of the Watershed

The Occoquan Watershed, shown in Figure 1, drains a total area of 570 square miles. The Occoquan Creek subwatershed drains a total area of approximately 343 square miles, while Bull Run drains approximately 185 square miles. Together they contribute more than 90% of the total discharge to the reservoir.

These two watersheds are very different in character. The Occoquan subwatershed is fed primarily by Cedar Run and Broad Run, both of which drain areas of primarily agricultural character.

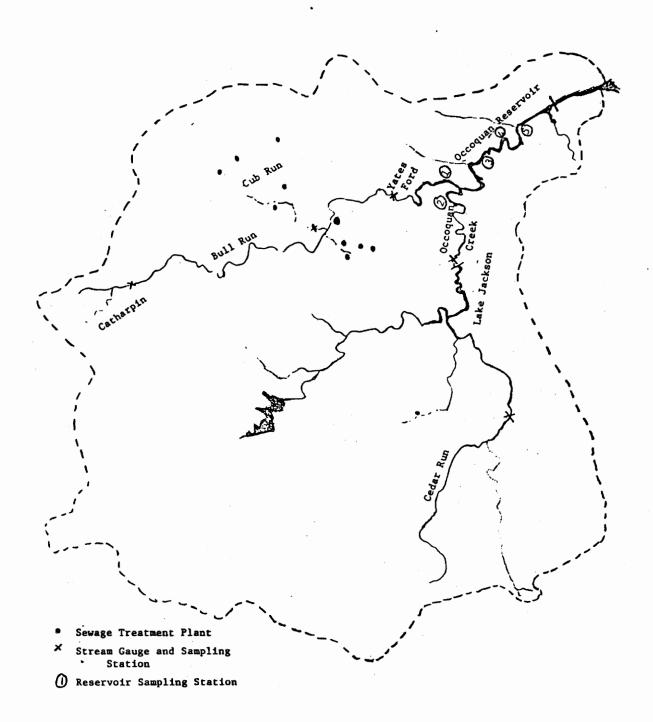


FIGURE 1: MAP OF THE OCCOQUAN WATERSHED

Between the confluence of Cedar Run and Broad Run and the head of the Occoquan Reservoir, Occoquan Creek is impounded by the Lake Jackson Dam. Lake Jackson is also a long, narrow, shallow lake, but unlike the reservoir, it is surrounded by cabins and various recreational facilities. This lake is highly eutrophic, with rapidly increasing sediment and encroaching aquatic plants, as well as early blooms of bluegreen algae.

The Bull Run subwatershed is quite different from that of Occoquan Creek. It drains from a somewhat remote, partially forested area near the community of Catharpin, Virginia, through the urban areas of Manassas, Manassas Park, and Yorkshire, Virginia. Within this same urbanized area, most of the 11 area sewage treatment facilities are located. Five of these treatment plants discharge to Cub Run, a small stream that enters the Bull Run flow immediately above the urban area. The largest area treatment plant, Greater Manassas Sanitary District (GMSD), discharges to Bull Run just below the confluence with Cub Run. Although these treatment facilities keep BOD levels down to the levels expected of secondary treatment, they had no provisions for phosphorus and nitrogen removal during the period of study. All treatment plant discharge points are more than 20 miles above the Fairfax County Water Authority intake point on the reservoir.

<u>Limnological Aspects of the Occoquan Reservoir</u>

The reservoir has total length more than 10 miles, typical width less than 1000 feet, and mean depth approximately 17 feet. The

maximum depth is 65 feet. In a previous survey of water quality (1) of the Occoquan, the Occoquan Reservoir was considered to behave hydraulically as a plug-flow system. On this basis the reservoir detention time has been estimated as varying from 10 days to 180 days. During spring particularly, the short detention time estimates are probably fairly reliable. However, the longer detention time estimates arise during dry periods when the reservoir level is below the crest of the high dam, and stably stratified. During these dry periods the only flow out of the reservoir occurs through the Water Authority pumps. Thus for most of the summer months and into the fall the assumption of complete vertical mixing is entirely untenable. However, plug flow within strata remains a strong possibility. Problems arise, however, in determining at which level in the stratification system plug flow may occur. There are two typical constraints that may restrict plug flow of strata. One is the presence of a submerged dam near the center point of the reservoir, and the other is an aeration system just upstream from the Water Authority raw water intake at the high dam. The submerged dam restricts flow in the hypolimnion and the aeration system causes some destratification near the high dam so that laminar flow is undetectable.

Although the reservoir is very shallow for much of its area, very little encroachment of aquatic plants is observed. This is probably the result of the wide variation in water level during the growing season.

The Occoquan Reservoir is highly considered by sportsmen as a

good fishing area. This probably reflects the highly productive nature of the lake. However, the extreme oxygen depletion that occurs annually in the hypolimnion could endanger this reputation.

Oxygen depletion occurs very rapidly and by the beginning of June most deep areas are devoid of dissolved oxygen. When thermal stratification is complete, all locations sampled below the confluence of Bull Run and Occoquan Creek typically have less than 1 ppm dissolved oxygen at depth of 10 feet until stratification is broken. This depletion of dissolved oxygen may be contrasted with the frequently observed supersaturation of dissolved oxygen that often occurs between the surface and 5-foot depth at the same locations.

As may be expected, high concentrations of bluegreen algae are a typical component of the algal flora of the Occoquan reservoir.

These become particularly noticeable during the summer and early fall at times of stable stratification. At such times the formation of floating mats of algal material are commonly seen in the upper reaches. Associated with the appearance of the bluegreen algae is a commonly observed disagreeable odor. If allowed to reach maximum proportions, the extensive formation of these algal mats will aggravate the condition of oxygen depletion and ultimately result in fish kills.

Study Objectives

Since most of the water quality problems encountered in the Occoquan Reservoir relate to the excessive production of bluegreen algae, it was considered important to investigate the relation between nutrient sources within the watershed and the occurrence of algal

bloom conditions. The specific objectives of the study are threefold:

(1) to quantify the algal nutrient contribution of land use areas
within the watershed, (2) to characterize the specific chemical
parameters which constitute these nutrient contributions, and predict their potential effectiveness in stimulating the development of
nuisance algae, and (3) to describe the dynamics of nutrient flux
from sources within watershed to the problem areas of the reservoir.

II. REVIEW OF LITERATURE

The term eutrophication has been used many different ways by different authors. Generally, eutrophication refers to the changes a body of water undergoes as it accumulates materials from its watershed. It has been recognized as a natural process, ultimately leading to extinction of the lacustrine environment and to bog formation (2, 3). The accumulation of nutritive materials leads directly to increased plant production, rooted aquatics in the littoral zone, phytoplankton in the limnetic zone, and benthic algae on submerged surfaces in the euphotic zone. Thus the primary accumulation process stimulates a secondary accumulation through the production of organic carbon from inorganic carbon dioxide. Generally, human activity in a watershed tends to increase the movement of nutritive materials into the watershed basin. This results in fertilization and plant production beyond the rate that would obtain in the absence of human activity.

Palmer (4) points out that algae are "common and normal inhabitants" of all surface waters and may be a help or a hindrance to the process of drinking water treatment. He points out that the presence of algae tends to oxygenate water; however, excessive quantities of certain algae tend to cause problems of taste and odor or clogging of filters. In addition to the deterioration of drinking water quality, Cole (5) indicates that cultural enrichment of streams and lakes could

stimulate productivity while decreasing consumer efficiency, causing diurnal oxygen fluctuation and ultimate changes in the macroinverte-brate and fish population. He indicates two obvious effects, over-population of nuisance-type algae - those that produce mats, taste, and odor, or clog filters - and underproduction of game fish.

The appearance of bluegreen algae has been a matter of great concern in recent years as this group includes the species having the greatest nuisance potential. In particular, Anabaena, Microcystis, and Aphanizomenon are typically predominant in highly enriched lakes. Certain species of bluegreen algae, in addition to causing taste and odor problems, have apparently caused animal deaths due to their production of toxic substances (6). Although these species have been well known for many years, they were previously described by Smith (7) as algae observed primarily in hard-water lakes. An example of the apparent increase of these bluegreen species is cited by Bartsch (8): Lake Washington in 1959 contained a maximum of 1.5 x 10⁶ micron³/ml, of which 15% were bluegreens. By 1963, the phytoplankton population increased tenfold, and the bluegreens comprised as much as 95% of the population. Today, bluegreen algae are a major component of the phytoplankton of nearly every man-made impoundment.

To date, two approaches have been used in attempting to control the overproduction of nuisance algae: (1) control and limiting of nutrient input, and (2) the use of algicide. The best known and most widely used algicide at present is copper sulfate. This compound is available in large quantities, has low toxicity to fish, and high

toxicity to bluegreen algae. The problem with its use is that it forms insoluble hydroxides and settles out. Thus copper sulfate is not effective for long periods and its use can be very expensive. A current search for newer and more effective algicides, specific to bluegreens, is outlined by Prows $et\ al.$ (9).

Even greater effort has been invested into control of algal nutrient. As man-made wastes appear to be the "primal cause" of over-production of algae (10), there has been intense study into which components of waste are directly responsible. A sharp controversy has raged for years over whether nitrogen or phosphorus or some minor element is the controlling factor in algal growth (11, 12, 13, 14, 15). An interesting note in this respect was provided by Krantz and Myers (16), who showed that only 75% of maximum growth yield of a nitrogen-fixing bluegreen could be obtained in the absence of soluble nitrogen salts.

The nitrogen or phosphorus controversy has been joined more recently by those who believe carbon may be the growth limiting substance in natural lake conditions. In particular, Kuentzal (17) suggests that only free CO₂ is available to algae for growth and that its concentration may in fact be too low in oligotrophic waters to provide adequate substrate for rapid algal growth. This concept was further modified by King (18) who agrees that algae require free CO₂ and suggests that green algae require higher concentration of free CO₂ than do bluegreen algae. Consequently, he suggests that bluegreen algae may out-compete the more favored green algae. That the concentration

of CO₂ and not just the pH is the critical factor in competitive growth of green and bluegreen algae is, however, not so obvious. A recent kinetic experiment by Goldman et al (19) indicates that some freshwater algae can grow near maximal rates at low concentration of CO₂ if bicarbonate is available.

Thus one is left with a muddle in which discussions rage as to which element should be controlled to prevent growth of excessive quantities of nuisance algae.

The technology is presently available to significantly reduce the levels of phosphate and nitrate from sewage treatment effluent. The expense of adding the required tertiary treatment process to secondary treatment facilities, of which there are still too few, is very high. Many investigators have questioned whether the investment is worthwhile if non-point sources of pollution remain uncontrolled (20). These background levels from agriculture (21) and urban runoff (22) in particular appear to contribute heavily to overall levels of eutrophication.

An extensive watershed water quality survey was conducted on the Occoquan watershed by Sawyer (1) during 1969. This study characterized the Occoquan Reservoir as an eutrophic impoundment on the basis of the rapid depletion of dissolved oxygen in the hypolimnion and the frequent occurrence of bluegreen algal blooms. Through extensive stream water analysis, this study further identified the sewage treatment plants within the basin as overwhelming nitrogen and phosphorus contributors. This study concentrated intensively upon composite sampling

of small stream drainages but resorted to only monthly sampling of the reservoir. All samples were analyzed thoroughly by standard chemical methods.

An alternate approach to chemical assay for algal nutrients is the algal growth potential assay (AGP). Several different assay procedures have evolved to assess the algal nutrient concentration of a water sample by measuring algal growth under controlled environmental conditions. Perhaps the most straightforward technique is the "bottle test." As used by Wang $et\ al.$ (31), it involves simply putting an unsterilized water sample into constant light and temperature conditions and measuring the total biomass produced. Its disadvantage arises from the diverse algal populations which may be present in the original sample. Since both the nutrient and biological community structure may vary from one sample to another, the results are difficult to interpret. The method does, however, provide an indication of how much algal material may be produced from a given water sample. Another bottle test technique was developed and standardized by the National Environmental Research Council (NERC) (24). It is amenable to simpler interpretation. In this technique water samples are first sterilized by autoclaving or filtering, then inoculated from a unialgal stock culture, supplied by the NERC laboratory. Results from this test are directly comparable between areas. Drawbacks still remain because one measures nutrient utilization to an unpredictable level by species which may differ significantly from those found in nature. A far more complex technique which may eventually be amenable to

standardization, and yet is more like the field situation, is the chemostat test. This test as used by Foree and Scroggin (32), is a simulation of natural conditions using a continuous flow system.

Measurements of algal yield are compared with feeding rates at steady state. The test may give more reliable and realistic results, but it is susceptible to upset due to the complex apparatus involved. For this reason the chemostat method of algal assay is not likely to gain the wide acceptance achieved by the "bottle test."

III. MATERIALS AND METHODS

Sampling

Sampling for this study was done as part of the routine sampling program of the Occoquan Watershed Monitoring Laboratory. All samples were collected using nonmetallic containers, such as a Kemmerer nonmetallic water sampler, or polyethylene bottles. At the time of this study, spring and summer of 1973, automatic stream samplers had not yet been implemented, so grab samples were taken at weekly intervals. All samples were stored on ice from time of sampling until returned to the laboratory facility. In the laboratory they were analyzed immediately or preserved by methods consistent with those suggested in Standard Methods for Water and Wastewater Analysis (23).

Samples for algal bioassay were split off the laboratory sample and stored separately in polypropylene bottles on ice until returned to the laboratory. Upon return, these samples were immediately autoclaved at 20 psi for 20 minutes and stored until time for bioassay analysis.

The watershed monitoring program at the time of this study had five stream stations each with a stream flow gaging station.

Maintenance and rating of the stream gages were handled by the State Water Control Board and the U. S. Geological Survey. The locations of the stream sampling stations as shown in Figure 1 were as follows:

Bull Run at Catharpin, Cub Run near confluence with Bull Run, Bull

Run at Yates Ford (near where it enters the reservoir). In the Occoquan subwatershed stream stations were located at Cedar Run near Aden, Virginia, and on Occoquan Creek just below the Lake Jackson Dam.

