# INTERACTIONS GOVERNING BIPYRIDINIUM PHYTOTOXICITY TO *Chlorella pyrenoidosa*, Chick.

By

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# INTRODUCTION

Paraquat (1,1'-dimethyl-4,4' bipyridinium cation) is an effective herbicide used for control of both aquatic and terrestial weeds. Low concentration levels cause chlorosis with rapid dessication and top kill in a wide range of plants. The unique characteristic of being very strongly absorbed by soil colloids results in difficulty for an evaluation of persistence and degradation of paraquat in soil. There is great need for characterization of factors affecting this herbicides activity, degradation and control. Although there are several theories concerning paraquat, little has been established at present concerning its specific phytotoxic activity and controls.

The objective of this study was concerned with determining the factors that govern rates and magnitude of paraquat phytotoxicity to C. pyrenoidosa and to determine means for control of this herbicide's effectiveness.

## LITERATURE REVIEW

The herbicidal properties of diquat, 6,7-dihydrodipyrido (1,2-a: 2',1'-c) pyrazidiinium salt and paraquat, 1,1'-dimethyl-4, 4'-bipyridinium salt were discovered by Brian et. al (7) in 1955. Some quaternary ammonium compounds have been used as redox indicators under the name of viologens since 1933 by Michaelis and Hill (13). It was reported that paraquat has relatively nonspecific action on plants. It is effective in control of giant duckweed (Spirodela polyrhyza (L.) Scheid), and to common duckweed (Lemna minor L.). The mode of action of paraquat is very rapid and two theories are proposed concerning paraquat phytotoxicity.

Homer (10) proposed that phytotoxicity and reduction reactions are related. The hypothesis was therefore considered that herbicidal activity depends on the ability of the active compounds to form free radicals by uptake of one electron to form toxic radicals within the plants and reacting in processes connected with photosynthesis and respiration. Homer further proposed that oxygen is essential for the function of dipyridyl quaternary salts. The interpretation is that not only reduction to a radical is necessary, but that a reoxidation is also involved.

Black (4) suggested that paraquat acts as a catalytic electron carrier which entrapped energy. Since the dipyridyls has the same oxidation-reduction potential as ferredoxin, the dipyridyls can replace ferredoxin in the reduction of cytochrome C in the presence of

TPN reductase and TPNH mechanism:

$$\text{Reducing Potential} \xrightarrow{\bar{e}} \text{Dipyridyl Reduced} \xrightarrow{+O_2} H_2O_2 + \text{Dipyridyl Oxidized}$$

The reducing potential from reduced pyridine nucleotide may be active both in light and dark.

Brian (6) agreed with Homer's (10) assumption and indicated that paraquat's final effect on plants is physical rather than metabolic. These effects were related to paraquat activity and the oxidation-reduction potential of the treated substances. It appears unlikely that if free radical chain reactions were involved, proteins and other large molecules in the protoplasm would be degraded. However, such an occurrence would be consistent with the nature and rapid appearance of the visible toxic symptoms in treated plants. The most recent, comprehensive review of the metabolism and decomposition properties of both diquat and paraquat have been presented by Funderburk and Bozarth (9). They indicated that paraquat moved in a acropetal direction and the degradation of paraquat can be caused by soil microorganism and ultraviolet light.

It was reported by Merkle et. al (11) that light, oxygen and temperature will influence the properties of paraquat. Light and oxygen are related to the destruction of a protective system that normally prevents photo-oxidation.

Coats et. al (8) have indicated that because of the cationic nature of both diquat and paraquat, soil may reduce their herbicidal activity. It was shown by Weber et. al (15) that paraquat is easily adsorbed by montomonillite and kaolinite and is then unavailable to the plants. Paraquat was adsorbed to approximately the cation

exchange capacity of the clay minerals.

In previous work, Barnes (2, 3) used the leaf disk of Phasolus vulgaris L. and Chlorella pyrenoidosa in bioassay studies to determine factors that influence paraquat activity. The unicellular green algae, C. pyrenoidosa, has many characteristics that are favorable for bioassay. Since the whole development process of this algae strain, including cell division, was not inhibited by light, it was possible to carry on these experiments with continuous light (14). Allen et. al (1) reported that C. pyrenoidosa illuminated cells had been found to form products containing unpaired electrons and that chlorophyll extraction had spectra absorbance peaks at 672 mμ and 680 mμ.

## MATERIALS AND METHODS

Chlorella pyrenoidosa Chick, IAC251, cultures were grown photolithotrophically in an inorganic distilled water medium. Continuous light of 500 Lumen/ft.$^2$ was supplied with equal illumination by daylight (GE) and standard Gro-lux (Sylvania) fluorescent lamps. Cultures were maintained at 25-30° C in 2.8 liter Erylenmeyer flasks and supplied with constant bubbling of saturated compressed air providing aeration and agitation.

The basic inorganic salt medium was composed of three grams each $NH_4NO_3$, $K_2SO_4$ and Phillips-Hart IV salt mixture per liter of distilled water. The P.H. IV salt mixture contained in percent by weight: $K_2HPO_4$ 32.2, $CaCO_3$ 30.0, NaCl 16.7, $MgSO_4$ 10.2, $CaHPO_4$ 7.5, ferric citrate 2.75, $MnSO_4$ .51, KI .08, $CuSO_4$ .03, $ZnCl_2$ .025 and cobalt chloride .005. The paraquat (1,1'-dimethyl-4,4' bipyridinium) solution used was the Chevron Chemical Corporation commercial formulation with 24% methyl sulfate salt active ingredient. Cation ratio salt solution media studies utilized the formula

$$\frac{K}{\sqrt{\frac{Ca + Mg}{2}}} = 1$$

with the elements expressed as milliequivalents and all supplied to the solution as sulfates. The $Cu^{++}$, $Mn^{++}$ and $Fe^{++}$ media solutions used were also sulfates.

Initial studies were to establish the growth response curve of the organism in response to the basic medium, light and temperature parameters. Determinations were then made to establish interations of

cell concentration, incubation time and media variations to paraquat activity and induced chlorsis.

Induced chlorosis with paraquat solutions was determined by quantitative herbicide addition to synchronized cell cultures with variation in pretreatment growth media composition, cell concentration and reaction time. Synchronized stock cultures were grown to an estimated maximum of the log phase then alternately placed in 12 hours darkness at 9° C and 12 hours continuous 500 Lumen/ft.$^2$ fluorescent light at 25-30° C for three complete cycles before cell washing, suspension in 0.2% sodium bicarbonate, differential salt solution and paraquat treatment. Individual cultures were 30 ml volume of adjusted cell concentration in 50 ml Erlenmeyer flasks. Flask openings were covered with transparent parafilm puncture to allow free gas exchange. These flask cultures were continuously shaken at approximately 120 oscillations per minute in an Eberbach culture shaker for the time, light and temperature conditions defined for each experiment.

Cell concentrations were determined as optical density at 600 mμ with a Bausch and Lomb Spectronic 20 spectrophotometer. Residual paraquat was determined using .2% sodium dithionite in .3N NaOH with quantitation at 394 mμ. Chlorella dry cell weight in mg. per ml. culture was determined following 24 hours dehydration at 80° C.

Extractable chlorophyll was determined with cell separation by centrifugation in a Sorvall SS-3 automatic superspeed centrifuge at 15 x $10^3$ rpm for 15 minutes, decanting the free culture liquid and freezing cells at -10° C overnight, then rupturing frozen cells with a tissue homogenizer. Cells separated from 30 ml cultures were

extracted for one hour with 10 ml methanol using a Model 150V Multi-Purpose Rotator (Scientific Ind.). These chlorophyll extracts were read at 660 mμ with the B. and L. spectrophotometer and full spectra absorbance, 350 mμ to 750 mμ, was recorded with a Perkin-Elmer Model 202 spectrophotometer. Differential absorbance peaks at 434 mμ and 670 mμ were attained from the full spectra recording for each culture, tabulated and related to culture treatments.

