



Massachusetts Department of Environmental Protection
Bureau of Resource Protection - Wetlands

WPA Form 3 – Notice of Intent

Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

Provided by MassDEP:

MassDEP File Number

Document Transaction Number

City/Town

Important:
When filling out forms on the computer, use only the tab key to move your cursor - do not use the return key.



Note:
Before completing this form consult your local Conservation Commission regarding any municipal bylaw or ordinance.

A. General Information

1. Project Location (**Note:** electronic filers will click on button to locate project site):

Buttonwood Park Pond

a. Street Address

New Bedford

b. City/Town

02740

c. Zip Code

Latitude and Longitude:

49

f. Assessors Map/Plat Number

41.63345

d. Latitude

-70.95430

e. Longitude

14

g. Parcel /Lot Number

2. Applicant:

Mary

a. First Name

Rapoza

b. Last Name

City of New Bedford Parks, Recreation, and beaches Department

c. Organization

181 Hillman Street, Bldg #3

d. Street Address

New Bedford

e. City/Town

MA

f. State

02740

g. Zip Code

508-961-3015

h. Phone Number

508-991-6175

i. Fax Number

infopr@newbedford-ma-gov

j. Email Address

3. Property owner (required if different from applicant): ☐ Check if more than one owner

a. First Name

b. Last Name

c. Organization

d. Street Address

e. City/Town

f. State

g. Zip Code

h. Phone Number

i. Fax Number

j. Email address

4. Representative (if any):

Jeff

a. First Name

Young

b. Last Name

Advanced Marine Technologies

c. Company

33 Cape Street

d. Street Address

New Bedford

e. City/Town

MA

f. State

02740

g. Zip Code

508-9915225

h. Phone Number

508-991-3033

i. Fax Number

Jeff@organicgem.com

j. Email address

5. Total WPA Fee Paid (from NOI Wetland Fee Transmittal Form):

-
a. Total Fee Paid

-
b. State Fee Paid

-
c. City/Town Fee Paid



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A. General Information (continued)

6. General Project Description:

Applicant is seeking approval for a pilot project to reduce the nitrogen content in the pond, thereby increasing the water quality of the pond and reducing the nuisance plant and algae growth, using only naturally derived de-nitrifying cultures (see Attachment B - Project Description)

7a. Project Type Checklist: (Limited Project Types see Section A. 7b.)

- | | |
|-----------------------------------------------------------------------|-----------------------------------------------------------|
| 1. <input type="checkbox"/> Single Family Home | 2. <input type="checkbox"/> Residential Subdivision |
| 3. <input type="checkbox"/> Commercial/Industrial | 4. <input type="checkbox"/> Dock/Pier |
| 5. <input type="checkbox"/> Utilities | 6. <input type="checkbox"/> Coastal engineering Structure |
| 7. <input type="checkbox"/> Agriculture (e.g., cranberries, forestry) | 8. <input type="checkbox"/> Transportation |
| 9. <input checked="" type="checkbox"/> Other | |

7b. Is any portion of the proposed activity eligible to be treated as a limited project (including Ecological Restoration Limited Project) subject to 310 CMR 10.24 (coastal) or 310 CMR 10.53 (inland)?

1. ☒ Yes ☐ No If yes, describe which limited project applies to this project. (See 310 CMR 10.24 and 10.53 for a complete list and description of limited project types)

310 CMR 10.53(4)(5) Improving the natural capacity of a Resource Area through the removal of aquatic nuisance vegetation to retard pond and lake eutrophication

If the proposed activity is eligible to be treated as an Ecological Restoration Limited Project (310 CMR 10.24(8), 310 CMR 10.53(4)), complete and attach Appendix A: Ecological Restoration Limited Project Checklist and Signed Certification.

8. Property recorded at the Registry of Deeds for:

a. County

b. Certificate # (if registered land)

c. Book

d. Page Number

B. Buffer Zone & Resource Area Impacts (temporary & permanent)

1. ☐ Buffer Zone Only – Check if the project is located only in the Buffer Zone of a Bordering Vegetated Wetland, Inland Bank, or Coastal Resource Area.
2. ☒ Inland Resource Areas (see 310 CMR 10.54-10.58; if not applicable, go to Section B.3, Coastal Resource Areas).

Check all that apply below. Attach narrative and any supporting documentation describing how the project will meet all performance standards for each of the resource areas altered, including standards requiring consideration of alternative project design or location.



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Bureau of Resource Protection - Wetlands

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Document Transaction Number

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B. Buffer Zone & Resource Area Impacts (temporary & permanent) (cont'd)

For all projects affecting other Resource Areas, please attach a narrative explaining how the resource area was delineated.

Resource Area	Size of Proposed Alteration	Proposed Replacement (if any)
a. <input type="checkbox"/> Bank	1. linear feet	2. linear feet
b. <input type="checkbox"/> Bordering Vegetated Wetland	1. square feet 435600	2. square feet
c. <input checked="" type="checkbox"/> Land Under Waterbodies and Waterways	1. square feet 3. cubic yards dredged	2. square feet

Resource Area	Size of Proposed Alteration	Proposed Replacement (if any)
d. <input type="checkbox"/> Bordering Land Subject to Flooding	1. square feet 3. cubic feet of flood storage lost	2. square feet 4. cubic feet replaced
e. <input type="checkbox"/> Isolated Land Subject to Flooding	1. square feet 2. cubic feet of flood storage lost	3. cubic feet replaced
f. <input type="checkbox"/> Riverfront Area	1. Name of Waterway (if available) - specify coastal or inland	
2. Width of Riverfront Area (check one):		
<input type="checkbox"/> 25 ft. - Designated Densely Developed Areas only		
<input type="checkbox"/> 100 ft. - New agricultural projects only		
<input type="checkbox"/> 200 ft. - All other projects		

3. Total area of Riverfront Area on the site of the proposed project: _____ square feet

4. Proposed alteration of the Riverfront Area:

a. total square feet _____ b. square feet within 100 ft. _____ c. square feet between 100 ft. and 200 ft. _____

5. Has an alternatives analysis been done and is it attached to this NOI? ☐ Yes ☐ No

6. Was the lot where the activity is proposed created prior to August 1, 1996? ☐ Yes ☐ No

3. ☐ Coastal Resource Areas: (See 310 CMR 10.25-10.35)

Note: for coastal riverfront areas, please complete **Section B.2.f.** above.



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Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

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B. Buffer Zone & Resource Area Impacts (temporary & permanent) (cont'd)

Check all that apply below. Attach narrative and supporting documentation describing how the project will meet all performance standards for each of the resource areas altered, including standards requiring consideration of alternative project design or location.

Online Users:
Include your
document
transaction
number
(provided on your
receipt page)
with all
supplementary
information you
submit to the
Department.

<u>Resource Area</u>	<u>Size of Proposed Alteration</u>	<u>Proposed Replacement (if any)</u>
a. <input type="checkbox"/> Designated Port Areas	Indicate size under Land Under the Ocean, below	
b. <input type="checkbox"/> Land Under the Ocean	1. square feet	
	2. cubic yards dredged	
c. <input type="checkbox"/> Barrier Beach	Indicate size under Coastal Beaches and/or Coastal Dunes below	
d. <input type="checkbox"/> Coastal Beaches	1. square feet	2. cubic yards beach nourishment
e. <input type="checkbox"/> Coastal Dunes	1. square feet	2. cubic yards dune nourishment
	<u>Size of Proposed Alteration</u>	<u>Proposed Replacement (if any)</u>
f. <input type="checkbox"/> Coastal Banks	1. linear feet	
g. <input type="checkbox"/> Rocky Intertidal Shores	1. square feet	
h. <input type="checkbox"/> Salt Marshes	1. square feet	2. sq ft restoration, rehab., creation
i. <input type="checkbox"/> Land Under Salt Ponds	1. square feet	
	2. cubic yards dredged	
j. <input type="checkbox"/> Land Containing Shellfish	1. square feet	
k. <input type="checkbox"/> Fish Runs	Indicate size under Coastal Banks, inland Bank, Land Under the Ocean, and/or inland Land Under Waterbodies and Waterways, above	
	1. cubic yards dredged	
l. <input type="checkbox"/> Land Subject to Coastal Storm Flowage	1. square feet	
4. <input type="checkbox"/> Restoration/Enhancement	If the project is for the purpose of restoring or enhancing a wetland resource area in addition to the square footage that has been entered in Section B.2.b or B.3.h above, please enter the additional amount here.	
	a. square feet of BVW	b. square feet of Salt Marsh
5. <input type="checkbox"/> Project Involves Stream Crossings		
	a. number of new stream crossings	b. number of replacement stream crossings



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C. Other Applicable Standards and Requirements

- ☒ This is a proposal for an Ecological Restoration Limited Project. Skip Section C and complete Appendix A: Ecological Restoration Limited Project Checklists – Required Actions (310 CMR 10.11).

Streamlined Massachusetts Endangered Species Act/Wetlands Protection Act Review

1. Is any portion of the proposed project located in **Estimated Habitat of Rare Wildlife** as indicated on the most recent Estimated Habitat Map of State-Listed Rare Wetland Wildlife published by the Natural Heritage and Endangered Species Program (NHESP)? To view habitat maps, see the *Massachusetts Natural Heritage Atlas* or go to http://maps.massgis.state.ma.us/PRI_EST_HAB/viewer.htm.

a. ☐ Yes ☐ No

If yes, include proof of mailing or hand delivery of NOI to:

Natural Heritage and Endangered Species Program
Division of Fisheries and Wildlife
1 Rabbit Hill Road
Westborough, MA 01581

b. Date of map

If yes, the project is also subject to Massachusetts Endangered Species Act (MESA) review (321 CMR 10.18). To qualify for a streamlined, 30-day, MESA/Wetlands Protection Act review, please complete Section C.1.c, and include requested materials with this Notice of Intent (NOI); OR complete Section C.2.f, if applicable. *If MESA supplemental information is not included with the NOI, by completing Section 1 of this form, the NHESP will require a separate MESA filing which may take up to 90 days to review (unless noted exceptions in Section 2 apply, see below).*

- c. Submit Supplemental Information for Endangered Species Review*

1. ☐ Percentage/acreage of property to be altered:

(a) within wetland Resource Area

percentage/acreage

(b) outside Resource Area

percentage/acreage

2. ☐ Assessor's Map or right-of-way plan of site

2. ☐ Project plans for entire project site, including wetland resource areas and areas outside of wetlands jurisdiction, showing existing and proposed conditions, existing and proposed tree/vegetation clearing line, and clearly demarcated limits of work **

(a) ☐ Project description (including description of impacts outside of wetland resource area & buffer zone)

(b) ☐ Photographs representative of the site

* Some projects **not** in Estimated Habitat may be located in Priority Habitat, and require NHESP review (see <http://www.mass.gov/eea/agencies/dfg/dfw/natural-heritage/regulatory-review/>). Priority Habitat includes habitat for state-listed plants and strictly upland species not protected by the Wetlands Protection Act.

** MESA projects may not be segmented (321 CMR 10.16). The applicant must disclose full development plans even if such plans are not required as part of the Notice of Intent process.



