Didymo Control Studies

Development of a Potential Didymo Control Tool

Sue Clearwater, Phillip Jellyman, Barry Biggs, Neil Blair, Chris Hickey, John Clayton
Objective of Control Study

“Provide methods to control didymo”

Elimination

Suppression

Ideally a:
  – Selective tool
  – Low risk
Objective of Control Study

“Provide methods to control didymo”

Challenges

– Fast-flowing rivers, complex environment
– High water volumes, short dose
– Dispersion, dilution, adsorption
– Thick mats
– Method development
– Physiology/ecology
– Effective
– Impacts
– Practical
– Low Risk
– Cost
Control Study

Didymo Control Trials

STALK DISRUPTION

Stage 1: SCREENING

BIOCIDES

Stage 1: SCREENING

Stage 2 – Phase 1
BIOCIDE TESTING & SELECTION

Stage 2 – Phase 2
INTENSIVE BIOCIDE TESTING

Stage 3
RIVER TRIALS

Effectiveness, impacts, risk, feasibility, cost assessed at each stage
Outline of Presentation

Review of Control Study - background

• Overview of Stage 1 and 2 Screening and Selection
• Stage 3 Field Trial results

Implications for didymo control using a biocide

Future directions
Didymo Control Trials

STALK DISRUPTION

Stage 1: SCREENING

BIOCIDES

Stage 1: SCREENING

Stage 2 – Phase 1
PRODUCT TESTING & SELECTION

Stage 2 – Phase 2
INTENSIVE PRODUCT TESTING

Stage 3
RIVER TRIALS
Biocide trial site

Waiau and Monowai Rivers, Southland
Biocide Stage 1

SCREENING
# Biocide Screening

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Mode of action</th>
<th>Response time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium hypochlorite</td>
<td>Photosynthetic inhibitor</td>
<td>Immediate</td>
</tr>
<tr>
<td>Chelated Copper (Gemex™)</td>
<td>Photosynthetic inhibitor</td>
<td>Immediate</td>
</tr>
<tr>
<td>Diquat</td>
<td>Photosynthetic inhibitor</td>
<td>Immediate</td>
</tr>
<tr>
<td>QAC (303 Clear All)</td>
<td>Membrane disruption</td>
<td>Immediate</td>
</tr>
<tr>
<td>Simazine</td>
<td>Metabolic disruption</td>
<td>Immediate</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>Cell toxicant</td>
<td>Immediate</td>
</tr>
<tr>
<td>Organic Interceptor™</td>
<td>Photosynthetic inhibitor</td>
<td>Immediate</td>
</tr>
<tr>
<td>Hydrothol®191</td>
<td>Photosynthetic inhibitor</td>
<td>Longer term</td>
</tr>
<tr>
<td>EDTA</td>
<td>Exfoliation &amp; degradation</td>
<td>Longer term</td>
</tr>
<tr>
<td>Germanium dioxide</td>
<td>Cell toxicant</td>
<td>Longer term</td>
</tr>
</tbody>
</table>
Biocide Screening

Long/short term effectiveness

Contact times  6 sec, 60 sec, 60 min
Biocide application - long & short-term effects
Experimental site in the Monowai River - longer-term biocide effects
Method development
-stain cells to assess viability
## Biocide Screening - Conclusions

Summary of the short- and long-term efficacy of the selected biocides.

<table>
<thead>
<tr>
<th>Biocide</th>
<th>Short-term</th>
<th>Longer-term</th>
<th>Accept/Reject</th>
<th>Reasons for acceptance/rejection</th>
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</thead>
<tbody>
<tr>
<td>Organic Interceptor™</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>highly effective short &amp; long-term</td>
</tr>
<tr>
<td>Chelated Copper (Gemex™)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>highly effective short &amp; long-term</td>
</tr>
<tr>
<td>EDTA</td>
<td>X</td>
<td>✓</td>
<td>✓</td>
<td>highly effective short &amp; long-term</td>
</tr>
<tr>
<td>Hydrothol®191</td>
<td>X</td>
<td>✓</td>
<td>?</td>
<td>effective long-term</td>
</tr>
<tr>
<td>Simazine</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>ineffective</td>
</tr>
<tr>
<td>Zinc sulphate</td>
<td>✓</td>
<td>X</td>
<td>?</td>
<td>ineffective long-term</td>
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<tr>
<td>Chlorine</td>
<td>✓</td>
<td>X</td>
<td>X</td>
<td>toxic, non-specific, ineffective long-term</td>
</tr>
<tr>
<td>Germanium</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>ineffective, expensive</td>
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<tr>
<td>QAC</td>
<td>✓</td>
<td>X</td>
<td>?</td>
<td>ineffective long-term</td>
</tr>
<tr>
<td>Diquat</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>ineffective</td>
</tr>
</tbody>
</table>
Biocide Screening