## RESULTS AND DISCUSSION

Growth response of this unicellular algae with the basic medium, light, and temperature conditions resulted in the semi log quadratic curve during fourteen days as shown in Figure 1. Calculated line of best fit was:

$$y = .1072x - 6 \cdot 10^{-6}x^2 - .093$$

with y as optical density of cell spectraphotometric readings at 600 mμ. Number of cells at O.D. 1.0, 600 mμ was reported by Meyers (12) as $50 \times 10^6$ cells per ml. Actual cell count was not determined in these studies. The near peak log growth phase of this organism, under the culture restraints of this study was estimated to be at approximately O.D. 1.0 attained at ten days.

The relationship of dry cell weight and culture O.D. at 600 mμ is shown in Figure 2. The general quadratic relationship shown as line of best fit with means from three replicate cultures was:

$$y = 1.9793x - 0.0027x^2 - 0.0038$$

with x as dry cell weight in milligrams per milliliter. This gave basis for assumed cell concentration at measured culture O.D. within the range 0.1 to 1.0. Old cultures with higher cell concentrations tended to flocculate and give erratic results from the O.D. turbidity measurements with this organism.

Chemical quantitation of paraquat utilizes the blue color reaction with sodium dithionite in an alkaline solution. The standard reference curve as percent transmission at 394 mμ is shown in

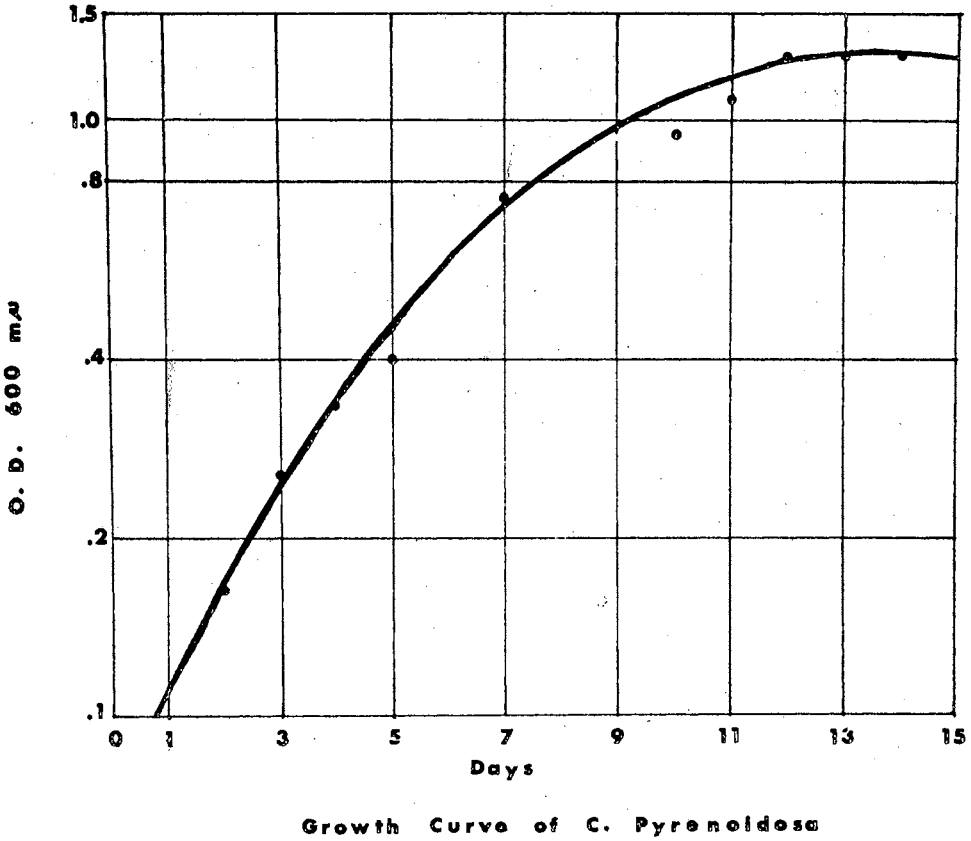


Figure 1. Fourteen Day Growth Curve of Chlorella pyrenoidosa at 500 Lumen/ft.$^2$

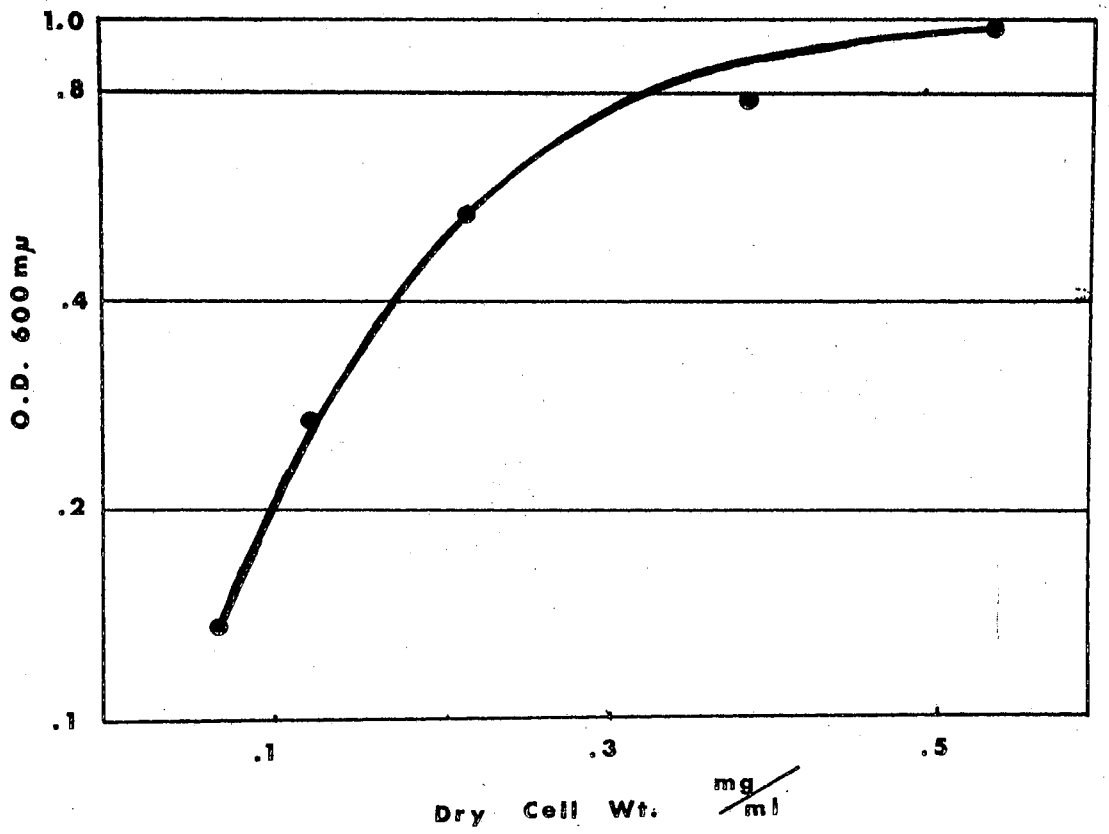


Figure 2. The Relationship of Dry Cell Weight and C. pyrenoidosa Cell Density (at 600 mμ).

Figure 3 and closely follows Beer's law $\log I/I_o$ = concentration paraquat, 0 to 5 ppm. This standard curve was used in the chemical determination for residual paraquat in these studies. However, the dithionite reaction is non-specific and other active oxidation-reduction reactions in extracts or media containing paraquat may result in errors as shown in Table IV.

