Improved Test Medium for the Evaluation of Algaecides and Algaestats for Swimming Pools

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An algaecide/algaestat test medium is proposed which contains sufficient nutrients for algal growth, has the hardness and alkalinity characteristics of properly balanced pool water and has an orthophosphate level more consistent with typical concentrations found in swimming pool waters. The chlorophyte alga Chlorella vulgaris was used to test the EC_{50} and Minimum Inhibitory Concentration (MIC) of ionic silver, copper and zinc in Allen's medium and a proposed algal test medium (PATM). Results indicate that both the EC_{50} and MIC of all three toxicants tested were lower in the proposed medium than in Allen's medium.

Introduction

In the 1950's and 1960's a great deal of work was done examining the efficacy of swimming pool algaecides under laboratory conditions. The algal growth medium primarily used was Allen's medium, a high phosphate, low hardness medium with no bicarbonate alkalinity. This medium bears little resemblance to chemically balanced swimming pool water. Swimming pools require sufficient hardness (200–400 ppm as $CaCO_3$) and alkalinity to prevent damage to pool surfaces and equipment. This, coupled with the fact that high phosphate levels have been shown to decrease the bactericidal and algaecidal efficacy of metal ions, suggests the need for an algaecide/algaestat test medium which more closely approximates the chemistry of pool water.

The majority of published research on swimming pool algaecides and algaestats was performed by George P. Fitzgerald, a researcher at the University of Wisconsin. Fitzgerald published a number of studies investigating the Minimum Inhibitory Concentration (MIC) of silver and copper ions against green algae (e.g., *Chlorella vulgaris, C. pyrenoidosa,* etc.) in Allen's medium. Other workers have done similar studies (see Table 1); few, however, are directly related to the swimming pool industry. Corral, et al., after investigating the efficacy of a number of commercial algaecides, voiced the need for a synthetic approximation of swimming pool water in which efficacy studies could be conducted.

Many investigations have been made into the EC_{50} and MIC of various metals of environmental concern (e.g., mercury, copper, lead, etc.). Stauber and Florence (1989), for example used synthetic soft water to test the activity of toxicants against algae in high nutrient media vs. a more natural low nutrient medium. Their goal was to "establish realistic water quality criteria" by using a test medium closer in composition to natural water than to traditional algal growth media. The goals of this study are to develop an algal test media and protocol which permits a more realistic evaluation of swimming pool algaecides; and to compare the MIC and EC_{50} of copper, silver and zinc against *Chlorella vulgaris* in this newly developed media to historical and experimental results in other media.

Table 1 is a summary of investigations which have been done regarding the effect of silver, copper and zinc ions on *Chlorella* species.

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Compound Tested	Organism	Test Medium	MIC (ppm)	EC50 (ppm)	Reference
Ag as $AgNO_3$	*C. pyrenoidosa*	Allen's	0.047	—	Fitzgerald, 1967
Ag as $AgNO_3$	*C. pyrenoidosa*	Allen's	0.063-0.094	—	Allen, 1952
Ag as $AgNO_3$	*C. pyrenoidosa*	Allen's	0.032-0.063	—	Fitzgerald & Faust, 1971
Cu as $CuSO_4$*$5H_2O$	*C. pyrenoidosa*	Allen's	0.75	0.13-0.25	Fitzgerald & Faust, 1963
Cu as $CuSO_4$*$5H_2O$	*C. pyrenoidosa*	EPA	>0.20	0.02-0.05	Stauber & Florence, 1989
Cu as $CuSO_4$*$5H_2O$	*C. pyrenoidosa*	Soft Water	0.10	0.01-0.02	Stauber & Florence, 1989
Cu as $CuSO_4$*$5H_2O$	*C. vulgaris*	Bristol's	>0.30	0.10-0.20*	Young & Lisk , 1972
Cu as $CuSO_4$*$5H_2O$	*C. vulgaris*	Bristol's	>0.32	0.18	Rosko & Rachlin, 1977
Zn as $ZnSO_4$	*C. vulgaris*	Bristol's	>10.0	2.4	Rachlin & Farran, 1974
Zn as ZnCl	*C. vulgaris*	Chu #10	>65	>65	Rai, et. al., 1981
Zn as ZnCl	*C. vulgaris*	Bristol's	>7.5	5.1	Rosko & Rachlin, 1977

