ALGAE CONTROL USING HALOGENS AND COPPER SULFATE AS ALGICIDES

by

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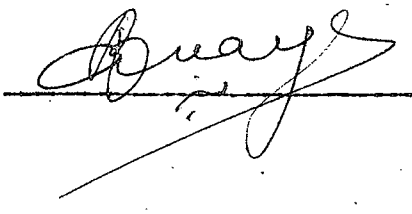
SIGNED: 

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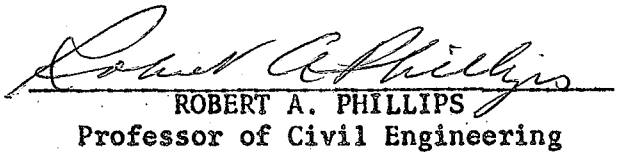

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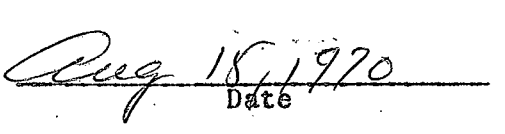

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ABSTRACT

Concentrations of chlorine and copper sulphate ranging from 0.3 to 3.0 mg/l were added, separately or combined, to pure cultures of chlorella pyrenoidosa. Using a haemacytometer the number of algae which survived the effects of these algicides for up to 25 hours were counted. It was noted that a combined concentration of 0.3 mg/l chlorine and 1.0 mg/l copper sulphate had the highest rate of kill.

Since this mixture of chlorine and copper sulphate solutions caused rapid algal death, it was selected and used in computing the amount of chemical required to treat the 9,500 million gallon Impounding Reservoir at Weija, Accra. To ensure that the additional cost of treating this reservoir is reasonable, the price per thousand gallons for obtaining 120 million gallons from the Weija and Kpong Reservoirs was computed. This was found to be 33 cents per 1,000 gallons excluding operating and maintenance costs. Using 33 cents per thousand gallons as the water rate, the imputed benefits for the Accra-Tema Metropolitan Municipal Water Supplies would be $1,290 million.

The computation of the above water rate was based upon the grand total construction costs for the Weija and Kpong sources of water supply and the cost of treating the algae bloom at the Weija Impounding Reservoir. The operating and maintenance costs which consist of the following items should be added to the above costs if the recommended rate of charge of 58 cents per thousand gallons is to be obtained:

1. Power cost for the various components of the water supply scheme.
2. Cost of chemicals used in water treatment plants.
3. Salaries and wages of all operating and maintenance staff.
4. Maintenance charges and costs of out-of-ordinary repair work.
5. Administration costs.

CHAPTER 1

INTRODUCTION

General Discussion

The control of the algae of lakes, ponds, reservoirs, and to a lesser extent of streams is an important and often vexing problem in water quality management. Among the nuisances created by the often sudden blooming of one or more algal genera are: nauseous odors and tastes, fish kills, interference with stock watering, shortened filter runs in water purification plants, growths in pipes and other water conduits, and interference with industrial water uses. Ordinarily, consumers complain when the concentration of odor-producing organisms lies above 500 to 1,000 areal standard units per 400 μ^2. However, some organisms become objectionable in much smaller concentrations. The bitter taste of Synura, for instance, becomes objectionable when the mere presence of this organism can be detected.

It is, therefore, not surprising that the Ghana Water and Sewerage Corporation is very much concerned about algae blooms in the Weija Impounding Reservoir. Tahal (Water Planning) Limited in a report submitted to the government of Ghana on the feasibility study for Water and Sewerage Works in the Accra-Tema Metropolitan Area confirmed this problem.

An extract from this report [1] reads as follows:

> Algae blooms continue to develop despite the application of copper sulphate to the extent of about 0.5 ppm (1965). This dose may be insufficient. The dose had recently been increased to 1.0 ppm in an attempt to see whether any improvement results. If not, other methods of combined treatments will have to be applied. (p. 34)

It is apparent from the above statement that it is not enough that the water plants deliver quantities commensurate with needs. The Ghana Water and Sewerage Corporation is, therefore, morally and lawfully bound to deliver water which will meet the standards of health and industry.

Purpose and Scope

Faced with the problem of algae blooms in most of the impounding reservoirs of the water supply systems in Ghana, the writer had deemed it necessary to investigate the possibility of using chlorine and copper sulphate, separately or combined, as algicides to control algae blooms. To the best of the knowledge of the writer, no attempt has been made as yet to control algae by using a mixture of chlorine and copper sulphate as algicides.

The absence of actual samples from the Weija Impounding Reservoir has produced an investigation of the various genera and species of algae responsible for algae bloom. As a result the investigation was limited to the effect of algicides on one specific alga, namely, Chlorella pyrenoidosa which is a unicellular green alga.

In an attempt to grow and control the activity of living organisms under laboratory conditions, it should be remembered that

natural environmental conditions cannot be duplicated. However, there is a wide range of conditions under which organisms normally will react. Since the object of this exercise is to apply laboratory results to field conditions, it was deemed necessary to carry out the investigation under the same general environmental factors such as temperature, light, pH, etc., as obtained in the Weija Impounding Reservoir.

The aim of the present study, therefore, is to compare the actions of chlorine and copper sulphate and determine the concentrations needed, separately or combined, for inhibition of growth or killing of Chlorella pyrenoidosa

From the results of this investigation the quantity, in pounds, of the most effective algicide required to control the algae blooms of the 9,500 million gallon Weija Impounding Reservoir could then be computed and the method to be used in the treatment of the impounding reservoir recommended.

Knowing the grand construction costs for the Weija and Kpong sources of water supply, the price per million gallons of the 120 mgd emanating from these sources could be found and the imputed benefits for the Accra-Tema Metropolitan Water Supplies calculated.

Environmental Factors Affecting the Growth of Algae

It will be noted from Table I--River Densu Raw Water Analysis--that the pH during the wet and dry seasons is 7.1 and 8.1 respectively. The annual maximum and minimum pH is 7.1 and 6.9 respectively, Table II. The wet and dry season temperatures are 24°C and 28°C, respectively.

Table I. River Densu Raw Water Analysis (1969)

Component	Unit	Season					
		Wet Season Depth (ft.)			Dry Season Depth (ft.)		
		0	6	12	0	6	12
Apparent Color		100	98	100	70	60	40
True Color		65	70	68	45	25	17
Turbidity	J.C.V.	38	32	36	32	31.0	33
pH		7.1	7.1	7.1	8.1	8.1	8.1
Carbon Dioxide	mg/l	14	10	13	5	3.0	5
Oxygen Abs. in 3 hours	mg/l	7.9	7.9	8.2	3.0	2.0	2.6
Nitrite Nitrogen	mg/l	Trace	Trace	Nil	0.01	0.001	0.002
Nitrate Nitrogen	mg/l	1.13	0.68	0.68	0.23	0.05	0.7
Albuminoid Nitrogen	mg/l	2.2	3.07	0.93	1.9	1.6	2.4
Ammonia Nitrogen	mg/l	0.23	1.0	0.92	2.4	2.2	2.2
Total Alkalinity	mg/l as C_oCO_3	80	84	80	118	-	-
Total Hardness	mg/l	62	54	54	158	150	154
Calcium Hardness	mg/l	44	33	33	92	80	83
Total Manganese	mg/l	Nil	Trace	Trace	Nil	Nil	Trace
Total Iron	mg/l	2.4	2.0	1.8	1.7	0.9	0.7
Sulphate	mg/l	20.3	19.3	21.1	21.2	13	14.5
Chloride	mg/l	30	29	31	41.0	39	40
Silica	mg/l	26.5	26.0	26.0	28.0	26.0	66.0

Table II. Weija Water Works Accra, Ghana Monthly Chemical Doses and pH of Water (1965)

Chemical Description mg/l	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual Max.	Annual Min.
Aluminium Sulphate	73.55	70.86	73.94	96.3	104.0	100.2	100.4	81.74	70.4	76.05	73.66	67.6	104.0	67.6
Hydrated Lime & Buxton Lime	24.8	19.2	21.1	24.6	7.0	11.6	9.6	6.0	10.7	21.8	20.0	10.1	24.8	6.0
Ammonium Sulphate	1.26	1.32	1.51	1.71	0.32	1.93	1.9	1.2	1.05	-	-	-	1.93	0.32
Bleaching Powder & Chlorine Gas	1.63	1.66	1.67	1.90	1.92	1.95	1.95	1.74	1.80	2.13	2.34	1.93	2.34	1.63
Copper Sulphate	0.38	0.45	0.41	0.33	0.31	0.35	0.34	0.10	0.93	-	1.90	0.95	1.90	0.10
pH of Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raw Water	7.1	7.1	7.1	7.0	7.1	6.9	6.9	7.0	6.9	7.1	6.9	6.9	7.1	6.9
Settled Water	6.5	6.3	6.5	6.3	6.3	6.1	6.3	6.4	6.3	6.5	6.3	6.3	6.5	6.1
Treated Water	8.1	8.0	8.1	8.1	8.2	8.1	8.2	8.2	8.1	8.2	8.2	8.1	8.2	8.0

Source of Information: Water Works Monthly Reports.