Extractable chlorophyll was found to be a reliable indicator of paraquat activity to C. pyrinoidosa with initial studies by Barnes (2). In these studies 660 mμ was used as quantitating wavelength. In later studies it was found that two absorption peaks, 670 mμ and 434 mμ, were apparent with the extraction procedures used. A correlation of readings at 670 mμ and 660 mμ is shown in Figure 4. Linear relationship was apparent as: $y = 1.638x - 0.378$. Values of $y$ = log O.D. 670 mμ and $x$ = log O.D. 660 mμ. This uniform relationship may be explained by the shape of the curves with 670 mμ as actual peaks of these recordings.

Figure 5 is percent absorbance reduction with differential paraquat concentrations at 434 mμ and 670 mμ. These two lines appear nearly parallel with this plotting. Rate of change in chlorosis from paraquat activity was apparently greater at 434 mμ ($y = 75.74 - 45.23x$) than at 670 mμ ($y = 42.36 - 29.72x$). Previous experiments in this study (2) used cell density, cell weight and extractable chlorophyll for measuring paraquat phytotoxicity. There was no consistent response measured with cell weight and cell density as influenced by paraquat concentration. Extractable chlorophyll quantitated at 434 mμ and 670 mμ was apparently the most reliable paraquat phytotoxic index.

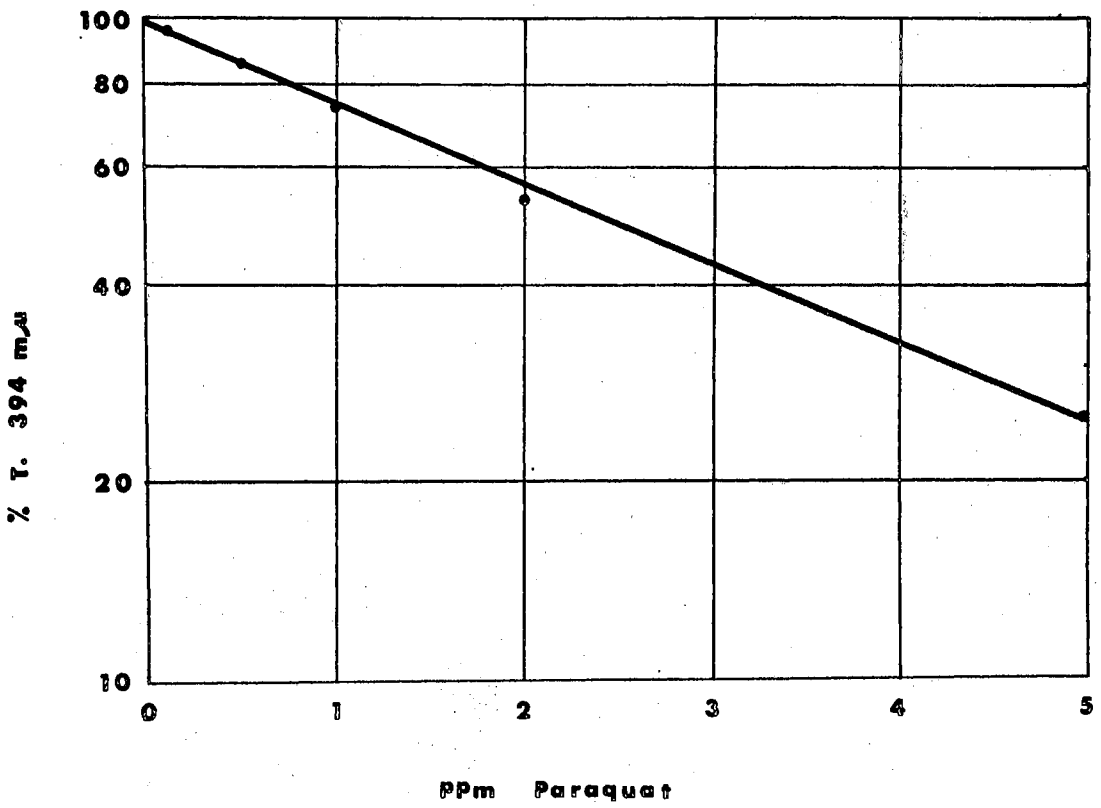


Figure 3. Standard Curve for Dithionite Chemical Quantitation at 394 mμ of Paraquat Levels.

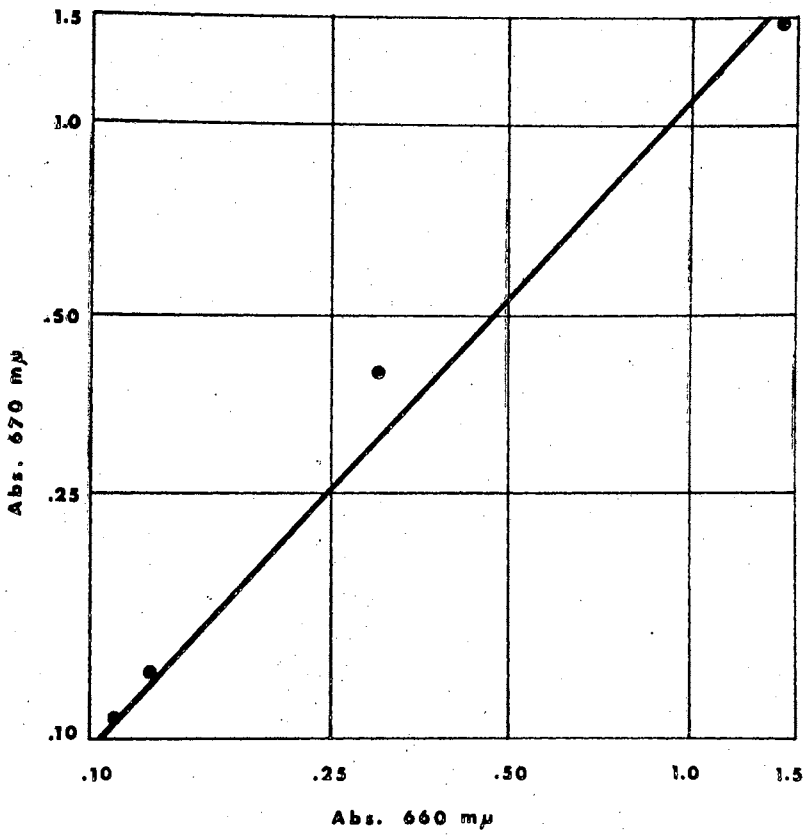


Figure 4. Extractable Chlorophyll Absorbance Relationship Between 660 mμ and 670 mμ for Chlorella pyrenoidosa.