Massachusetts Department of Environmental Protection
Bureau of Resource Protection - Wetlands

WPA Form 3 – Notice of Intent

Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

Provided by MassDEP:

MassDEP File Number

Document Transaction Number

City/Town

C. Other Applicable Standards and Requirements (cont'd)

- (c) ☐ MESA filing fee (fee information available at http://www.mass.gov/dfwele/dfw/nhesp/regulatory_review/ mesa/ mesa_fee_schedule.htm). Make check payable to "Commonwealth of Massachusetts - NHESP" and **mail to NHESP** at above address

Projects altering 10 or more acres of land, also submit:

- (d) ☐ Vegetation cover type map of site
- (e) ☐ Project plans showing Priority & Estimated Habitat boundaries
- (f) OR Check One of the Following
1. ☐ Project is exempt from MESA review.
Attach applicant letter indicating which MESA exemption applies. (See 321 CMR 10.14, http://www.mass.gov/dfwele/dfw/nhesp/regulatory_review/ mesa/ mesa_exemptions.htm; the NOI must still be sent to NHESP if the project is within estimated habitat pursuant to 310 CMR 10.37 and 10.59.)
 2. ☐ Separate MESA review ongoing. a. NHESP Tracking # _____ b. Date submitted to NHESP _____
 3. ☐ Separate MESA review completed.
Include copy of NHESP "no Take" determination or valid Conservation & Management Permit with approved plan.
3. For coastal projects only, is any portion of the proposed project located below the mean high water line or in a fish run?
- a. ☐ Not applicable – project is in inland resource area only b. ☐ Yes ☐ No

If yes, include proof of mailing, hand delivery, or electronic delivery of NOI to either:

South Shore - Cohasset to Rhode Island border, and the Cape & Islands:

Division of Marine Fisheries -
Southeast Marine Fisheries Station
Attn: Environmental Reviewer
836 South Rodney French Blvd.
New Bedford, MA 02744
Email: DMF.EnvReview-South@state.ma.us

North Shore - Hull to New Hampshire border:

Division of Marine Fisheries -
North Shore Office
Attn: Environmental Reviewer
30 Emerson Avenue
Gloucester, MA 01930
Email: DMF.EnvReview-North@state.ma.us

Also if yes, the project may require a Chapter 91 license. For coastal towns in the Northeast Region, please contact MassDEP's Boston Office. For coastal towns in the Southeast Region, please contact MassDEP's Southeast Regional Office.



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Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

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C. Other Applicable Standards and Requirements (cont'd)

Online Users:
Include your document transaction number (provided on your receipt page) with all supplementary information you submit to the Department.

4. Is any portion of the proposed project within an Area of Critical Environmental Concern (ACEC)?
- a. ☐ Yes ☐ No If yes, provide name of ACEC (see instructions to WPA Form 3 or MassDEP Website for ACEC locations). **Note:** electronic filers click on Website.
- b. ACEC
5. Is any portion of the proposed project within an area designated as an Outstanding Resource Water (ORW) as designated in the Massachusetts Surface Water Quality Standards, 314 CMR 4.00?
- a. ☐ Yes ☐ No
6. Is any portion of the site subject to a Wetlands Restriction Order under the Inland Wetlands Restriction Act (M.G.L. c. 131, § 40A) or the Coastal Wetlands Restriction Act (M.G.L. c. 130, § 105)?
- a. ☐ Yes ☐ No
7. Is this project subject to provisions of the MassDEP Stormwater Management Standards?
- a. ☐ Yes. Attach a copy of the Stormwater Report as required by the Stormwater Management Standards per 310 CMR 10.05(6)(k)-(q) and check if:
1. ☐ Applying for Low Impact Development (LID) site design credits (as described in Stormwater Management Handbook Vol. 2, Chapter 3)
 2. ☐ A portion of the site constitutes redevelopment
 3. ☐ Proprietary BMPs are included in the Stormwater Management System.
- b. ☐ No. Check why the project is exempt:
1. ☐ Single-family house
 2. ☐ Emergency road repair
 3. ☐ Small Residential Subdivision (less than or equal to 4 single-family houses or less than or equal to 4 units in multi-family housing project) with no discharge to Critical Areas.

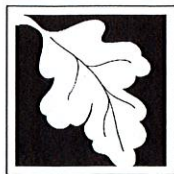
D. Additional Information

- ☒ This is a proposal for an Ecological Restoration Limited Project. Skip Section D and complete Appendix A: Ecological Restoration Notice of Intent – Minimum Required Documents (310 CMR 10.12).

Applicants must include the following with this Notice of Intent (NOI). See instructions for details.

Online Users: Attach the document transaction number (provided on your receipt page) for any of the following information you submit to the Department.

1. ☐ USGS or other map of the area (along with a narrative description, if necessary) containing sufficient information for the Conservation Commission and the Department to locate the site. (Electronic filers may omit this item.)
2. ☐ Plans identifying the location of proposed activities (including activities proposed to serve as a Bordering Vegetated Wetland [BVW] replication area or other mitigating measure) relative to the boundaries of each affected resource area.



Massachusetts Department of Environmental Protection
Bureau of Resource Protection - Wetlands

WPA Form 3 – Notice of Intent

Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

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D. Additional Information (cont'd)

3. ☒ Identify the method for BVW and other resource area boundary delineations (MassDEP BVW Field Data Form(s), Determination of Applicability, Order of Resource Area Delineation, etc.), and attach documentation of the methodology.

4. ☐ List the titles and dates for all plans and other materials submitted with this NOI.

Assessor Map 049

a. Plan Title

b. Prepared By

c. Signed and Stamped by

d. Final Revision Date

e. Scale

f. Additional Plan or Document Title

g. Date

5. ☐ If there is more than one property owner, please attach a list of these property owners not listed on this form.
6. ☐ Attach proof of mailing for Natural Heritage and Endangered Species Program, if needed.
7. ☐ Attach proof of mailing for Massachusetts Division of Marine Fisheries, if needed.
8. ☐ Attach NOI Wetland Fee Transmittal Form
9. ☐ Attach Stormwater Report, if needed.

E. Fees

1. ☒ Fee Exempt: No filing fee shall be assessed for projects of any city, town, county, or district of the Commonwealth, federally recognized Indian tribe housing authority, municipal housing authority, or the Massachusetts Bay Transportation Authority.

Applicants must submit the following information (in addition to pages 1 and 2 of the NOI Wetland Fee Transmittal Form) to confirm fee payment:

2. Municipal Check Number

3. Check date

4. State Check Number

5. Check date

6. Payor name on check: First Name

7. Payor name on check: Last Name



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Bureau of Resource Protection - Wetlands

WPA Form 3 – Notice of Intent

Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

Provided by MassDEP:

MassDEP File Number

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F. Signatures and Submittal Requirements

I hereby certify under the penalties of perjury that the foregoing Notice of Intent and accompanying plans, documents, and supporting data are true and complete to the best of my knowledge. I understand that the Conservation Commission will place notification of this Notice in a local newspaper at the expense of the applicant in accordance with the wetlands regulations, 310 CMR 10.05(5)(a).

I further certify under penalties of perjury that all abutters were notified of this application, pursuant to the requirements of M.G.L. c. 131, § 40. Notice must be made by Certificate of Mailing or in writing by hand delivery or certified mail (return receipt requested) to all abutters within 100 feet of the property line of the project location.

1. Signature of Applicant

2. Date

3. Signature of Property Owner (if different)

4. Date

5. Signature of Representative (if any)

6. Date

For Conservation Commission:

Two copies of the completed Notice of Intent (Form 3), including supporting plans and documents, two copies of the NOI Wetland Fee Transmittal Form, and the city/town fee payment, to the Conservation Commission by certified mail or hand delivery.

For MassDEP:

One copy of the completed Notice of Intent (Form 3), including supporting plans and documents, one copy of the NOI Wetland Fee Transmittal Form, and a **copy** of the state fee payment to the MassDEP Regional Office (see Instructions) by certified mail or hand delivery.

Other:

If the applicant has checked the "yes" box in any part of Section C, Item 3, above, refer to that section and the Instructions for additional submittal requirements.

The original and copies must be sent simultaneously. Failure by the applicant to send copies in a timely manner may result in dismissal of the Notice of Intent.



Massachusetts Department of Environmental Protection
Bureau of Resource Protection - Wetlands
NOI Wetland Fee Transmittal Form
Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

Important: When filling out forms on the computer, use only the tab key to move your cursor - do not use the return key.



A. Applicant Information

1. Location of Project:

Buttonwood Park Pond

a. Street Address

-

c. Check number

New Bedford

b. City/Town

-

d. Fee amount

2. Applicant Mailing Address:

Mary

a. First Name

Rapoza

b. Last Name

City of New Bedford Parks, Recreation, and Beaches Department

c. Organization

181 Hillman Street, Bldg #3

d. Mailing Address

New Bedford

e. City/Town

MA

f. State

02740

g. Zip Code

508-961-3015

h. Phone Number

508-991-6175

i. Fax Number

infopr@newbedford-ma.gov

j. Email Address

3. Property Owner (if different):

a. First Name

b. Last Name

c. Organization

d. Mailing Address

e. City/Town

f. State

g. Zip Code

h. Phone Number

i. Fax Number

j. Email Address

B. Fees

Fee should be calculated using the following process & worksheet. **Please see Instructions before filling out worksheet.**

Step 1/Type of Activity: Describe each type of activity that will occur in wetland resource area and buffer zone.

Step 2/Number of Activities: Identify the number of each type of activity.

Step 3/Individual Activity Fee: Identify each activity fee from the six project categories listed in the instructions.

Step 4/Subtotal Activity Fee: Multiply the number of activities (identified in Step 2) times the fee per category (identified in Step 3) to reach a subtotal fee amount. Note: If any of these activities are in a Riverfront Area in addition to another Resource Area or the Buffer Zone, the fee per activity should be multiplied by 1.5 and then added to the subtotal amount.

Step 5/Total Project Fee: Determine the total project fee by adding the subtotal amounts from Step 4.

Step 6/Fee Payments: To calculate the state share of the fee, divide the total fee in half and subtract \$12.50. To calculate the city/town share of the fee, divide the total fee in half and add \$12.50.

To calculate filing fees, refer to the category fee list and examples in the instructions for filling out WPA Form 3 (Notice of Intent).



Massachusetts Department of Environmental Protection
Bureau of Resource Protection - Wetlands
NOI Wetland Fee Transmittal Form
Massachusetts Wetlands Protection Act M.G.L. c. 131, §40

B. Fees (continued)

Step 1/Type of Activity	Step 2/Number of Activities	Step 3/Individual Activity Fee	Step 4/Subtotal Activity Fee
Cat. 2e: Inland Limited Project			
Fee exempt: Municipal	1	\$0	\$0

Step 5/Total Project Fee: _____

Step 6/Fee Payments:

Total Project Fee:	\$0
	a. Total Fee from Step 5
State share of filing Fee:	\$0
	b. 1/2 Total Fee less \$12.50
City/Town share of filling Fee:	\$0
	c. 1/2 Total Fee plus \$12.50