Since pH, temperature and light are the main environmental factors affecting the growth of algae, the investigation was carried out under the above workable conditions.

The foregoing discussion will enable the reader to appreciate the reasons why the environmental factors affecting the growth of algae should be taken into account in the control of algae blooms.

The successful introduction of algae into new localities by streams, birds and winds depends upon their being transferred to a suitable habitat. Water is essential for growth of the alga in the new location, but there are many other factors, any one of which may prevent its growth. Chiefly among these are light, temperature, and the chemical composition and pH of the water. Attempts to evaluate the effects of these various factors by biologists, have been based upon two general methods of investigation: (1) growth of algae in pure culture, and (2) chemical and physical studies of various types of habitat.

The results obtained by either method are so inconclusive and contradictory that one can make only very broad generalizations concerning the effects of the various factors [2].

Light

Light is an essential for photosynthesis, but algae differ markedly in respect to their tolerance of light intensity. Some of the aerial algae, such as terrestial species of Vaucheria, are indifferent to the intensity of light; other aerial algae, particularly many Myxophyceae, grow only in shaded habitats. Aqualic algae of unshaded pools are usually considered as growing in full sunlight, but the more

deeply submerged are not so strongly illuminated as those at the surface, because there is a geometrical decrease in intensity of illumination with an arithmetic increase in depth. Despite this, many algae show a greater rate of photosynthesis at a certain depth than they do at the surface.

There is a qualitative penetration of light in water and a greater absorption at the red end of the spectrum. However, the extent to which the various rays penetrate the water is affected by the color of the water, its turbidity, and the amount of dissolved salts.

Temperature

Temperature rarely plays a direct role in the acclimatization of algae in new localities, but it has a very important effect in its acceleration or retardation of growth and reproduction. Under exceptional conditions, as in thermal algae and cryovegetation, the temperature of the habitat restricts the algal population to certain species. A few algae, such as Hydrurus, are found in very cold waters. In most cases, temperature is not a factor determining the nature of algal flora, since most species are able to develop if other conditions are favorable.

Temperature is probably the limiting factor for the algae restricted to tropical and subtropical regions. This is shown by the known cases where tropical algae have been introduced into colder regions and where they developed luxuriantly the first summer but were unable to withstand winter in the colder climate.

Inorganic Compounds

The elements essential for growth of the algae are the same as those necessary for the growth of higher plants. Calcium and silicon are essential at times for the growth of algae and diatoms, respectively.

The marked differences in the algal flora of various regions are directly correlated with the amount of calcium. The algae of soft-water regions (calciphobes) are largely desmids and certain species of Chrysophyceae, Myxophyceae, and Chlorophyceae. These algae are rarely found in the calciphilic flora of hard-water regions. The distinction between calciphilic and calciphobic algae rests more upon the pH value of the water than upon the amount of calcium since it has been shown that the bottom and littoral flora of lakes in limestone regions may have a rich calciphobic element if suitable conditions of acidity prevail. The pH value of the water has been shown to be a limiting factor for many algae, especially species of desmids, but these observations must be repeated for many localities before one can make generalizations upon the distribution of particular species.

Calcium and magnesium are also of importance in their influence upon the total number of algae present, because their bicarbonates furnish a supplemental supply of carbon dioxide for photosynthesis. The greater abundance of algae in hard-water lakes as compared with their abundance in soft-water lakes, is traceable directly to a utilization of dissolved bicarbonates in photosynthesis.

Laboratory experiments have shown that small quantities of many elements are toxic to algae, but iron is the only one of these that

is of importance in nature. Most algae grow best when the Fe_2O_3 content of the water is 0.2 to 2.0 mg/ℓ, and there is a distinct toxic effect when the amount of available iron is over 5 mg. Many natural waters have a total iron content of more than 5 mg/ℓ, but these waters are not toxic because of the buffer action of organic compounds or of calcium salts.

Many of the higher plants show a decided preference for nitrates as the source of nitrogen. A majority of the algae grow equally well when their nitrogen is obtained from nitrates, nitrites, or ammonium compounds.

Organic compounds dissolved in the water affect the nature of the algal flora, but the problem is so complex that it is impossible to determine their effect upon the flora. This is especially true of peaty moors and swampy areas where characteristic flora is undoubtedly due in considerable part to the dissolved organic materials.

Weija Impounding Reservoir

At this juncture, one might be interested to know how the storage capacity of the Weija Impounding Reservoir [1] was determined. The following computations are quite explanatory.

Storage Capacity

The following components were taken into account in computing the required gross storage capacity Vg of the Weija Impounding Reservoir [1]:

1. Vn = Net storage for supply.
2. Vl = Evaporation and seepage losses.
3. Vd = Dead storage, silting and bed load.

Net Storage for Supply

The reservoir should be able to supply 40 mgd during a dry season of a maximum duration of 6 consecutive months (182 days) in a year.

Then,

$$Vn = 182 \text{ days} \times 40 \text{ mgd} = 7{,}280 \text{ mil/gal}$$

say $$Vn = 7{,}500 \text{ mil/gal.}$$

Evaporation and Seepage Losses

It was assumed that the losses would amount to an average of 6 inches per month.

1. Average reservoir area is 2.9 square miles.
2. One inch per square mile is equivalent to 14.3 mil/gal.
3. Vl = 6 x 14.3 x 2.9 x 6 = 1,500 mil/gal.

Dead Storage Silting and Bed Load

The required gross storage capacity amounts to:

$$Vg = Vn + V = Vd = 7{,}500 + 1{,}500 + 500 = 9{,}500 \text{ mil/gal}$$

It will be noted from the above computation that a net capacity of 7,500 mil/gal. is required to meet a demand of 40 mgd during a critical dry year with a recurrence frequency of lone in 100 years. The corresponding gross capacity of 9,500 mil/gal. allowing for dead

storage, silting and evaporation would be obtained by raising the normal water level in the existing reservoir to an elevation of 38 feet. The area capacity curve for the dam site is shown in Figure 1.

The surface area of the Weija Impounding Reservoir at normal water level of 38 feet will be 4,000 acres (Figure 2).