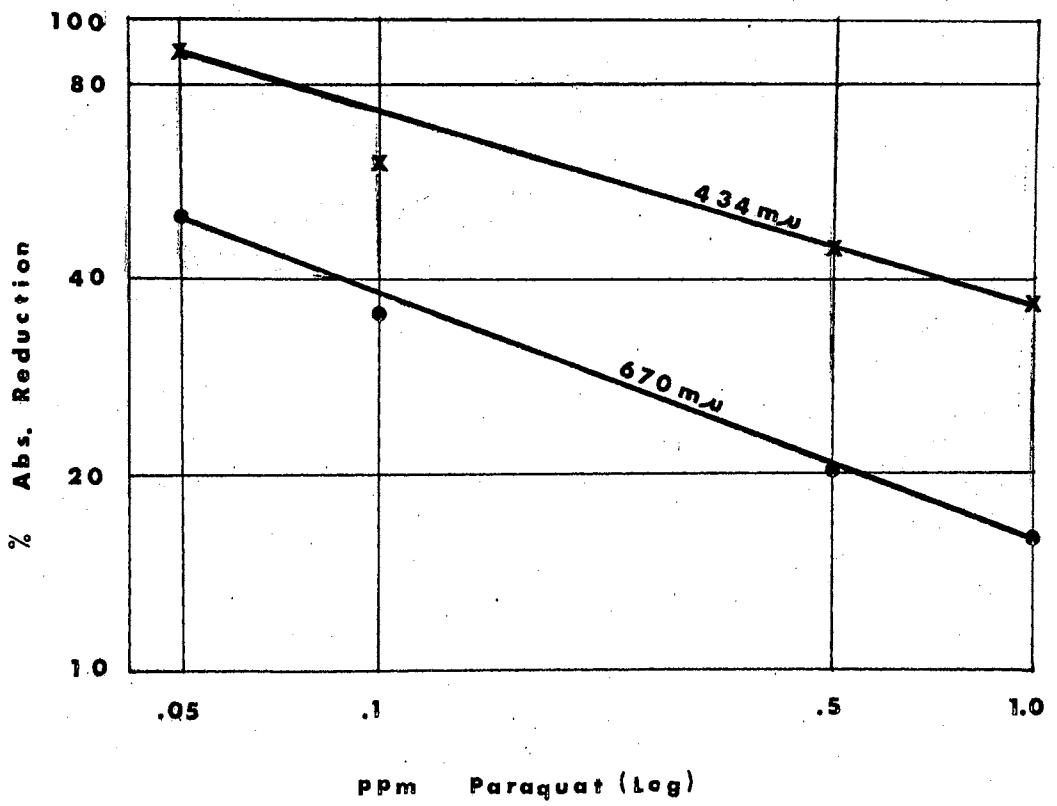


Figure 5. Effect of Paraquat on Percent Absorbance Reduction of Chlorella Chlorophyll Extracts at 434 mμ and 670 mμ.

Relative sensitivity of a range in Chlorella cell concentration to paraquat at 1 ppm for 24 hours continuous illumination is shown in Table I. Cell concentration is shown as O.D. at 600 mμ at time 0. The cell concentration as O.D. with corresponding dry cell weights mg/ml were: 1.00 (.54), .53 (.26), .25 (.14), and .08 (.02). Extractable chlorophyll absorbance at 434 mμ for these respective cell concentrations following 1 ppm paraquat addition and 24 hours continuous light with corresponding percent transmission reduction were: 1.5 (1.0), .655 (19.2), .140 (69.2), and .115 (73.5). A relatively higher rate of change was apparent at 670 mμ: 1.5 (0), .36 (40.4), .13 (70.9), and .11 (74.4). Results from this study indicated cell concentration with corresponding O.D. values from .53 to 1.0 as most suitable for desired reaction levels in subsequent cultural studies.

Studies by Barnes (2) indicated a governing influence of medium composition on response of Chlorella to paraquat phytotoxicity. Trial and error preliminary studies also indicated an influence of the cations $K^{+}$, $Mg^{++}$, and $Ca^{++}$ and their ratio in growth medium. Other nutrient ions apparently influential on Chlorella reactions were $Cu^{++}$, $Mn^{++}$ and $Fe^{++}$.

Results from initial studies with growth responses of washed synchronized Chlorella cells in media having factorial combinations of cation salt and paraquat concentrations are shown in Figure 6 and 7. Time of incubation was 24 hours.

Cation salt concentrations of 0, 50, 100 and 200 ppm were supplied in the ratio of

$$\frac{K^{+}}{\sqrt{\frac{Ca^{++} + Mg^{++}}{2}}} = 1, \text{ expressed in}$$

TABLE I

RELATIONSHIP OF *Chlorella pyrenoidosa* CELL CONCENTRATION TO EXTRACTABLE CHLOROPHYLL SPECTRA WITH 1 ppm PARAQUAT, 24 HOURS[1]

| *Chlorella* | 434 mμ | | 670 mμ | |
|---|---|---|---|---|
| Cell O.D. | Abs | % T Reduction | Abs | % T Reduction |
| 1.00 | 1.500 | 0 | 1.50 | 0 |
| .53 | .655 | 19.2 | .36 | 40.4 |
| .25 | .140 | 69.2 | .13 | 70.9 |
| .08 | .115 | 73.5 | .11 | 74.4 |

[1]Figures are means of duplicate cultures with incubation under 500 Lumen/ft.$^2$ at 25-30° C.

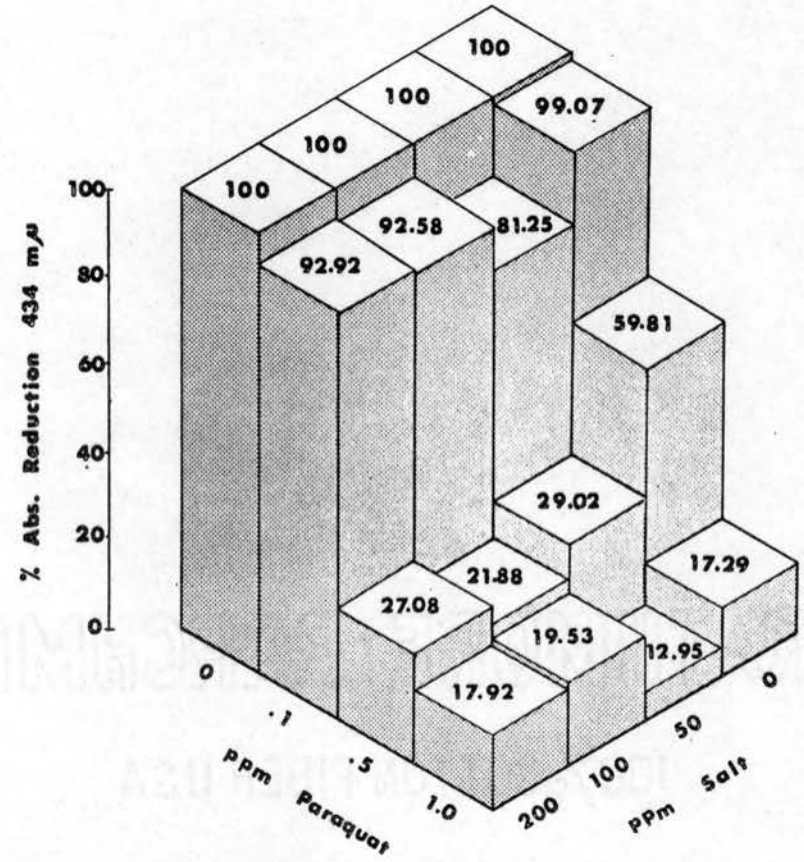


Figure 6. Effect of Salt and Paraquat Levels in Extractable Chlorophyll Percent Absorbance Reduction in C. pyrenoidosa at 434 mμ.