Five locations on the reservoir were also sampled on a weekly basis. At each station surface and bottom samples were handled separately. Station 1 is located in the Bull Run arm approximately one mile above the confluence with Occoquan. Station 2 is in the Occoquan Creek arm approximately the same distance above the confluence. Station 3 is on the main body of the reservoir approximately six miles above the high dam. The actual location is approximately 100 yards upstream from Ryan's Dam, a submerged dam. Station 4 is in a 90 degree bend approximately four miles above the high dam. Station 5 is located two miles above the high dam near a cove where Sandy Run enters the reservoir. All sites were sampled mid-channel. A sixth station used for the routine was in the aeration area immediately above the high dam. However, this station was not of interest to the present study because it is not typical of the main volume of the reservoir.

Bioassay Procedure

The bioassay procedure was basically that suggested in the National Eutrophication Research Program "Algal Assay Procedure Bottle Test" (1971) (24). The only modification introduced for the present study was a reduction in sample size from 40 ml to 2 ml. This modification is similar to that suggested by Forsberg (25).

Three test species of algae were obtained from the Environmental

Protection Agency laboratory in Corvalis, Oregon: Anabaena flos-aquae, Microcystis aeruginosa, and Selenastrum capricornutum. Anabaena and Microcystis are typical bluegreen components of the nuisance blooms commonly observed on the Occoquan reservoir and were therefore of great interest in the present study. The third species, Selenastrum, was chosen because it is a well studied green alga that grows particularly well in vitro. During the course of this investigation bioassay with the Microcystis species was discontinued as it was found to be very difficult to maintain in the laboratory and gave erratic results. It would seem that some condition present in the laboratory caused undue stress to the Microcystis culture. Consequently, repeatability was minimal with this species. In addition the morphology observed in the laboratory for this species never conformed to that observed in nature. For these reasons it was decided to rely on results from studies with Anabaena and Selenastrum.

Growth Conditions

Stock cultures of test species were maintained in the synthetic medium recommended in the bottle test procedure. (See Appendix for composition of synthetic medium.) Small samples of the test species were kept in test tubes under refrigeration. Forty ml samples of test species culture were also kept under constant light conditions in the growth chamber in 125 ml Erlenmeyer flasks. Ten ml aliquots of stock algae were transferred on a weekly basis to 400 ml of synthetic media in Roux bottles. The Roux bottles were stored in the growth

chamber under constant light conditions and bubbled with compressed air obtained from an aquarium air pump. Generally within one week cells were harvested from Roux bottle by centrifugation to be used as inoculum for the algal assay. The harvesting procedure included washing with NaHCO₃, 15 mg/liter. After washing the algae were resuspended in NaHCO₃ and allowed to remain in this solution for 10-12 hours prior to use as inoculum for the bioassay.

A growth chamber was constructed from 3/4" plywood. Overall dimensions were 3' x 3' x 2'. The interior was divided into three sections with vertical partitions that sectioned the cabinet from front to back. Across the back was a single chamber where cool white fluorescent lamps were mounted vertically. Lights were positioned so that groups of one or two lamps could illuminate only one of the front chambers. Ballast transformers for the fluorescent lights were placed outside the growth chamber and a thermostated fan was placed near the bottom of the light chamber for temperature control. Clear lucite shelves were constructed to hold 6" test tubes at an eight degree angle from horizontal and located in each front chamber.

Fluorescence Assay

A Photovolt photofluorimeter Model 520 was obtained from government surplus and reconditioned for use in measuring fluorescence of chlorophyll a. In order to adapt a standard fluorimeter for use in measuring chlorophyll a it is necessary to restrict excitation energy to the blue absorption band of chlorophyll and to measure

emission in the wavelength region above 680 nm. This requires careful selection of primary and secondary filter combinations and the use of a light detector that has high quantum efficiency in the far red region. If these requirements are not adequately considered, (a) sensitivity of measurement will be impaired, or (b) fluorescence measurements for low density samples will be unduly affected by stray light particularly in the presence of sample turbidity. Another problem which restricts the use of certain filters is that occasionally the filter pigment or glass itself may fluoresce and cause a reduction in the sensitivity of the measuring technique.

With these considerations in mind the fluorimeter was modified in the following way: the standard S-10 photomultiplier that is stock with the Photovolt fluorimeter was replaced by an EMI 9781R selected for high red response. To obtain adequate sensitivity with this photomultiplier tube and retain the low noise typical of the photovolt instrument it was necessary to replace the high voltage circuit of the instrument with an external power supply capable of maintaining a low-current highly-regulated voltage in the range of 700 to 1000 volts. For this purpose a Hewlett Packard Model 6515A DC power supply was employed.

Three blue filters were combined as a primary filter. The secondary filter consisted of a Corning #2403 followed by a 710 nm interference filter. This combination reduced stray light to 20% of full scale on the highest sensitivity range of the instrument.

As the samples used were typically somewhat turbid, a

correction was always made for the stray light reading obtained from measurement of the uninoculated water sample.

The light source used to obtain fluorescence emission was the low pressure mercury arc lamp supplied with the photovolt instrument. Fluorescence measurements were made using a photovolt cuvette that required minimum sample volume of 1 ml.

This instrument as adapted was found to have overall sensitivity similar to or better than other chlorophyll measuring fluorimeters commercially available. The only disadvantage found was that the photovolt amplifier unit has sensitivity ranges that vary by decades and therefore maintaining linearity between scales was more difficult than with those units which vary by multiples of three.

Calibration of Fluorescence

Each test species was grown in Roux bottles under constant light conditions with compressed air bubbling in synthetic medium. After one week's growth, 100 ml of cell suspension were removed with a transfer pipette and filtered through a prewashed and tared 40 micron Millipore filter. The filter was then dried to constant weight at 90° C. From the original stock culture aliquots were taken for serial dilution and fluorescence measurement. As cell densities observed from synthetic media were never as high as those observed in some test samples, concentration of sample was necessary for calibration on the lowest sensitivity range of the instrument. As precision in the highest range of concentrations was thought to be of minimal

importance, an indirect technique was used, as follows: after initial calibration of the low concentration range was done, stock cell suspension was concentrated by centrifugation. Dilutions were then made until two or more fluorescence points fell in the range of the more precise points. The more highly concentrated samples were then assayed for fluorescence and a third order regression curve was fitted in the complete set of data by a linear least squares technique. The above calibration procedure was done twice during the period of study, once at the beginning of the period in April and again after the primary filter combinations were improved in May. The results of these calibrations can be seen in Figures 2 and 3.

Assay Procedure and Growth Conditions

Because fluorescence assay is an extremely sensitive technique for measuring algal growth (26, 27) it is not necessary to maintain large volume cultures. For this reason sample size was reduced to 2 ml. In place of Erlenmeyer flasks, 16 x 150 mm disposable Pyrex test tubes were used. In order to ensure a large surface area for gaseous exchange, the culture vessels were covered with plastic caps and placed in the growth chamber at an 8 degree slant from the horizontal. Illumination for Selenastrum was 400 ft-candles provided by two cool white fluorescent lamps. For Anabaena, one cool white fluorescent lamp with the addition of a 25-watt incandescent lamp were used for a total illumination of approximately 200 ft-candles.

Temperature was maintained at 24 ± 4 degrees C. In many cases,

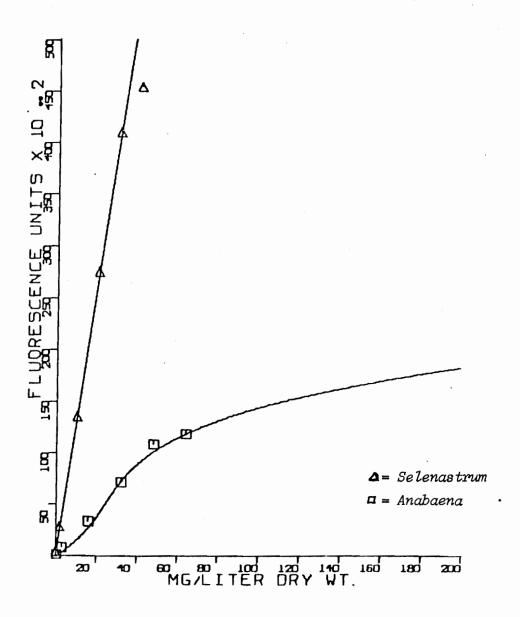


FIGURE 2: INSTRUMENT CALIBRATION USED DURING APRIL, 1973

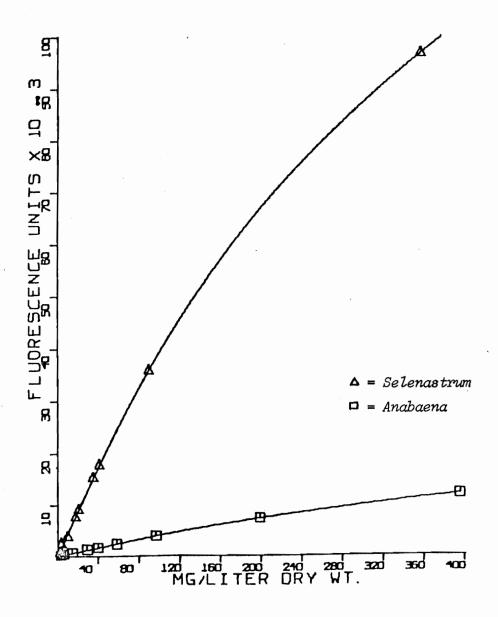


FIGURE 3: INSTRUMENT CALIBRATION USED MAY THROUGH SEPTEMBER, 1973

temperature control was a severe problem, occasionally causing termination of an experiment because the room temperature went out of this temperature range.

Actual assay procedure was as follows. After autoclaving, water samples from the various sampling stations were allowed to stand overnight to reduce turbidity from settleable solids. Four ml aliquots were transferred from sampling containers to growth tubes. Each growth tube was inoculated with test algae resuspended in 15 g/liter NaHCO₃. Inoculation volumes varied between .01 and .2 ml. The inoculated four ml sample was thoroughly mixed and divided into two approximately equal samples. Thus replicate samples were obtained with the same initial concentration of algae. Initial fluorescence for Anabaena cultures was typically 200-300 whereas for Selenastrum initial fluorescence was approximately 1000. As can be seen in the calibration curves of Figures 2 and 3, the quantum yield for fluorescence of Selenastrum was very much higher than that of Anabaena. Microcystis fluorescence yield was found to be considerably lower than that of Anabaena (results not shown).

At twelve hour intervals after inoculation, samples were removed from the growth chamber and assayed for fluorescence. The culture tube was mixed thoroughly on the vortex mixer, then part of the 2 ml growth sample was poured into the fluorescence cuvette, measured, and poured back into the growth vessel. Between sets of replicate samples the cuvette was rinsed with distilled water. Between assay of different species the cuvette was rinsed five times

each with 10% HCl, tap water, then distilled water. Using this procedure no contamination of successive samples was found by fluorescence measurement or microscopic analysis. It was typically found that maximum fluorescence was achieved in less than five days growth. The assay was terminated when all samples had shown the same value for fluorescence for consecutive 12 hour measurements.

Growth yields reported were obtained by taking the maximum fluorescence observed and converting to mg dry weight using the calibration regression equation. To estimate growth rates the entire data set obtained was arbitrarily edited to include only those points which were not obviously in the lag or stationary phase. A linear regression of log fluorescence versus time was then done on this edited data. The growth rate data are included in the present data only for descriptive purposes and are not considered to be highly reliable for any use but comparison of extreme values.

Analysis of Data

The compiled bioassay data were analyzed as a multifactor experiment, the treatments of which were as follows: test species used as inoculum, season of sample collection, and replication during assay. Individual sample collections and subsequent assays which occurred throughout the period of study were considered as replication. A regression technique was used to adjust the observed data for varying cell means in the presence of varying cell frequencies. The resulting sums of squares accounted for by regression coefficients were then tested in an analysis of variance. The error mean square

derived from this analysis of variance was used to calculate the <u>least</u> significant range for the Duncan's Multiple Range analysis. All calculations used in the statistical analyses were done using procedures from a statistical programming package (33).

Algal Identification and Enumeration

Seventy-five ml samples were taken from surface water at three reservoir stations each week throughout the period of study. These samples were immediately preserved with Lugol's solution. An additional small sample was split off the algal assay sample prior to autoclaving and centrifuged. The centrifugate was then analyzed microscopically to identify algal types to aid the later enumeration of algal species in the preserved samples. Preserved samples were placed in combination settling chambers to allow deposition of algal material on a glass coverslip which was at the bottom of the chamber. After 12 hours or more the settling chamber was removed from the counting chamber and enumeration was done on a Wilde Model 40 inverted microscope. Counts were made of dominant algal types with identification to species wherever possible. See the Appendix for complete listing of species observed. In addition to dominant types those species which are considered in the category of nuisance types were also counted even when their numbers were inadequate to rely on the number obtained. All counts were reported as number of individuals whether the plants were multicellular or unicellular.

IV. RESULTS

Algal Population Dynamics

Of the wide variety of species observed in the reservoir, fifteen to twenty species were highly prominent at some time during the study. These species are shown in Table I. A complete list of all species identified is shown in Table XVI of the Appendix. species distribution was not uniform throughout the lake nor through time. During spring when the reservoir was nearly homothermous, stations 2 through 5 were very similar in species distribution and number. However, station 1 was noticeably different. In particular, Asterionella formosa was found in high density at stations 2 through 5 at the start of this study, April 18, but absent at station 1. With heavy rains the peak A. formosa population density was seen to shift toward downstream stations. The A. formosa population was essentially eliminated from the entire reservoir by the beginning of June. During this entire period, although as many as 650 colonies per ml were recorded at other locations, A. formosa was never observed at station 1 in the Bull Run arm of the reservoir.