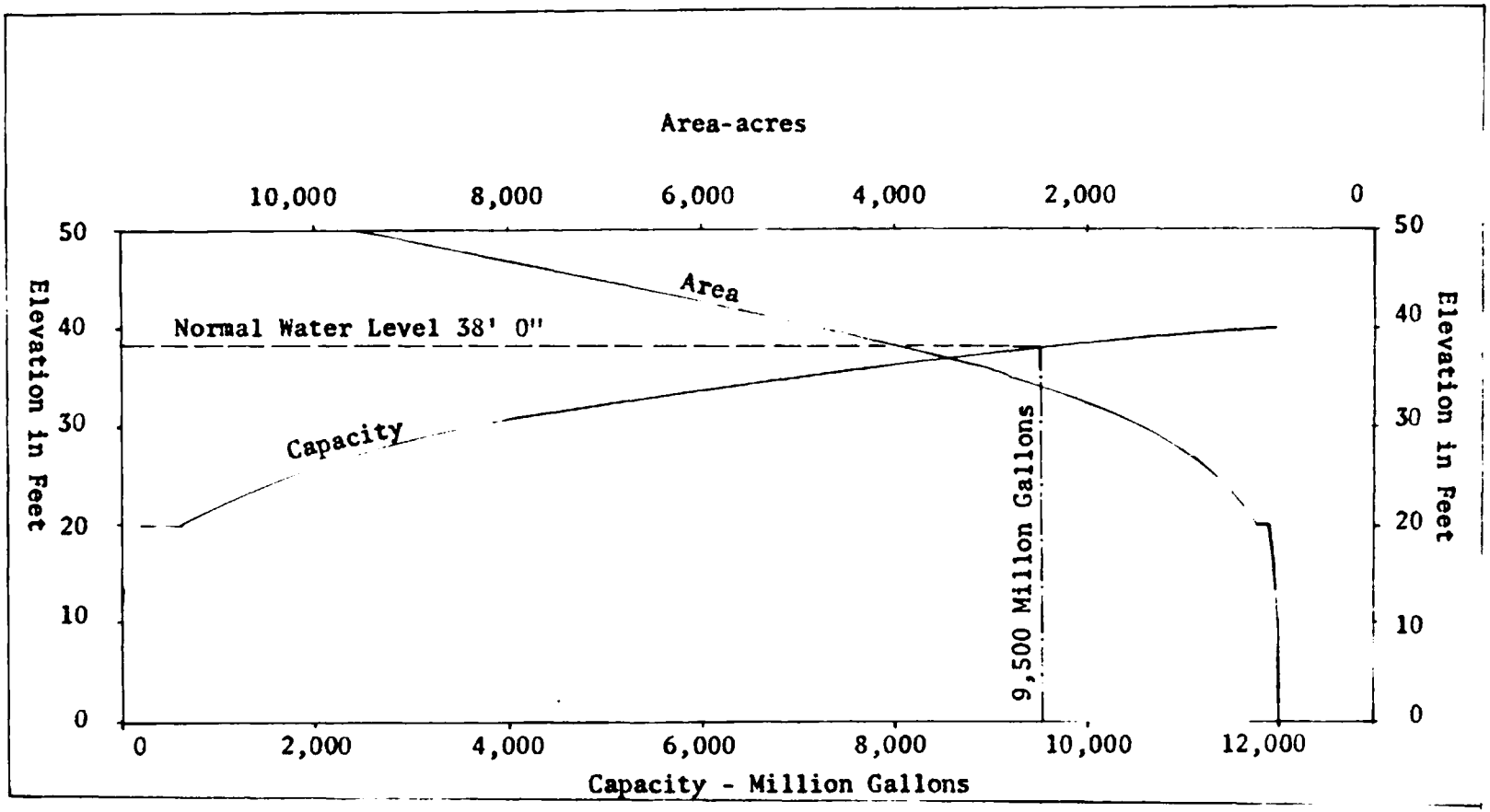


Figure 1. Area-Capacity Curves

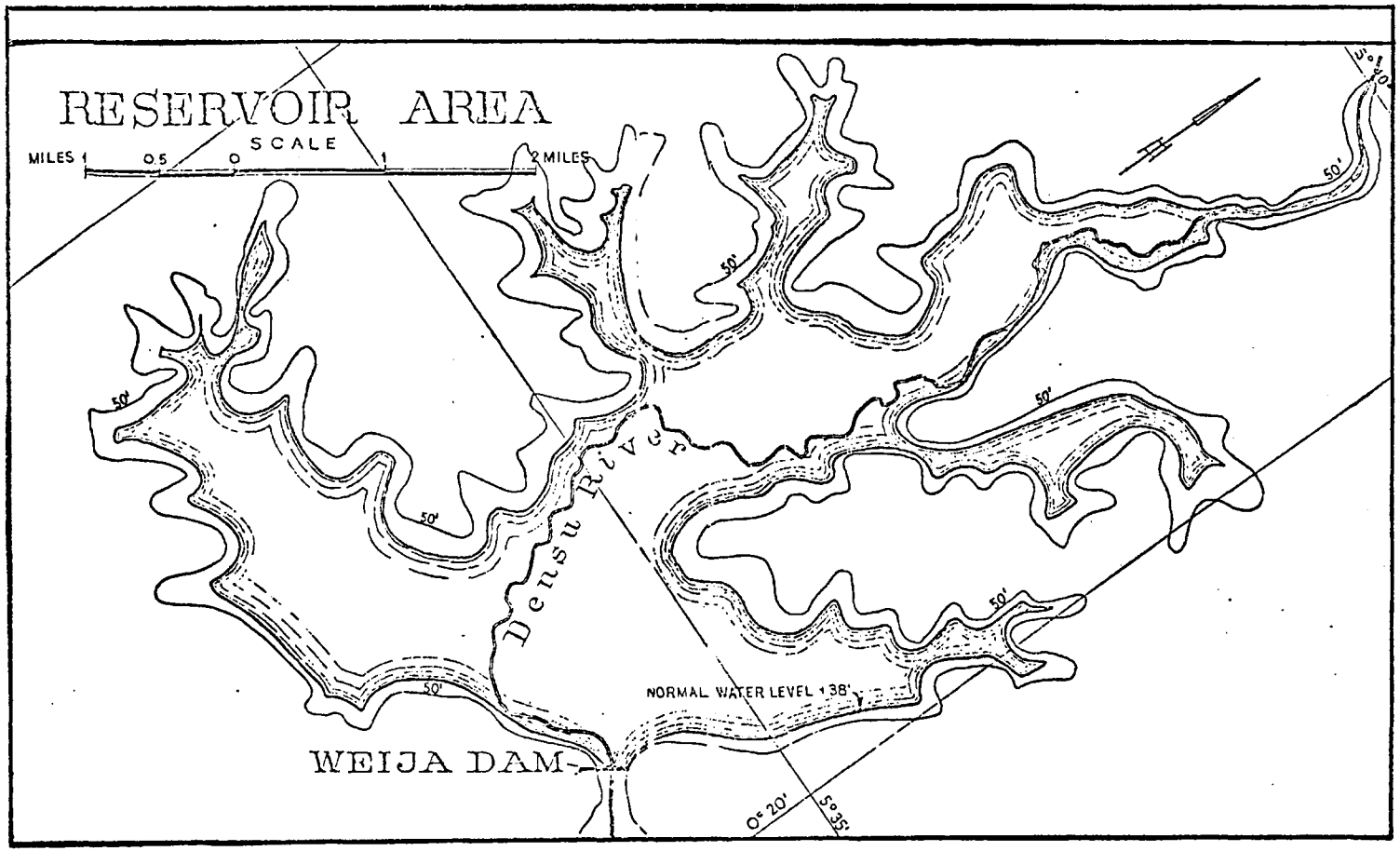


Figure 2. Reservoir Area

CHAPTER 2

REVIEW OF LITERATURE

Effect of Halogens on Algae-Chlorella Sorokiniana

The halogens (chlorine, bromine and iodine) have been investigated as bactericidal by various workers. For more than 60 years, chlorine has been most frequently used. The great majority of data available led to the use of the general concept of disinfection as a synonym for chlorination.

Although bactericidal effects of bromine and iodine seemed to be promising in water technology, it does not seem likely that they will replace the widespread practice of chlorination. This postulation can be supported in spite of the fact that chlorination practice all over the world has shown that many times chlorine, being unstable, does not affect planktonic algae, nor filamentous strains growing in open channels or swimming pools.

The most comprehensive study made to date concerning the effect on algae of bromine and chlorine separately or combined is that of Kutt and Edlis [4]. In their investigation, Kutt and Edlis demonstrated that bromine concentrations of 0.2 to 1.0 mg/l reduced the algal concentration of Chlorella sorokiniana from 16 to 80 percent respectively within 2 to 4 hours. However, regrowth rapidly occurred following the initial kill, indicating that no residuals remained to control further growth.

Regrowth was prevented on a second set of experiments by additional bromine dosages at 4 to 5 hour intervals. This also resulted in a further decline in numbers of cells following each dosage.

Chlorine at a concentration of 0.4 mg/l produced similar results with regrowth occurring unless further additions were made. A second addition of chlorine caused no additional kill.

It was demonstrated that an effective algal kill was achieved only temporarily with one initial dose of mixed halogens--0.25 mg/l chlorine and 0.75 mg/l bromine. For a given total concentration there was no difference seen in action of different ratios of bromine to chlorine. However, a higher kill was achieved at higher dosages.

All the above mentioned experiments were performed in the light. Since algal metabolism changes in the dark, the action of halogens on Chlorella in the dark was also investigated by Kutt and Edlis.

Table III summarizes results obtained in experiments in the absence of light. It may be seen that a better algal kill was achieved by using chlorine and a lower percentage of algal kill was recorded by using bromine. No similar comparison under otherwise identical conditions was made between chlorine and bromine in the light.

In this study, it was found that the addition of various higher concentrations of bromine caused increasing algicidic effects lasting as long as low concentrations of bromine were still present in the water, after which slow growth began. It is estimated that new growth occurred between 5 and 8 hours after the experiment started; and another addition of halogen caused total algal death. The chlorine action has

Table III. Effect of Halogens on Chlorella in Dark Conditions

Halogen Introduced	Conc. (mg/l)	Kill (%)
Chlorine	0.2	16.9
	0.2*	26.8
	0.6	40.0
	0.6*	43.0
Bromine	0.2	6.7
	0.2*	17.8
	0.6	24.0
	0.6*	28.5

* Halogen concentration checked and readjusted after 8 hours of contact time.

Experimental conditions: Initial number of algae: 225/mm^3; Volume of experimental vessel: 1 liter; Temperature: 30°C; Contact time: 20 hours.

been shown to be algistatic in nature and an additional concentration of chlorine added to the system did not cause any acceleration in Chlorella kill.

It was found in this investigation that one addition of a mixture of halogens to the algal culture caused rapid and total death. This effect was much more pronounced than when any one of the halogens was used.

Rate of Algal Kill

It has been shown that the application of an algicide to a sample of Chlorella pyrenoidosa is in essence a disinfection of the sample. Chick's Formula for disinfection of bacterium will, therefore, be used in determing the rate of algal kill. Chick's investigation of disinfection revealed that the factors that determine the efficiency of disinfection are the nature of the disinfection agent, the concentration of this agent and the time allowed for its action, the number of organisms present and the temperature.