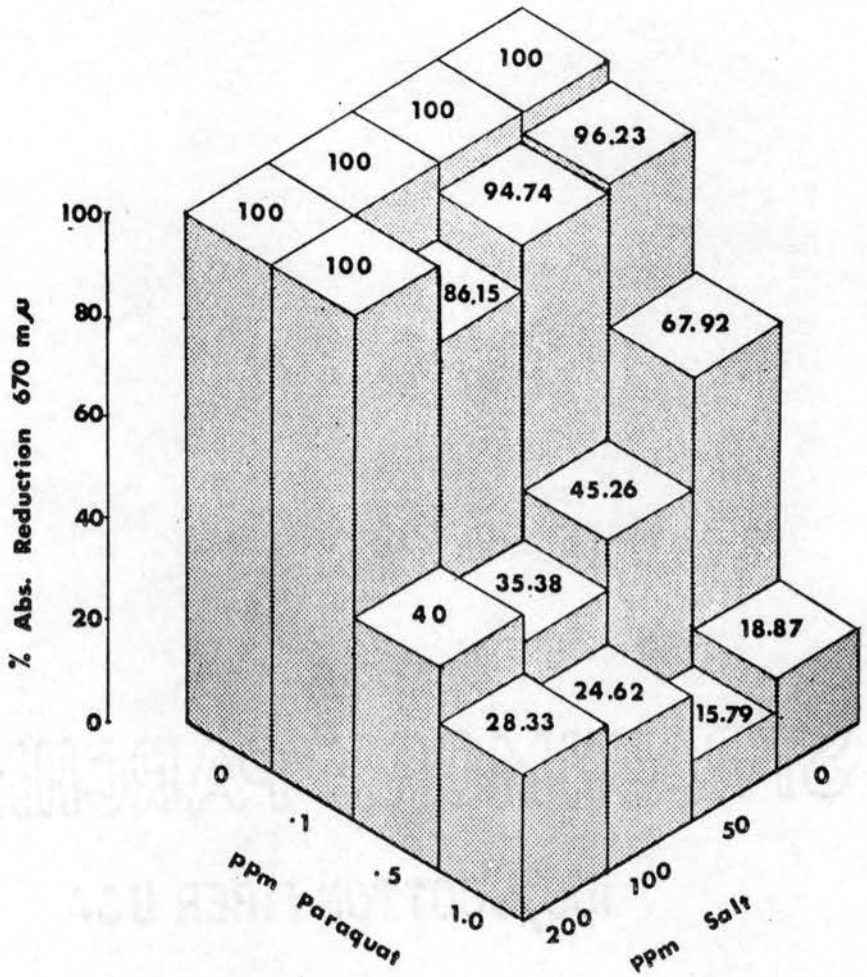


Figure 7. Effect of Salt and Paraquat Levels on Extractable Chlorophyll Percent Absorbance Reduction in C. pyrenoidosa at 670 mμ.

milliequivalents and all were supplied as sulfates. The percent absorbance reduction as influenced by combinations with paraquat concentrations at 0, .1, .5 and 1.0 ppm at 434 mμ is shown in Figure 6 and at 670 mμ is shown in Figure 7. Absorbance readings at 0 paraquat levels were used as 100% base for induced chlorosis at each salt concentration.

Influence of salt concentrations on paraquat activity recorded at 434 mμ was somewhat erratic; with C.V. 8%, highly significant F values were obtained for paraquat level 522.6**, and for paraquat x salt interaction 7.31**. A real trend for salt levels ameliorating paraquat activity was not apparent at this wavelength.

Chlorosis at 1.0 ppm was apparently reduced with 100 and 200 ppm salt levels as indicated at 670 mμ shown in Figure 7. Treatment F values were highly significant, paraquat levels = 874.45**, salt levels = 41.45**, and paraquat x salt interaction = 133.27** with C.V. 6%. The physical appearance of the algae cultures indicated a favorable influence of the higher salt levels that was not reflected in the chlorophyll extracts. The cells tend to coagulate into clusters at the high paraquat levels without salt addition. The precipitated cells could not be resuspended even with vigorous shaking. These same paraquat levels combined with the salt levels resulted in the cultures staying in suspension within the media and apparently were normal in physical appearance.

The influence of the salt mixture at 100 ppm on paraquat quantitation with the dithionite analysis method is shown in Table II. Readings of optical density at 394 mμ indicated no real or consistent differences between paraquat only and paraquat plus salt additions.

TABLE II

DITHIONITE DETERMINATIONS OF PARAQUAT WITH AND WITHOUT MEDIA SUPPLEMENTAL CATION SALT MIXTURE AT 100 ppm

| Paraquat ppm | O.D. at 394 mμ | |
|---|---|---|
| | Without Salt | With Sulfate Salt As $\frac{K}{\sqrt{\frac{Ca^{++} + Mg^{++}}{2}}} = 1$ |
| 0 | 0 | 0 |
| .1 | .017 | .014 |
| .5 | .070 | .083 |
| 1.0 | .185 | .193 |
| 2.0 | .333 | .295 |
| 5.0 | .680 | .700 |

Figures are means of duplicate cultures, C.V. = 3.9%
F values:
Replication = 0.361
Treatment Combination = 1246.2**
Paraquat = 2865**
Salt = 0.00049
Paraquat x salt = 4.9705**
**Significant at .05 level.

These data indicate that there were no apparent chemical or physical influences of the salt concentrations on paraquat chemical activity within culture solutions in these studies.

The influence of time 0 to 30 hours of culture incubation on 1 ppm paraquat reactions with Chlorella, with and without 100 ppm salt solutions, at 434 mμ and 670 mμ is shown in Figure 8. Percent absorbance reduction was increased with time for all salt treatments. However, at both wavelengths the salt levels reduced the paraquat effectiveness consistently for all sampling periods in this study. Treatment F value at 434 mμ was 5.623** with C.V. 17%, and at 670 mμ the F value was 16.30** with C.V. 17%. These results indicated a real influence of this salt combination on rates of paraquat activity with the culture techniques used in these studies. These data indicate more effective paraquat inhibition detected at 434 mμ than at 670 mμ. Differential effects of salt on paraquat activity levels were apparently also of greater magnitude at 434 mμ.

Preliminary studies in variation of media composition influencing response of Chlorella to paraquat indicated an effect of $Cu^{++}$, $Mn^{++}$ and $Fe^{++}$. Results from a comprehensive factorial study with $Cu^{++}$ as sulfate at 2 ppm combined with and without the $\frac{K}{\sqrt{\frac{Ca^{++} + Mg^{++}}{2}}} = 1$ sulfate salt are shown in Figure 9. The salt component was based on results from the culture studies shown in Figures 6, 7 and 8 and Table II. The salt ratio of 1 supplied at 100 ppm resulted in less absorption reduction at 670 mμ, with and without 1.0 ppm paraquat, following 40 hours incubation. $Cu^{++}$ combined with the salt levels resulted in even greater inhibition of paraquat activity. The

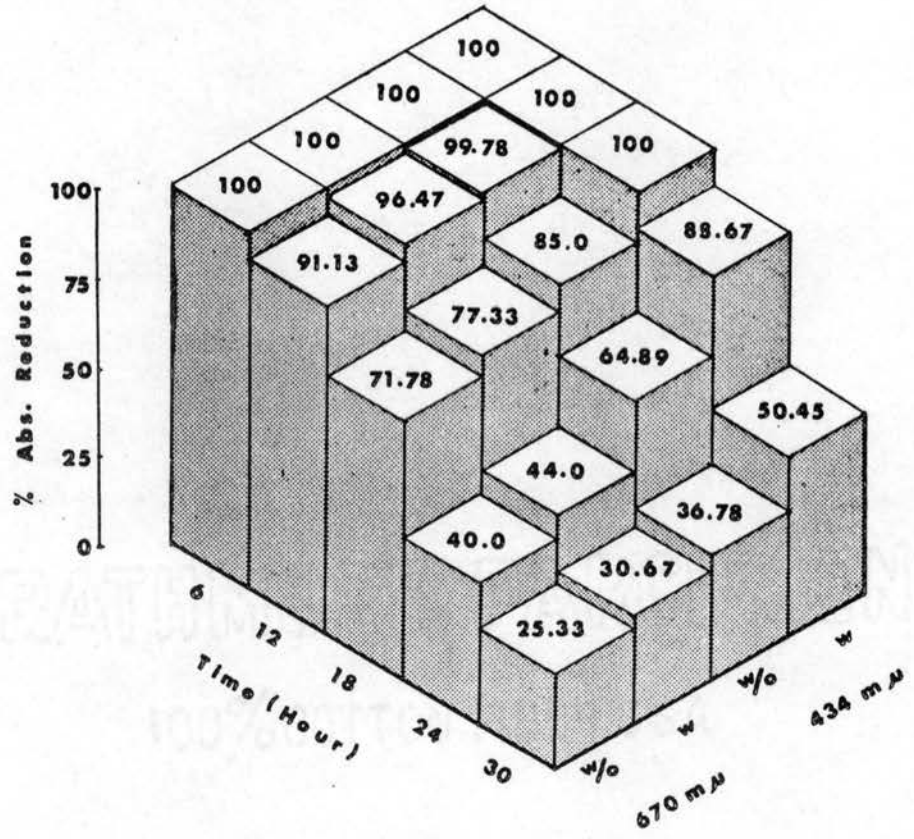


Figure 8. Effect of Time and Cation Salt on Chlorophyll Percent Absorbance Reduction in C. pyrenoidosa at 434 mμ and 670 mμ .