Long/short term effectiveness

Organic Interceptor™
Gemex™
Hydrothol®-191
EDTA

Contact times 6 sec, 60 sec, 60 min
Control Study

Didymo Control Trials

STALK DISRUPTION
- Stage 1: SCREENING

BIOCIDES
- Stage 1: SCREENING

Stage 2 – Phase 1
PRODUCT TESTING & SELECTION

Stage 2 – Phase 2
INTENSIVE PRODUCT TESTING

Stage 3
RIVER TRIALS
Stage 2 Phase 1

BIOCIDES TESTING

Artificial channel trials – Monowai
Non-target toxicity testing – Hamilton
Monowai Trials – Artificial Channels

Effects on didymo

Four biocides, 3 concentrations

- Organic Interceptor™
- Gemex™
- Hydrothol®-191
- EDTA

One contact time 60 min

Fish (rainbow trout, bullies)
Monowai Experimental Facility (MEF) (at Monowai Power Station)
Artificial substrates colonised with didymo
Artificial substrates growing in Waiau River

Fully colonised artificial substrates in channels
Application method

Biocides applied for 1 hr
- 4 biocides @ 3 concs.
Non-target toxicity testing
Hamilton
Laboratory trials
Non-target toxicity tests

Green alga
*Pseudokirchneriella subcapitata*

Water flea
*Daphnia magna*
Juvenile rainbow trout
1 h exposures, 4 d survival
RESULTS


Ideal Ecotox Profile

Dose Response Relationship
Hydrothol®-191

% Survival

Concentration (mg a.e./L)

Fish-Channels

Invertebrates

Didymo

Algae
Didymo Control Trials

STALK DISRUPTION
Stage 1: SCREENING

BIOCIDES
Stage 1: SCREENING
Stage 2 – Phase 1
PRODUCT TESTING & SELECTION

SCREENING
Stage 2 – Phase 2
INTENSIVE PRODUCT TESTING
Stage 3
RIVER TRIALS

Control Study
Biocide Stage 2 Phase 1

Further product screening
....at Monowai
Stage 2 Phase 1 Rapid screening

Products
- Hydrothol®-191
- Organic Interceptor™
- Gemex™
- Chlorine

New Product
- KTEA™ (chelated copper)

High concentrations
Multiple exposures
Combinations
High Concentrations, Multiple exposures Hydrothol®-191 ineffective on didymo

![Graph showing the percentage of viable cells against concentration (mg a.e./L). The graph compares single and multiple exposures of Hydrothol®-191.](image)
Combinations, less effective

![Graph showing combinations of Gemex™, Gemex™/Organic Interceptor™, Gemex™/Hydrothol®-191, and previous Gemex™ data vs. concentration (mg Cu/L) and percentage (%) of viable cells. The graph illustrates a decrease in viable cell percentage with increasing concentration for each combination.](image-url)
Rapid screening

- Hydrothol®-191
- Organic Interceptor™
- Gemex™
- Chlorine

New Product
- KTEA™

- High concentrations
- Multiple exposures
- Combinations
Control Study

Didymo Control Trials

STALK DISRUPTION

Stage 1: SCREENING

BIOCIDES

Stage 1: SCREENING

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PRODUCT TESTING & SELECTION

Stage 2 – Phase 2
INTENSIVE PRODUCT TESTING

Stage 3
RIVER TRIALS
Artificial substrates 10-20 mm mats
Treatment Approaches

1 h application of Gemex™

Testing
  • concentration
  • velocity
  • multiple applications

Non-target species
  • Invertebrates
  • Fish

Target Efficacy
  <2% survival
RESULTS

Clearwater et al., (2007a). Didymosphenia geminata experimental control trials: Stage Two Phase Two testing the effectiveness of Gemex™, a chelated copper formulation.