The motile green and motile non-green algae were prominent in all locations of the reservoir at most times. Maximum density was typically seen at station 1 in the Bull Run arm. In particular, Chlamydomonas sp. reached density greater than 2000 per ml at two occasions at station 1, June 26 and July 24. At station 2 this alga never exceeded a density of 700 per ml. Densities of

TABLE I

PREDOMINANT ALGAL SPECIES OBSERVED

IN OCCOQUAN RESERVOIR: JULY THROUGH SEPTEMBER, 1973

Diatoms

Asterionella formosa Nitzschia sp. Melosira varians

Motile Green Algae

Chlamydomonas sp. Pandorina morum Eudorina elegans

Nongreen Motile Algae

Cryptomonas erosa Trachelomonas sp. Euglena sp.

Green Algae

Coelastrum microporum Scenedesmus quadricauda Pediastrum simplex P. duplex Sphaerocystis schroeteri Radiococcus nimbatus

Blue Green Algae

Anabaena affinis
A. circinalis
A. spiroides
Aphanizomenon flos-aquae
Microcystis aeruginosa

Chlamydomonas varied at other stations to a maximum of 350 per m1, observed at station 3, July 3.

The dynamics of the bluegreen algae were highly complex and were further complicated by the frequent addition of copper sulfate to the lake surface by the Fairfax County Water Authority. The first bluegreens noted were Anabaena affinis at station 2, and Anabaena spiroides at station 1, both on June 19. The A. affinis population had reached a density of 1000 colonies per ml at station 2 by July 17. The A. spiroides population at station 1 remained at low density and was displaced later in August by A. circinalis and another form that resembled the description of A. flos-aquae. Immediately after the first Anabaena bloom on the Occoquan Creek arm this area was treated heavily with copper sulfate. Bloom conditions did not again occur in this area until fall overturn in late September. The Anabaena population in the Bull Run arm, however, remained at a low level until August from which time it gradually increased to a maximum of 5000 per ml during September.

Farther upstream on the Bull Run arm where standing water first occurs, extremely heavy mat conditions were observed at the beginning of July. The mats were composed of a diverse population including Anabaena circinalis, Aphanizomenon flos-aquae, Microcystis aeruginosa, filamentous and nonfilamentous diatoms, and Hydrodictyon reticulatum. The mat was broken up by distributing copper sulfate from an automatic feeder upstream at Yates Ford.

Prior to July only very low densities of bluegreen algae were observed on the main body of the reservoir. However, from the first time these were seen copper sulfate was applied approximately once a week from the confluence of Bull Run and Occoquan Creek to a few hundred yards above the high dam. Immediately following the first extensive application of copper sulfate, a large population of small green algae developed. Particularly prominant in this population were Scenedesmus quadricauda and Coelastrum microporum. Until mid-August few Anabaena were observed in this area, although Aphanizomenon flos-aquae reached a density of 330 colonies per ml, June 19, near station 3. Through the latter part of August and through September and October, particularly after the first heavy rain, Anabaena, Microcystis, and Aphanizomenon reached bloom proportions in all areas of the reservoir. Continued application of copper sulfate was not effective at this time.

Later in September after the first cold windy spell there was some equilibration of surface and bottom temperatures and the largest bloom of the entire season occurred. This was the first time *Micro-cystis* was seen to account for a major portion of the algal population. Total bluegreen algal density exceeded 8000 colonies per ml at station 1, and reached 2500 at station 3.

Algal Bioassay

The complete data set for AGP yield including results from both test species was subjected to an analysis of variance. The analysis of variance is shown in Table II. As might be expected, the two test species were shown to have significantly different mean yield values. At least one station had a mean AGP value that is not equal to the overall population mean, and AGP varied significantly from spring to summer. In addition, a significant species x station effect was noted. This indicates that the AGP yield for different stations is not entirely predictable within the terms of the model. Similarly, the significant station x season interaction indicates that variation in AGP at the different stations varies between spring and summer.

The data set was next subdivided and stream data and reservoir data were analyzed separately. The middle portion of Table II shows that species and station effects constitute highly significant sources of variation in the stream data. The season effect, however, was less pronounced in this subset than in the complete data set. Once again the analysis indicates that at least one stream sampling station had AGP yield significantly different from that of the complete group of stream stations. As replication was previously found to be nonsignificant in the previous analysis, it was omitted from this analysis. All other possible interactions were tested and rejected due to a nonsignificant F value.

The analysis for reservoir AGP data is shown in the lower portion of Table II. Since the surface and bottom samples were handled separately during the bioassay procedure, an additional classification variable, level, was added to the analysis. As before, significant variation was detected between species and between stations. The

TABLE II: ANALYSIS OF VARIANCE FOR AGP YIELD

	Source of Variation	Degrees of Freedom	Sum of Squares	F Value	Mean
Entire Watershed	Species Station Season Rep Species x Station Station x Season Error	1 10 1 1 10 10 498	136705 1005489 10216 40 342790 35239 632439	107.64** 79.17** 8.04** 0.03 26.99** 2.77**	39.38
Stream Stations	Species Station Season Species x Station Error	1 4 1 4 129	148271 806258 18586 243564 3677	40.32** 54.82** 5.05* 16.56**	68.59
Lake Stations	Species Station Level Season Species x Station Level x Season Error	1 4 1 4 1 335	16563 55960 7133 24 7617 7663 100661	55.12** 46.55** 23.74** 0.08 6.33** 25.50**	23.71

^{**}Significance level greater than 99% *Significance level greater than 95%

season effect, however, was found to be nonsignificant. Surface and bottom samples also do not appear to share a common mean. Although the season effect was found to be nonsignificant, the significant interaction of level x station indicates that the difference between surface and bottom samples varies from spring to summer. Finally, it may be noted that a significant species x station effect persists throughout the analysis and is not explained by the difference between stream and lake sampling stations.

To detect statistically which stations were responsible for the distinct station effect noted throughout the previous analysis, the pooled data set was subjected to a Duncan's Multiple Range test. This test uses the error mean square as a basis for developing critical values at which consecutive means may be resolved at a given level of significance. From this test, the data is grouped into a series of overlapping groups such that all members of a group have means that are not significantly distinguishable.

The results for the Duncan's Multiple Range test at the 5% level of significance are shown diagrammatically for Anabaena AGP yield in Figure 4. Five overlapping groups were detected. Group I includes only the Cub Run station, which has the highest mean AGP yield. Group II includes only the Bull Run station at Yates Ford. Group III includes both surface and bottom at station 1 in the Bull Run arm of the reservoir. Group IV includes all reservoir stations except station 1 bottom, which is exclusively in Group III, and station 5 surface, which is exclusively in Group V. Also included in Group IV is Cedar

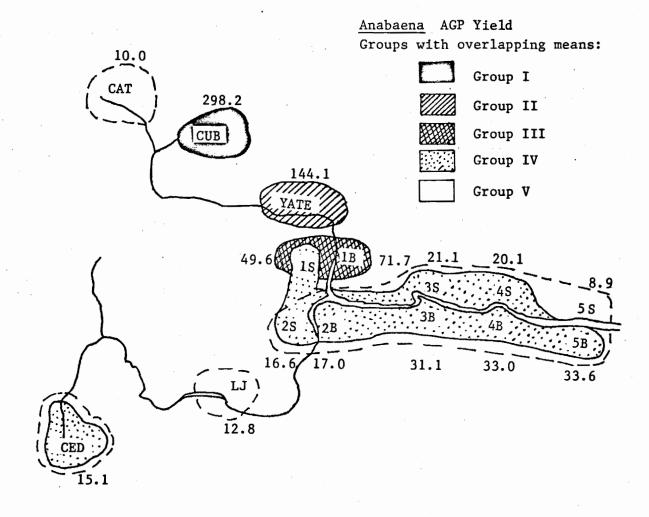


FIGURE 4: RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR OVERLAPPING MEANS OF ANABAENA AGP YIELD. LEVEL OF SIGNIFICANCE IS .05.

Run, the sampling station in the agricultural area of the watershed.

Group V, which had the lowest mean AGP yield, includes all stream

stations except Cub Run and Yates Ford, and all lake stations with

the exception of station 1 bottom. Thus we see that Cub Run and

Yates Ford sampling stations were distinct, having higher AGP yield,

from all three other stream stations, and reservoir station 1 bottom

has AGP values significantly higher than all other reservoir stations.

The results of the Duncan's Multiple Range test for Selenastrum AGP yield are shown in Figure 5. In this case only three distinct groupings were noted. As with Anabaena AGP yield, Group 1 includes only Cub Run. Group II, however, includes Bull Run at Yates Ford, surface and bottom samples at station 1 in the Bull Run arm of the reservoir, and bottom samples from reservoir stations 3, 4, and 5. Group III, with the lowest AGP yield values, includes all reservoir stations, surface and bottom, plus all stream stations except Cub Run and Yates Ford. This is essentially the same as the result obtained with Anabaena as test species. The pronounced species x station interaction will be discussed in a later section.

In planning the AGP assay, it was hoped that careful measurement of growth rate would provide similar information to that obtained from maximum yield, but with less investment of time. As stated in the Methods section, fluorescence measurements were taken at 12-hour intervals and used to evaluate growth rates. A similar analysis to that done on the AGP yield data was performed. Shown in Table III is the result of the analysis of variance on growth rate data obtained

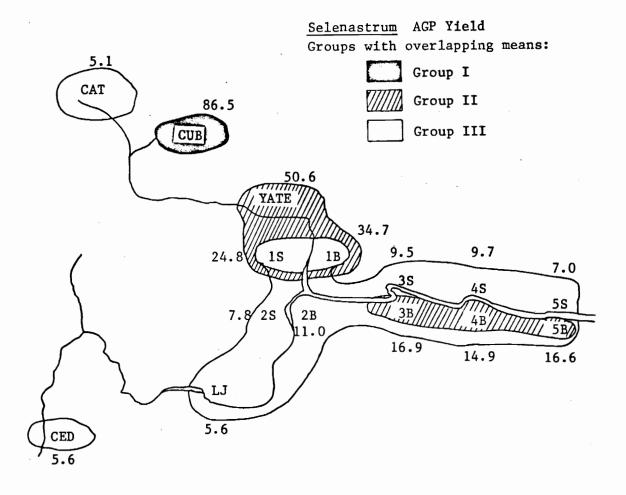


FIGURE 5: RESULTS OF DUNCAN'S MULTIPLE RANGE TEST FOR OVERLAPPING MEANS OF SELENASTRUM AGP YIELD. SIGNIFICANCE LEVEL IS .05.

TABLE III: ANALYSIS OF VARIANCE FOR ALGAL GROWTH RATE

	Source of Variation	Degrees of Freedom	Sum of Squares	F Value	Mean
Entire Watershed	Species Station Season Rep Station x Season Species x Season Error	1 10 1 1 10 10 1 507	2.81 14.08 13.83 0.06 6.42 2.08 105.50	13.50** 6.77** 66.49** 0.32 3.08** 10.02**	1.28
Stream Stations	Species Station Season Error	1 4 1 133	1.37 15.56 1.11 23.86	7.64** 21.69** 6.22*	1.36
Lake Stations	Species Station Level Season Level x Season Error	1 4 1 1 1 347	1.48 2.19 1.98 30.55 2.47 117.19	6.56* 2.42 8.78** 134.90** 10.90**	1.25

^{**}Significance level greater than 99%
*Significance level greater than 95%

simultaneously with the AGP yield data.

The analysis of AGP growth rate runs parallel to the previous analysis of AGP yield. The same main effects were noted, species, station and season. The main difference between the yield analysis and the rate analysis is first observed in the interactions. Only the station x season and the species x season interactions were found to be significant at the 5% level. Among the stream data no interactions were found to be significant, and within the reservoir data, sampling station was found to be nonsignificant. The most apparent result is that the season of sample collection and analysis introduced the greatest source of variation. This result is taken with some reservation as severe temperature control problems were encountered during the bioassay procedure. It is not unlikely that this may account for the distinct seasonal variations. In general, however, the AGP growth rate analysis may be useful but appears to have lower sensitivity than the maximum yield analysis.

A Duncan's Multiple Range analysis is not shown for growth rate as too much overlap among groups was encountered.

Chemical Nutrient Analysis

Chemical and bioassay mean values and observed ranges are shown by sampling station in Tables XVII - XXVI in the Appendix. The nutrient and bioassay data are summarized to include only the parameters of interest in Table IV. These data indicate that Cub Run stream samples typically have the highest concentration of all

TABLE IV: MEAN CONCENTRATION OF NUTRIENT AND AGP

OCCOQUAN WATERSHED: WEEKLY SAMPLING APRIL THROUGH SEPTEMBER, 1973

NUTRIENT MEANS - mg/1

					
	Orthophosphate Phosphorus	Total Phosphate Phosphorus	Nitrate Nitrogen	Selenastrum AGP Yield	Anabaena AGP Yield
Bull Run Sub-Watershed					
Bull Run/Catharpin	0.017	0.044	0.279	5.1	10.1
Cub Run	0.886	0.973	1.725	86.5	298.2
Bull Run/Yates Ford	0.450	0.492	0.796	50.6	144.1
1 Bottom	0.170	0.200	0.426	34.7	71.7
1 Surface	0.108	0.147	0.291	24.8	49.6
Occoquan Creek Sub-Waters	shed				
Cedar Run	0.013	0.041	0.476	5.6	15.1
Occoquan below Lake Jackson	0.010	0.030	0.313	5.6	12.8
2 Bottom	0.059	0.088	0.199	11.0	17.0
2 Surface	0.018	0.041	0.148	7.8	16.6
Main Reservoir					
3 Bottom	0.157	0.193	0.188	16.9	31.1
3 Surface	0.018	0.046	0.130	9.5	21.7
4 Bottom	0.137	0.169	0.090	14.9	33.0
4 Surface	0.013	0.047	0.073	9.7	20.8
5 Bottom	0.072	0.091	0.158	16.6	33.6
5 Surface	0.014	0.041	0.099	7.0	9.2

potential algal nutrients. Next in order of typical nutrient concentrations were Bull Run at Yates Ford and bottom samples at reservoir station 1.