C. Submittal Requirements

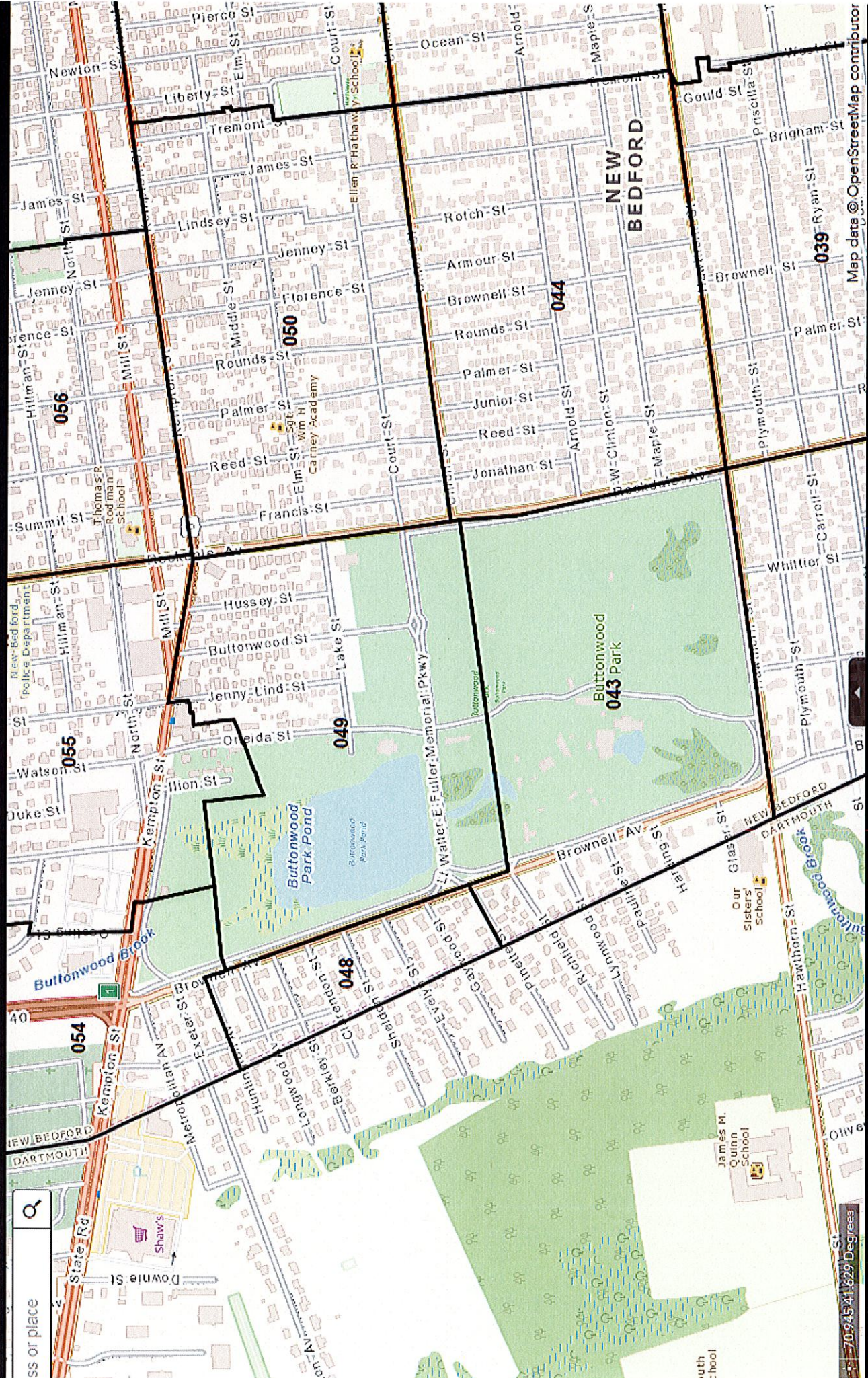
- a.) Complete pages 1 and 2 and send with a check or money order for the state share of the fee, payable to the Commonwealth of Massachusetts.

Department of Environmental Protection
Box 4062
Boston, MA 02211

- b.) **To the Conservation Commission:** Send the Notice of Intent or Abbreviated Notice of Intent; a **copy** of this form; and the city/town fee payment.

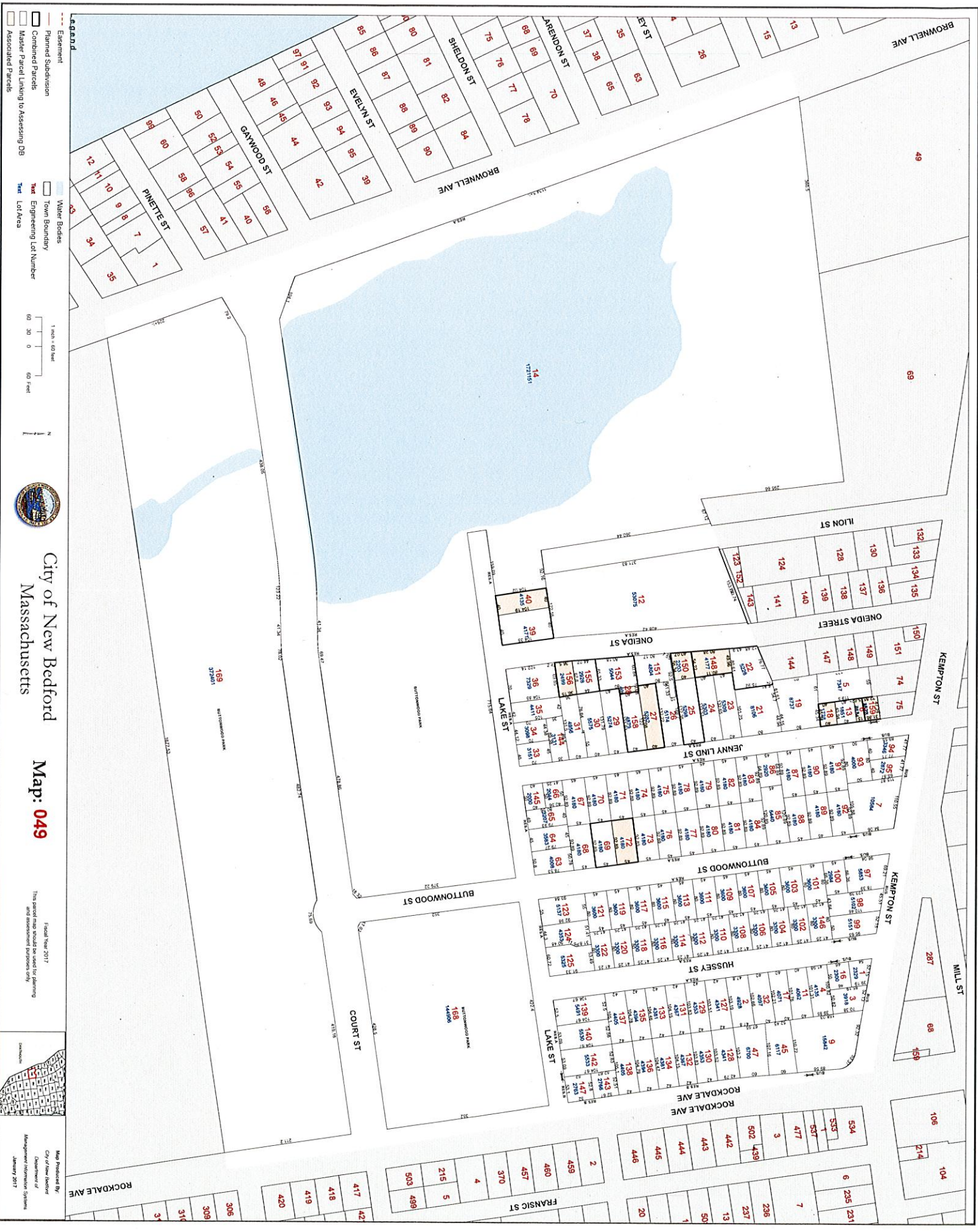
To MassDEP Regional Office (see Instructions): Send a copy of the Notice of Intent or Abbreviated Notice of Intent; a **copy** of this form; and a **copy** of the state fee payment. (E-filers of Notices of Intent may submit these electronically.)

Parcel Information Click on Parcel to open Assessors information



All rights reserved

Map data © OpenStreetMap contributor



- Easement
- Planned Subdivision
- Combined Parcels
- Master Parcel Linking to Assessing DB
- Associated Parcels
- Water Bodies
- Town Boundary
- Engineering Lot Number
- Lot Area



City of New Bedford
Massachusetts
Map: 049

Printed: 10/17/2017
This map is for informational purposes only.
It is not intended for use in any legal proceeding.



Map Produced By:
City of New Bedford
Department of Information Systems
January 2017

Ecological Laboratories, Inc.
Acute Toxicity Evaluation

With the water flea *Daphnia magna* and the freshwater fish *P.promelas*

Microbe-Lift Industrial SA

Conducted For:

ECOLOGICAL LABORATORIES, INC
2525 NE 9th Ave
Cape Coral, FL 33909

Conducted and Prepared By:

ENVIROSCIENCE, INC.
5070 Stow Road
Stow, OH 44224



Katherine L. Hansler

, Aquatic Biologist

April 3, 2018

Ms. Delvia Lukito
Ecological Laboratories, Inc.
2525 NE 9th Ave
Cape Coral, FL 33909

Re: Acute Toxicity Test Results for Microbe-Lift Industrial SA

Dear Ms. Lukito:

Enclosed is a copy of EnviroScience's final report for the following toxicity tests which were initiated on March 28, 2018:

- (1) 48-hour static, non-renewal acute bioassay using *Daphnia magna* (water flea).
- (1) 96-hour static, non-renewal acute bioassay using *Pimephales promelas* (fathead minnow).

The sample identified as Microbe-Lift Industrial SA was received in the EnviroScience laboratory in March 2018. The samples were tested for LC₅₀ values (i.e., the concentration that is lethal to 50% of the exposed organisms). The following report details the testing events for each species and the respective toxicity endpoints.

Acute endpoints for Industrial SA (mg/L)		
Ecological Laboratories	<i>Daphnia magna</i>	<i>Pimephales promelas</i> (FHM)
	48-hour LC ₅₀	96-hour LC ₅₀
Industrial SA	>100 mg/L	>100 mg/L

Please call me if you have any questions.

Sincerely,

Katherine L. Hansler

Katherine L. Hansler, Aquatic Biologist

enclosures



5070 Stow Road
Stow, OH 44224

TABLE OF CONTENTS

1.0	INTRODUCTION.....	1
2.0	METHODS	1
	2.1 Test design.	1
	2.2 Preparation of test solutions.	1
	2.3 Data collection.	2
	2.4 Data analysis.	3
3.0	RESULTS.....	4

APPENDIX

Bench sheets, data analysis, Standard Reference Toxicant (SRT) Control Charts

LIST OF TABLES

Table 2.1	Summary of acute toxicity test conditions.....	3
Table 3.1	LC ₅₀ endpoints.	4
Table 3.2	Water quality data.....	4
Table 3.3	Microbe-Lift Industrial SA acute toxicity test results.....	5

1.0 INTRODUCTION

The acute toxicity of Ecological Laboratories, Inc. product labeled Microbe-Lift Industrial SA was evaluated for two aquatic species common to freshwater toxicity test methods. These methods are used by the National Pollutant Discharge Elimination System (NPDES) regulatory program for monitoring discharges of industrial and municipal sources of wastewater in the United States.

One invertebrate species, *Daphnia magna* (water flea) and one vertebrate species, *P. promelas* were exposed to the product using a static, non-renewal testing procedure. This report describes the results of the toxicity tests conducted at EnviroScience Inc., 5070 Stow Rd., Stow, OH 44224, during the period March 28-April 1, 2018.

2.0 METHODS

Toxicity test methods followed EnviroScience's written standard operating procedures (SOPs), which were derived from USEPA guidelines found in their documents titled *Methods for Measuring the Acute Toxicity of Effluents and Receiving waters to Freshwater and Marine Organisms* (EPA-821-R-02-012) and *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA-821-R02-013).

2.1 Test design

Table 2.1 lists testing conditions for both species and types of tests, including test dates

2.2 Preparation of test solutions

The test used concentrations set up with aliquots of a stock solution of product equal to 100 mg/L product. This served as the highest concentration tested for the product. Each concentration was made using a portion of stock solution that was measured out to 100, 50, 25, 12.5, and 6.25 mg/L

and each was brought to 1000 milliliters utilizing MHRW. The dilution water (MHRW) was prepared by dissolving four reagent grade salts (KCl, MgSO₄, CaSO₄·2H₂O, and NaHCO₃) in ELGA Pureflex II de-ionized water, and was continuously aerated before use. Specimens exposed to an aliquot of diluent served as the test control. The stock solution was prepared by measuring Microbe-Lift Industrial SA and mixing it for 30 minutes with MHRW with a Teflon coated magnetic stir bar.

Labeled test vessels were filled with test solutions, covered with a sheet of plexiglass, and placed in an environmental chamber set to operate at the desired test conditions until solutions reached test temperature (25±1 °C for the minnows; 20±1 °C for the fleas).

Approximately 50 milliliters of each test solution was poured into a labeled plastic beaker for analysis of the initial water quality (dissolved oxygen concentration, pH, and conductivity). Temperature was measured directly in test solutions immediately prior to loading specimens into the prepared test vessels.