Effects of Concentration

Threshold at 10 mg/L
Effects of water velocity

![Graph showing the effects of water velocity on % viable cells over days. The graph compares fast, medium, and slow velocities. The fast group shows a significant decrease in % viable cells, while the medium and slow groups show a minimal decrease.]
Effects of multiple applications

![Graph showing the effects of single and multiple applications of 10 mg/L on viable cells over 35 days. The graph indicates a decrease in viable cells with time for both conditions, with multiple applications showing a more rapid decline.]
Comparison of mats post-Gemex™

10-20mm mats
Effects on non-target organisms
Summary of eco-tox profile for Gemex™

- Fish-Lab data
- Stream Invertebrates
- Algae
- Pond Invertebrates
- Didymo

Concentration (mg Cu/L)

% Survival

Summary of eco-tox profile for Gemex™
Overall Conclusions on Gemex™

Tested
• Effective concentration (5, 10, 15, 20 mg Cu/L)
• Effect of water velocity –more effective in higher flows
• Multiple applications (3 x 1 h applications, 24 h apart) –increase didymo mortality
• Good native fish survival
• Minimal/moderate effects on river invertebrates

Field Validate 20 mg Cu/L, 1 hour
Control Study

Didymo Control Trials

- STALK DISRUPTION
  - Stage 1: SCREENING

- BIOCIDES
  - Stage 1: SCREENING
  - Stage 2 – Phase 1
    - PRODUCT TESTING & SELECTION
  - Stage 2 – Phase 2
    - INTENSIVE PRODUCT TESTING

- PREPARATION FOR NORTH ISLAND INCURSION

- Stage 3
  - RIVER TRIALS
Stage 3 Field Trial Approach

• Gemex™ one hour application 20 mg Cu/L (target dose)

APPLICATION METHOD

• Intensive monitoring DURING Gemex™ application = DOSE

• Pre- and post-treatment monitoring = RESPONSE

Gemex™ treatment profile for didymo control

Dose-response relationship
Stage 3
Field Trial

Princhester Creek
Upstream area clear of didymo
Stage 3 Field Trial

Princhester Creek

- Resource consent
- ERMA Permits
## Stage 3 Trial
Pre- & post-treatment monitoring

<table>
<thead>
<tr>
<th>Day after treatment</th>
<th>Algae</th>
<th>Inverts</th>
<th>Fish</th>
<th>Water</th>
<th>Sediments</th>
</tr>
</thead>
<tbody>
<tr>
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<td>78</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>
Application method - Diffuser
Prepared for North Island Incursion – Oct ‘06

- Gemex™ Treatment Contingency Plan
- Updated with Control Study progress
- “How to” manual
- Version 3 Available to Partners from MAF BNZ on request
APPLICATION METHOD
Diffuser in river
Even application of biocide, well-mixed
Flow meter for accurate dosing
Gemex™ Application Method

Photo: B. Jarvie
Prepared for North Island Incursion - equipment is easy to transport
Stage 3 Field Trial

- One hour application 20 mg Cu/L (target dose) APPLICATION METHOD

- Intensive monitoring DURING Gemex™ application = DOSE

- Pre- and post-treatment monitoring = RESPONSE

Gemex™ treatment profile for didymo control - efficacy
Field Trial DOSE
Monitoring during treatment

Dispersion

Dilution

Adsorption

“Target dose” ——> “Off-target” dose
Field Trial ACCURATE DOSE

- Practice run
- Only tracer dye (100 µg/L)
Field Trial ACCURATE DOSE

- Practice run
- Only tracer dye (100 µg/L)
Field Trial – Transporting Gemex™

Photos: B. Jarvie
Field Trial ACCURATE DOSE

- One hour application Gemex™ 20 mg Cu/L (target dose)
- Tracer dye (20 µg/L)
Field Trial ACCURATE DOSE

- One hour application Gemex™ 20 mg Cu/L (target dose)
- Tracer dye (20 μg/L)
Field Trial DOSE MEASUREMENT

- One hour application 20 mg Cu/L (target dose)
- Plus tracer dye (20 µg/L)

Clearwater et al. (2007). *Didymosphenia geminata* control trials: Stage 3, Phase One...
NIWA Client Report. www.biosecurity.nz
Stage 3 Trial DOSE MEASUREMENT

- One hour application 20 mg Cu/L (target dose)
- Plus tracer dye (20 µg/L)