Rate of Disinfection

The rate at which disinfection proceeds with time under ideal conditions, namely, constant concentration of disinfecting agent, constant temperature, and the presence of a single species of bacterium of which all individuals are equally susceptible to the agent, is given by Chick's Law [5],

$$\log \frac{N}{No} = -rt$$

where No and N represent the numbers of organisms present at the beginning and after t minutes, respectively, and r is a constant which measures the rapidity of the kill. It is not surprising to find that few experimental studies conform exactly to Chick's Law.

Generally, the rate of kill decreases as time goes on--this reflects the survival of the more resistant individuals, the decline in concentration of the active agent, and a number of other interfering factors. More complicated formulae have been devised to express results of individual studies but their applicability is limited.

The important point to be made is that the killing of organisms by disinfecting agents follows a logarithmic law--that it is the ratio of the numbers of bacteria present before and after disinfection that is significant, not the numbers themselves.

The terminology "time required to kill 50 percent, or 90 percent of the organisms present" has a real significance, and the total number of organisms is of less importance.

Concentration of Disinfectant

An examination of data from many sources shows that the time required to effect a constant percentage of kill of bacteria or other organisms by means of any disinfectant is dependent on the concentration of the disinfectant in the following manner:

$$C^n t = \text{constant}$$

where C represents the concentration of disinfectant, t represents the time required to kill a selected percentage of the organisms, and n is

a numerical value which depends on the disinfectant, the organism, and the temperature. This equation is purely empirical and has as yet no theoretical justification [6]. The constant n is a positive number, and its numerical value ranges from 0.75 to 2.00 with 1.00 as the more common value. It is a measure of the change of killing efficiency of the disinfectant with a change of concentration.

Temperature

The effect of increased temperature on disinfection is to increase the kill; the rate of increase follows the law which governs the rate of increase of all chemical reactions with temperature, namely [5]

$$\log \frac{t_1}{t_2} = \frac{E\ (T_1 - T_2)}{4.575\ (T_1 T_2)}$$

where the t values represent times required for equal percentages of kill at the absolute temperature T_1 and T_2, and E is a constant characteristic of the disinfectant and organisms.

This is a cumbersome expression and can be simplified by taking the temperature interval as 10°C. The right-hand side of the equation becomes nearly constant and the ratio t_1/t_2, called Q_{10}, represents the relative increase in disinfection rate for a 10° increase in temperature.

For example, if $Q_{10} = 2.00$, the rate is doubled for every 10° rise. Some values of Q_{10} for E. coli, computed by Fair, Geyer, and Okun [7] from the work of Butterfield are given in Table 4.

Table IV. Values of Q_{10} for E. Coli

Aqueous Chlorine		Chloramines	
pH	Q_{10}	pH	Q_{10}
7.0	1.65	7.0	2.08
8.5	1.42	8.5	2.28
9.8	2.13	9.5	3.35
10.7	2.50		

Effect of Copper Sulphate on Algae

McKee and Wolf [8] have extensively reviewed the literature covering the effect of copper sulphate on aquatic life. The following pertinent statements are taken from that reference:

> Because of variable toxicity of copper to living organisms, the killing concentrations of copper sulphate reported by independent workers vary over a wide range. (p. 42)

For example:

1. Concentrations from 0.01 to 20 mg/l of copper sulphate have been used to control different aquatic fauna, from insect larvae to snails.

2. Concentrations from 0.05 to 10.0 mg/l have been effective agains different kinds of algae, protozoa and weeds.

The concentration of copper sulphate required to control plankton in water supply reservoirs varies from as little as 0.05 or 0.10 mg/l for some organisms to as much as 12 mg/l for very resistant species. Most algae and protozoa succumb to dosages of 1.0 mg/l or less. Duckweed and pondweed were controlled by 0.6 to 1.0 mg/l of $CuSO_4.5H_2O$, but a few cattails and tules survived this residual concentration.

The following rates of application of copper sulphate have been recommended to control specific types of algae in farm ponds:

Type	mg/l
Anabaena	0.09
Beggiatoa	5.0
Chara	0.2 - 5.0
Cladophora	1.0
Cladothrix	0.2
Conferva	0.4
Navicula	0.07
Oscillatoria	0.1 - 0.4
Scenedesmus	5.0 - 10.0
Spirogyra	0.05 - 0.3
Ulothrix	0.2
Volvox	0.25

The amount of copper sulphate required to control algae varies with the temperature, increasing about 2.5 percent for each degree below 15°C.

Tests were conducted with 30 different cultures of algae to determine the concentration of $CuSO_4.5H_2O$ required for control.

a. None of the species was controlled by 0.25 mg/l of the salt

b. Thirteen percent were controlled by 0.50 mg/l

c. Thirty-seven percent by 1.00 mg/l, and

d. Fifty-three percent by 2.00 mg/l."

CHAPTER 3

METHODS OF CULTURE AND GENERAL PROCEDURE

Algal Culture

Test Organism

The geometric shape of cells of Chlorella vary from small and spherical to broadly ellipsoidal. They have a single parietal chloroplast that is usually cup-shaped but may be a curved band. Pyrenoids are usually lacking.

The only method of reproduction is by means of autospores, 2, 4, 8 or 16 of which are formed within a cell and are liberated by rupture of the parent-cell wall.

Media

Trace Solution. Holm-Hansen and Lewen trace element solution [9] was prepared by weighing the salts listed below in the amount indicated and dissolving in one liter of distilled water,

H_3BO_3	2.85g	$CuCl_2.2H_2O$	0.027g
$MnCl_2.4H_2O$	1.80g	$ZnCl_2$	0.021g
FeSO4	1.36g	$C_oCl_2.6H_2O$	0.040g
Sodium tartrate	1.77g	$Na_2M_oO_4.2H_2O$	0.025g

Distilled water 1000 ml.

Bold's Defined Medium. Bold's Defined Medium [10] was prepared from six stock solutions, 400 ml in volume, each containing one of the following salts in the amount indicated.

$NaNO_3$	10g
$CaCl_2$	1g
$MgSO_4 \cdot 7H_2O$	3g
K_2HPO_4	3g
KH_2PO_4	7g
NaCl	1g

Ten ml of each stock solution was added to 940 ml of distilled water along with 1 percent $FeCl_3$ and 1 ml of Trace Solution. By means of a pipet, 100 ml of the prepared Bold's Defined Medium were transferred to each of fifty 250 ml Erlenmeyer flasks. The mixtures were then autoclaved for 20 minutes at 15 psig. on a slow-exhaust cycle.

Growth of Culture

Fresh agar cultures were prepared as follows:

1. A generous quantity of green inoculum was transferred with a sterile bacteriological loop into sterile water contained in a test tube. The transfer was repeated several times until the color turned light green.
2. Using a sterilized pipet containing cotton wool, a pipet full of inoculum was transferred into each flask containing 100 ml of Bold's Defined Medium.

3. The cultures were then placed on an orbital shaker and maintained and handled in a lighted constant temperature room. It must be noted that as long as cultures receive light of 200-400 ft-c intensity and are maintained at a temperature of 20-25°C, they should exhibit normal growth and activity. Cool white standard fluorescent tubes provided a suitable light source when placed within 8-10 inches of a single flask. The lighting system was controlled by a clock device.
4. After at least one month of culturing the Chlorella, sufficient cells were obtained for the investigation of the effect of algicides on Chlorella pyrenoidosa.

Algae Counting

A haemacytometer was used for counting the Chlorella cells [11]. The cells were counted in the four corner squares and the central square of the central large square of each of the two counting chambers.

A typical pattern of counting the algae is shown in the following diagram:

52		49	= 213 cells
	41		
27		44	
30		53	= 212 cells
	61		
33		35	

Average = 213 cells

Total = 213 x 50 where 50 is the combined conversion factor to convert to total count per mm^3.

$$= 10{,}650 \frac{\text{cells}}{mm^3}$$

CHAPTER 4

EXPERIMENTAL PROCEDURE AND DISCUSSION OF RESULTS

Experimental Procedure

For a test run, six culture flasks were selected from the shaker and diluted to 200 ml with distilled water and the pH was adjusted to pH 7 with phosphate buffer. Five of the flasks were dosed with soldium hypochlorite solution to give a chlorine content of 0.3 mg/l. Copper sulphate was added to give concentrations of 0.3, 0.5, 0.8, 1.0 and 3.0 mg/l. The sixth flask was used as a control without added chemicals.

At intervals of about 0, 1, 2, 3, 10, 15 and 25 hour samples were withdrawn from each flask and the viable cell count made, using the haemacytometer. Healthy green cells were considered viable. Dead cells appeared devoid of chlorophyll.

This general procedure was repeated for the second and third sets of samples using chlorine alone and copper sulphate alone, respectively. The disinfection temperature in all cased was 23°C.