2.3 Data collection

Tests were initiated as organisms were randomly selected and placed into test vessels. Each chamber was monitored daily at 24 ±2 hour intervals from the time of test initiation (±1 hour at test termination). The number of mortalities and the total number of adversely affected specimens (cumulative mortality plus specimens showing a behavioral effect) were recorded on bench sheets. Solution temperature was recorded from one replicate vessel per test level. Dissolved oxygen concentration, pH, and conductivity were monitored daily. The following methods and instruments were used in chemical analysis:

Dissolved Oxygen: APHA (1998, 20th ed.) 4500-O G., YSI 5100

pH: APHA (1998, 20th ed.) 4500-H⁺ B., Orion 920A/Orion 2Star

Conductivity: APHA (1998, 20th ed.) 2510 B., Orion 160

2.4 Data analysis

The median lethal concentration (LC₅₀) was computed from mortality data using the Spearman-Kärber, trimmed Spearman-Kärber, or binomial methods with the computer program Cetis 1.9.1. The LC₅₀ endpoint represents the concentration of Microbe-Lift Industrial SA that would be expected to cause 50% mortality during the specified exposure period, typically 48 hours for the water fleas and 96 hours for the fathead minnows.

Table 2.1. Summary of toxicity test conditions for testing with <i>Daphnia magna</i> and <i>Pimephales promelas</i> .		
	<i>Daphnia magna</i>	<i>Pimephales promelas</i>
1. Test period:	03/28/18-1430 to 03/30/18-1330	03/28/18-1340 to 04/01/18-1350
2. Test type and duration:	static, non-renewal, 48 hours	static, non-renewal, 96-hours
3. Age, source of test organisms:	<24 hours, EnviroScience 03/27/18-1450	7 days (within 24 hrs), ES 03/19/18-1520
4. Photoperiod:	16 hours light / 8 hours dark	16 hours light / 8 hours dark
5. Light quality:	fluorescent light, 50-100fc	fluorescent light, 50-100fc
6. Test solution temperatures, °C:	20±1	25±1
7. Feeding regime:	fed alga <i>Selenastrum capricornutum</i> and YAT prior to test only	fed approximately 500 brine shrimp/vessel at 48 hours
8. Size of test vessel:	30 ml plastic cup	600 ml glass beaker
9. Volume/depth of test solutions:	15 ml and 24 mm	300 ml and 5.8 cm
10. No. of test organisms/vessel:	5	10 (except where noted)
11. No. of vessels per solution:	4	2
12. No. organisms/test level:	20	20 (except where noted)
13. Test concentrations: mg/L MHRW	6.25, 12.5, 25, 50, 100	6.25, 12.5, 25, 50, 100
14. Dilution and control water:	moderately hard reconstituted water (MHRW)	moderately hard reconstituted water (MHRW)
15. Aeration:	none	none
16. Test acceptability criterion:	≥90% survival in control	≥90% survival in control
17. Endpoints:	mortality - no movement with gentle prodding (LC ₅₀)	mortality - no movement with gentle prodding (LC ₅₀)

3.0 RESULTS

Toxicity test results and associated water quality data are summarized in Tables 3.1 through 3.3. Table 3.1 lists LC₅₀ endpoints for each species. Table 3.2 lists the initial water quality data for samples of diluent and selected concentrations of freshly prepared test solutions. Water quality data collected at 24-hour intervals and at test termination are included in the Appendix. Table 3.3 lists percent mortality and percent adversely affected in test solutions for each 24-hour period and provides the 95% confidence interval (C.I.) estimates associated with the calculated LC₅₀ value for the acute test.

Table 3.1 Acute endpoints for Industrial SA (mg/L)		
Ecological Laboratories	<i>Daphnia magna</i>	<i>Pimephales promelas</i> (FHM)
	48-hour LC ₅₀	96-hour LC ₅₀
Industrial SA	>100 mg/L	>100 mg/L

Table 3.2 Water quality data for dilution water and selected test concentrations for freshly prepared test solution.						
Test solution	Date of first use in tests	DO mg/L	pH s.u.	conductivity µmho/cm	alkalinity mg/L CaCO ₃	hardness mg/L CaCO ₃
MHRW	03/28/18	8.6	7.4	282	58	88
Industrial SA	03/28/18	8.6	7.2	296	NA	NA

Table 3.3. Product Industrial SA toxicity test results. Cumulative mortality and percent affected, LC ₅₀ and 95% confidence interval, and method of analysis.									
Concentration milligrams product/ L MHRW	D. magna (water flea) tested 03/28/18 to 03/30/18		P. promelas (fathead minnow) tested 03/28/18 to 04/01/18						
	24-hours % mortality (% affected)	48-hours % mortality (% affected)	Conc. milligrams product/ L MHRW	24-hours % mortality (% affected)	48-hours % mortality (% affected)	72-hours % mortality (% affected)	96-hours % mortality (% affected)		
0, MHRW	0 (0)	0 (0)	0, MHRW	0 (0)	5 (5)	5 (5)	5 (5)		
6.25	0 (0)	0 (0)	6.25	0 (0)	10 (10)	15 (15)	20 (20)		
12.5	0 (0)	0 (0)	12.5	0 (0)	0 (0)	0 (0)	5 (5)		
25	0 (0)	0 (0)	25	0 (0)	0 (0)	0 (0)	5 (5)		
50	0 (0)	0 (0)	50	0 (0)	0 (0)	0 (0)	0 (0)		
100	0 (0)	5 (5)	100	0 (0)	0 (0)	5 (5)	5 (5)		
LC₅₀		>100 mg/L	LC₅₀				>100 mg/L		
95% C.I.			95% C.I.						
Method/Comments: None needed.									

APPENDIX

Bench Sheets

Data Analysis

Standard Reference Toxicant (SRT) Control Charts



ACUTE, STATIC-NON RENEWAL BIOASSAY:
 CLIENT: Microbe SA
 START DATE: 032818 TIME: 1430
 END DATE: 033018 TIME: 1330
 DILUTION WATER: MHR

ORGANISM: D. Magna
 ORIGIN: ES cultures
 HATCH: 032718 1450
 AGE: 24hrs. old
 ORGANISMS PER VESSEL: 5

Test Levels		Biological Parameters			Chemistry and Physical Parameters													
		Rep	Ni	Dead / Affected		Temperature (Celsius)			Dissolved Oxygen (mg/l)			pH (s.u.)			Conductivity (µmhos/cm)			
				24 hr	48 hr	0	24	48	0	24	48	0	24	48	0	24	48	
Control: <u>MHR</u>	A	5	0 / 0	0 / 0	20.9	20.7	21.0	8.3		8.6	7.4		7.3	286		301		
	B	5	0 / 0	0 / 0	Alkalinity: ___ mL (Mg/L CaCO ₃)													
	C	5	0 / 0	0 / 0	Hardness: ___ mL (Mg/L CaCO ₃) (Mg/L CaCO ₃)													
	D	5	0 / 0	0 / 0														
<u>6.25</u> mg/L	A	5	0 / 0	0 / 0	20.1	21.0	21.0	8.6		8.6	7.3		7.2	288		310		
	B	5	0 / 0	0 / 0														
	C	5	0 / 0	0 / 0														
	D	5	0 / 0	0 / 0														
<u>12.5</u> mg/L	A	5	0 / 0	0 / 0	19.6	21.0	21.0	8.6		8.6	7.2		7.3	289		311		
	B	5	0 / 0	0 / 0														
	C	5	0 / 0	0 / 0														
	D	5	0 / 0	0 / 0														
<u>25</u> mg/L	A	5	0 / 0	0 / 0	19.6	21.0	21.0	8.6		8.6	7.2		7.3	289		308		
	B	5	0 / 0	0 / 0														
	C	5	0 / 0	0 / 0														
	D	5	0 / 0	0 / 0														
<u>50</u> mg/L	A	5	0 / 0	0 / 0	19.5	21.0	21.0	8.6		8.6	7.2		7.3	292		312		
	B	5	0 / 0	0 / 0														
	C	5	0 / 0	0 / 0														
	D	5	0 / 0	0 / 0														
<u>100</u> mg/L	A	5	0 / 0	0 / 0	19.9	21.0	21.0	8.6		8.6	7.2		7.3	296		320		
	B	5	0 / 0	0 / 0														
	C	5	0 / 0	0 / 0														
	D	5	0 / 0	1 / 1														
___ mg/L	A		1	1														
	B		1	1														
	C		1	1														
	D		1	1														
___ mg/L	A		1	1														
	B		1	1														
	C		1	1														
	D		1	1														
Time	1430		1425		1330		1230		1335		1230		1335		1230		1335	
Tech	TM		AM		TM		MT		CB		MT		CB		MT		CB	
Comments: <u>144</u>					2	4	4	YSI 100			Orion 920			Orion 160				
					ID or <input type="checkbox"/> instrument used			<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		
					Other:			Orion 2Star			Other:							



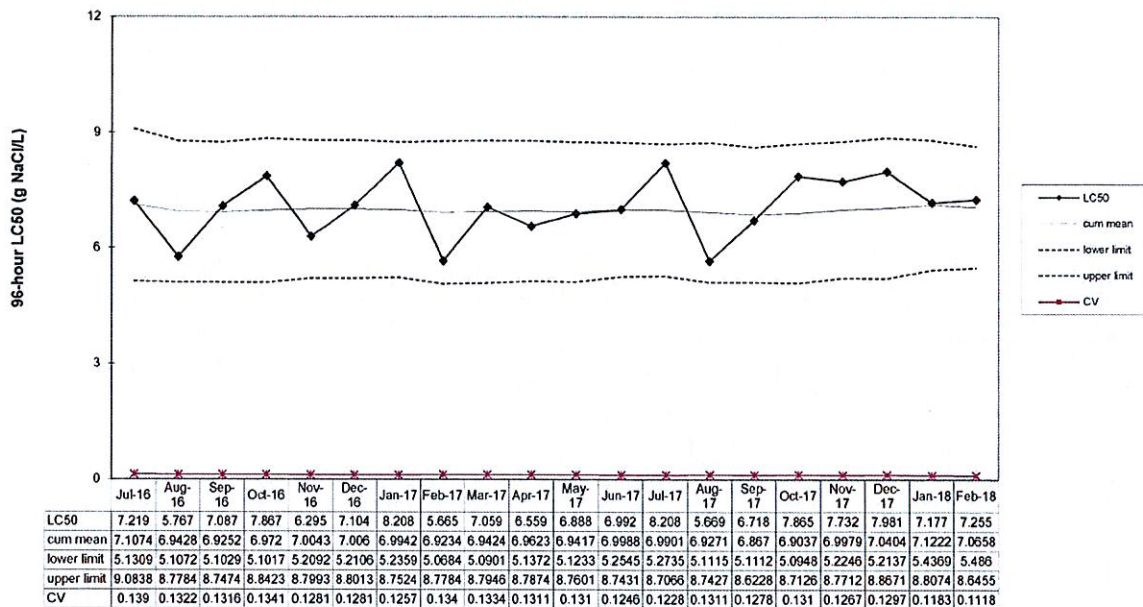
ACUTE, STATIC-NON RENEWAL BIOASSAY:
CLIENT: Microbe SA
START DATE: 032818 TIME: 1340
END DATE: 040118 TIME: 1350
DILUTION WATER: MHE

ORGANISM: FHM
ORIGIN: ES cultures
HATCH: 031918 1520
AGE: 7 days
ORGANISMS PER VESSEL: 10