Table 1 – Summary of Investigations Regarding the Effect of Silver, Copper and Zinc Ions on *Chlorella* Species

Materials and Methods

Algal Cultures

The algae culture used in this study was *Chlorella vulgaris* (Carolina Biological Supply). This culture was maintained on a proteose agar slant. Sub–cultures for algal assays were grown in 250 ml Erlenmeyer flasks containing 100 ml of the growth media in which the assay was to be carried out. All growth media were filter sterilized using 0.45μm cellulose acetate filters. Sub–cultures were incubated on an orbital shaker (150 rpm) under 200 ft–candles of illumination at 25± 3° C. Shaking and illumination were continuous.

Algal sub–cultures were incubated between six and seven days before use in assays. Cell density was measured microscopically using a haemocytometer.

Algal Assays

Two different media were utilized for each assay.

(1) Allen's Medium (In 1L of Type 1 reagent grade water: 329 mg $K_2HPO_4.3H_2O$; 66 mg $CaCl_2.2H_2O$; 50 mg NH_4Cl; 1 g $NaNO_3$; 513 mg $MgSO_4.7H_2O$; 5 mg $FeCl_3.6H_2O$.)

(2) Proposed Algal Test Medium (In 1L of Type 1 reagent grade water: 294 mg $CaCl_2.2H_2O$; 246 mg $MgSO_4.7H_2O$; 37 mg KCl;1.18 mg $K_2HPO_4.3H_2O$; 61 mg $NaNO_3$; 137 mg $NaHCO_3$; 0.145 mg $FeCl_3.6H_2O$; 0.360 mg $MnCl_2.4H_2O$.)

All assays were carried out in 50 ml borosilicate glass Erlenmeyer flasks containing 25 ml of sterile medium (filter sterilized using 0.45μm cellulose acetate filters). All glassware was acid washed before use in a 1:1 HNO_3 bath, rinsed four times with Type 1 reagent grade water and sterilized in an autoclave at 121°C and 15 psi. Addition of metals was made with $AgNO_3$, $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$ stock solutions which were filter sterilized using 0.45μm PTFE filters. Additions of algal inoculum were made aseptically to give a final cell density in each flask of $3x10^5$ cells/ml. Optical absorbance at 600 nm was prior to incubation at 25± 3°C on an orbital shaker (150 rpm) under 200 ft–candles of illumination. Growth was measured after four days of incubation by optical absorbance at 600 nm. Specific growth rate (μ) was calculated according to the following equation (from Standard Methods for the Examination of Water and Wastewater 8111 G, 19th edition):

$$\text{Specific Growth Rate}(\mu) = \frac{\ln(X_2/X_1)}{t_2-t_1} \quad [=]\ d^{-1}$$

where:

X_2 = Absorbance at 600 nm at end of selected time interval,

X_1 = Absorbance at 600 nm at beginning of selected time interval, and

t_2-t_1 = elapsed time between selected intervals, d.

Specific growth rates of test flasks were compared to the specific growth rate of a control flask (medium only, no toxicants) to determine the % inhibition of growth as follows:

% inhibition of growth =

$$\left[1 - \frac{(\text{Specific Growth Rate})_{\text{test flask}}}{(\text{Specific Growth Rate})_{\text{control flask}}}\right] \times 100.$$

Determination of EC_{50}

Three metal ion concentrations were chosen and tested for each of the metals Ag, Cu and Zn. These concentrations were based on preliminary experiments which gave an indication of the EC_{50} and MIC values for each toxicant. Percent inhibition of growth was plotted against a logarithmic scale of toxicant concentration. Linear regression was used to generate an equation to describe the straight line which best fit the data. To determine the EC_{50}, a "y" value of 50 ("y" being the variable describing percent inhibition of growth) was inserted and the equation solved for "x" (the variable describing toxicant concentration). To determine MIC, a "y" value of 100 was inserted and the equation solved for "x".