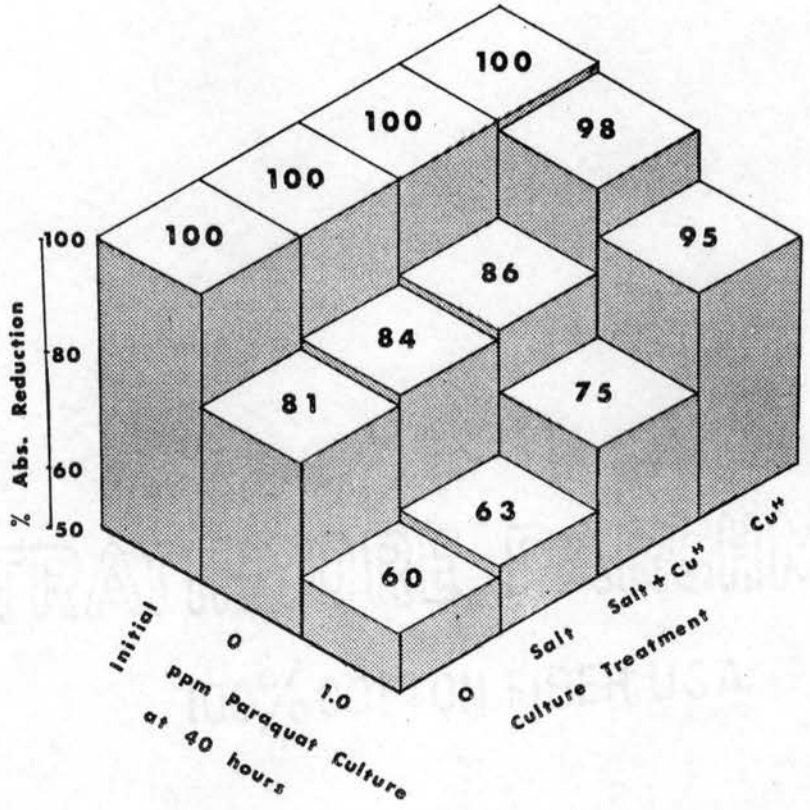


Figure 9. Effect of Time, Paraquat, Cation Salt and $Cu^{++}$ on Extractable Chlorophyll Percent Absorbance Reduction in C. pyrenoidosa at 670 mμ.

addition of $Cu^{++}$ at 2 ppm only was highly effective in reducing the apparent chlorosis induced with paraquat. Treatment F 47.41** was highly significant with C.V. of 3%. These results indicated a governing influence of $Cu^{++}$ as sulfate salt for modification of interactions on some reactions involved with the effectiveness of paraquat to this algae in the culture conditions of these experiments.

Results of combinations of $Fe^{++}$ and $Mn^{++}$ as sulfates at 2 ppm with and without the $\frac{K}{\sqrt{\frac{Ca^{++} + Mg^{++}}{2}}} = 1$ salt mixture and paraquat additions are shown in Table III. Figures shown are the relative percentage change in absorbance at 434 mμ and 670 mμ after 40 hours incubation as compared to initial absorbance at time 0 of the experiment. Statistical analyses of these results indicated highly significant results from paraquat treatment with C.V. 5% at 434 mμ and 13% at 670 mμ. $Fe^{++}$ additions alone apparently had little influence on herbicide effectiveness, $Mn^{++}$ treatments were slightly higher than the cation salt only additions. Combined salt and $Fe^{++}$ or $Mn^{++}$ treatments were apparently more effective. The $Fe^{++}$ and salt combination apparently was most effective at both wavelengths. The magnitude of difference was not within a range comparable to results with the $Cu^{++}$ treatments, however, and further work was concentrated on the $Cu^{++}$ factorial.

The results from comprehensive studies with levels of $Cu^{++}$ as sulfate and paraquat level in factorial combinations are presented in Figures 10 and 11. Sum of absorbance from duplicate cultures at 434 mμ are given in Figure 10 with paraquat levels 0, .1, .5, 1.0

TABLE III

PERCENT CHANGE IN SPECTRAL ABSORPTIONS OF CHLOROPHYLL EXTRACTS FROM VARIOUSLY TREATED *C. pyrenoidosa* CULTURES WITH 40 HOURS INCUBATION

| Treatment | 434 mμ | | 670 mμ | |
|---|---|---|---|---|
| | 0 | 1 ppm Paraquat | 0 | 1 ppm Paraquat |
| Check | 114.5 | 2.3 | 106.6 | 3.3 |
| Salt | 105.6 | 9.1 | 115.8 | 11.1 |
| $Fe^{++}$ | 100.0 | 4.0 | 100.0 | 2.5 |
| $Mn^{++}$ | 114.5 | 14.5 | 111.6 | 13.0 |
| $Fe^{++}$ + Salt | 100.0 | 36.6 | 115.3 | 33.0 |
| $Mn^{++}$ + Salt | 100.0 | 25.3 | 118.8 | 15.5 |

434 mμ =
C.V. = 5%
Replication F = 1.01
Treatment combination F = 600**
Paraquat F = 7000**
Media F = 29.81**
Paraquat x Media F = 28.3**

670 mμ =
C.V. = 13%
Replication F = 2.23
Treatment combination F = 70**
Paraquat F = 700**
Media F = 11.24**
Paraquat x Media F = 1.89

$$\text{Salt} = \frac{K}{\sqrt{\frac{Ca^{++} + Mg^{++}}{2}}} = 1 \quad \text{at 100 ppm}$$

$Fe^{++} = FeSO_4$ at 2 ppm
$Mn^{++} = MnSO_4$ at 2 ppm
**Significant at .05 level.
Figures are means from duplicate cultures with incubation under 500 Lumen/ft.$^2$ at 25-30° C.