Test Levels		Biological Parameters					Chemistry and Physical Parameters																			
		Rep	Ni	Dead / Affected			Temperature			Dissolved Oxygen (mg/l)			pH (s.u.)			Conductivity										
				24h	48h	72h	96h	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96				
Control: <u>pH</u>		A	10	0/0	0/0	0/0	0/0	0/0	24.0	24.0	24.0	24.0	8.3	8.6	8.4	8.6	7.4	7.4	7.3	7.2	286	308	303	313	308	
		B	10	0/0	1/1	1/1	1/1	1/1																		
<u>12.5</u> mg/L		A	10	0/0	1/1	2/2	3/3	1/1	24.0	24.0	24.0	24.0	8.6	8.6	8.4	8.6	7.3	7.3	7.2	7.2	288	313	308	324	326	
		B	10	0/0	1/1	1/1	1/1	1/1																		
<u>12.5</u> mg/L		A	10	0/0	0/0	0/0	1/1	1/1	24.0	24.0	24.0	24.0	8.6	8.6	8.4	8.5	7.2	7.2	7.2	7.2	289	307	319	330	324	
		B	10	0/0	0/0	0/0	0/0	0/0																		
<u>25</u> mg/L		A	10	0/0	0/0	0/0	0/0	0/0	24.0	24.0	24.0	24.0	8.6	8.6	8.4	8.6	7.2	7.2	7.1	7.2	289	330	331	338	336	
		B	10	0/0	0/0	0/0	0/0	1/1																		
<u>50</u> mg/L		A	10	0/0	0/0	0/0	0/0	0/0	24.0	24.0	24.0	24.0	8.6	8.6	8.4	8.4	7.2	7.2	7.1	7.2	292	317	315	327	324	
		B	10	0/0	0/0	0/0	0/0	0/0																		
<u>100</u> mg/L		A	10	0/0	0/0	0/0	0/0	0/0	24.0	24.0	24.0	24.0	8.6	8.6	8.4	8.3	7.2	7.2	7.1	7.2	290	327	329	337	334	
		B	10	0/0	0/0	1/1	1/1	1/1																		
<u>mg/L</u>		A		/	/	/	/	/																		
		B		/	/	/	/	/																		
<u>mg/L</u>		A		/	/	/	/	/																		
		B		/	/	/	/	/																		
<u>mg/L</u>		A		/	/	/	/	/																		
		B		/	/	/	/	/																		
Time		1340	1330	1305	1250	1230	1350		1	1	1	1	YSIS100			Orion 920			Orion 160							
Tech		AM	AM	SL	CB	MT			ID or Instrument used	MT			CB	SL	MT	MT	CB	CB	MT	MT	CB	CB	SL	MT	MT	
Comments:									Other:			Other:			Other:			Other:								

VHL

EnviroScience QC Chart - *Pimephales promelas*
Acute Toxicity Endpoint - 25 C

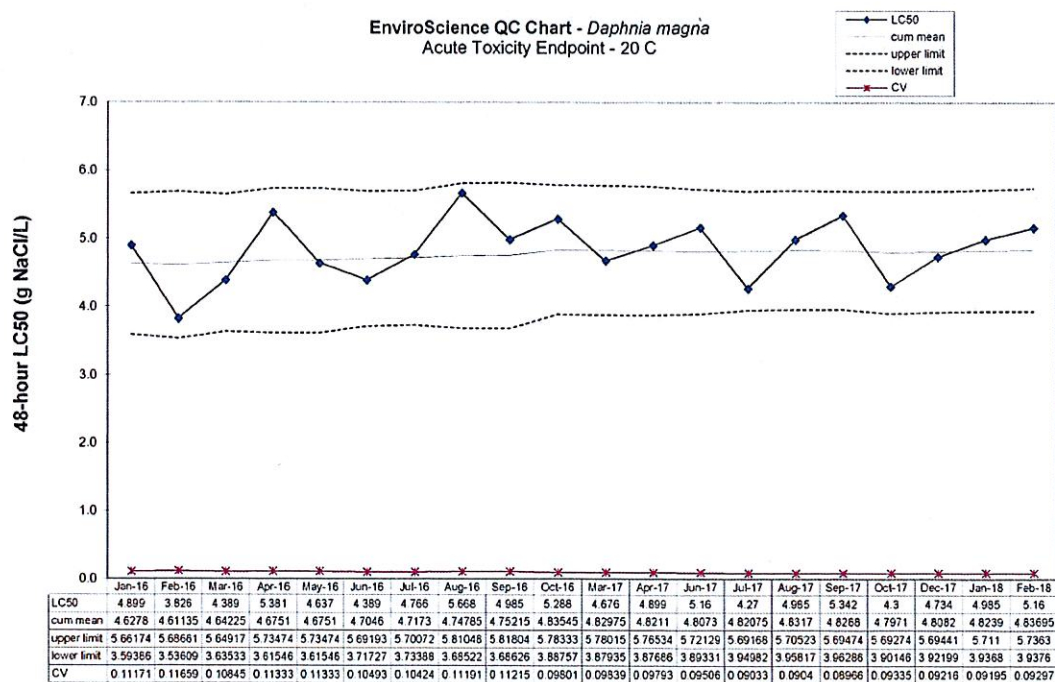


Test Date (MM.YY)

Tests conducted at Stow, OH facility

EnviroScience QC/SRT Chart rev. 03/06/18

EnviroScience QC Chart - *Daphnia magna*
Acute Toxicity Endpoint - 20 C



Test Date

Tests conducted at Stow, OH facility

EnviroScience QC/SRT Chart rev. 03/06/18

Ecological Laboratories, Inc.
Acute Toxicity Evaluation

with Water Flea Species *Daphnia magna*

Microbe-Lift IND HI-Count

Conducted For:

ECOLOGICAL LABORATORIES, INC
2525 NE 9th Ave
Cape Coral, FL 33909

Conducted and Prepared By:

ENVIROSCIENCE, INC.
5070 Stow Road
Stow, OH 44224



Katherine L. Hansler, Laboratory Manager

April 3, 2018

Ms. Delvia Lukito
Ecological Laboratories, Inc.
2525 NE 9th Ave
Cape Coral, FL 33909

Re: Acute Toxicity Test Results for Microbe-Lift IND HI-Count

Dear Ms. Lukito:

Enclosed is a copy of EnviroScience's final report for the following toxicity test which was initiated on March 28, 2018:

(1) 48-hour static, non-renewal acute bioassay using *Daphnia magna* (water flea).

The product identified as Microbe-Lift IND HI-Count was received in the EnviroScience laboratory in March 2018. The product was tested for LC₅₀ values (i.e., the concentration that is lethal to 50% of the exposed organisms). The following report details the testing events for the product and the respective toxicity endpoints.

Acute endpoint for IND HI-Count (mg/L)	
Ecological Laboratories	<i>Daphnia magna</i>
	48-hour LC ₅₀
IND HI-Count	>100 mg/L

Please call me if you have any questions.

Sincerely,

Katherine L. Hansler

Katherine L. Hansler, Laboratory Manager

enclosures



5070 Stow Road
Stow, OH 44224

TABLE OF CONTENTS

1.0	INTRODUCTION	1
2.0	METHODS	1
	2.1 Test design.....	1
	2.2 Preparation of test solutions.....	2
	2.3 Data collection.....	2
	2.4 Data analysis.....	3
3.0	RESULTS	4
APPENDIX		
	Bench sheets, data analysis, Standard Reference Toxicant (SRT) Control Charts	

LIST OF TABLES

Table 2.1	Summary of acute toxicity test conditions	3
Table 3.1	LC ₅₀ endpoints.	4
Table 3.2	Water quality data	4
Table 3.3	Product IND Hi-Count acute toxicity test results.....	5

1.0 INTRODUCTION

The acute toxicity of Ecological Laboratories, Inc. product labeled Microbe-Lift IND HI-Count was evaluated for one aquatic species common to freshwater toxicity test methods. These methods are used by the National Pollutant Discharge Elimination System (NPDES) regulatory program for monitoring discharges of industrial and municipal sources of wastewater in the United States.

One invertebrate species, *Daphnia magna* (water flea) was exposed to IND HI-Count using a static, non-renewal testing procedure for the acute toxicity. This report describes the results of the toxicity test conducted at EnviroScience Inc., 5070 Stow Rd., Stow, OH 44224, during the period March 28-30, 2018.

2.0 METHODS

Toxicity test methods followed EnviroScience's written standard operating procedures (SOPs), which were derived from USEPA guidelines found in their documents titled *Methods for Measuring the Acute Toxicity of Effluents and Receiving waters to Freshwater and Marine Organisms* (EPA-821-R-02-012) and *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms* (EPA-821-R02-013).

2.1 Test design

Table 2.1 lists testing conditions for *Daphnia magna*, including test dates and product concentrations.

2.2 Preparation of test solutions

A stock solution was made equal to 100 mg/L of product. This served as the highest concentration tested for the product. Each concentration was made using a portion of stock solution that measured out to 6.25, 12.5, 25, 50, and 100 mg/L and each was brought to 200 milliliters utilizing MHRW. The dilution water was prepared by dissolving four reagent grade salts (KCl, MgSO₄, CaSO₄•2H₂O, and NaHCO₃) in ELGA Pureflex II de-ionized water, and was continuously aerated before use. Specimens exposed to an aliquot of diluent served as the test control.

Labeled test vessels were filled with test solutions, covered with a sheet of plexiglass, and placed in an environmental chamber set to operate at the desired test conditions until solutions reached test temperature (20±1 °C).

Approximately 50 milliliters of each test solution was poured into a labeled plastic beaker for analysis of the initial water quality (dissolved oxygen concentration, pH, and conductivity). Temperature was measured directly in test solutions immediately prior to loading specimens into the prepared test vessels.

2.3 Data collection

The test was initiated as organisms were randomly selected and placed into test vessels. Each chamber was monitored daily at 24 ±2 hour intervals from the time of test initiation (±1 hour at test termination). The number of mortalities and the total number of adversely affected specimens (cumulative mortality plus specimens showing a behavioral effect) were recorded on bench sheets. Solution temperature was recorded from one replicate vessel per test level. Dissolved oxygen concentration, pH, and conductivity were monitored daily. The following methods and instruments were used in chemical analysis:

Dissolved Oxygen: APHA (1998, 20th ed.) 4500-O G., YSI 5100
pH: APHA (1998, 20th ed.) 4500-H⁺ B., Orion 920A/Orion 2Star
Conductivity: APHA (1998, 20th ed.) 2510 B., Orion 160

2.4 Data analysis

The median lethal concentration (LC₅₀) was computed from mortality data using the Spearman-Kärber, trimmed Spearman-Kärber, or binomial methods with the computer program Cetus 1.9.1. The LC₅₀ endpoint represents the concentration of product that would be expected to cause 50% mortality during the specified exposure period, typically 48 hours for the *Daphnia magna*.

Table 2.1. Summary of acute toxicity test conditions for testing with <i>Daphnia magna</i> .	
1. Test period:	03/28/18-1420 to 03/30/18-1325
2. Test type and duration:	static, non-renewal, 48-hours
3. Age, source of test organisms:	<24 hours, EnviroScience 03/27/18-1450
4. Photoperiod:	16 hours light / 8 hours dark
5. Light quality:	fluorescent light, 50-100fc
6. Test solution temperatures, °C:	20±1
7. Feeding regime:	fed alga <i>Selenastrum capricornutum</i> and YAT prior to test only
8. Size of test vessel:	30 ml plastic cup
9. Volume/depth of test solutions:	15 ml and 24 mm
10. No. of test organisms/vessel:	5
11. No. of vessels per solution:	4
12. No. organisms/test level:	20
13. Test concentrations: mg/L MHRW	6.25, 12.5, 25, 50, 100
14. Dilution and control water:	moderately hard reconstituted water (MHRW)
15. Aeration:	none
16. Test acceptability criterion:	≥90% survival in control
17. Endpoints:	mortality - no movement with gentle prodding (LC ₅₀)

3.0 RESULTS

Toxicity test results and associated water quality data are summarized in Tables 3.1 through 3.3. Table 3.1 lists LC₅₀ endpoints. Table 3.2 lists the initial water quality data for samples of diluent and selected concentrations of freshly prepared test solutions. Water quality data collected at test termination are included in the Appendix. Table 3.3 lists percent mortality and percent adversely affected in test solutions for each 24-hour period and provide the 95% confidence interval (C.I.) estimates associated with the calculated LC₅₀ value for each acute test.