The mean value for nitrate-nitrogen was approximately four times higher at Cub Run than at any point in the Occoquan subwatershed. On only one occasion during the period of study nitrate nitrogen reached concentrations in the Occoquan subwatershed which were comparable to those of the Bull Run subwatershed. This occurred during the last week of June. This nitrate spike was observed to coincide with a bloom of Anabaena and green motile algae on Lake Jackson. A light bloom of Anabaena was also noted at reservoir station 2 at this time.

Concentrations of orthophosphate-phosphorus ranged as much as 30 times higher at the Cub Run sampling station than at any sampling point in the Occoquan subwatershed. One may note also from Table IV that the phosphate concentrations encountered in bottom samples from reservoir station 3 are considerably higher than those observed at station 2. This indicates the strong influence of Bull Run on phosphate levels in the reservoir. The nitrate levels of station 2 and station 3 are of similar magnitude, and differ from those observed at station 1. This is likely due to the similarity of depth for stations 2 and 3.

Analysis of Correlations

The entire set of chemical and physical parameters reported by the Occoquan Watershed Monitoring Laboratory during the period of this study was merged with the algal bioassay data and examined for correlations at the 5% level of significance. These results are presented in Table V. All correlation coefficients accepted at the 5% level of significance were also significant at the 1% level. Selenastrum AGP yield showed the highest correlation with nitrate-nitrogen, whereas Anabaena AGP yield was most highly correlated with total phosphate-phosphorus. It is of interest that no significant correlation was observed between concentration of ammonia and AGP yield for either species. The low but significant positive correlation observed between Anabaena AGP yield and alkalinity was not considered to be very meaningful as the samples were autoclaved prior to analysis of AGP.

The above data set was subdivided by station and level and again analyzed for correlation. All significant correlations observed at stream sampling stations are shown in Table VI. Although few significant correlations were observed between Selenastrum AGP yield and the chemical and physical parameters, Anabaena AGP yield was found to correlate highly with nitrate, nitrite, and phosphate concentrations at four of the five stations. Of particular interest is the high correlation between Anabaena AGP yield and total phosphate concentration at the Yates Ford and Cedar Run sampling stations. A strong negative correlation between Anabaena AGP yield and the observed dissolved oxygen concentration is also noted at the Cub Run and Cedar Run sampling stations. This negative correlation is probably a result of a seasonal effect. Summer values for Anabaena AGP yield were considerably higher at these two stations while dissolved oxygen

TABLE V
ENTIRE WATERSHED CORRELATION COEFFICIENTS
FOR CHEMICAL PARAMETERS AND AGP YIELD

AGP Yield

Station	Parameter	Selenastrum	Anabaena		
Entire Watershed	NO ₃ Ortho-PO ₄ Total PO ₄ Alkalinity NO ₂ Non-Ortho-PO ₄ NH ₃ Total Kjeldahl-N	.863324* .759471* .782588* NS .810414* .357041* NS	.817637* .867040* .881898* .396910* .805225* .343727* NS		

Level of Significance .05

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^{*}Level of Significance .01

NS = Nonsignificant at .05 level.

TABLE VI

STREAM STATIONS CORRELATION COEFFICIENTS
FOR CHEMICAL PARAMETERS AND AGP YIELD

AGP Yield

Station	Parameter	Selenastrum	Anabaena
Bull Run at Catharpin		NS	NS
Cub Run near Bull Run	NO ₂ Dissolved O ₂	NS NS	.865562* 717477
Bull Run at Yates Ford	NO ₃ Ortho-PO ₄ Total PO ₄	.837260 NS NS	.824000 .856476 .879057*
Cedar Run	NO ₂ Dissolved O ₂ Total PO ₄ Non-Ortho-PO ₄	NS NS NS NS	.968698* 816658 .905139* .846466
Occoquan Creek below Lake Jackson	NO 3 Total PO ₄	NS NS	NS .822991

Level of Significance .05 *Level of Significance .01

NS = Nonsignificant at .05 level

concentrations were typically lower during the summer season. The possibility of correlation between *Anabaena* yield and BOD may be considered, however, since a similar negative correlation was not observed at the Catharpin sampling station. Unfortunately, the data were not available to test this hypothesis.

Results of the correlation analysis for Selenastrum and Anabaena AGP yields with physical and chemical parameters at reservoir stations are presented in Table VII. Examining the surface data for correlations with Anabaena AGP yield, one can see that high correlation is obtained between AGP yield and phosphate concentration at four of the five stations, while significant correlation with nitrate is observed at only two stations. No significant correlation is observed between Anabaena AGP yield and ammonia concentration at any of the surface stations. One may infer from the negative correlation with dissolved oxygen at surface station 2 that indigenous algal populations are responsible for the lowered AGP values obtained.

At bottom stations one observes significant positive correlation between Anabaena AGP yield and ammonia only at station 1, and significant correlation with phosphate only at station 5. No significant correlation between Anabaena AGP yield and nitrate occurs at any bottom station. Correlation with nitrite, however, was noted at station 3.

The negative correlations between dissolved oxygen and AGP yield observed in bottom samples result from the obvious increase in algal nutrients that obtains during periods of low dissolved oxygen

TABLE VII RESERVOIR STATIONS CORRELATION COEFFICIENTS FOR CHEMICAL PARAMETERS AND AGP YIELD

		AGP Y	
Station	Parameter	Selenastrum	Anabaena
1-Surface	Non-Ortho PO4	NS	.85381
	Dissolved 0 ₂ 4	84044*	NS NS
	NO ₂	.64956	NS NS
}	Alkalinity	 75370	NS NS
	Temperature	86816*	NS NS
	NO ₂	.694365	NS
1-Bottom	Dissolved 02	NS	610179
	Alkalinity ~	NS	.852900
	NH ₃	NS	.82301
	Ortho-PO	68994	NS
	Temperature	NS	.607144
2-Surface	Ortho-PO ₄	.75975	.66206
·	Dissolved 02	NS	67031
	NO ₃	.93099	.61383
	NO ₂	.704348	NS
2-Bottom	NO ₃	.77267*	NS
	NO ₂	.644382*	NS
3-Surface	Total Kjeldahl-N		.92457
	Ortho-PO ₄	.81998*	NS
	Dissolved 02	67593*	NS
	NO 2	.95156*	NS
	Total PO ₄	NS	.808247*
·	NH ₃	.998090*	NS
3-Bottom	Temperature	NS	.64111
	NO ₂	.795167*	.728582
4-Surface		NS	NS
4-Bottom	NO ₃	99974	NS
5-Surface	Ortho-PO ₄ Temperature	NS	70927
	Temperature	821113	70784
	NO ₃	.89583	NS
5-Bottom	Total PO ₄	.867214	.906221
	Ortho-PO,	.89062	.93143
	NH ₃	NS	NS ·

NS = Nonsignificant at .05 level Level of Significance .05

^{*}Level of Significance .01

concentration in the hypolimnion.

Examining the correlation between surface sampling data and Selenastrum AGP yield, it is noted that high correlation with phosphates is obtained at only two of the five sampling stations. ever, nitrates correlate significantly with Selenastrum yield at four of the five surface stations. It is particularly interesting to note that at several surface stations, negative correlations with dissolved oxygen are found, and that at station 1 negative correlations are obtained for alkalinity and temperature as well as dissolved oxygen. The negative correlation between dissolved oxygen and Selenastrum AGP yield observed in surface samples probably indicates that the indigenous algal populations were responsible for reducing nutrient levels. As temperature and alkalinity increased, the population at station 1 was observed to contain greater proportions of bluegreen algae. phenomenon is well known and has been discussed extensively in the works of King (18), Goldman (28), Kuentzal (17), and others. present data are inadequate to suggest any cause-effect relationship.

Bottom sampling data show few significant correlations for Selenastrum AGP yields. Apparent, however, are the high correlations observed with nitrate and nitrite at stations 2 and 3, and the high correlation with phosphate at station 5. The negative correlations coefficient observed between nitrate and Selenastrum yield at station 4 was rejected because it results from only four data points. The negative correlation for orthophosphate and Selenastrum yield at station 1 remains unexplained.

V. DISCUSSION 1

Prediction of AGP Yields from Nutrient Levels

In the previous section nitrate and phosphate were found to be the chemical nutrients with highest correlation coefficients with respect to AGP yield. With this in mind it is presumed that AGP yield can be predicted given the observed concentrations of nitrate and phosphate.

AGP yield was plotted as a function of nitrate concentration. The complete data set was arbitrarily grouped by level of phosphate concentration. Selenastrum AGP yield for nitrate concentration and phosphate level is shown in Figure 6. One can see from this curve that at orthophosphate-phosphorus concentrations less than 0.010 mg-P/liter, AGP is distinctly limited. AGP yield at phosphate 0 - 0.010 mg-P/l has been fit with a second order polynomial by the method of least squares. At phosphate levels between 0.010 mg-P/liter and 0.100 mg-P/liter, the second order polynomial gives only poor fit. However, when all points for phosphate concentration greater than 0.010 mg-P/liter are combined good fit is observed for a straight line. This analysis predicts a yield of 60 mg Selenastrum per mg nitratenitrogen when orthophosphate-phosphorus concentration is greater than 0.010 mg-P/liter.

A more sensitive analysis for nutrient effects at the chosen phosphate levels is shown in the correlation analysis of Table VIII.

At phosphate levels below 0.010 mg-P/liter, significant correlation is

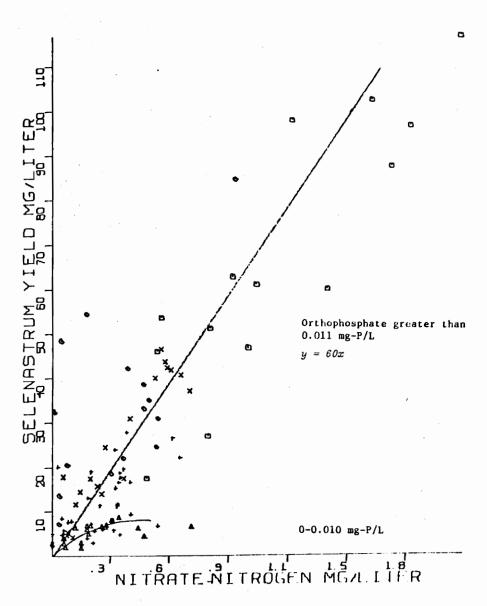


FIGURE 6: OBSERVED GROWTH OF SELENASTRUM AS A FUNCTION OF NITRATE

CONCENTRATION AND ORTHOPHOSPHATE LEVEL

```
△ = less than .010 mg-P/liter
+ = .011 to .050 mg-P/liter
× = .051 to .100 mg-P/liter
◇ = .101 to .200 mg-P/liter
□ = greater than .200 mg-P/liter
```

TABLE VIII

CORRELATION COEFFICIENTS

Nutrient Concentrations with Selenastrum AGP Yield at Orthophosphate Level

Orthophosphate level	orthophosphate Phosphorus	Total Phosphate Phosphorus	Nitrate Nitrogen	Nitrite Nitrogen	Ammonia Nitrogen	Total Kjeldahl Nitrogen	
less than .010 mg/1	NS	NS	.4817	NS	NS	NS	
.011050 mg/1	. 3955	NS	.5484*	.5735*	NS	NS	
.051100 mg/1	NS	NS	.9283*	.7144*	.7685	NS	
.101200 mg/1	NS	NS	.5755	.4996	NS	NS	
greater than .200 mg/1	NS	.5356	.8742*	NS	8316	NS	

Level of Significance .05

^{*}Level of Significance .01

NS = Nonsignificant at .05 level

observed only with respect to nitrate. Although this correlation coefficient is significant, its magnitude is low (0.4817). At phosphate concentrations greater than 0.010 mg-P/liter but less than 0.051 mg-P/liter, some significant correlation with orthophosphate and nitrite concentrations is observed. This range apparently includes the maximum level at which phosphate controls the AGP yield of Selenastrum.

It is of interest to note in Table VIII high, significant negative correlation between *Selenastrum* AGP yield and ammonia concentration at high phosphate and nitrate combined concentrations.

A similar analysis to that above was attempted plotting Selenastrum AGP yield versus orthophosphate-phosphorus concentration at nitrate levels. As can be seen in Figure 7, no obvious relationships were observed for nitrate levels. However, the first order linear regression yields the following estimate of AGP yield for a given concentration of orthophosphate-phosphorus: 0.189 mg/liter Selenastrum per microgram orthophospate-phosphorus. This value is low when compared with the recently published value, 0.43 mg/liter Selenastrum per microgram orthophosphate-phosphorus per liter (29).

