

Final Report Prepared for Modec

**To test the efficacy of MDF 500
for treating swimming pool tile contaminated
with the green alga, *Oocystis*, or the black
alga, *Phormidium***

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Introduction:

This study was conducted to compare the effect of MDF 500™ on Marzite swimming pool tile contaminated with the green alga, *Oocystis* or with the black alga, *Phormidium*. Both of these algae are common contaminants of commercial and private swimming pools.

Methods:

Preparation of alga inocula:

Oocystis sp. (UTEX 80), *Phormidium inundatum* (LB 2517) and *P. foveolarum* (UTEX 427) were obtained from the University of Texas—The Culture Collection of Algae. *Oocystis* was transferred to agar-solidified slants and culture flasks of Blue/Green algae medium (please see below for details of the medium). *Phormidium inundatum* and *P. foveolarum* were transferred to 250-mL flasks containing 50mL of Bold's 3N medium (please see below for composition of the medium). Slants were incubated at room temperature for 7 days in alternating light/dark cycles of fluorescent laboratory lighting. Cultures were transferred to fresh medium weekly.

Just prior to use, agar-solidified culture flasks were flooded with Blue/Green Medium (for *Oocystis*) and the dark green suspension used as described below. Culture flasks of *Phormidium* were well mixed to loosen the adherent dark algae from the sides of the flasks and used directly to inoculate tiles.

Preparation of Marzite squares: Marzite tile squares were obtained from Modec as ~4 x 4 inch x 0.5 inch squares. Squares were sterilized by exposure to UV light on each side for ~24 hours at room temperature. Squares were hydrated by soaking overnight in an excess of sterile distilled water. Squares were allowed to surface dry for 1 hour at room temperature in a sterile laminar air-flow hood.

Inoculation of tiles: Tile squares were individually placed in 6" square Tupperware containers containing with Blue/green algae medium (2 tiles) or Bold's 3N medium (2 tiles) sufficient to cover the surface of each tiles—approximately 250 mL. Each Tupperware containers were inoculated with either *Phormidium inundatum*/*P. foveolarum* or with *Oocystis sp.* Each container was loosely covered with plastic wrap (so as to permit gas exchange) and incubated without shaking at room temperature with alternating light/dark cycles of ambient fluorescent illumination.

Tiles were incubated for 10 to 21 days, with fresh medium added from time to time until visible growth of each alga was noted. Each tile was photographed using a digital camera prior to treatment.

Treatment of tile squares infected with each alga:

Squares were heavily infected with each alga as evidenced by abundant visible growth—please see relevant photographs. Squares were carefully removed from the incubation containers and placed on sterile absorbent paper with plastic backing in a chemical hood venting to the outside. Each square was treated with a solution of freshly prepared MDF 500 by spraying to wetness using commercially available plastic spray bottles. The solutions were allowed to stand for ~10 minutes, drained briefly, rinsed with sterile distilled water, and tiles individually placed into labeled, fresh Tupperware containers with ~250 mL unspent sterile medium (appropriate to the alga being tested). Each container was loosely covered with plastic wrap and incubated at room temperature without shaking in alternating light/dark cycles for 21 days.

At that time, the squares were examined for visible growth, if any. Photographs were also taken of each square.

Results:

The results of the study are presented in the following Tables for observations taken after ~3 weeks of post-treatment incubation.

Three weeks post-treatment

treatment	<i>Oocystis</i>	<i>Phormidium</i>
MDF 500	No visible growth (-)	No visible growth (-)

These results are also shown in the accompanying photographs taken after 3 weeks of post-treatment incubation. .

It is important to note, that both the visual impressions and the photographs reveal the following:

1. After 3 weeks of treatment, MDF500 was very efficacious in treating tile squares and preventing regrowth (even in the presence of growth medium) of both algae.

Conclusion:

Based on the results of this study, MDF 500 was very efficacious in treating pool tile squares heavily contaminated with either *Oocystis* or with *Phormidium*. Treated squares incubated in fresh algal growth medium showed no evidence of regrowth of either alga.

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APPENDIX: GROWTH MEDIA

Bold's 3N medium

Modification of Bold's recipe. General purpose freshwater medium used for xenic cultures, especially blue-greens and reds.

Preparation: To 940 mL of glass-distilled water, add:

mL	stock solution	g/400 mL H ₂ O
30	NaNO ₃	10.0
10	CaCl ₂ ·2H ₂ O	1.0
10	MgSO ₄ ·7H ₂ O	3.0
10	K ₂ HPO ₄	3.0
10	KH ₂ PO ₄	7.0
10	NaCl	1.0
1	vitamin B ₁₂	60.0 × 10 ⁻⁶
6	PIV metal solution	
40	soilwater (GR+) supernatant	

PIV metal solution.

To 1000 mL of glass-distilled water, add 0.750 g of Na₂EDTA, and dissolve fully. Add the following salts in the amounts indicated:

FeCl ₃ ·6H ₂ O	97 mg
MnCl ₂ ·4H ₂ O	41 mg
ZnCl ₂	5 mg
CoCl ₂ ·6H ₂ O	2 mg
Na ₂ MoO ₄ ·2H ₂ O	4 mg

Soilwater as a Medium Supplement

Soilwater (GR+) Supernatant:

The supernatant of soilwater (GR+) medium is included as an ingredient of some media. Soil was obtained from UTEX.

Preparation: In one-half pint bottles, combine:

Garden soil (dry)	1 teaspoon
CaCO ₃	1 pinch
Glass-distilled water	200 mL

Cover the medium container and steam for 2 h on each of 2 consecutive days. Refrigerate (10° C) 24 h or more and bring to room temperature before using. Decant the supernatant, and add the required amount to the medium.

Blue Green Algae Broth Recipe

1. Dissolved the following in 850ml of distilled water:

- 1.5g NaNO₃
 - 0.04g K₂HPO₄
 - 0.075g MgSO₄-7H₂O
 - 0.036g CaCl₂-2H₂O
 - 0.006g Citric acid
 - 0.006g Ferric ammonium citrate
 - 0.001g EDTA
 - 0.02g Na₂CO₃
 - 1.0ml Trace metal mix
2. Adjust pH to 7.1
 3. Bring to 1000ml with distilled water
 4. filter sterilized.

Trace metal mix

1. Dissolved the following in 850ml of distilled water:
 - 2.86g H₃BO₃
 - 1.81g MnCl₂-4H₂O
 - 0.222g ZnSO₄-7H₂O
 - 0.39g Na₂MoO₄-2H₂O
 - 0.079g CuSO₄-5H₂O
 - 0.0494g Co(NO₃)₂-6H₂O



Black Alga - Pretreatment



Black Alga – MDF-500



Green Alga - Pretreatment



Green Alga - MDF-500