Table 3.1 Acute endpoint for IND HI-Count (mg/L)	
Ecological Laboratories	<i>Daphnia magna</i>
	48-hour LC ₅₀
IND HI-Count	>100 mg/L

Table 3.2 Water quality data for dilution water (MHRW) and highest test concentrations for each product.						
Test solution	Date of first use in tests	DO mg/L	pH s.u.	conductivity µmho/cm	alkalinity mg/L CaCO ₃	hardness mg/L CaCO ₃
MHRW	03/28/18	8.6	7.4	282	58	88
IND HI-COunt	03/28/18	8.6	7.1	289	NA	NA

NA=not analyzed

Table 3.3. Product IND HI-Count toxicity test results. Cumulative mortality and percent affected, LC ₅₀ and 95% confidence interval, and method of analysis.		
Concentration milliliters product/ gal MHRW	<i>D. magna</i> (water flea) tested 03/28/18 to 03/30/18	
	24-hours % mortality (% affected)	48-hours % mortality (% affected)
0, MHRW	0 (0)	0 (0)
6.25	0 (0)	0 (0)
12.5	0 (0)	0 (0)
25	0 (0)	0 (0)
50	0 (0)	0 (0)
100	0 (0)	0 (0)
LC ₅₀		>100 mg/L
95% C.I.		
Methods/Comments: None needed.		

APPENDIX

Bench Sheets
Data Analysis
Standard Reference Toxicant (SRT) Control Charts

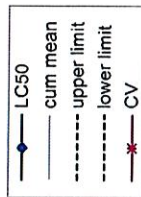


ACUTE, STATIC-NON RENEWAL BIOASSAY:

CLIENT: Microbe INDSTART DATE: 032818 TIME: 1420END DATE: 033018 TIME: 1325DILUTION WATER: MHRORGANISM: E. D. magnaORIGIN: ES culturesHATCH: 032718 1450AGE: 424hrs oldORGANISMS PER VESSEL: 5

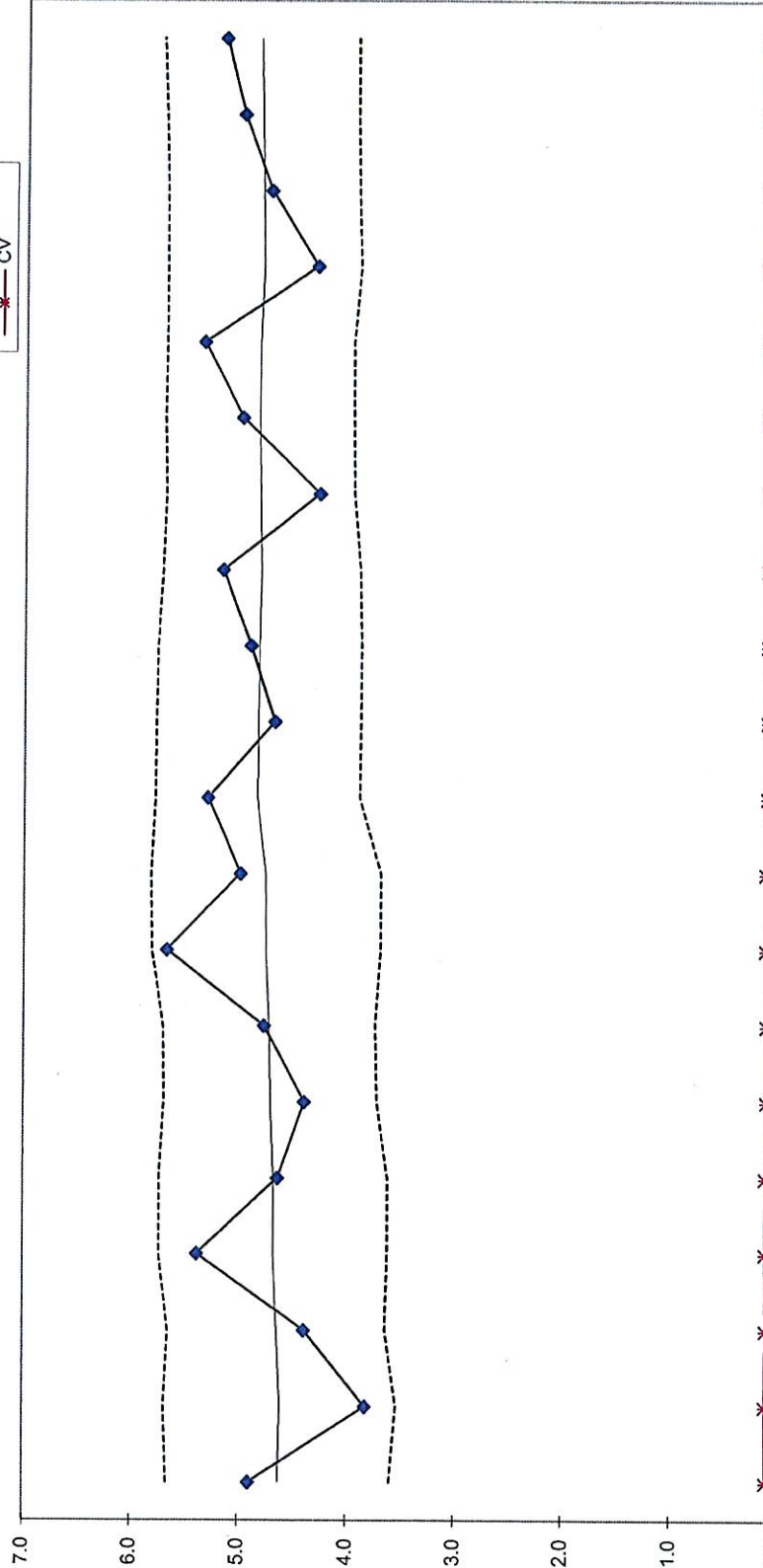
Test Levels		Biological Parameters			Chemistry and Physical Parameters														
		Rep	Ni	Dead / Affected		Temperature (Celsius)			Dissolved Oxygen (mg/l)			pH (s.u.)			Conductivity (µmhos/cm)				
				24 hr	48 hr	0	24	48	0	24	48	0	24	48	0	24	48		
Control: <u>MHR</u>	A	5	0 / 0	0 / 0	19.0	19.0	21.0	8.3		8.6	7.4	7.5	7.5	286		322			
	B	5	0 / 0	0 / 0	Alkalinity: ___ mL (Mg/L CaCO ₃)														
	C	5	0 / 0	0 / 0	Hardness: ___ mL (Mg/L CaCO ₃) (Mg/L CaCO ₃)														
	D	5	0 / 0	0 / 0															
<u>6.25</u> mg/L	A	5	0 / 0	0 / 0	19.0	19.5	20.9	8.6		8.6	7.2	7.5	7.5	275		297			
	B	5	0 / 0	0 / 0															
	C	5	0 / 0	0 / 0															
	D	5	0 / 0	0 / 0															
<u>12.5</u> mg/L	A	5	0 / 0	0 / 0	19.2	20.0	20.9	8.6		8.6	7.1		7.4	286		302			
	B	5	0 / 0	0 / 0															
	C	5	0 / 0	0 / 0															
	D	5	0 / 0	0 / 0															
<u>25</u> mg/L	A	5	0 / 0	0 / 0	20.0	20.2	20.9	8.6		8.6	7.1		7.4	286		301			
	B	5	0 / 0	0 / 0															
	C	5	0 / 0	0 / 0															
	D	5	0 / 0	0 / 0															
<u>50</u> mg/L	A	5	0 / 0	0 / 0	20.3	20.4	20.7	8.6		8.6	7.1		7.4	287		301			
	B	5	0 / 0	0 / 0															
	C	5	0 / 0	0 / 0															
	D	5	0 / 0	0 / 0															
<u>100</u> mg/L	A	5	0 / 0	0 / 0	20.2	20.4	20.5	8.6		8.6	7.1		7.3	289		317			
	B	5	0 / 0	0 / 0															
	C	5	0 / 0	0 / 0															
	D	5	0 / 0	0 / 0															
___ mg/L	A		L	L															
	B		L	L															
	C		L	L															
	D		L	L															
___ mg/L	A		L	L															
	B		L	L															
	C		L	L															
	D		L	L															
Time		1420	1425	1325	1420	1425	1325	1230		1330	1230		1330	1230	13	1330			
Tech		TM	AM	TM	TM	AM	TM	MT		CB	MT		CB	MT		CB			
Comments:	<u>44</u>				2			4			YSI5100			Orion 920			Orion 160		
					ID or <input type="checkbox"/> instrument used														
					Other:						Orion 2Star			Other:					

EnviroScience QC Chart - *Daphnia magna* Acute Toxicity Endpoint - 20 C



48-hour LC50 (g NaCl/L)

7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0



	Jan-16	Feb-16	Mar-16	Apr-16	May-16	Jun-16	Jul-16	Aug-16	Sep-16	Oct-16	Mar-17	Apr-17	May-17	Jun-17	Jul-17	Aug-17	Sep-17	Oct-17	Dec-17	Jan-18	Feb-18
LC50	4.899	3.826	4.389	5.381	4.637	4.389	4.766	5.668	4.985	5.288	4.676	4.899	4.899	5.16	4.27	4.985	5.342	4.3	4.734	4.985	5.16
cum mean	4.6278	4.61135	4.64225	4.6751	4.6751	4.7046	4.7173	4.74785	4.75215	4.83545	4.82975	4.8211	4.8073	4.8073	4.82075	4.8317	4.8288	4.7971	4.8082	4.8239	4.83695
upper limit	5.66174	5.68661	5.64917	5.73474	5.73474	5.69193	5.70072	5.81048	5.81804	5.78333	5.78015	5.76534	5.72129	5.69168	5.69168	5.70523	5.69474	5.69274	5.69441	5.711	5.7363
lower limit	3.59386	3.53609	3.63533	3.61546	3.61546	3.71727	3.73388	3.68522	3.68626	3.88757	3.87935	3.87686	3.89331	3.94982	3.94982	3.95817	3.96286	3.90146	3.92199	3.9368	3.9376
CV	0.11171	0.11659	0.10845	0.11333	0.11333	0.10493	0.10424	0.11191	0.11215	0.09801	0.09839	0.09793	0.09506	0.09033	0.09033	0.0904	0.08966	0.09335	0.09216	0.09195	0.09297

Test Date



Ecological Laboratories INC.

Providing Aquatic Solutions Since 1976

SAFETY DATA SHEET

VERSION 1.2

Revision Date : September 2018

Section 1 : IDENTIFICATION

Product Identifier : MICROBE LIFT PROFESSIONAL BLEND LIQUID

Brand : MICROBE-LIFT

Product Number is based on Product Size. For more information request for brochure/ refer one.

Manufacturer: Ecological Laboratories Inc.

Address : 2525 N.E. 9th Avenue
Cape Coral, FL 33909

Phone : (800)645-2976

Emergency : (800) 424-9300

Outside USA : (202) 483 7616

Recommended Use: Read Label

Section 2 : HAZARD(S) IDENTIFICATION

Hazard Classification : Skin & Eye Irritant

Signal Word : Caution

Hazard Statement: Avoid contact with eyes. Flush immediately for fifteen minutes if needed. Use gloves to avoid contact with skin and open wounds. If exposed wash with soap and water. Do not use internally. Keep out of reach of children.

Pictograms :



Precautionary Statements : NA

Description of hazard not otherwise classified : NA

Mixture containing an ingredient with unknown toxicity : NA

Section 3 : COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name: MICROBE LIFT PBL

Common Name & Synonyms : Microbe Lift PBL

Chemical Abstracts Service (CAS) number and unique identifiers: NA

Impurities & Stabilizing Additives : NA

Chemical Name and Concentration of all ingredients (classified as health hazard) : .NA

Section 4 : FIRST AID MEASURES

Note: The product has been classified as a non-hazardous substance.

If inhaled

Prolonged contact may cause slight irritation. Supply fresh air immediately. Rinse mouth and nose with water. Contact a physician if necessary.

In case of skin contact

Prolonged contact may cause skin irritation. Rinse with water. Slight redness on hands and forearms if individual has a history of dermal allergic reactions. Dermatitis and skin sensitization can develop after repeated and/or prolonged contact. Wash the material off the skin with soap and water. If symptoms persist, call a physician. Remove contaminated clothing and footwear. Wash clothing and footwear before reuse.

In case of eye contact

This material may cause eye irritation. Rinse with plenty of lukewarm water, also under eyelids for fifteen minutes. If symptoms persist, call a physician.