AGP yield for Anabaena was also analyzed as a function of nitrate concentration at phosphate level. This is shown in Figure 8. Since total phosphate-phosphorus appears to be the more highly correlated parameter for Anabaena AGP yield, total phosphate level was used in this analysis. It is obvious from inspection of Figure 8 that the nitrate effect on Anabaena AGP yield is very different from its

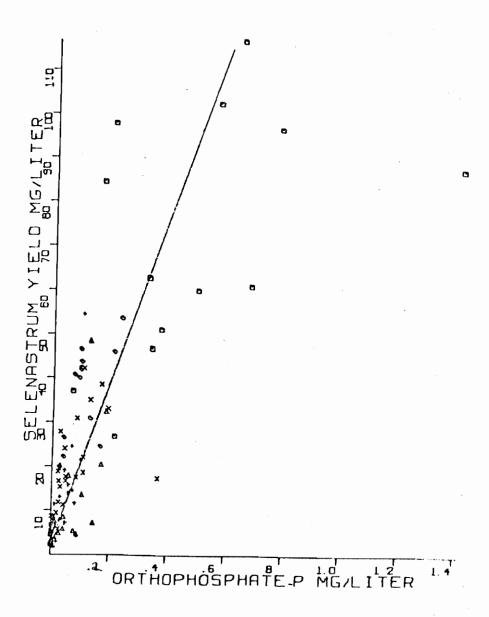


FIGURE 7: OBSERVED GROWTH OF SELENASTRUM AS A FUNCTION OF ORTHO-PHOSPHATE CONCENTRATION AND NITRATE LEVEL.

```
△ = 0 - 0.100 mg-N/liter
+ = .101 - .300 mg-N/liter
X = .301 - .500 mg-N/liter
♦ = .501 - .700 mg-N/liter
□ = greater than .701 mg-N/liter
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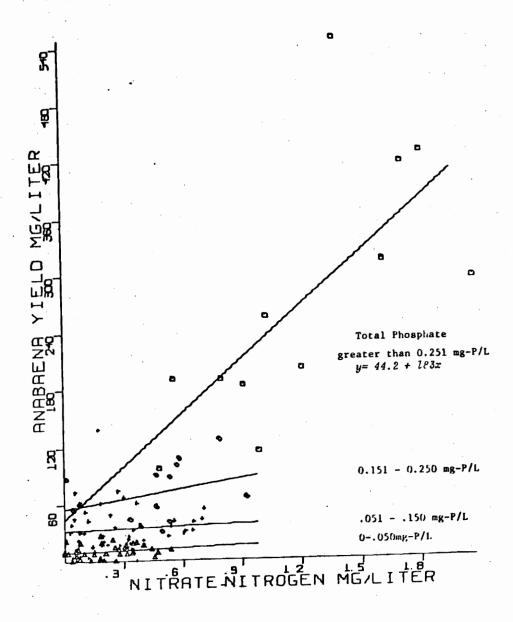


FIGURE 8: OBSERVED GROWTH OF ANABAENA AS A FUNCTION OF NITRATE

CONCENTRATION AND TOTAL PHOSPHATE LEVEL

 $[\]triangle$ = less than .050 mg-P/liter

 $^{+ = .051 \}text{ to } .150 \text{ mg-P/liter}$

^{♦ = .151} to .250 mg-P/liter

D = greater than .251 mg-P/liter

effect on the *Selenastrum* AGP yield. The various levels of phosphate showed AGP yields that were almost independent of nitrate concentration except at high phosphate and nitrate combined concentrations. It appears that phosphate level alone determines the AGP yield at low to medium concentrations of phosphate (less than 0.150 mg-P/liter). At high concentrations, particularly above 0.250 mg-P/liter, nitrate apparently has a stimulatory effect on AGP yield. This stimulation occurs to the extent of 183 mg AGP yield/liter per mg-N/liter.

Similar results obtain when total phosphate levels are subjected to correlation analysis. These results are shown in Table IX.

From this analysis it is obvious that only phosphorus was effective in controlling AGP yield for *Anabaena* at levels below 0.250 mg-P/liter.

Anabaena AGP yield is shown plotted as a function of orthophosphate in Figure 9. No definite nitrate effect was discernible.

A linear least squares regression gives good fit for a straight line.

The resultant yield is 590 mg Anabaena per mg orthophosphate-phosphorus.

This is almost precisely the value recently reported by the National Environmental Research Center (29).

On the basis of these AGP yield curves one can predict that Anabaena AGP yield is unaffected by nitrate concentration when phosphates are below 0.150 mg-P/liter, but at the concentrations of phosphate greater than this they act synergistically. Selenastrum, however, is totally dependent upon nitrate concentration when the phospate level is greater than 0.01 mg-P/liter. This value is consistent with the currently accepted maximum for phosphate limitation

TABLE IX
CORRELATION COEFFICIENTS

Nutrient Concentrations with Anabaena AGP Yield at Total Phosphate Level

Total Phosphate Level	Orthophosphate Phosphorus	Total Phosphate Phosphorus	Nitrate Nitrogen	Nitrite Nitrogen	Ammonia Nitrogen	Total Kjeldahl Nitrogen	
less than .050 mg/l	.4494*	NS	NS	NS	NS	NS	
.051150 mg/1	NS	.4096*	NS	NS	NS	NS	
.151250 mg/1	NS	.7681*	NS	NS	NS	NS	
greater than .251 mg/1	.5942	.6022	.6921	.8354*	NS	NS	

Level of Significance .05

^{*}Level of Significance .01

NS = Nonsignificant at .05 level

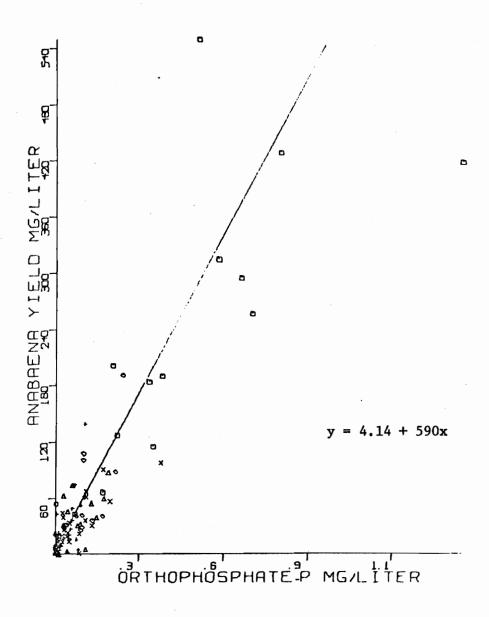


FIGURE 9: OBSERVED GROWTH OF ANABAENA AS A FUNCTION OF ORTHOPHOSPHATE

CONCENTRATION AND NITRATE LEVEL

△ = 0 - 0.100 mg-N/liter + = .100 - .300 mg-N/liter X = .300 - .500 mg-N/liter ♦ = .500 - .700 mg-N/liter □ = greater than .700 mg-N/liter to algal growth (30). One may assume on the basis of this analysis that *Anabaena* is limited by phosphate concentration to at least a concentration level of 0.150 mg-P/liter. This suggests that control of growth of bluegreen algae which can fix atmospheric nitrogen depends on control of phosphate concentration.

Nutrient Loadings at Watershed Tributaries

Average daily nutrient loadings for the main tributaries to the Occoquan Watershed for the period April through September, 1973, are shown in Table X. The values shown for loading at Bull Run/ Yates Ford and Occoquan Creek below Lake Jackson represent the amounts discharged to the reservoir from the two subwatersheds, respectively. Note that the total quantity of nitrate entering the reservoir from the Occoquan subwatershed exceeds that discharged from the Bull Run subwatershed. However, total nitrogen load to the reservoir from Bull Run was higher than from Occoquan. The quantity of ammonia was nearly four times higher from Bull Run than from Occoquan. Nearly ten times as much orthophosphate was discharged to the reservoir from Bull Run as from Occoquan, although the amounts of non-orthophosphate was nearly These chemical nutrient levels were reflected in the AGP the same. values. The Anabaena AGP load was approximately three times higher from Bull Run than that from Occoquan. Selenastrum AGP load was only twice as high. These nutrient load values at Bull Run obtain even though its total discharge was only one half that of Occoquan Creek.

Several quick calculations can be made to contrast the water

TABLE X

AVERAGE DAILY NUTRIENT LOADINGS - Lbs./Day

Occoquan Watershed, Weekly Samples April Through September, 1973

	Nitrate Nitrogen	Orthophosphate Phosphorus	Non-Orthophosphate Phosphorus	Ammonia Nitrogen	Nitrite Nitrogen	Total Kjeldahl Nitrogen	Total Nitrogen	Anabaena AGP	Selenastrum AGP	Discharge Million Gal/Day
Bull Run/Catharpin	31.0	1.7	3:0	4.6	.4	2.2	34.4	1021	573	10.8
Cub Run	205.0	58.0	13.0	72.0	8.2	39.0	285.0	25121	13629	33.0
Bull Run/Yates Ford	608.0	193.0	41.7	235.0	43.9	259.0	878.0	70045	31273	120.0
Cedar Run	431.0	15.0	35.8	51.0	8.0	87.0	490.0	7700	3400	100.0
Occoquan below Lake Jackson	703.0	21.0	46.9	68.0	13.9	48.0	785.0	23000	17000	255.0
Total to Reservoir	1311.0	216.0	88.6	303.0	57.8	307.0	1663.0	93045	48273	375.0

quality of Cub Run and Bull Run with all other streams. The ratio of nitrate-nitrogen to orthophosphate-phosphorus at Cub Run and Yates Ford ranged between 3.0 and 4.0, whereas at all other stations it varied from 18.3 to 33.5. Also, the ratio of orthophosphate to non-orthophosphate (the numerical difference between orthophosphate-phosphorus and total phosphate-phosphorus) is typically ten times higher at Cub Run and Yates Ford than at any other sampling station. Although the chemical analyses at Cub Run and Yates Ford show similar results in many respects, it is interesting to note the disproportionate increase in total Kjeldahl-nitrogen load from Cub Run to Yates Ford.

The mean daily nutrient loading values and discharge values shown in Table X have been used to estimate the relative contribution of each area sampled. These values are shown as percent of total load entering the reservoir in Table XI. One may note that Cub Run, although it contributed only 8.8% of the total discharge entering the reservoir, contributed 27.76% of all orthophosphate. The Bull Run/Yates Ford sampling station showed 52.8% of all nitrogen, and 90.19% of all orthophosphate, although its discharge only accounted for 32.2% of the total. AGP values also were well out of the expected range predicted by watershed area. Yates Ford accounted for 75% of all Anabaena AGP and 65% of all Selenastrum AGP. Although Cedar Run and Occoquan stations showed considerable amounts of nitrate, they contributed very little orthophosphate or ammonia.

TABLE XI

PERCENT OF TOTAL LOADS TO LAKE

Occoquan Watershed, Weekly Sampling April Through September, 1973

	Nitrate Nitrogen	Orthophosphate Phosphorus	Non-Orthophosphate Phosphorus	Ammonia Nitrogen	Nitrite Nitrogen	Total Kjeldahl Nitrogen	Total Nitrogen	Anabaena AGP	Selenastrum AGP	Discharge Million Gal./Day
Bull Run/Catharpin	2.36	.79	3.39	1.53	0.69	0.72	2.07	1.10	1.19	2.88
Cub Run	15.64	27.10	14.67	23.76	14.19	12.70	17.14	14.65	28.23	8.80
Bull Run/Yates Ford	46.38	90.19	47.06	77.56	75.95	84.36	52.80	75.28	-64.78	32.00
Cedar Run	38.11	7.01	40.41	16.83	13.84	28.34	29.46	8.28	7.04	26.67
Occoquan below Lake Jackson	53.63	9.81	52.93	22.44	24.05	15.74	47.20	24.72	35.22	68.00
Total lbs./day to Reservoir	1311	214	88.6	303	57.8	307	1663	93045	48273	375

VI. DISCUSSION 2: NUTRIENT SOURCES AND PATTERNS OF FLOW

Point Sources

The Bull Run watershed includes 11 sewage treatment plants that very in size from 0.1 to 2 million gallons per day. All are considered to be capable of secondary treatment, with no provisions for nitrate or phosphate removal. When operating properly, these plants will discharge high concentrations of nitrate because most of the nitrogen which enters the treatment process is oxidized to this form. Some reduction of phosphorus level is possible if the treatment process has efficient solids removal and careful management of anaerobic digesters. Table XII shows typical discharge analysis for treatment plants of the Bull Run watershed. One may note that the GMSD plant, which discharges directly into Bull Run, has effluent character typical of an efficient secondary treatment process.

Cub Run enters Bull Run in the vicinity of Manassas Park. All six other treatment plants discharge to Bull Run within the same one mile reach of stream that includes the confluence with Cub Run. The combined mean flow of all treatment plants from June and July, 1974, shown in Table XII was greater than five million gallons per day. Base flow for Bull Run during this season is typically 20 to 30 million gallons per day, so one can see these treatment plant effluents constitute a considerable portion of all nutrients present in the Bull Run drainage. In a recent study of nutrient contributions, shown in Table XIII, these treatment plants were seen to account for 47% of all

TABLE XII

SEWAGE TREATMENT DISCHARGE DATA

Occoquan Watershed, July Through September, 1974

		во	05	·	ss	T-Pho	sphorus	Nit	rate *	Ammo	mia*	T-Nitr	rogen*