Results

The effect of different media on the inhibitory effect of silver, copper and zinc ions against *C. vulgaris* is shown in Table 2. For each of the metals tested the estimated EC_{50} and MIC in Allen's medium was significantly higher than that in the proposed medium. The greatest difference was seen in comparing the effect of copper ions in the two media. In this instance it took almost 6 times as much copper in the Allen's medium as in the proposed medium to inhibit 50% of algal growth. The MIC of copper in Allen's medium was more than 10 times that of the MIC in PATM. Similar trends were seen for both zinc and silver. These trends are shown graphically in Figures 1–3.

Discussion

Stauber and Florence (1989) concluded that the use of full nutrient media in algal toxicity assays could lead to inaccurate EC_{50} values for metal contaminants. They felt that the inclusion of complexing agents such as EDTA and iron in the test media reduced the toxicity of metals to algae. Chambers et al (1960) found that high phosphate concentrations (63 ppm) interfered with the bactericidal action of silver. Other workers (Zevenhuizen, et al 1979 and Landeen, et al, 1989) saw a similar reduction in the activity of copper with phosphates present. Phosphate is known to have metal complexing properties. Being that Allen's medium contains 250 times more phosphate than PATM (125 ppm as compared to 0.5 ppm respectively), this could have reduced the toxicity of all three metals tested towards *C. vulgaris*.

The rationale behind reducing the concentration of phosphate in the proposed medium was to more accurately reflect concentrations commonly found in swimming pool water. Other constituents found in algal growth media (e.g., nitrates, sulfates, chloride) were adjusted to approximate the source water used to fill swimming pools (McGowan, 1988). Typical calcium hardness and alkalinity requirements for pools consistent with guidelines proposed by the National Pool and Spa Institute (1997) were also taken into account when formulating the proposed medium. A comparison of the major constituents of Allen's medium and PATM is given in Table 3.

The composition of PATM proved to be adequate to support growth over the short term (the four day period of the assay). The average specific growth rate(μ) of PATM controls was 0.278, as compared to Allen's controls where the average specific growth rate was 0.447. These data show that while Allen's me-

	Algal Growth Media	EC_{50}(ppm)	MIC (ppm)
Ag	PATM Allen's	0.007 0.019	0.018 0.122
Cu	PATM Allen's	0.020 0.120	0.042 0.450
Zn	PATM Allen's	0.402 1.8	6.0 30.0