If swallowed

Irritation of the mouth, pharynx, esophagus and stomach can develop. Rinse mouth with water. Induce vomiting only if advised by physician or poison control center. If uneasy symptoms persist, call a physician. Call poison control.

Recommendation for immediate medical care: Call poison control if ingested. Immediately flush with water for 15 minutes if it comes in contact with eyes. Seek medical attention if irritation persists. Remove contaminated clothing and footwear; wash with soap and water if it comes in contact with skin.

Section 5 : FIRE FIGHTING MEASURES

Suitable extinguishing media

Use extinguishing measures that are appropriate to local circumstances and the environment.

Special hazards arising from the substance or mixture

NA

Advice for firefighters

Fire fighters must wear fire resistant personnel protective equipment.

Further information

Product is not considered to be a Fire Hazard.

Section 6 : ACCIDENTAL RELEASE MEASURES

Personal Precautions, Protective Equipment and Emergency Procedures

Not Required

Environmental Precautions

NA

Methods and materials for containment and cleaning up

Where possible, flush down drain to waste treatment sewer, otherwise on small spills, use chemical absorbent and sweep up. For large spills, contain and collect for reuse or disposal.

Reference to other sections

See Section 7 for information on safe handling.

See Section 8 for information on PPE

See Section 13 for disposal information.

Section 7 : HANDLING & STORAGE

Precautions for safe handling

Prevent skin and eye contact. Wash hands thoroughly with soap and water after use. Avoid contact with eyes.

Conditions for safe storage, including any incompatibilities

Store material at temperatures below 120 F and above 32 F

Specific end use(s)

Keep out of reach of children.

Section 8 : EXPOSURE CONTROLS/PERSONAL PROTECTION

Control parameters

None

Components with workplace control parameters

Contains no substance with occupational exposure limit values.

Exposure controls

Material transfer should be done under conditions of local exhaust ventilation.

Permissible Exposure Limit (PELs)

None

Threshold Limit Values (TLVs)

None

Appropriate engineering controls

Open air/Ventilation

Personal protective equipment

Eye/face protection

Tightly fitting safety goggles recommended when handling large quantities.

Skin protection

Skin should be covered by clothing at a minimum. Avoid excessive skin contact. Rubber gloves-recommended

Body protection

Same as above

Respiratory protection

Dusk masks recommended

Control of environmental exposure

Minimized environmental exposure.

Section 9 : PHYSICAL & CHEMICAL PROPERTIES

Appearance

Light pink to red liquid.

Odor

Pungent and distinctive odor.

pH

6.9 to 7.5

Melting point/freezing point

NA

Initial Boiling Point

100 C

Flash Point

NA

Flammability (solid, gas)

No

Vapor Pressure & Density

Equivalent to water

Water Solubility

Soluble. Disperses in water.

Specific gravity

0.9958

Section 10 : STABILITY AND REACTIVITY

Reactivity

NA

Chemical stability

Stable under normal conditions.

Condition to avoid

none

Incompatible materials

NA

Hazardous polymerization

Will not occur

Hazardous decomposition products

NA

Section 11 : TOXICOLOGICAL INFORMATION

Acute Toxicity

NA

Chronic Toxicity

NA

Reproductive Toxicity

NA

Additional Information

NA

Section 12 : ECOLOGICAL INFORMATION

Toxicity

Not classified.

Persistence and degradability

None

Bio-accumulative potential

NA

Mobility in soil

NA

Other adverse effects

This product is believed not to be dangerous to the environment with respect to mobility, persistency and degradability, bio accumulative potential, aquatic toxicity and other data relating to Eco toxicity

Section 13 : DISPOSAL CONSIDERATIONS

Waste Treatment Methods

Disposal in accordance with federal, state and local regulations.

Section 14 : TRANSPORT INFORMATION

DOT (US)

UN Number

NA

Class

NA

Packing Group

NA

Proper Shipping Name

NA

Section 15 : REGULATORY INFORMATION

There are no safety, health and environmental regulations/legislation specific for the substance or mixture

Section 16 : OTHER INFORMATION

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named manufacturer nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

This product conforms on its chemical description and is reasonably fit for the purpose referred to in the directions for use when used in accordance with the directions under normal conditions. However the manufacturer could withdraw the chemical composition of the product on the basis of it being a trade secret.

29 CFR 1910.1200 (I)(1)

The safety data sheet indicates that the specific chemical identity and/or percentage of composition is being withheld as a trade secret; The specific chemical identity and percentage is made available to health professionals, employees, and designated representatives in accordance with the applicable provisions.

Seller makes no other warranty or representation of any kind, express or implied, concerning the product, including NO IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS OF THE GOODS FOR ANY OTHER PARTICULAR PURPOSE. No such warranties shall be implied by law and no agent of seller is authorized to alter this warranty in any way except in writing with a specific reference to this warranty. The exclusive remedy against seller shall be in a claim for damages not to exceed the purchase price of the product, without regard to whether such a claim is based upon breach of warranty or tort. Any controversy or claim arising out or relating to this contract, or breach thereof, shall be settled by arbitration in accordance with the commercial arbitration rules of the American Arbitration Association, and judgment upon the rendered by the Arbitrator(s) may be entered in any court having jurisdiction thereof.



Ecological Laboratories INC.

Providing Aquatic Solutions Since 1976

SAFETY DATA SHEET

VERSION 1.2

Revision Date : May 2017

Section 1 : IDENTIFICATION

Product Identifier : Sludge Away

Brand : MICROBE-LIFT

Product Number is based on Product Size. For more information request for brochure/ refer one.

Manufacturer: Ecological Laboratories Inc.

Address : 2525 N.E. 9th Avenue
Cape Coral, FL 33909

Phone : (800)645-2976

Emergency : (800) 424-9300

Outside USA : (202) 483 7616

Recommended Use: Removal of organic bottom solids that are slow to degrade.

Section 2 : HAZARD(S) IDENTIFICATION

Hazard Classification : Skin & Eye Irritant

Signal Word : Caution

Hazard Statement: Avoid contact with eyes. Flush immediately if needed. Use gloves to avoid contact with skin and open wounds. If exposed wash with soap and water. Do not use internally. Keep out of reach of children.

Pictograms :



Precautionary Statements : NA

Description of hazard not otherwise classified : NA

Mixture containing an ingredient with unknown toxicity : NA

Section 3 : COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name : Microbe Lift Sludge Away

Common Name & Synonyms : Microbe Lift Sludge Away

Chemical Abstracts Service (CAS) number and unique identifiers: NA

Impurities & Stabilizing Additives : NA

Chemical Name and Concentration of all ingredients (classified as health hazard) : NA

Section 4 : FIRST AID MEASURES

Note: The product has been classified as a non-hazardous substance.

If inhaled

Prolonged contact may cause slight irritation. Supply fresh air immediately. Rinse mouth and nose with water. Contact a physician if necessary.

In case of skin contact

Rinse with water. Slight redness on hands and forearms if individual has a history of dermal allergic reactions. Dermatitis and skin sensitization can develop after repeated and/or prolonged contact. If symptoms persist, call a physician. Remove contaminated clothing and footwear.

In case of eye contact

This material may cause eye irritation. Rinse with plenty of lukewarm water, also under eyelids for fifteen minutes. If symptoms persist, call a physician.

If swallowed

No effect if ingested in small amounts. A single dose of this product is rarely toxic by ingestion. Irritation of the mouth, pharynx, esophagus and stomach can develop. Rinse mouth with water. If uneasy symptoms persist, call a physician. Call poison control.

Recommendation for immediate medical care: Call poison control if ingested. Immediately flush with water for 15 minutes if it comes in contact with eyes. Seek medical attention if irritation persists. Remove contaminated clothing and footwear; wash with soap and water if it comes in contact with skin.

Section 5 : FIRE FIGHTING MEASURES

Suitable extinguishing media

Use extinguishing measures that are appropriate to local circumstances and the environment.

Special hazards arising from the substance or mixture

NA

Advice for firefighters

Fire fighters must wear fire resistant personnel protective equipment.

Further information

Product is not considered to be a Fire Hazard.

Section 6 : ACCIDENTAL RELEASE MEASURES

Personal Precautions, Protective Equipment and Emergency Procedures

Not Required

Environmental Precautions

NA

Methods and materials for containment and cleaning up

Flush spilled materials into sanitary or storm sewers. Use chemical absorbent and sweep up small spills. Contain and collect large spills.

Reference to other sections

See Section 7 for information on safe handling.

See Section 8 for information on PPE

See Section 13 for disposal information.

Section 7 : HANDLING & STORAGE

Precautions for safe handling

Minimize exposure in accordance with good hygiene practice.

Conditions for safe storage, including any incompatibilities

To maintain shelf life, avoid prolonged exposure or low temperatures and humidity. Avoid temperatures above 110 F and keep from freezing.

Specific end use(s)

Keep out of reach of children.

Section 8 : EXPOSURE CONTROLS/PERSONAL PROTECTION

Control parameters

None

Components with workplace control parameters

Contains no substance with occupational exposure limit values.

Exposure controls

Material transfer should be done under conditions of local exhaust ventilation to avoid breathing mist.

Permissible Exposure Limit (PELs)

None

Threshold Limit Values (TLVs)

None

Appropriate engineering controls

Open air/Ventilation

Personal protective equipment

Eye/face protection

Tightly fitting safety goggles recommended

Skin protection

Skin should be covered by clothing at a minimum. Avoid excessive skin contact. Rubber gloves-recommended.

Body protection

Same as above

Respiratory protection

Not recommended

Control of environmental exposure

Minimized environmental exposure.

Section 9 : PHYSICAL & CHEMICAL PROPERTIES

Appearance

Brown/black liquid.

Odor

Pungent distinctive earthy odor.

pH

Range 6.5 to 8.0

Melting point/freezing point

NA

Initial Boiling Point

100 C

Flash Point

NA

Flammability (solid, gas)

No

Vapor Pressure & Density

Equivalent to water

Water Solubility

99% Soluble; Disperse in water.

Specific gravity

1.04 approximately

Section 10 : STABILITY AND REACTIVITY

Reactivity

NA

Chemical stability

Stable under normal conditions.

Possibility of hazardous reactions

Condition to avoid

Strong acids or alkali compounds may inactivate bio cultures.

Incompatible materials

Strong acids or alkali compounds.

Hazardous decomposition products

NA

Section 11 : TOXICOLOGICAL INFORMATION

Acute Toxicity

NA

Chronic Toxicity

NA

Reproductive Toxicity

NA

Additional Information

None

Section 12 : ECOLOGICAL INFORMATION

Toxicity

Not classified.

Persistence and degradability

None

Bio-accumulative potential

NA

Mobility in soil

NA

Other adverse effects

None

Section 13 : DISPOSAL CONSIDERATIONS

Waste Treatment Methods

Disposal in accordance with federal, state and local regulations.

Section 14 : TRANSPORT INFORMATION

DOT (US)

UN Number

NA

Class

NA

Packing Group

NA

Proper Shipping Name

NA

Section 15 : REGULATORY INFORMATION

There are no safety, health and environmental regulations/legislation specific for the substance or mixture

Section 16 : OTHER INFORMATION

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named manufacturer nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

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29 CFR 1910.1200 (I)(1)

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Seller makes no other warranty or representation of any kind, express or implied, concerning the product, including NO IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS OF THE GOODS FOR ANY OTHER PARTICULAR PURPOSE. No such warranties shall be implied by law and no agent of seller is authorized to alter this warranty in any way except in writing with a specific reference to this warranty. The exclusive remedy against seller shall be in a claim for damages not to exceed the purchase price of the product, without regard to whether such a claim is based upon breach of warranty or tort. Any controversy or claim arising out or relating to this contract, or breach thereof, shall be settle by arbitration in accordance with the commercial arbitration rules of the American Arbitration Association, and judgment upon the rendered by the Arbitrator(s) may be entered in any court having jurisdiction thereof.