Treatment Plant	Flow MGD		Load lb/day	Conc.		Conc.	Load lb/day	Conc.	Load lb/day	Conc.	Load lb/day	Conc.	
					CUB	RUN SOUR	CES	-					
Greenbriar	0.667	14.3	79.7	20.0	111.0	2.7	15.2	0.4	2.3	15.9	89.6	21.0	118.4
Big Rocky	0.190	5.3	8.4	16.2	25.8	3.5	5.5	0.3	0.5	22.6	35.8	26.5	42.0
Flatlick	0.337	2.7	7.5	5.0	14.1	1.0	2.9	2.2	5.9	7.8	21.1	11.0	29.7
Middle Cub Run	0.562	5.0	23.4	14.7	68.8	2.8	13.0	0.4	1.9	16.9	77.9	19.9	91.9
Upper Cub Kun	0.172	1.3	1.9	12.7	18.2	0.7	1.0	0.5	0.7	12.6	19.0	15.6	22.4
Totals	1.928		120.9		238.0		37.6		11.3		243.4		304.4
					BULL	RUN SOUI	RCES						
Manassas Park #	1 0.175	6.5	9.6	7.5	11.1								
Manassas Park	2 0.146	3.5	4.6	4.0	4.9								
Westgate	1.04	6.0	51.9	7.8	67.2	1.0	8.3	2.2	18.7	-	-	6.7	57.7
ಇ ಟಾ	1.43	2.3	27.2	4.7	56.0	0.6	7.7	7.6	90.4	-	-	8.6	102.3
Liberia	0.189	7.0	11.1	14.4	22.8	0.5	0.8	6.3	9.9	-	-	13.0	20.5
Northside	0.425	7.9	28.0	17.7	62.7	0.6	2.2	2.7	9.5	-	-	20.2	71.7
Totals	3.408		132.4		224.7								
Grand Total	5.336		253.3		462.6								

^{*}Cub Run data for July only.

TABLE XIII

COMPARISON OF AVERAGE NUTRIENT AND SOLIDS LOADINGS FROM POINT AND

DIFFUSE SOURCES: From Weekly Sampling July Through September, 1974

			1bs	s/day			
	Total phosphate Phosphorus	Percent		Total Nitrogen	Percent	Total suspended Solids	Percent
Occoquan Creek	27	23		291	33	1059	46
Lower Bull Run	35	30		23	3	753	33
Sewage Treatment Plants	56	47		557	64	463	21
Total	118			871		2275	

phosphorus and 64% of all nitrogen entering the reservoir during the period June and July, 1974. Consequently, as previously noted, mean nitrate and phosphate concentrations were highest at the Cub Run sampling station, while the greatest phosphate load was observed below these treatment plants at Yates Ford.

Non-Point Sources

In Table XIV, the nutrient loadings for watershed areas monitored by stream gages are considered as uniformly distributed non-point nutrient sources. The Catharpin subwatershed stands out as having the lowest observed nutrient loading per acre of watershed. Cub Run, which is predominantly affected by sewage treatment plant effluent, shows the highest values for nitrate and very high values for phosphate. The lower Bull Run gaging station, which includes the drainage of Catharpin, Cub Run, six additional treatment plants, a large urban area and an undetermined amount of forested land, shows the highest per acre phosphate loadings and a very high per acre nitrate loading. In the Occoquan subwatershed at Cedar Run and Occoquan Creek below Lake Jackson, phosphate loadings are not much higher than that in the forested region at Catharpin; however, the mean per acre daily nitrate loading is as much as 40 times higher than that of Catharpin and approaches the levels of Bull Run at Yates Ford. Cedar Run watershed, as previously mentioned, is considered to be primarily agricultural drainage.

TABLE XIV
MEAN NUTRIENT CONTRIBUTIONS OF LAND USE AREAS

Source	Area (acres)	(1bs. day -1)		(1bs.acre ⁻¹ day ⁻¹)	
		<u>NO</u> 3	TP	NO ₃	TP
Forested Area:					
Bull Run/Catharpin	1660	31	4.7	.0187	.00283
Sewage Treatment and Forest:					
Cub Run near Bull Run	3170	205	58.0	.0654	.0183
Sewage Treatment, Urban Drainage, Forest: Lower Bull Run/Yates Ford	11830	608	276.7	.0515	.0234
Agricultural Area: Cedar Run	9920	431	50.8	.0435	.00513
ocal Rail		,,,,			-
Agricultural, Forest, Light Residential:					
Occoquan below Lake Jackson	21800	703	67.9	.0322	.00311

Nitrate Flux

Although the mean daily nitrate loadings for the Occoquan subwatershed were lower than those of Bull Run, when examined on a daily basis, the reverse situation often obtains. As can be seen in Figure 10, the flux of nitrate in the Occoquan sub-basin is strongly influenced by precipitation. (See discharge records, Figures 13 and 14 in the Appendix.) This effect is typical of areas that are predominantly influenced by non-point nutrient sources such as land application of fertilizer. During the period of heavy rains in April, May, and the beginning of June, nitrate flux in the Occoquan sub-basin is seen to rise and fall sharply, whereas the Bull Run pattern is more uniform. Although Cub Run is smaller and therefore more flashy than Bull Run, its NO₃ flux is seen to be more stable than that of lower Bull Run at Yates Ford. This suggests that there may be significant influence of non-point nitrate source in the Bull Run subwatershed also.

The highest daily nitrate flux observed in the Occoquan sub-basin occurred in April during a period of heavy rain. The total discharge in the first week of April was greater than the estimated 9.8 billion gallons contained in the reservoir at normal pool level. Three more storms of similar intensity occurred during April, May, and the beginning of June. As the reservoir was unstratified during this season, it is reasonable to assume that this nitrate-rich water flowed through the reservoir uniformly, completely filling it at the end of the rainy season.

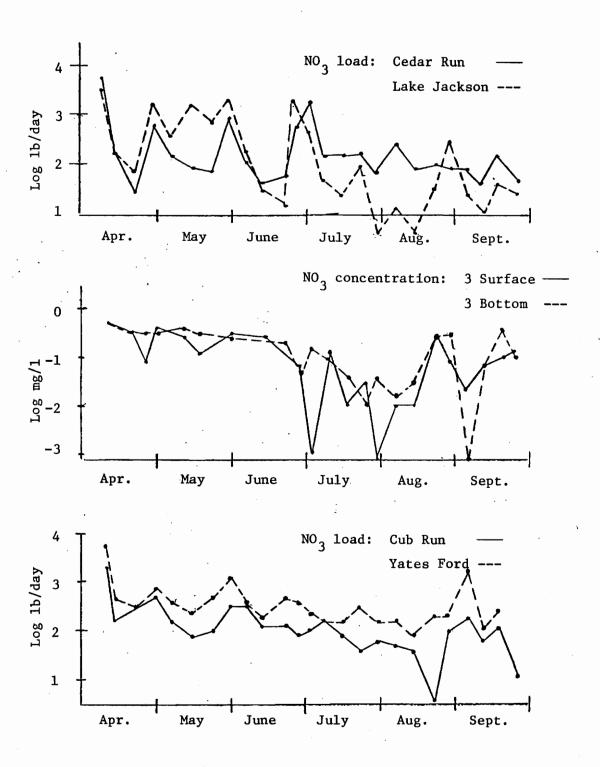


FIGURE 10: NITRATE FLUX IN OCCOQUAN SUBWATERSHED, BULL RUN SUBWATER-SHED, AND RESERVOIR STATION 3, 1973

The middle graph in Figure 10 confirms this assumption. One may note the sudden drop in nitrate concentration in the surface water of station 3 during the first week of July. This sudden disappearance of nitrate corresponds with the first significant algal bloom.

Considering the nitrate flux in Occoquan Creek, one may note that during spring nitrate nitrogen flux was always greater at the downstream station below Lake Jackson than that upstream at Cedar Run; however, during the summer there was an apparent loss of nitrate at the downstream station. This apparent loss of nitrate ensues as a result of stratification and high algal growth on Lake Jackson.

Nitrate is taken up by the algae present in the epilimnion of Lake Jackson leaving a nutrient—depleted water to flow off over the dam to Occoquan reservoir. This low nutrient water, however, typically contained large numbers of algae. Three distinct storms occurred during the summer. Only one of these was intense enough to increase the nitrate flux below Lake Jackson above the level observed at the upstream station. Two of these storm periods were followed by distinct periods of algal bloom at station 3.

This analysis probably overstates the extent to which Occoquan sub-basin influences bloom conditions on the reservoir. One may note that during the summer period, nitrate flux from Bull Run was typically higher than that from Occoquan. As flow rates were extremely low during the entire summer period, this high nitrate flux only caused extremely high productivity in the upper reaches of the reservoir. The lower reaches of the reservoir were typically free

of algal blooms and low in nutrient level until the occurrence of heavy rains in August.

Phosphate Flux

Examining Figure 11, one sees a pattern of phosphate loadings that closely resembles that for nitrate. The result is perhaps somewhat misleading as 90% of the phosphate observed in Bull Run was in the orthophosphate form whereas less than 30% of the phosphate observed in Occoquan Creek was in this quickly metabolizable form. Nevertheless, one can see that the Occoquan subwatershed contributes significant quantities of phosphate to the reservoir during periods of storm.

The middle graph of phosphate concentration at station 3 clearly indicates that during periods of stratification, in midsummer, phosphate concentration increases in the hypolimnion and remained low in the epilimnion. Whether this accumulation of soluble phosphates in the depths of the reservoir results from bottom release or from settling and degradation of algal material cannot be clearly decided. One may presume, however, that a significant portion of this hypolimnion phosphate originated in the epilimnion during the season of study.

The hypolimnion of the Occoquan reservoir has been estimated to contain as much as 5 billion gallons (see Figure 15 in the Appendix) below a depth of 10 feet. If the mean concentration of phosphate-phosphorus is approximately 0.150 mg/liter (a low estimate) it corresponds to a potential of approximately 6300 lbs of phosphate-

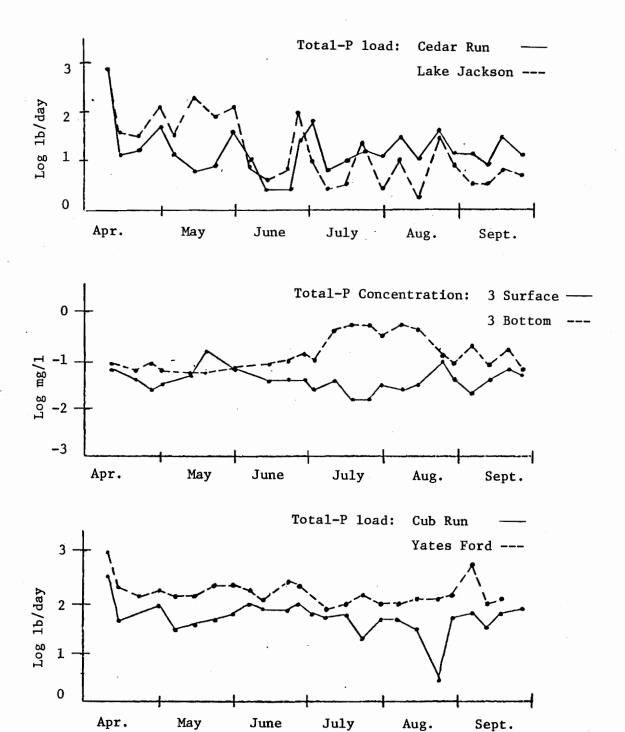


FIGURE 11: PHOSPHATE FLUX IN OCCOQUAN SUBWATERSHED, BULL RUN SUBWATERSHED, AND RESERVOIR STATION 3, 1973

phosphorus, which, if transported to the surface, would be sufficient to produce as much as 190 tons of *Anabaena*.

The preceding calculation is not very realistic, as it is impossible for the entire content of the hypolimnion to appear at once in the euphotic zone. The actual exchange of nutrient from hypolimnion to epilimnion during periods of stratification is difficult to estimate. Because concentration gradient between surface and bottom concentrations is very large, the possibility of exchange was considered an important parameter. The exchange of material between surface and bottom was estimated in the following way:

It was first assumed that the flow of nutrient material between surface and bottom would be analogous to the flow of heat, that is, amount exchanged would be proportional to the gradient observed.

$$\frac{dH}{dt} = k_T (T_B - T_S)$$

(2)
$$\frac{dN}{dt} = k_N (C_B - C_S)$$

where: dH = amount of heat

dN = amount of nutrient exchanged

dt = infinitesimal unit of time

k, = transfer coefficient for heat

k_N = transfer coefficient for nutrient

 C_{B} , C_{S} = concentrations of nutrient in bottom and surface

The two transfer coefficients are next considered to be characteristic of the system, both estimates of eddy diffusivity. Heat transfer was

then adjusted for unit volume and used as temperature in degrees

Centigrade, and amount of nutrient was also adjusted for unit volume

and represented as concentration. This gives the following solution

form:

(3)
$$\frac{\Delta T}{\Delta t} = k(T_B - T_S)$$

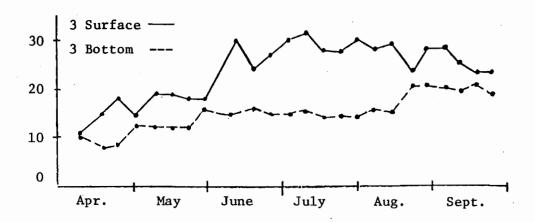