Table 2 – Comparison of EC_{50} and MIC of Metals in Allen's Medium and PATM

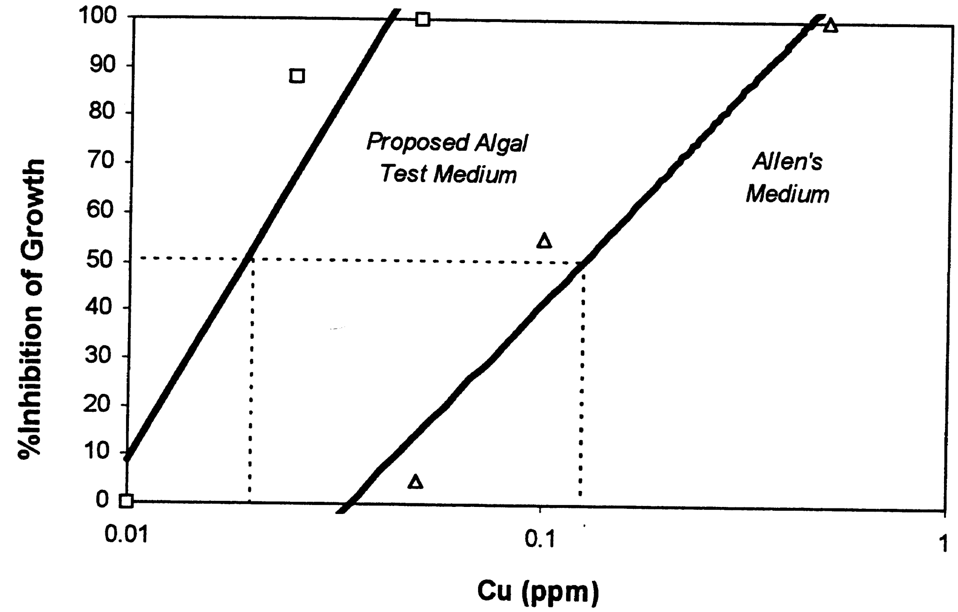

Figure 1 – Percent Inhibition of Growth of *C. vulgaris* vs. Copper Ion Concentration

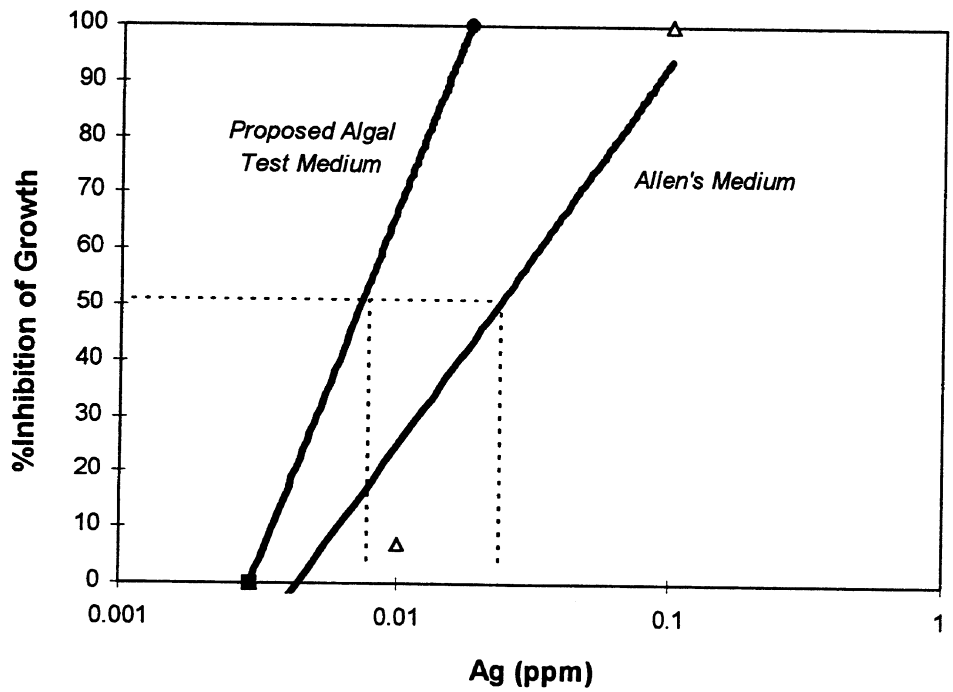

Figure 2 – Percent Inhibition of Growth of *C. vulgaris* vs. Silver Ion Concentration

dium supports more robust growth over the short term, PATM is more than adequate for use in the four day toxicity test employed in this study.

Conclusions

In developing algaecidal compounds and systems for use in swimming pools, it is important to strike a balance between simulating real world conditions and maintaining the control of laboratory experiments. Controlled experiments are necessary for meaningful data, but if the conditions are far removed from those of typical pool water chemistries the data may be misleading with regard to the efficacy of the final product. For example, when tests are done in Allen's medium, an estimated MIC for silver ions of 0.122 ppm is obtained. If this high a level is deemed necessary to inhibit the growth of green algae, a common problem in swimming pools, silver could be ruled out as a practical choice. The MIC test results from PATM, however, indicate that a silver ion concentration of approximately 0.02 ppm would be effective at inhibiting the growth of *C. vulgaris*.