Environmental Monitor
New Bedford Office of Environmental Stewardship
133 William Street, Room 304
New Bedford, MA 02740

RE: Notification of Filing an NOI for Buttonwood Park Pond

May 23, 2019

Introduction:

Advanced Marine Technologies ('AMT') of New Bedford, MA, in partnership with Ecological Labs, Inc. ('ELI'), of Cape Coral, FL, and City of New Bedford Parks, Recreation and Beaches Department are seeking permission to execute a pilot demonstration to reverse the eutrophication of Buttonwood Pond and thus eliminate its annual algal blooms, and possible fish kills.

This project will be utilizing ELI's MICROBE-LIFT™ technology, which is a consortium of natural microbe cultures that reduce nutrient loading, and improve water quality through vegetative microbe growth, denitrification, and most importantly, through mimicking (and outcompeting) algae's mode of action: needing only CO₂ as a carbon source.

History:

ELI's microbes have been used in household fish tanks for 40 years. For 20 years, they have been perfecting using a proprietary blend of microbes/cultures to clean up waterways. They have used their technology to clean up large waterways in China, Malaysia and Canada. They have also done extensive work in FL including projects that were monitored and validated by the FL DEP including the 11-acre Hourglass Pond at Isleworth Golf Course and the Cabot Canal in Jacksonville.

ELI is currently conducting an 18 month, 9 pond project jointly with the FL DEP, FL EPA, the City of Jacksonville, and the FL DOT. *Please also see the attached summary of their work on Occum Pond at Dartmouth College, NH.*

AMT first worked with ELI in 2009 as part of the Isleworth Golf Course project. AMT's product, ORGANIC GEM™, Liquid Fish Fertilizer was used along with ELI's Quantum™ microbes to create a 50 foot bio-barrier around Hourglass pond to successfully arrest the nutrient run-off from the golf course, before it could enter the pond.

Site Description:

Buttonwood Park Pond is a 10-acre waterbody located within a residential area of New Bedford (see attachment). The pond's watershed is small (544 acres) with a main source of water being surficial runoff from the drainage basin that extends Northward to US Interstate 195. There is a man-made outlet structure at the southern end of the pond adjacent to Lieutenant Walter E. Fuller memorial Parkway. Outflow from the pond continues southward as Buttonwood Brook before entering Apponagansett Bay in Dartmouth, MA. The shoreline of Buttonwood Park is primarily park-land and established deciduous trees. It is used for passive wildlife viewing.

Buttonwood Park Pond	
Surface Area	10.0 acres
Est. Mean Depth	5.0 feet
Maximum Reported Depth	9.0 feet
Estimated Volume	50 ac-ft (16.3 million gal.)
Dominant Plant Species	Filamentous algae, Pondweeds, Duckweed, White Waterlily

The historic record shows that in the summer months, a little over half of Buttonwood Wood Pond's surface can be covered with dense, topped out filamentous algae. The focus of this demonstration will be in this area that typically encompasses the southern half of the pond.

Project Design:

Application:

AMT will apply two different liquid microbe products by dispersing from them a flat bottom boat within the treated area of the pond. The two products are MICROBE-LIFT™ - Professional Blend Liquid ('M/L PBL') and MICROBE-LIFT™ - Sludge Away ('M/L SA') - see attached SDS's for each.

Application rates and dates are subject to small adjustments based on water test results, rainfall amounts and other unexpected weather or nutrient conditions. Following is the proposal for 2019:

	M/L PB	M/L SA
June 13 th - Initial Application	60 gals	18 gals
1 week later	14 gals	6 gals
2 weeks later	14 gals	6 gals
3 weeks later	14 gals	6 gals
4 weeks later	14 gals	6 gals
5 weeks later	14 gals	6 gals
Aug. 1 - 1 st monthly application	6 gals	2 gals
Sep. 1 - 2 nd monthly application	6 gals	2 gals
Oct. 1 - 3 rd monthly application	6 gals	2 gals

Important: The initial application of cultures will work quickly to turn around the water quality in Buttonwood Pond. To avoid an algal bloom or possible fish kill in 2019, it is important to do the first application before June 15th.

Containment:

ELI has proven through testing that MICROBE-LIFT™ cultures attach themselves to the nutrient sources either in the water column or in the sludge, and do not leave the treated area. The cultures, either alive, vegetative or dead, do not in any way contribute to the creation or growth of any pathogen. In fact their use in waterways has proven to LOWER pathogen levels.

Nevertheless, a floating, permeable turbidity curtain will be placed over the man-made outlet structure at the southern end of the pond of the pond that feeds into Buttonwood Brook. The turbidity curtain will be installed before any product is applied and kept in during the entire trial. The purpose of this barrier is to isolate the treatment area from the rest of the outflowing water.



Turbidity Curtain Installed



Turbidity Curtain



ELI has also proven their cultures to be safe for the environment. Please see attached toxicity test results.

Testing:

All Buttonwood Pond sampling and testing will be coordinated through UMASS SMAST.

In general, the testing to be performed will be for BOD, COD, VSS, nitrogen compounds, D.O., chlorophyll, secchi depth, and sludge depth.

Pre-Application:

- Base line data will be established and will supplement the baseline data that was already taken in fall of 2018.
- To help prove that ELI's cultures do not travel outside the application area, samples will be taken 15 minutes prior to the first application and one hour following the first application. Samples will be taken within the application area as well as in Buttonwood Brook below the turbidity curtain. These samples will be sent over-night to ELI's lab for identification testing of the cultures.

Post Application:

- Periodic sampling and testing will be done throughout the summer and fall.
- It is expected that during the summer, looking only at the nutrient test results, it may appear that conditions are NOT improving. This is actually an indication that the cultures are indeed working and releasing the nutrients in the water.
- The chlorophyll tests will be an accurate measuring stick for the reduction of algae content in the pond.
- Sludge depth and secchi depth will be another indicator of progress.
- The D.O. content will not drop as a result of the cultures' activity.

Fish Kills:

Often associated with low oxygen amounts in the water, fish kills are also often the result of hydrogen sulfide production by anaerobic microbes degrading bottom sludge. ELI's cultures do not lower the dissolved oxygen content of ponds and also quickly eliminate the production and release of hydrogen sulfide.

ELI has done testing in hundreds of waterways, many in high heat conditions, over 20 years and has never experienced a fish kill during any trial.

Jeffrey Young
Director

Ecological Laboratories, Inc.

The following information provides an overview and basic explanation of Ecological Laboratories, Inc.'s. Biological aquatic ecosystem restoration technology, with review of the biological processes, functions, process pathways and covers the overall capabilities of the novel technologies ability to restore water quality while controlling excessive aquatic weed growth and related green water events.

One of the function specific to achieving restoration is related to the bio-technologies capability to biologically eliminate a primary nutrient responsible for ecosystem loss, and for excessive weed and green water events. This is accomplished through the denitrification process, referred to as anoxic respiration, thereby promoting the removal of nitrate via biological anoxic respiration that occurs within the anaerobic zones, within littoral and benthic zones containing accumulated organic matter.

Denitrification is a key function in our technologies capability and water management efforts for restoration and control of green water events and excessive aquatic weed growth. Nitrogen removal is key to algae control, but it comprises not only denitrification of nitrate but nitrification of ammonia to nitrate since algae can grow on both nitrogen types.

With nitrate as one of the primary factors and requirements for algae growth and existence, the objective is to explain the nitrate removal process and the biological functions associated with the use of our products allowing for a reasonable explanation of the successful control or suppression on algae.

Phosphate is another issue altogether. Some algae, like cyanobacteria, can fix nitrogen needing only phosphate for growth, so it may be necessary to chemically and mechanically remove phosphate from a system to permanently control algae. This is outlined in the presentation as chemical technology may be required should phosphate levels exceed microbial cellular up-take and plant levels.

The primary and the secondary essential process to algae control involves the technologies capability or the functions associated with the products photosynthetic microorganisms. Their pathways and processes can mirror the image of algae as the food source in the environment is depleted by the biological restoration process, thereby limiting available food for the developing microorganisms.

This places microorganisms within the ecosystem in the declining phase, or death phase where they normally go through lysis recycling their byproduct back to the environment, this consist of; carbon, nitrogen, and phosphate, resulting in material to support continued biology, with the released nutrients promoting algae and aquatic weed growth, remember all algae requires is; light, nutrients and Carbon Dioxide.

PHOTOSYNTHESIS

The novel technology incorporate photosynthetic microorganisms that can function like and mirror the image of algae, they can utilize light, carbon dioxide and nutrients in place of organic carbon during the declining phase, that would normally cause standard bio technology (competative biological agents) and the ecosystems indigenous microorganisms to release and recycle their material thereby fostering a return of algae.

The explanation theory:

1. These concepts apply to bacteria that can fix carbon dioxide and the non-autotrophic bacteria important to the remediation process, these microorganisms will still be subject to starvation and lysis. We are dealing with an aquatic ecosystem that has a constant organic loading from influent water, run off, and air-born carbon. What is required to achieve ecosystem restoration and algae control during lysis is to achieve a delicate balance in the declining phase, in a MICROBE-LIFT treated ecosystem vs that of non-treated ecosystems the difference in results obtained is based on scientific data and also considers empirical observations of environments treated with our technology; i.e., thousands of aquariums, Koi ponds and lake remediation projects where ecosystem restoration is achieved and maintained via the technologies use.
2. Carbon is usually not the limiting nutrient to autotrophs, the limiting nutrient is typically nitrogen, phosphorus or a micronutrient like iron. The autotrophs will grow until this critical nutrient is depleted and then be subject to lysis by protozoal grazers, regardless of their ability to fix carbon. At the same time algae is almost always present in an aquatic ecosystem, it's percentage is the primary factor that often interrupts ecosystem balance, this is dependent on available nutrients, light and carbon dioxide. The use of our microbial technology effects the restoration process of the entire ecosystem while improving the ratio of biological balance via denitrification and the photosynthetic function outlined in the explanation.

The reasonable explanation is that we do not build up a high amount of nutrient-retaining biomass (sludge) to see measurable results, this is due in-part to high substrate affinities and low growth yield of our organisms. The substrate is primarily being catabolized to energy rather than being converted into biomass. In comparison to the biomass of a harmful algal bloom, the contribution to solids from our bacteria is very small. Once cells reach death phase, further mineralization will occur during growth of surviving bacteria on lysis products.

Understanding the biological declining phase (biological death)

Lysis will continue during the final stages of ecosystem restoration until the F to M ration is achieved and returns to a steady state. During the declining period the resulting byproduct nutrients still need to be controlled via cellular up-take of other microorganisms, denitrification, and plant adsorption or algae will return and become dominant.

Aquatic ecosystems adequately treated with our MICROBE-LIFT bio-technology for restoration purposes on average has shown to balance and control aquatic ecosystems following restoration with controlled green water events.

- While competitive biological products used in restoration programs terminate in failure due to the inability to control lysis that occurs in the declining phase of the restoration process as food supplies are reduced and the biological community declines and releases their stored nutrient load, resulting in an increase of nutrient release of nitrate as an end product.

Summary

During the initial start of biological restoration the microorganisms will take up nitrate and phosphate as a cellular requirement thereby limiting algae and aquatic weed growth, however once the biological enhancement process goes beyond steady-state and the food supply is limited, the microorganisms will go into decline to match the food supply, this is the point where our technology photosynthetic function contribute with their capability to provide unmatched restoration functions via denitrification, carbon dioxide utilization, and use of light energy.

Without the use of this classification of microorganisms and microbial pathways, a biological restoration process would see good results during early use, to be followed by an increase in green water events and aquatic weed growth as all of the nutrients removed during the cultures log growth, and through steady state would then be recycled back to the environment due to lysis, with the release of stored nutrients, as the ecosystem food source was reduced and more cultures existed than food (F to M ratio).

You will find the above information outlined and covered in the attached power point session. You can eliminate or remove the information related to the comparison of dredging of an ecosystem and or the use of chemical agents in comparison to biological restoration for a much shorter presentation and understanding of the microbial technology

- **Following your review should you have questions please contact me at your convenience.**

Regards
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