(4)
$$\frac{\Delta C}{\Delta t} = k(C_B - C_S)$$

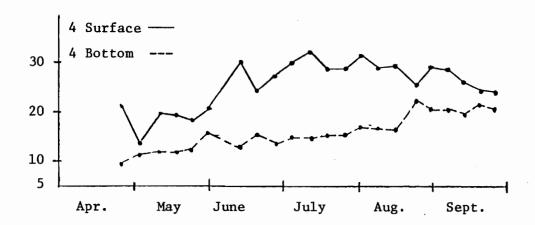
where: ΔT = average change in temperature in degrees Centigrade Δt = 1 day

 ΔC = average change in concentration in ppm

The values for T, T_B , and T_S were obtained from Figure 12. Temperature changes at station 5 were used to estimate the coefficient of eddy diffusivity, k, using equation 3. The value for k obtained indicated an exchange rate of $0.01~\rm day^{-1}$. This value for k was then substituted into equation 4, assuming a concentration gradient $(T_B - T_S)$ equal to 0.150 ppm. The result is a predicted exchange rate of 0.0015 ppm day $^{-1}$. This is an amount of phosphate that could allow production of only 0.075 mg/liter of Anabaena as predicted from AGP analysis. One can therefore assume that essentially no transfer of materials from hypolimnion to epilimnion occurs during periods of stable stratification.

The first really intense bluegreen algae bloom was noted on the main body of the reservoir at the beginning of September shortly after a period of heavy rain late in August that seemed to mix the entire





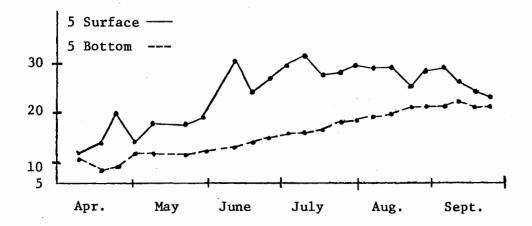


FIGURE 12: WEEKLY TEMPERATURE VARIATION OBSERVED AT STATIONS 3, 4, & 5

ON OCCOQUAN RESERVOIR, 1973

One may note in Figure 11 that surface and bottom concentrations of phosphate were approximately equal during the third week of August. The average change in surface phosphate concentration observed at stations 3, 4, and 5 was approximately 0.080 mg-P/liter above the previous week's value. This corresponds to approximately 3360 lbs of phosphate-phosphorus added to the epilimnion in one storm, assuming epilimnion volume equal to 5 billion gallons. During the storm a total phosphate flux including both Occoquan and Bull Run reached a maximum of 177 lbs-phosphate-phosphorus/day. If this loading is assumed to have occurred continually for the full three-day storm period, an estimated 351 lbs of phosphate-phosphorus added to the reservoir from tributaries. Therefore, one can see that the greatest effect of storms results from mixing the contents of the hypolimnion into the epilimnion and making them available for algal production. Since the mean daily loading of phosphate-phosphorus for the summer period, June through September, 1973, was 185 lbs per day. It took approximately 18 days to accumulate the amount of phosphorus which was suddenly mixed uniformly into the epilimnion. Naturally, bloom conditions soon followed.

VII. CONCLUSIONS

- The major sources of algal nutrient in the Occoquan Watershed are sewage treatment plants located in the Cub Run and Lower Bull Run drainage basins. These contribute extremely high loads of both nitrogen and phosphorus at a uniform rate throughout the spring and summer seasons.
- The Occoquan Creek sub-basin contributes large amounts of nitrate and lesser amounts of phosphate to the reservoir during the spring season. During summer, contribution of nitrates and phosphates from this region is negligible due to the influence of Lake Jackson.
- 3. Phosphates derived from the Bull Run basin are predominantly orthophosphate, whereas phosphates from the Occoquan sub-basin are predominantly forms other than orthophosphate and are therefore less readily metabolized.
- 4. Large quantities of phosphate are stored in the hypolimnion and released periodically to the epilimnion due to the influence of storm flow mixing.
- 5. The growth of green algae as represented by Selenastrum capricornutum is entirely dependent on nitrate concentration when orthophosphate concentration is greater than 0.010 mg-P/liter.
- 6. At low concentrations of phosphate, the production of nitrogenfixing bluegreens, as represented by Anabaena flos-aquae, is
 independent of nitrate concentration. However, at concentration

- of phosphate-phosphorus greater than 0.150 mg-P/liter, nitrate tends to stimulate the production of bluegreen algae.
- 7. The appearance of a significant non-point source of nitrates and phosphates in the lower Bull Run basin is suggested.
- 8. Occoquan reservoir is at present in a highly advanced state of eutrophication. The elimination of all sewage treatment plant effluent from the Bull Run drainage will significantly reduce the level of bluegreen algal growth by reducing the phosphate contribution. However, this will probably not eliminate the problem entirely, since significant amounts of phosphates from non-point sources will still be stored in the hypolimnion.

Considering the short flow-through time observed during storm periods, it seems likely that the first intense summer storm will release algal nutrients from the hypolimnion and create bloom conditions. However, due to the low level of base nutrient loading from non-point sources, the nutrient storage available in the hypolimnion may not be replenished during the summer. One may thus anticipate improvement in late summer and fall water quality conditions with only moderate usage of algicide.

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IX. APPENDIX

DATA FROM OCCOQUAN WATERSHED APRIL THROUGH SEPTEMBER, 1973

TABLE XV
SYNTHETIC ALGAL NUTRIENT MEDIUM

Macronutrien	ts		Micronutrients	
Compound	Concentration	(mg/1)	Compound	Concentration (mg/1)
NaNO ₃	25.500		H ₃ BO ₃	185.520
K2HPO4	1.044		MnC1 ₂	264.264
MgC1 ₂	5.700		ZnCl ₂	32.709
MgSO ₄ .7H ₂ O	14.700		CoC1 ₂	0.780
CaC1 ₂ .2H ₂ O	4.410		CuCl ₂	0.009
NaHCO ₃	15.000		Na ₂ MoO ₄ .2H ₂ O	7.260
3			FeC1 ₃	96.000
			Na ₂ EDTA.2H ₂ O	300.000

TABLE XVI

COMPLETE LIST OF SPECIES OBSERVED IN OCCOQUAN RESERVOIR

COMPLETE LIST OF	SPECIES	OBSERVED IN COCCOUNT RES	LIVOIR
	Station		Station
CYANOPHYTA		CHLOROPHYTA	1235
Anabaena affinis	1 2 3 5	Actinastrum sp.	Ŧ Ŧ Ŧ Ŧ
A. catenula	+	Ankistrodesmus sp.	++++
A. circinalis	+ +	Carteria sp.	+ ++
A. constricta	+ +	Chlamydomonas sp.	++++
A. spiroides	+++	Chlorella ellipsoida	++++
Anabaena sp.	++++	Chlorococcum humicola	+
Aphanizomenon flos-aquae	++++	Chlorogonium sp.	+
Aphanocapsa sp.	++++	Closterium sp.	+++
Coelosphaerium sp.	++ +	Coelastrum angustae	+ +
Gomphosphaeria sp.	+ +	C. microporum	++++
Merismopedia punctata	+++	Cosmarium sp.	+ ++
Microcystis seruginosa	++++	Dictyosphaerium sp.	+ ++
Oscillatoria sp.	++++	Eudorina elegans	++++
Trichodesmium lacustre	+	Conium pectorale	+++
		Kirchneriella subsolitaria	++++
EUGLENOPHYTA		Micractinium sp.	++++
Euglena elastica	++++	Microspora sp.	+
Euglena sp.	++++	Oocystis sp.	+ + + +
Lepocinclis sp.	++	Pandorina morum	++++
Phacus tortus	+	Pediastrum duplex	++++
Trachelomonas sp.	++,++	P. simplex	+ + + +
PYRRHOPHYTA		P. tetras	++++
Ceratium hirundinella	++++	Pediastrum sp.	++++
Peridinium sp.	+ +	Platydorina sp.	+ -
·		Pleodorina sp.	+
CRYPTOPHYTA		Protococcus sp.	+ ++
Cryptomonas erosa	++++	Radiococcus nimbatus	++++
CHRYSOPHYTA		Scenedesmus acuminatus	+ + +
Xanthophyceae		S. arcuatus	+ + •
Pseudotetraedron neglectum	+++	S. quadricouda	+ + + +
m 411 and a share as		Scenedesmus sp.	++++
Bacillariophyceae		Selcnastrum capricornutum	+++
Achnanthes sp.	+	Sphaerocystia achroeteri	++++
Amphora sp.	++	Staurastrum chaetoceras	+
Asterionella formosa	+++	S. tetracerum	+ -
Cocconeis sp.	, , ,	Staurastrum sp	++++
Cyclotella sp.	+ + +	Ulothrix sp.	. +
Cymbella ventricosa	+++	Volvox tertius	++++
Eunotia sp.	+		
Fragilaria ap.	++++		
Comphonema parvulum	++		
Cyrosigma sp.	++		
Helosira granulata	++++		
M. varians Helosira sp.	## 		
Heridion circulare	* * * *		
Navicula sp.			
Nitzschia sp.	T T		
Pinnuluria ap.	4 T T		
Rhoicosphania curvata	4.		
Stephenodiscus sp.	` _		
Surirella sp.	. .		
Synedra sp.	T T		
Бупеста вр. Tabellaria sp.	-		
rancitating ab.	7 T		
Chrysophyceae			
Dinobryon sp.	++		
Mallomonas sp.	+++		

Synura lapponica

TABLE XVII
BULL RUN AT CATHARPIN

Bull Run Sub-Watershed

Parameter	Range - mg/1	Mean - mg/1
Orthophosphate Phosphorus	0.0 - 0.135	0.017
Non-Orthophosphate Phosphorus	0.0 - 0.155	0.026
Total Phosphate Phosphorus	0.010 - 0.290	0.044
Nitrate Nitrogen	0.100 - 0.592	0.278
Nitrite Nitrogen	0.0 - 0.013	0.002
Ammonia Nitrogen	0.0 - 0.158	0.037
Total Kjeldahl Nitrogen	0.037 - 0.648	0.198
Dissolved Oxygen	6.4 - 12.0	9.33
рН	6.1 - 8.3	7.34
Alkalinity*	23.0 - 59.5	38.81
Temperature	8.0 - 27.0	20.28
Anabaena AGP Yield	0.3 - 19.9	10.07
Anabaena Growth Rate	0.249 - 1.672**	1.159**
Selenastrum AGP Yield	3.1 - 8.8	5.10
Selenastrum Growth Rate	0.123 - 2.215**	1.026**

^{*}Expressed as CaCO₃
**Day

TABLE XVIII

CUB RUN NEAR BULL RUN

Bull Run Sub-Watershed

Parameter	Range - mg/l	Mean - mg/1
Orthophosphate Phosphorus	0.050 - 1.830	0.886
Non-Orthophosphate Phosphorus	0.0 - 0.250	0.086
Total Phosphate Phosphorus	0.090 - 1.900	0.973
Nitrate Nitrogen	0.296 - 4.615	1.725
Nitrite Nitrogen	0.009 - 0.250	0.089
Ammonia Nitrogen	0.011 - 0.350	0.107
Total Kjeldahl Nitrogen	0.110 - 0.517	0.310
Dissolved Oxygen	6.7 - 10.6	8.33
рН	6.4 - 8.2	7.31
Alkalinity*	24.0 - 58.0	41.74
Temperature	8.5 - 27.0	20.50
Anabaena AGP Yield	66.7 -549.3	298.20
Anabaena Growth Rate	1.197 - 2.226**	1.805**
Selenastrum AGP Yield	46.8 -116.5	86.45
Selenastrum Growth Rate	1.097 - 2.334**	1.636**

^{*}Expressed as CaCO₃
**Day

TABLE XIX
BULL RUN AT YATES FORD
Bull Run Sub-Watershed

Parameter	Range - mg/1	Mean - mg/l
Orthophosphate Phosphorus	0.066 - 1.350	0.450
Non-Orthophosphate Phosphorus	0.0 - 0.140	0.040
Total Phosphate Phosphorus	0.110 - 1.380	0.492
Nitrate Nitrogen	0.470 - 1.650	0.796
Nitrite Nitrogen	0.010 - 0.190	0.090
Ammonia Nitrogen	0.082 - 0.591	0.277
Total Kjeldahl Nitrogen	0.270 - 1.550	0.597
Dissolved Oxygen	5.4 - 11.5	7.22
рН	6.2 - 8.1	7.27
Alkalinity*	22.0 - 68.0	41.88
Temperature	9.0 - 27.0	20.52
Anabaena AGP Yield	42.2 -256.2	144.12
Anabaena Growth Rate	1.125 - 2.349**	1.892**
Selenastrum AGP Yield	33.1 - 62.7	50.62
Selenastrum Growth Rate	1.246 - 2.199**	1.668**

^{*}Expressed as CaCO₃
**Day

TABLE XX
OCCOQUAN RESERVOIR STATION 1