Results

The detailed results obtained for the various concentrations of algicide on Chlorella are tabulated and compiled in the Appendix.

It was evident from these data that cell reductions occurred only within about the first three hours following chemical addition.

After this time, cell growth rapidly took place at a rate about equal to that of the control. This overall effect is consistent with the observations of Kutt and Edlis [4].

Adequate comparisons in the algicidal effects of the various dosages were therefore, only evident for the 3 hour period of observed cell destruction. Data for this period were selected and calculated on the basis of the surviving organisms at any time as a percent of the initial number. The log of the percent survivors was then plotted against the square of the time in hours. This was done in order to obtain a straighter line than can be obtained assuming strict adherence to a first-order relationship as described by Chick's Law.

Figure 3 shows the results for disinfection at 23°C using a constant chlorine dosage of 0.3 mg/l and varying copper sulphate concentrations from 0.3 to 3 mg/l.

It can be seen that although there are inconsistencies in the data, there appears to be increased cell destruction as the concentration of copper sulphate is increased from 0.3 to 3.0 mg/l along with a constant chlorine dosage of 0.3 mg/l.

Data for the effect of copper sulphate were similarly treated and are shown in Figure 4. Quite wide variations occur with these data and it appears that there is no particular effect for additions of copper sulphate up to 3 mg/l. Variations are probably within the experimental error.

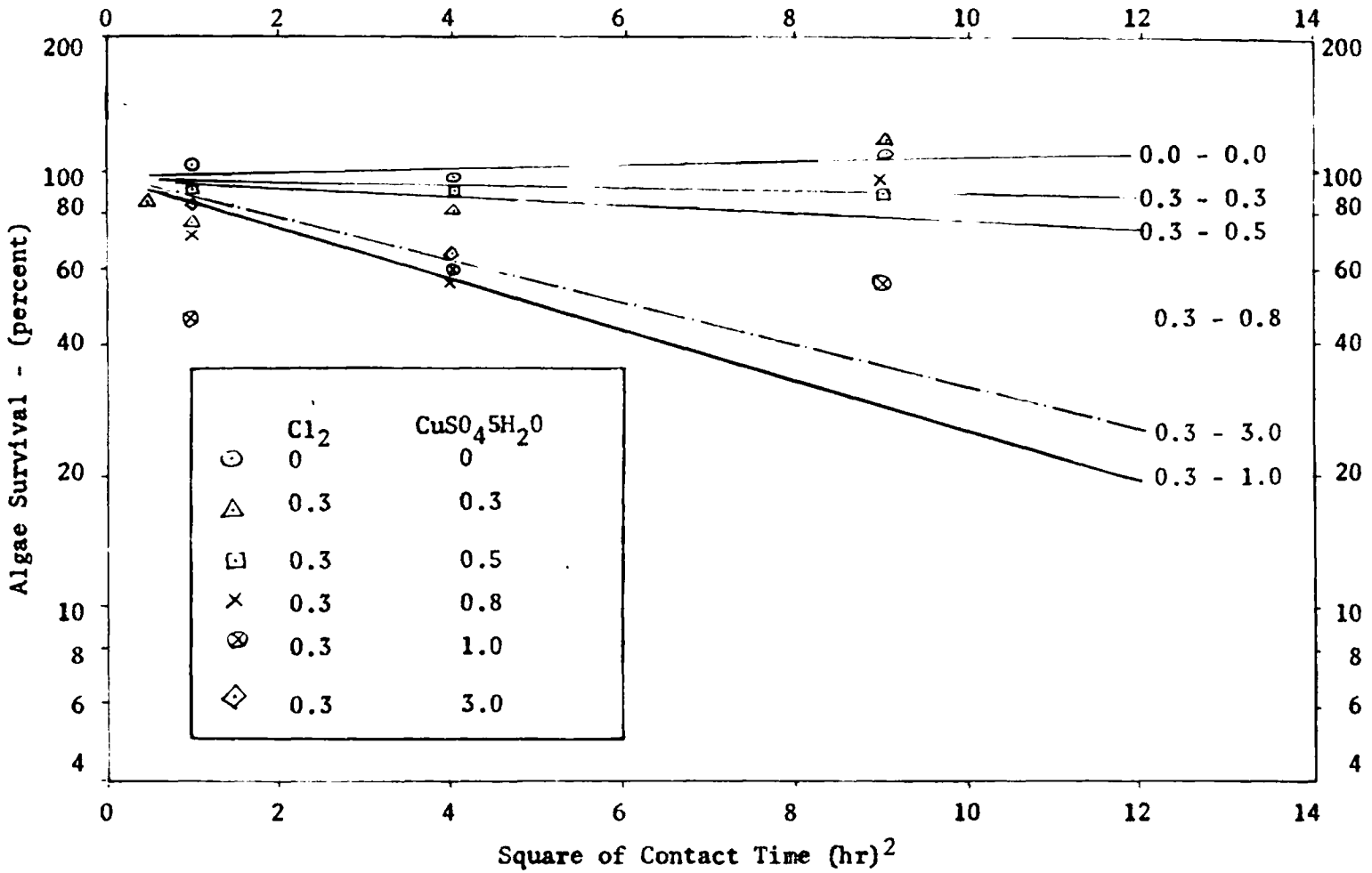


Figure 3. Effect of Various Concentrations of Chlorine and Copper Sulphate Mixture on the Growth of Chlorella - 23°C

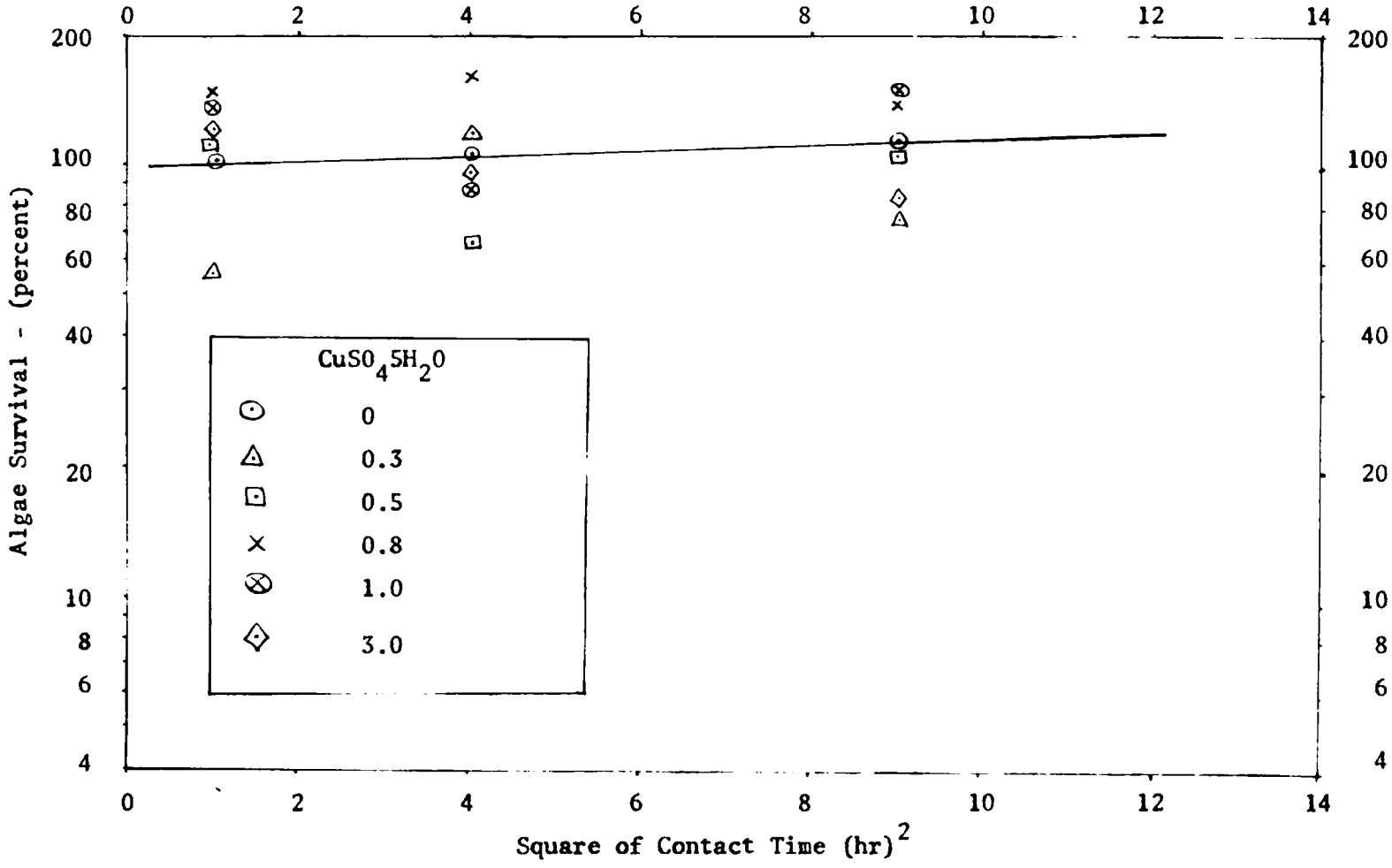


Figure 4. Effect of Various Concentrations of Copper Sulphate on Growth of Chlorella - 23°C

Data for additions of chlorine alone at concentrations up to 3.0 mg/l are shown in Figure 5. Again, there appears to be no particular cell destruction within the chlorine range used.