The results regarding copper ions and their effectiveness against green algae also raise questions concerning conclusions reached from historical experimental data. Traditionally it was believed that copper levels of 0.5 – 1.0 ppm were necessary for effective control of algae. Recommended dosages of commercially available copper based algaecides provide for copper concentrations in this range. The estimated MIC attained in this study from the use of the high nutrient Allen's medium (0.45 ppm) would do little to question the need for such high levels of copper. Results from tests with PATM, however, indicate a MIC more than ten times lower (0.042 ppm) and may call for a reevaluation of the levels of copper required in swimming pools to effectively control algae.

Given the results of this study, it is clear that a distinction must be drawn between algaecidal testing done in high nutrient media, bearing little resemblance to swimming pool water, and the results of studies which more closely approximate the typi-

	Proposed Algal Test Medium	Allen's Medium
NH_4	–	17
NO_3	44	730
PO_4	0.5	136
SO_4	96	200
K	19	114
Ca	80	18
Mg	24	51
Fe	Trace	1
Mn	Trace	–
Bicarbonate Alkalinity	100	–

Table 3 – Nutrient Levels in PATM and Allen's Media

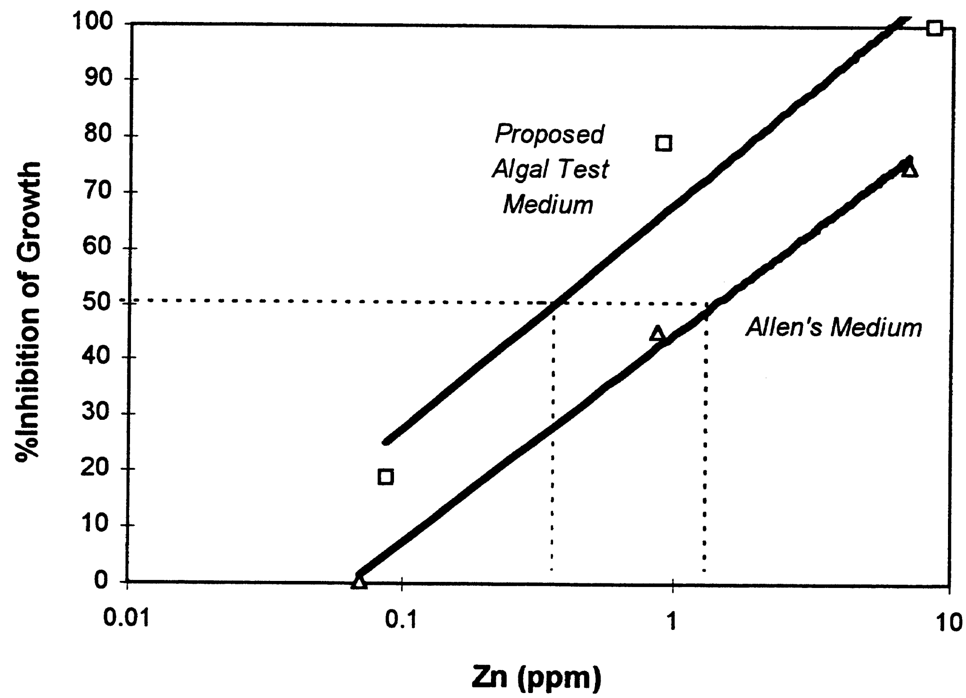

Figure 3 – Percent Inhibition of Growth of *C. vulgaris* vs. Zinc Ion Concentration

cal water chemistry found in pools. Conclusions made regarding the effective level of a given algaecide must be made in view of the test protocol and medium used. Results of tests in high nutrient media (e.g., Allen's medium) should not be considered the standard by which to use an algaecidal chemical.

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