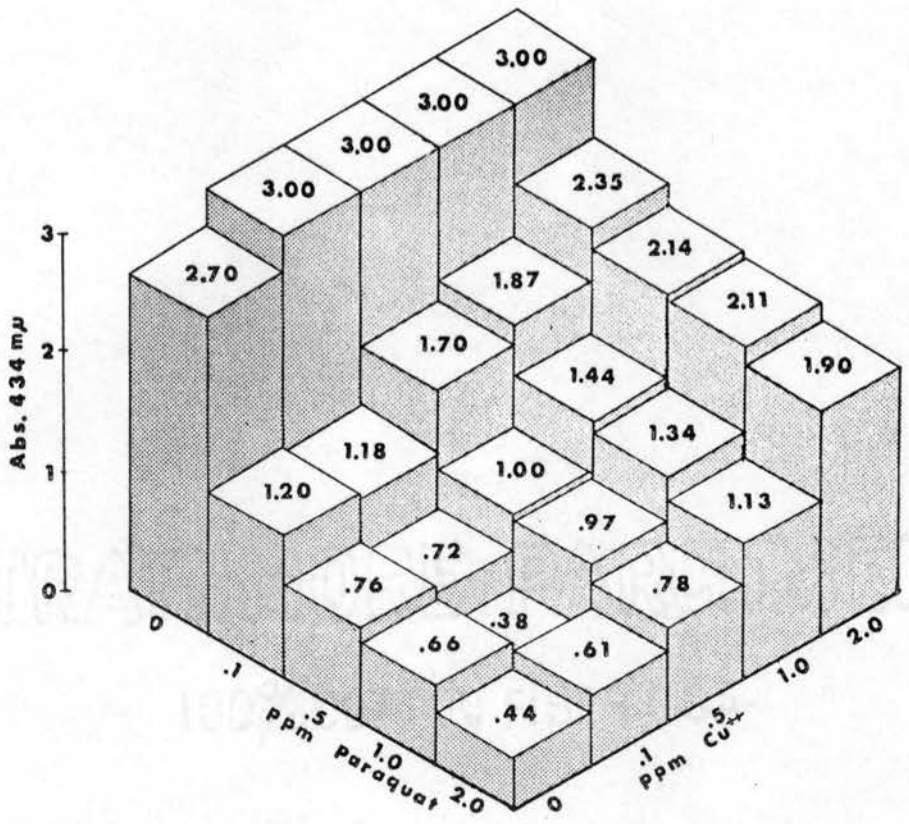


Figure 10. Effect of Differential $Cu^{++}$ and Paraquat Levels on Extractable Chlorophyll Absorbance in C. pyrenoidosa at 434 mμ.

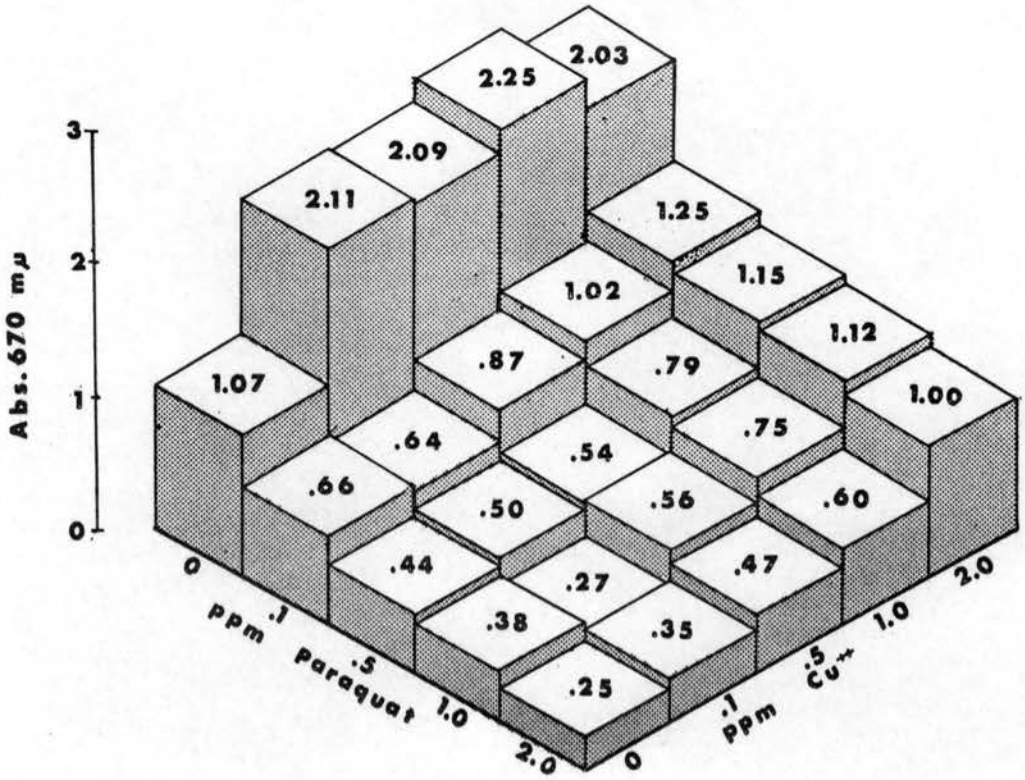


Figure 11. Effect of Differential $Cu^{++}$ and Paraquat Levels on Extractable Chlorophyll Absorbence in C. pyrenoidosa at 670 mμ.

and 2.0 ppm in factorial combination with $Cu^{++}$ levels also supplied at these same levels of 0, .1, .5, 1.0 and 2.0 ppm. Although some deviations were apparent, a general trend for decrease in paraquat effectiveness resulted with increase of $Cu^{++}$ concentration at all levels. Highly significant F values were apparent for paraquat level 305.72**, for $Cu^{++}$ level 85.26**, and for interactions 12.99** with C.V. of 9%.

A somewhat uniform trend, indicating progressive paraquat inhibition with increased $Cu^{++}$ concentration, was apparent at 670 mμ as shown in Figure 11. Highly significant F values were attained for paraquat level 241.88**, $Cu^{++}$ level 61.95** and interaction 6.217** with C.V. 10%.

Presentation of absorption recordings for the full spectra of 350 to 750 mμ obtained in the paraquat x $Cu^{++}$ factorial experiments are shown in Figures 12 and 13. Relative chlorosis as reflected with absorbance after 40 hours continuous light incubation with levels of paraquat only are shown in Figure 12. Near complete chlorosis was attained at the 2.0 ppm level with only small deviations in absorbance noted. The zero paraquat level recorded the two maximum absorbance peaks estimated at 434 mμ and 670 mμ. A decrease in these peak recordings occurred with increased paraquat additions. Actual rates of change in magnitude of absorbance as a function of increased herbicide concentrations were greatest at the 434 mμ wavelength in this study.

Addition of 2.0 ppm $Cu^{++}$ to culture paraquat treatments of 0, .1, .5, 1.0, and 2.0 ppm resulted in extractable chlorophyll spectra shown in Figure 13. Inhibition of paraquat phytotoxicity to Chlorella