Discussion

It is apparent from the data that concentrations of chlorine alone of up to 3 mg/l and copper sulphate alone of up to 3.0 mg/l will not produce a destruction of cells of Chlorella pyrenoidosa. However, a combination of these two chemicals using 0.3 mg/l of available chlorine and with increasing dosage of copper sulphate up to 3.0 mg/l produced a destruction of Chlorella in the first three hours following disinfection. This destruction was only a temporary effect, however, and is followed thereafter by a cell increase at about the same rate as an untreated sample.

In treating the Weija Impounding Reservoir, it is assumed that only the first two feet of depth would be considered in the calculation of total dosage. From the area-capacity curves for this reservoir, it can be calculated that there are about 1500 million gallons contained in the first two feet of depth.

If calcium hypochlorite were used which contains 70 percent available chlorine, it can be calculated that for a 0.3 mg/l dosage, 5,370 pounds of calcium hypochlorite will be required. Similarly, for 1 mg/l of copper sulphate (as the pentahydrate), 17,900 pounds will be needed.

A difficulty arises in calculating chemical costs for these treatments when considering their application for the Weija Reservoir

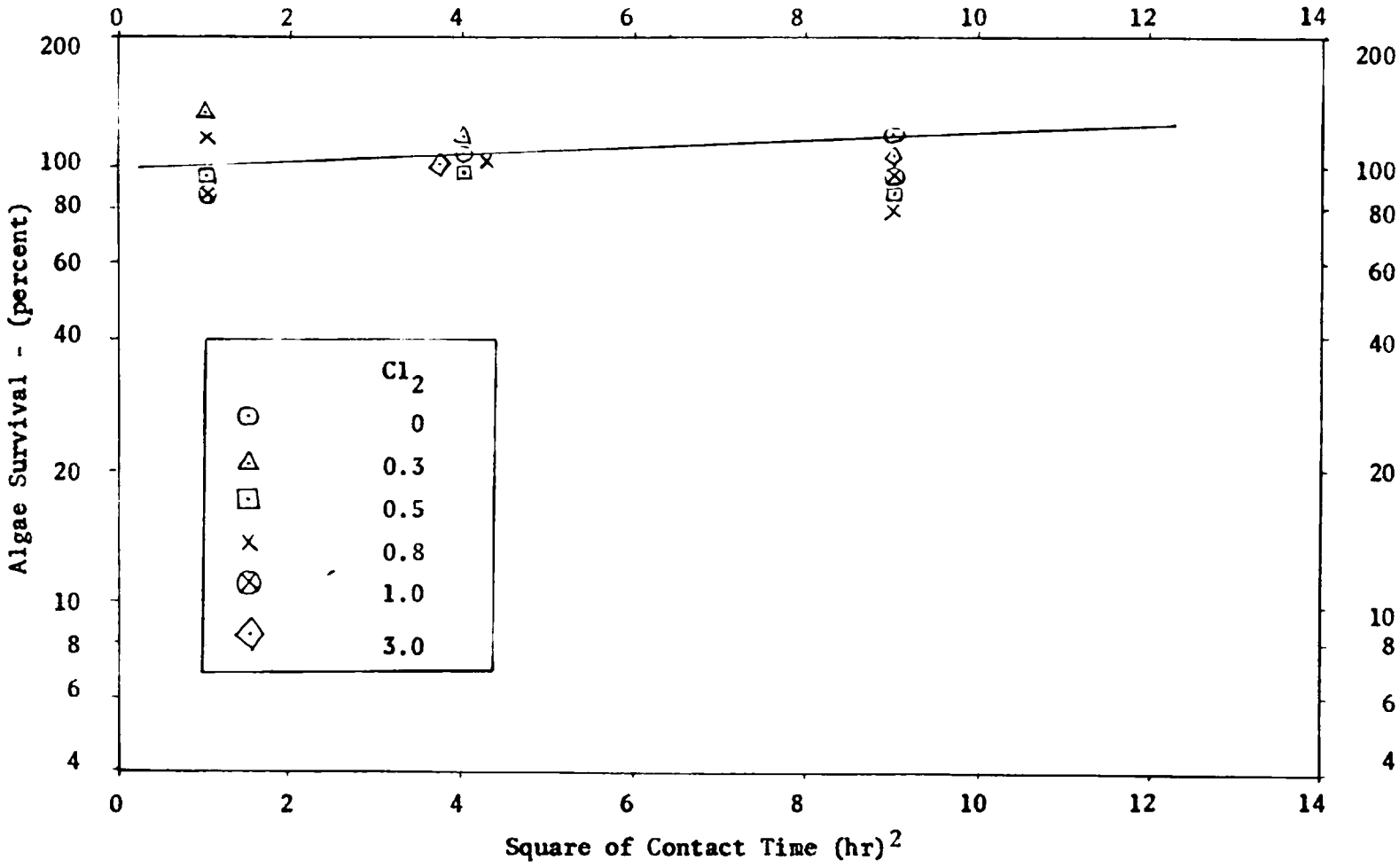


Figure 5. Effect of Various Concentrations of Chlorine on Growth of Chlorella - 23°C

because of the uncertainty as to their availability in a large quantity in Ghana, and added costs of transportation, etc.

For purposes of calculation, it might be assumed that $ .50 per pound would be adequate estimates for each of these chemicals. Certainly, it would be no greater than this. This, then, would give chemical costs per treatment of $2,685 for chlorine and $8,950 for copper sulphate or a total cost of $11,635 for a single weekly treatment.

CHAPTER 5

IMPUTED BENEFITS OF MUNICIPAL WATER SUPPLY

Introduction

In designed water resource systems, the difficult problem of assigning benefits to a municipal water supply is usually handled by *ad hoc* methods, or it may even be completely by-passed. One device for assignment of benefits in common use--that of equating benefit to the cost of the least expensive alternative supply--not frequently underestimates the benefits as measured by the criterion of "willingness to pay." In some project planning, water demand is treated as a constraint. That is, a demand for water is predicated which must be met without consideration of water needs for other purposes.

The model of this chapter shows that in a multipurpose project in which at least one of the other purposes has (or is assigned) monetary benefits, then the predication of a stated level of municipal water demand, is tantamount to the predication of an imputed benefit of the water is equal to the increase in benefits accruing to the other water uses that would occur if the municipal water requirement were reduced by one unit and this unit routed (optionally) to the other uses. It is of interest to compare the imputed benefits thus computed with prices charged for domestic water in comparable situations in adjacent regions. Also it may be of interest to compare the imputed benefit with the cost of the least expensive alternative supply--if such a

supply exists. Comparisons of this sort may serve as a guide of judgement of planners in assessing whether or not the constraining water requirement has been set at a reasonable and justifiable level. Such assessment may be particularly useful in designs where the water requirement has been projected for many years into the future. In designs in which the imputed water supply benefit appears to be excessively large, maintaining the requirement at the level set may result in an uneconomic pre-emption of water from other uses.

On the other hand, if planners are not in a position to attach an independent rational estimate of benefits to uses such as water supply, water may in some cases be pre-empted for other uses in multi-purpose projects to which explicit benefits have been assigned. This could happen if the water supply requirement had been set too low. Such pre-emption may be undesirable if benefits assigned to some of the other purposes are not directly market-connected, but have been set more or less arbitrarily. If it were possible to assign a benefit figure to municipal water use rather than treating the municipal need as a constraint, the design of the most efficient system might be quite different. In this situation calculation of the imputed benefit may again provide a guid to judgement.