Below Confluence of Bull Run & Occoquan

Parameter	Range - mg/1	Mean - mg/l
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.030 - 0.280 0.062 - 0.290 0.048 - 0.660 0.0 - 0.062 0.0 - 0.444 0.036 - 0.665 5.9 - 14.1 6.8 - 9.5 18.0 - 43.0 10.0 - 31.0	0.108 0.037 0.147 0.290 0.017 0.129 0.331 11.59 7.98 32.20 22.89
Anabaena AGP Yield	29.1 -100.6	49.65
Anabaena Growth Rate	0.649 - 2.010**	1.381**
Selenastrum AGP Yield	4.2 - 48.4	24.75
Selenastrum Growth Rate	0.218 - 1.796**	1.214**
Bottom		
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.055 - 0.375 0.0 - 0.135 0.060 - 0.420 0.050 - 0.896 0.0 - 0.112 0.260 - 1.950 0.589 - 2.595 0.1 - 10.0 6.8 - 8.5 18.0 - 63.0 9.0 - 23.5	0.170 0.027 0.200 0.426 0.031 0.806 1.239 2.70 7.17 37.88 17.79
Anabaena AGP Yield	22.3 -139.2	71.67
Anabaena Growth Rate	0.934 - 1.907**	1.478**
Selenastrum AGP Yield	17.6 - 54.5	34.68
Selenastrum Growth Rate	0.681 - 1.983**	1.478**

^{*}Expressed as CaCO₃

TABLE XXI CEDAR RUN

Occoquan Creek Watershed

Parameter	Range - mg/l	Mean - mg/l
Orthophosphate Phosphorus	0.0 0.060	0.013
Non-Orthophosphate Phosphorus	0.005 - 0.086	0.028
Total Phosphate Phosphorus	0.015 - 0.090	0.041
Nitrate Nitrogen	0.062 - 1.958	0.476
Nitrite Nitrogen	0.0 - 0.102	0.010
Ammonia Nitrogen	0.0 - 0.362	0.077
Total Kjeldahl Nitrogen	0.049 - 0.645	0.249
Dissolved Oxygen	5.6 - 10.9	7.63
pH	6.1 - 8.2	7.28
Alkalinity*	21.0 - 50.0	36.10
Temperature	9.0 - 26.5	20.72
Anabaena AGP Yield	4.1 - 54.9	15.07
Anabaena Growth Rate	0.605 - 1.692**	1.130**
Selenastrum AGP Yield	2.8 - 7.0	5.65
Selenastrum Growth Rate	0.205 - 1.714**	0.936

^{*}Expressed as CaCO₃
**Day

TABLE XXII
OCCOQUAN CREEK BELOW LAKE JACKSON
Occoquan Creek Watershed

Range - mg/1	Mean - mg/l
0.0 - 0.050	0.010
0.005 - 0.060	0.020
0.010 - 0.085	0.030
0.040 - 1.958	0.313
0.0 - 0.102	0.008
0.0 - 0.392	0.084
0.056 - 0.576	0.267
7.3 - 11.2	8.62
6.0 - 8.4	7.33
19.0 - 43.5	32.12
10.0 - 28.0	22.42
3.1 - 23.2	12.76
0.330 - 1.988**	1.233**
2.4 - 8.1	5.59
0.197 - 1.824**	0.946**
	0.0 - 0.050 0.005 - 0.060 0.010 - 0.085 0.040 - 1.958 0.0 - 0.102 0.0 - 0.392 0.056 - 0.576 7.3 - 11.2 6.0 - 8.4 19.0 - 43.5 10.0 - 28.0 3.1 - 23.2 0.330 - 1.988**

^{*}Expressed as CaCO₃
**Day

TABLE XXIII OCCOQUAN RESERVOIR STATION 2 Below Confluence of Bull Run & Occoquan

Surface

Parameter	Range - mg/1	Mean - mg/l
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.0 - 0.130 0.0 - 0.120 0.015 - 0.135 0.0 - 0.656 0.0 - 0.024 0.0 - 0.350 0.054 - 0.450 5.2 - 14.2 6.3 - 9.5 17.5 - 40.0 10.5 - 32.5	0.018 0.023 0.041 0.148 0.003 0.077 0.244 9.90 7.70 29.57 23.60
Anabaena AGP Yeild	1.2 - 31.9	16.57
Anabaena Growth Rate	0.448 - 1.985**	1.250**
Selenastrum AGP Yield	2.0 - 22.2	7.84
Selenastrum Growth Rate	0.279 - 1.558**	1.041**
Bottom		
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.0 - 0.185 0.0 - 0.115 0.019 - 0.220 0.026 - 0.618 0.0 - 0.040 0.118 - 2.525 0.329 - 5.760 0.0 - 10.7 6.3 - 7.6 19.0 - 86.5 10.0 - 21.5	0.059 0.028 0.088 0.199 0.150 1.133 1.727 2.82 6.93 47.00 15.69
Anabaena AGP Yield	0.7 - 39.9	17.01
Anabaena Growth Rate	0.114 - 1.968**	1.207**
Selenastrum AGP Yield	1.9 - 27.9	11.00
Selenastrum Growth Rate	0.344 - 1.811**	1.194**

^{*}Expressed as CaCO3

TABLE XXIV OCCOQUAN RESERVOIR STATION 3

Below Confluence of Bull Run & Occoquan

Parameter	Range - mg/1	Mean - mg/1
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.0 - 0.060 0.0 - 0.145 0.015 - 0.150 0.0 - 0.431 0.0 - 0.027 0.0 - 0.376 0.087 - 0.695 5.7 - 13.2 6.4 - 9.4 16.0 - 37.0 11.0 - 31.5	0.018 0.027 0.046 0.130 0.005 0.060 0.232 10.46 7.91 30.46 23.65
Anabaena AGP Yield Anabaena Growth Rate	0.0 - 49.5 0.340 - 2.249**	21.68 1.203**
Selenastrum AGP Yield Selenastrum Growth Rate	3.7 - 17.8 0.214 - 1.797**	9.48 1.104**
Bottom		
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.025 - 0.465 0.0 - 0.140 0.055 - 0.540 0.0 - 0.454 0.0 - 0.046 0.104 - 2.230 0.430 - 3.100 0.1 - 9.4 6.3 - 7.8 16.0 - 68.0 8.0 - 20.5	0.157 0.031 0.193 0.188 0.007 1.037 1.363 2.58 7.05 39.62 15.06
Anabaena AGP Yield Anabaena Growth Rate	5.1 - 74.6 0.489 - 2.123**	31.06 1.469**
Selenastrum AGP Yield Selenastrum Growth Rate	11.3 - 24.6 0.507 - 2.070**	16.90 1.399**

^{*}Expressed as CaCO₃

TABLE XXV

OCCOQUAN RESERVOIR STATION 4

Below Confluence of Bull Run & Occoquan

Pamameter	Range - mg/1	Mean - mg/l
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.0 - 0.050 0.0 - 0.185 0.019 - 0.190 0.0 - 0.368 0.0 - 0.012 0.0 - 0.330 0.036 - 0.553 3.5 - 13.0 6.9 - 9.3 15.0 - 40.0 13.5 - 32.0	0.013 0.028 0.047 0.073 0.002 0.047 0.222 9.85 8.05 32.42 25.22
Anabaena AGP Yield	0.0 - 45.8	20.81
Anabaena Growth Rate	0.708 - 1.652**	1.130**
Selenastrum AGP Yield	3.3 - 19.7	9.70
Selenastrum Growth Rate	0.317 - 1.521**	1.088**
Bottom		
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.030 - 0.400 0.0 - 0.100 0.040 - 0.455 0.0 - 0.332 0.0 - 0.018 0.370 - 1.550 0.512 - 2.087 0.0 - 8.0 6.5 - 8.1 14.0 - 54.5 9.0 - 22.0	0.137 0.024 0.169 0.090 0.006 0.919 1.127 2.15 7.13 39.20 15.68
Anabaena AGP Yield	5.2 - 73.9	33.62
Anabaena Growth Rate	0.935 - 2.573**	1.350**
Selenastrum AGP Yield	9.6 - 20.3	16.65
Selenastrum Growth Rate	0.933 - 1.557**	1.136**

^{*}Expressed as CaCO₃

TABLE XXVI OCCOQUAN RESERVOIR STATION 5

Below Confluence of Bull Run & Occoquan

Parameter	Range - mg/1	Mean - mg/1
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.0 - 0.060 0.0 - 0.145 0.015 - 0.155 0.0 - 0.374 0.0 - 0.011 0.0 - 0.500 0.036 - 0.630 4.2 - 13.2 6.5 - 9.3 13.0 - 37.0 12.0 - 31.5	0.014 0.025 0.041 0.099 0.002 0.077 0.565 9.83 7.39 29.92 24.15
Anabaena AGP Yield	0.0 - 36.2	9.24
Anabaena Growth Rate	0.042 - 3.057**	1.257**
Selenastrum AGP Yield	2.8 - 18.9	7.01
Selenastrum Growth Rate	0.120 - 1.708**	0.898**
Bottom		
Orthophosphate Phosphorus Non-Orthophosphate Phosphorus Total Phosphate Phosphorus Nitrate Nitrogen Nitrite Nitrogen Ammonia Nitrogen Total Kjeldahl Nitrogen Dissolved Oxygen pH Alkalinity* Temperature	0.013 - 0.190 0.0 - 0.050 0.035 - 0.190 0.0 - 0.336 0.0 - 0.024 0.0 - 1.820 0.397 - 2.195 0.1 - 9.5 6.5 - 8.1 12.0 - 51.5 8.5 - 22.0	0.072 0.016 0.091 0.158 0.006 0.671 0.993 2.15 7.13 33.04 15.75
Anabaena AGP Yield	1.8 - 87.8	33.62
Anabaena Growth Rate	0.559 - 2.476**	1.350**
Selenastrum AGP Yield	5.1 - 32.5	16.65
Selenastrum Growth Rate	0.318 - 1.510**	1.136**

^{*}Expressed as CaCO₃

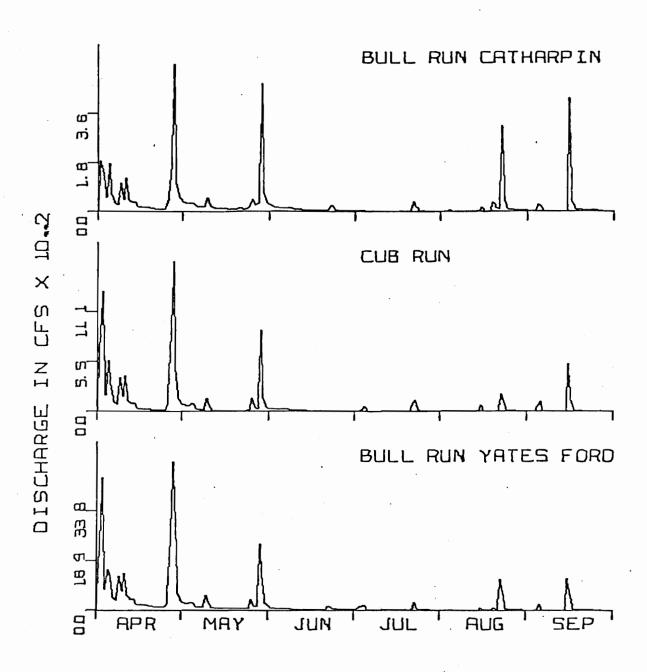
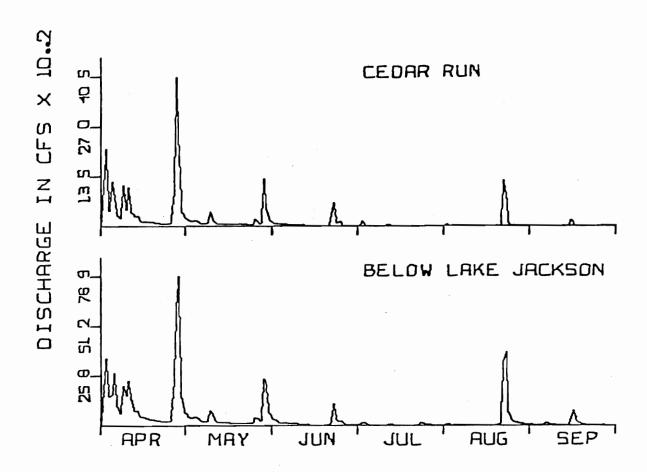


FIGURE 13: DISCHARGE RECORD FOR BULL RUN SUBWATERSHED



· FIGURE 14: DISCHARGE RECORD FOR OCCOQUAN SUBWATERSHED

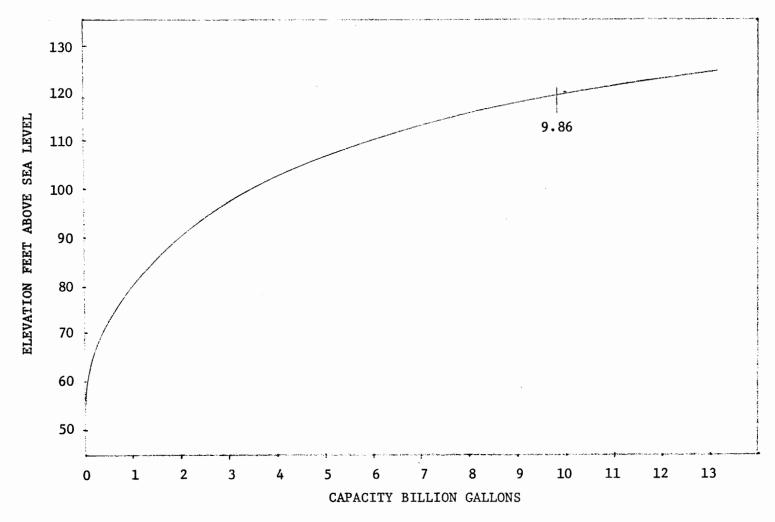


FIGURE 15: CAPACITY CURVE FOR THE OCCOQUAN RESERVOIR
(FAIRFAX COUNTY WATER AUTHORITY 1975)

VITA

The author, Michael Smolen, was born August 7, 1944, son of Rose and Daniel Smolen. He graduated from Derby High School, Derby, Connecticut, in 1962; received his B.S. in Biology with minor in Chemistry, at the University of Rochester, 1967; and his M.S. in Botany with minor in Biochemistry, from the University of Tennessee, 1970.

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ALGAL NUTRIENTS: SOURCES AND PATTERNS OF FLOW IN THE OCCOQUAN WATERSHED

by

Michael Smolen

(ABSTRACT)

The movement of algal nutrients was studied in the Occoquan Watershed. During the period April through September 1973, weekly water samples collected by the Occoquan Monitoring Laboratory were split for chemical analysis and algal growth potential (AGP) assay.

Variation in AGP observed between stations indicated that the major source of algal nutrients was sewage treatment plants in the Bull Run portion of the Occoquan Watershed. Chemical analyses, likewise, indicated that the Bull Run waters were highest in phosphate and nitrate concentration.

Both phosphate and nitrate concentrations were found to correlate highly with AGP for green and bluegreen test species. For the green alga Selenastrum capricornutum, the chemical nutrient most highly correlated with AGP was nitrate, whereas for the bluegreen alga Anabaena flos-aquae, total phosphate gave the highest correlation coefficient. A graphic analysis of AGP versus nitrate-nitrogen concentration indicated that Selenastrum yield does not increase with nitrate concentration when orthophosphate concentration is less than 0.010 mg-P/liter. At concentrations greater than this, however, nitrate appears to control the Selenastrum AGP. A similar analysis of Anabaena AGP versus nitrate-nitrogen indicated that nitrate

concentration does not affect *Anabaena* AGP unless the total phosphate concentration is greater than 0.150 mg-P/liter. At total phosphate concentration greater than 0.250 mg-P/liter a definite stimulation of *Anabaena* yield due to nitrate concentration was observed.

The weekly changes in chemical nutrient flux were examined through spring and summer seasons. The nitrate and phosphate loadings of Bull Run, which receives sewage treatment effluent, were found to be high and relatively constant throughout spring and summer. The agricultural region of Occoquan Creek, however, showed high nitrate loadings only during periods of stormflow. At baseflow, Occoquan Creek nutrient loadings were found to be insignificant. A decrease in nutrient content of the Occoquan Creek tributary was observed during summer due to the influence of a small recreational lake in Occoquan Creek upstream from the reservoir.

Finally, analysis of material and energy transfer between water strata in the stratified reservoir indicated that hypolimnetic nutrients do not enter the epilimnion at a rate sufficient to cause algal bloom conditions. Storm events were observed to cause mixing of the reservoir and so provided the nutrient concentration necessary for algal blooms.

It is suggested that removal of phosphates from all sewage treatment effluent in the Occoquan Watershed will reduce the frequency of bluegreen algal blooms and allow more effective control with copper sulfate.