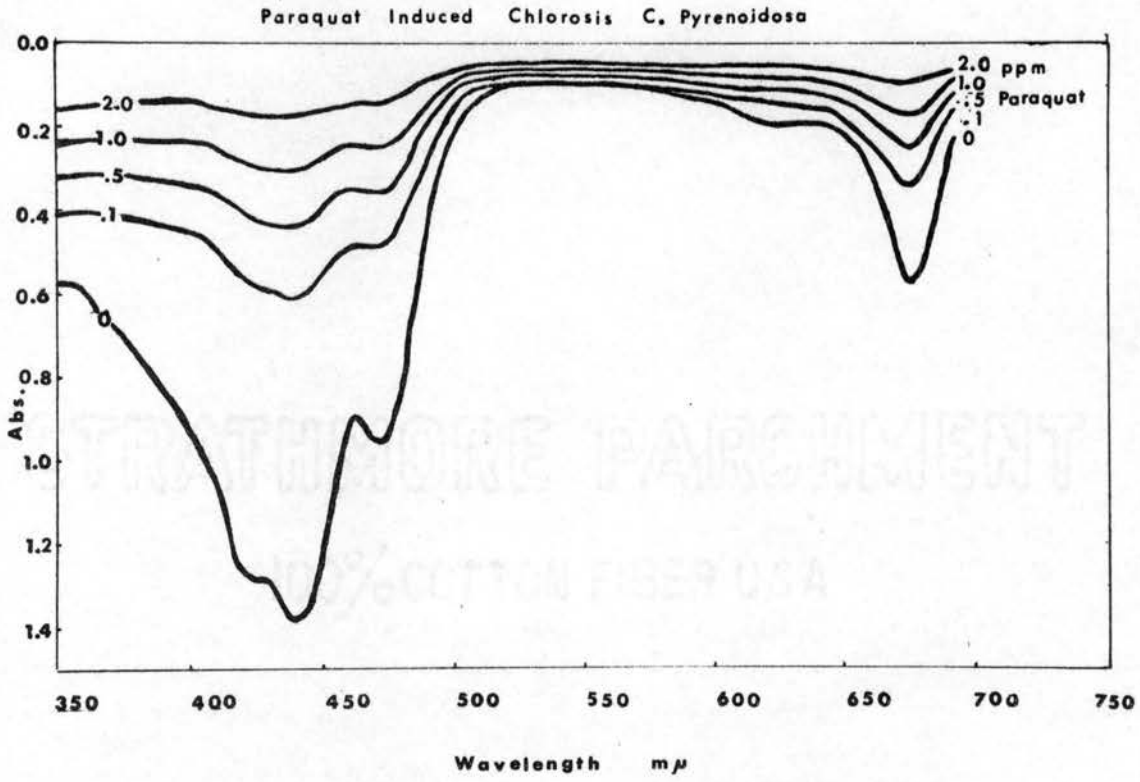


Figure 12. Extractable Chlorophyll Absorption Spectra at 40 Hours with Paraquat Levels

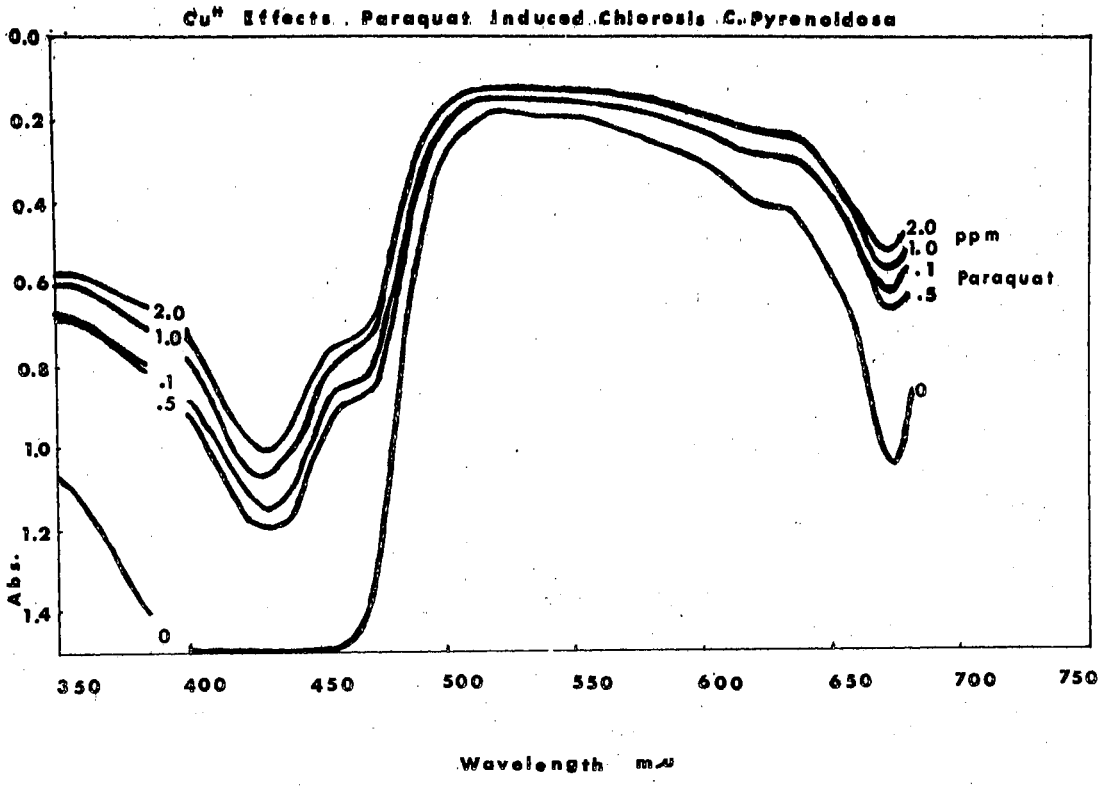


Figure 13. Extractable Chlorophyll Absorption Spectra from $Cu^{++}$ Pretreatment of Cultures at 40 Hours with Paraquat Levels.

by $Cu^{++}$ additions was apparent at all herbicide concentrations. Rate of change at the two peak frequencies was apparently about the same for these cultures with greatly reduced magnitudes of difference apparent between paraquat concentrations as contrasted to the paraquat only series (no $Cu^{++}$) shown in Figure 12.

Results of chemical dithionite determinations for residual paraquat from the paraquat x $Cu^{++}$ factorial cultures are summarized in Table IV. Values shown are means from two replicate cultures expressed as ppm paraquat. Very close duplication of initial paraquat culture additions was recovered in the residual media with no apparent relationship to $Cu^{++}$ treatment or induced chlorosis of the individual Chlorella cultures. Initial treatment level with range in ppm paraquat recovered in residual media were: .1, .06-.16; .5, .46-.56; 1.0, .86-1.06; and 2.0, 1.65-2.05. Values recovered above those added initially may be explained as resulting from metabolism intermediates or excretion products from the organism that also reacted in the non-specific dithionite reactions.

TABLE IV

RESIDUAL MEDIA DITHIONITE DETERMINATION OF PARAQUAT FOLLOWING *C. pyrenoidosa* GROWTH AT FIVE $Cu^{++}$ LEVELS

| Initial paraquat ppm / Initial $Cu^{++}$ ppm | 0 | .1 | .5 | 1.0 | 2.0 |
|---|---|---|---|---|---|
| | ppm Residual Paraquat | | | | |
| 0 | 0 | .06 | .50 | 1.02 | 2.16 |
| .1 | 0 | .10 | .56 | 1.09 | 2.195 |
| .5 | 0 | .085 | .42 | .95 | 2.13 |
| 1.0 | 0 | .26 | .56 | 1.12 | 2.13 |
| 2.0 | 0 | .225 | .49 | .88 | 1.76 |

Figures are means of duplicate cultures C.V. = 5.7%

F values:

Replication = 5.52**

Treatment Combination = 650.50**

Paraquat = 3845.10**

$Cu^{++}$ = 17.86**

Paraquat x $Cu^{++}$ = 10.12**

**Significant at .05 level.

## SUMMARY AND CONCLUSIONS

The object of this study was to determine factors that influence rates and magnitude of paraquat phytotoxicity to the unicellular photolithotrophic organism Chlorella pyrenoidosa Chick IAC251. Culture composition variables with cations in these studies were all supplied as sulfates. Initial Chlorella cell density at 600 mμ was selected for concentrations equivalent to 0.53-1.0 O.D. after synchronization near peak log phase of growth. Paraquat concentrations ranged from 0.1 ppm to 2.0 ppm.

Cell density and cell weight varied from paraquat treatments because of coagulation effects with the paraquat treated Chlorella cells. Extractable chlorophyll was found to be a precise index for paraquat activity with this organism.

Reduction of extractable chlorophyll in Chlorella cultures was closely related to paraquat concentration. Cation sulfate salts of $Cu^{++}$, $Fe^{++}$, $Mn^{++}$ were all found to influence paraquat activity. $Cu^{++}$ levels were particularly effective with apparent inhibition of paraquat phytotoxicity reflected in chlorophyll spectral determinations at 434 mμ and 670 mμ.

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