Model

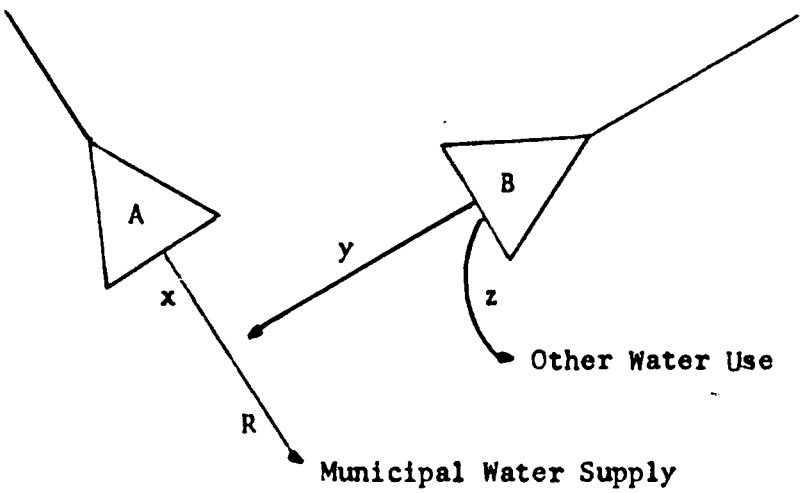
The following simple model of a water resource systems involves two reservoirs and two uses. One use is for domestic water supply for which no monetary benefit function is available but a water demand is

specified; the other use is one for which there is a direct market value. The problem is:

a. To determine the optimal design of the system to maximize economic efficiency subject to the constraint of the water supply requirement, and

b. To compute the imputed benefits.

The solution is obtained using the Lagrange method of undetermined multipliers [12]. The two reservoirs are shown in the accompanying sketch together with outflow vectors. The outflow from Reservoir A can only be used for meeting the water supply requirement. The outflow from Reservoir B can be routed to either or both uses. All flows are



assumed to be non-stochastic. The following cost and benefit functions are assumed.

The cost function for Reservoir A is $C_1 = a + x^2$ dollars, where x is the safe yield for this reservoir. The cost function for Reservoir B is $C_2 = b + (y + z)^2/2$ where y is the portion of outflow from B routed

to the municipal supply, and z is the portion routed to the other use. The benefit function for the other use is $B = pz$ where p is the price per unit of flow. The price is assumed to be independent of flow. The municipal water requirement is:

$$R = x + y$$

Optimum Design: Both Uses

The Lagrangian function to be maximized is:

$$L = B(z) - C_1(x) - C_2(y + z) + k(x + y - R) = 0 \qquad (1)$$

where k is the Lagrangian undetermined multiplier.

By setting

$$\frac{dL}{dx} = \frac{dL}{dy} = \frac{dL}{dz} = 0$$

it is found that

$$\frac{dB}{dz} = \frac{dC}{dx} = \frac{dC_2}{dy} = \frac{dC_2}{dz} = k = p \qquad (2)$$

and for the optimum design

$$x = P/2,\ y = R - P/2,\ z = (3/2)P - R \qquad (3)$$

The net benefit will be

$$N = P[(3/2)P - R] - [a + (P/2)^2] - [b + P^2/2] = (3/4)P^2 - PR - (a + b) \qquad (4)$$

Optimum Design: Water Supply Only

The design objective is to meet the requirement with minimum cost,

$$Ct = C_1(x) + C_2(y)$$

Note:

$$C_2(y) = b + y^2/2$$

$$L = C_1(x) + C_2(y) + k(x + y - R)$$

$$\frac{dL}{dx} = \frac{dC_1}{dx} + k = 0 \tag{5}$$

$$\frac{dL}{dy} = \frac{dC_2}{dy} + k = 0 \tag{6}$$

From (5) and (6) $2x = y$ or $x = y/2$ and $R = x + y = 3/2y$; $x = R/3$; $y = 2R/3$; $C_t = a + R/3^2 + b + (1/2)\ 2R/3^2$; $C_t = a + b + R^2/3$.

Optimum Design: Other Use Only

The design objective is to maximize net benefits

$$N = B(z) - C_2(z) = pz - b + z^2/2$$

Note:

$$C_2(z) = b + \frac{z^2}{2}$$

$$\frac{dN}{dz} = p - z = 0$$

$$N = p^2 - b + p^2/2 = p^2/2 - b$$

Application of Model to Accra-Tema Metropolitan Water System

Long-Range Water Development Program

The Master Plan for the Accra-Tema Metropolitan Water Supply recommended a phased development program which consists of three development stages. The capacities at various phases are shown in Table 5. The third stage is divided into five phases which are expected to become operational by 1976, 1981, 1984, 1990 and 1995, respectively. However, the time of implementation of each stage or phase would be advanced or retarded, to meet the needs of the metropolitan area.

The computation of the imputed benefits will be based on the total capacity at the end of the year, 1980.

The following is an extract from Tahal Ltd. Report [3] confirming the use of water drawn from the Volta River for other purposes.

> The Third Stage will also draw water from the Volta River. For reasons of economy it is proposed to combine the conveyance of water for municipal supply with the delivery of irrigation water to the Accra Plains according to the regional irrigation project drawn up by Kaiser Engineers and Constructors, Inc. Under this project water from the Volta River will be conveyed through a network of canals serving the Accra Plains, with the main canal reaching the outskirts of the Accra-Tema Metropolitan Area. Under the proposed combination of the municipal water supply scheme with the irrigation project, it will be necessary to design the canal system to carry both the irrigation supply and the Metropolitan Area water supply. The planners of the irrigation project envisaged that the Accra Plains Irrigation Project would become operational in the early 'seventies'; however, no firm dates have, as yet, been set for the implementation of the project. The time schedule arrived at on the basis of design demand is summarized in Table 5. (p. 37)

Table V. Water Development Program

Scheme	Source	First Year of Operation According to: Design Demand Projection	Minimum Demand Projection*	Capacity (mgd)
Existing Weija Works**	Densu River at Weija	19	-	10
First Stage	Volta River at Kpong	1967	1967	40
Second Stage	Densu River at Weija	1972	1976	30
Third Stage:	Accra Plains Canal			
Phase One)	System from Volta	1976	1984	30
Phase Two)	River at Kpong	1981	1990	15
Phase Three)	Accra Plains Canal	1984		45
Phase Four)	System from Volta	1990		65
Phase Five)	River at Akosombo	1995		90
Total capacity of existing and proposed schemes				325

* No dates have been assigned for implementation of Phases Three, Four and Five of the Third Stage under the minimum demand projection.

** After retirement of obsolete works.

It is estimated that by 1980 the water obtained from the Volta River at Kpong will be used as follows:

Average Domestic Consumption	48.6 mgd
Industrial Consumption	22.5 mgd
Public and Commercial Consumption	15.63 mgd
	86.73 mgd

The water for Industrial and Public and Commercial Consumptions includes the water for the Irrigation Project. The sum of these is estimated to be 40 mgd. The Average Domestic Consumption is estimated as 50 mgd. It is, therefore, proposed that 40 mgd out of 90 mgd of water obtained from Kpong will be used for other purposes. The remaining 50 mgd will be used for water supply only. This will supplement the 30 mgd obtained from the Densu River at Weija which is intended for water supply only. The sketch below is self-explanatory.

Cost Estimates

The computation of the imputed benefits is based on the grand total construction costs and the cost of treating the Weija Impounding Reservoir. These are estimated as $30,038,000 and $1,505,000 respectively. The total cost of producing 30 mgd from the Weija Impounding Reservoir is, therefore, $31,543,000.

The grand total construction costs of the scheme are obtained by adding to the construction costs (inclusive of contingencies and cost of escalation) the cost of the following items: interest during construction, head office supervision, supervision on site, legal and administration charges, and distribution system accessories.

The term "construction costs" in this report refers to the sum of costs of principal individual units of the main scheme plus the main, secondary and tertiary distribution grids and their accessories. These costs are inclusive of contingencies and cost of escalation.

The actual grand total construction costs for Stages 1, 2 and 3, are as detailed below:

Stage	Phase	Source	Mgd	Cost ($)
1st	-	Kpong	40	43,691,000
2nd	-	Weija	30	31,543,000
3rd	1	Kpong	30	63,093,000
	2	Kpong	15	
			115	138,327,000

The details of the estimated costs of the model, assuming that the cost of producing 90 mgd from Kpong is $113,000,000 is as follows:

Case	Source	Yield Mgd	Symbol of Cost	Cost Million Dollars
Water Supply Only	Weija	30	$C_1(x)$	32.0
Water Supply Only	Kpong	50	$C_2(y)$	62.8
Other Use Only	Kpong	40	$C_2(Z)$	50.2
Both Uses (x,y,z)		120		145.0

Computation of Imputed Benefits - Model

Optimum Design: Both Uses

The cost function for Weija Water Supply only is given by

$$C_1(x) = a + x^2$$

For the optimum design

$$x = p/2,\ y = R - p/2,\ Z = 3/2p - R$$

$$C_1(x)\ a + (p/2)^2$$

$$a = C_1(x) - p^2/4$$

$$a = (32.0 - 0.25p^2)$$ where p is the cost per million gallon

Optimum Design: Other Use Only

The cost function for Kpong Other Use Only is given by

$$C_2(z) = b + Z^2/2$$

For the optimum design

$$Z = p$$

$$b = C_2(Z) - Z^2/2$$

$$b = (50.2 - 0.5p^2$$

Optimum Design: Water Supply Only

The cost function for Weija and Kpong Water Supply only is given by:

$$C_T = C_1(x) + C_2(y)$$

$$= 32.0 + 62.8$$

$$= 94.8 \text{ million dollars}$$

For optimum design

$x = y/2$; $R = x + y - 3/2y$

$x = R/3$; $y = 2/3R$

Tabulation of Optimal Designs - Benefits and Costs

Using the values given above the optimal designs together with benefits and costs are computed and tabulated as shown in Table VI.

Table VI. Optimal Design - Benefits and Costs

Case	Yield A (x)	Yield B (y+Z)	Other Uses (Z)	Cost C	Benefit (pZ+W)	Net Benefit (pZ+W-C)
1. Both Uses	p/2	p	3/2p-80	145.0	$3/2p^2$-80p	$3/2p^2$-80p
2. W.S. Only	26.7	53.4	0	94.8	W	W-94.8
3. Other Use	0	p	p	50.2	p^2	p^2-50.2

Imputed Benefit of the Accra-Tema Metropolitan Water Supply

From Table VI it is evident that the design of Case 1 is preferable to that of Case 2 since the net benefit is greater for all values of the water supply benefit. Hence Case 2 may be eliminated from further consideration. Now if the final decision is made to construct the design of Case 1 instead of Case 3, the lower bound for the total benefit imputed to water supply will be:

$$1.5p^2 - 80p + W - 145 = p^2 - 50.2$$

$$W - 94.8 + 80p - 0.5p^2 \qquad (10)$$

where W = Gross Water Supply Benefit

In a more general way, we can reason as follows. If the unknown water supply benefit is included in Equation 4 and if the design of Case 1 is preferred to that of Case 3 (i.e., we prefer the system where both uses obtain rather than only the "other use"), then

$$W + 0.75p^2 - pR - (a+b) = 0.5\,p^2 - b$$

$$W = pR - 0.25p^2 + a \qquad (11)$$

The marginal benefit of water supply is given by

$$\frac{dW}{dR} = p \qquad (12)$$

$$\frac{94.8 + 80p - 0.5p^2}{80} = p$$

$$P = 13.78 \qquad (13)$$

$$= \frac{\$13,780,000}{\text{mil gal}}$$

Substituting equation (13) in (10)

$$W = 94.8 + (80 \times 13.78) - 0.5(13.78)^2$$

$$= \underline{1290} \text{ million dollars}$$

The imputed benefit of the Accra-Tema Metropolitan Water Supply is $1,290,000,000.

From the total cost for Both Uses per year of $14,500,000 and a yearly flow from both sources to 43,800 million gallons the production cost can be calculated at $0.33 per 1000 gals.

General Discussion

The total cost for treating the Weija Impounding Reservoir weekly for the first three months, followed by a monthly treatment for the period 1970 to 1980 is $1,505,000. This is 10.3 per cent of the total cost. The cost of treatment is, therefore, insignificant as compared to the total cost. It is, therefore, proposed that the reservoir be treated as recommended in the report.

CHAPTER 6

SUMMARY AND CONCLUSION

Summary

The Weija Impounding Reservoir has been vexed with intermittent heavy algal bloom, necessitating its control. Addition to the water of copper sulphate to a concentration of 0.5 mg/l proved unsuccessful; an increase to 1.0 mg/l produced variable results.

A laboratory study was initiated to investigate the simultaneous addition of chlorine with copper sulphate for algae control using Chlorella pyrenoidosa as the test organism.

From laboratory data, effective concentration has been suggested and chemical requirements and costs based on the dosages have been calculated.

An economic analysis of the Accra-Tema Metropolitan Water Supply system was made to determine the impact of algal control on the estimated cost of production.

Conclusion

From these studies, the following conclusions may be drawn:

1. A mixture of chlorine and copper sulphate at 0.3 mg/l Cl_2 and 1.0 mg/l $CuSO_4.5H_2O$ have been shown to successfully reduce the population of green alga, Chlorella pyrenoidosa.

2. Concentration of chlorine alone of 0.3 to 3.0 mg/l and of copper sulphate (pentahydrate) showed no significant effect on the test organism.
3. Wherever an initial reduction occurred, after chemical addition, the growth of the algae progressed at about that of the untreated samples after 2 to 3 hours.
4. The cost of chemical treatment of the Weija Impounding Reservoir has been calculated to be 10.3 per cent of the total production cost.

APPENDIX

EFFECT OF THE MIXTURES OF CHLORINE AND COPPER SULPHATE CONCENTRATIONS ON ALGAE - 23°C

Table A-I. Effect of Mixture of Chlorine and Copper Sulphate Concentration on Algae - 23°C

Contact Time (hrs)	Algae Count (cells/mm^3)					
	Chlorine and Copper Sulphate Concentrations (mg/l) ($Cl_2/CuSO_4.5H_2O$)					
	0/0	0.3/0.3	0.3/0.5	0.3/0.8	0.3/1.0	0.3/3.0
0	5,850	7,000	4,450	10,300	4,350	2,600
0.5		6,200				
1	5,950	5,500	3,950	7,200	1,950	2,200
2	5,550	5,750	3,900	5,800	2,500	1,500
3		7,750	3,650	9,050	2,400	
7					2,350	1,700
13		6,750				
14	6,250					
15				6,500		
16			3,800			
24			5,800			
25	9,150	6,050		5,300		
29						2,100
30					2,650	

Table A-II. Effect of Copper Sulphate Concentration on Algae - 23°C

Contact Time (hrs)	Algae Count (cells/mm^3)					
	Copper Sulphate Concentration (mg/l) (as pentahydrate)					
	0	0.3	0.5	0.8	1.0	3.0
0	1,600	1,900	2,150	1,700	1,600	1,600
1	1,650	1,050	2,250	2,500	2,150	1,900
2	1,700	2,250	1,400	2,650	1,400	1,550
3	1,800	1,450	2,350	2,350	2,300	1,200
4	1,550	1,850		2,400	2,150	
6			1,550			
9		1,950	1,950			
10	1,450					
13						1,350
14				2,850	1,900	
24						1,150
25			2,750	2,700	2,000	
26		1,350				
27	1,550					

Table A-III. Effect of Chlorine Concentration on Algae - 23°C

Algae Count (cells/mm^3)

Contact Time (hrs)	Chlorine Concentration (mg/l)					
	0	0.3	0.5	0.8	1.0	3.0
0	1,500	1,700	2,600	1,500	1,950	2,000
1		2,250	2,400	1,750	1,750	
2	1,600		2,700	1,500		2,050
3		2,000	2,200			2,050
4					1,850	
5	1,750	2,050		1,250		
6		2,050				
7	1,700	1,600				
8	1,500					
9						2,100
10					2,100	2,300
11				1,400	1,550	
12				1,500		
15			1,900			
19		2,550				
24			1,350			
26						2,850
27					1,300	
28		2,100		2,700		
30	1,950					

LIST OF REFERENCES

1. *Feasibility Study for Water and Sewerage Works in the Accra-Tema Metropolitan Area, Vol. 2,* Engineering Report, Tahal (Water Planning) Ltd., in association with Engineering Science, Inc., (1966).

2. Smith, G. M., *Fresh-Water Algae of the United States,* 2nd Ed., McGraw-Hill, New York, (1950).

3. *Feasibility Study for Water and Sewerage Works in the Accra-Tema Metropolitan Area, Vol. 1,* Engineering Report, Tahal (Water Planning) Ltd., in association with Engineering Science, Inc., (1966).

4. Kutt, Y. and Edlis, J., "The Effect of Halogens on Algae - Chlorella sorokiniana," *Water Research, 3,* 251, (1969).

5. Moore, E. W., *Fundamentals of Chlorination of Sewage and Waste,* Water and Sewage Works, *Vol. 98, No. 3,* (March 1951).

6. Amer. Public Health Assn., *Standard Methods for the Examination of Water and Wastewater,* 12th Ed., APHA, New York, (1965).

7. Fair, G. M., Geyer, J. C. and Okun, D. A., *Water and Waste Water Engineering, Vol. 2,* John Wiley and Sons, New York, (1968).

8. McKee, J. E. and Wolf, A. W., "Water Quality Criteria," 2nd Ed., The Resources Agency of California, State Water Quality Control Board, Publication No. 3-A, (1963).

9. Starr, R. C., *The Culture Collection of Algae at Indiana University, Amer. Jour. Botany, 51, 67,* (1964).

10. Hostetter III, H. P., *Growth, Reproduction and Survival of Diatoms,* Ph.D. Dissertation, University of Arizona, (1969).

11. Page, L. B. and Culver, P. J., *A Syllabus of Laboratory Examinations in Clinical Diagnosis,* Revised Edition, Harvard University Press, Cambridge, Mass., (1960).

12. Maass, A. et al., *Design of Water-Resource Systems,* Harvard University Press, Cambridge, Mass., (1962).