Clearwater et al. (2007). Didymosphenia geminata control trials: Stage 3, Phase One...
NIWA Client Report. www.biosecurity.nz

- Detailed dose information at each site
- Dose description needs concentration and time
• Target dose 15 mg/L, 50 min
• pH decrease to 4
• Gemex™:rhodamine ratio <1
Site 5 (2 km)

- Off-target dose 5 mg/L, 60 min
- pH ~circumneutral at 6.5
- Gemex™:rhodamine ratio >1 = adsorption
Stage 3 Trial DOSE MEASUREMENT

- One hour application 20 mg Cu/L (target dose)
- Plus tracer dye (20 µg/L)

- Low concentrations, longer exposures downstream

“Target dose” ——> “Off-target” dose

Clearwater et al. (2007). *Didymosphenia geminata* control trials: Stage 3, Phase One...
NIWA Client Report. www.biosecurity.nz
Stage 3 Trial  RESPONSE
Pre- & post-treatment monitoring
Didymo assessment
Stage 3 Trial RESPONSE - Didymo viability

- Transects (riffles)
- Runs and pools
- **Viability, biomass, chlorophyll a, ash free dry weight**
Field Trial RESPONSE - Didymo viability
Non-visible, early stage 0.3 km d/s

Site 3 (0.3 km d/s)
Early stage infestation – small colonies, mostly not visible
“Target dose” of Gemex™
“Elimination possible”

Only 4 dead cells found on entire transect
Field Trial RESPONSE - Didymo viability
High % cover, late stage 3 km d/s

Site 6 (3.4 km d/s)

Late infestation, 60% cover
1-6 mm thick

"Off-target dose" of Gemex™
Stage 3 Trials Princhester Creek

**RESPONSE - Didymo**

- **Site 8 (8.7 km d/s)**
  - Dried out

- **Site 7 (4.2 km d/s - below confluence with East Branch Princhester)**
  - Remained immersed
  - Only 4 dead cells found on entire transect

- **Site 6 (3.4 km d/s)**
  - Dried out

- **Site 5 (1.9 km d/s)**
  - Dried out

- **Site 4 (0.9 km d/s)**
  - Remained immersed

- **Site 3 (0.3 km d/s)**
  - Remained immersed
Field Trial RESPONSE - Didymo
Live cell density

Riffles

Live cells/mm²

Day -6
Day 21

Site
Field Trial RESPONSE

Didymo
21 day, live cell density

Suppression for 21 d
Field Trial RESPONSE  Handspraying
Stage 3 Trial RESPONSE

Photo: B. Jarvie
## Field Trial RESPONSE
### Pre- & post-treatment monitoring

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<tr>
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<tr>
<td>78</td>
<td>√</td>
<td>√</td>
<td></td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>
Invertebrate Community

- 3 Hess samples/site
- 8 Sites
  - Total density
  - Total # species
  - Density/species
  - % Taxa
  - MCI score
  - %EPT
Invertebrate Community

- Density -NSD
- Total # species -NSD

Clearwater et al. (2007). *Didymosphenia geminata* control trials: Stage 3, Phase One...
NIWA Client Report. www.biosecurity.nz
Invertebrate Community

Fish – Rainbow trout, Galaxiids

- Resident community, electric fishing surveys (species, size, density)
- Caged fish (rainbow trout, galaxiids) monitored during treatment

Photos: B. Jarvie
Fish survival – cages 9 days post-trt

Fish density - resident community

Rainbow trout
- Definite decrease 2-3 km downstream, still evident 77 d post-trt

Galaxiids
- Decreased density 2 km downstream, 20 d post-trt
- NSD 77 d post-trt
Fate of Gemex™-Algal mats

**Graph:**
- **X-axis:** Site Number (1-8)
- **Y-axis:** Algal Cu concentration (mg/kg dry weight ± s.e.)
- **Bars:**
  - Day -6
  - Day 1
  - Day 21
  - Day 42

**Legend:**
- Spirogyra

**Significance:**
- *: Significant difference

**Footnote:**
Clearwater et al. (2007). *Didymosphenia geminata* control trials: Stage Three Phase One...
NIWA Client Report
www.biosecurity.nz
Fate of Gemex™-Sediments

Clearwater et al. (2007). *Didymosphenia geminata* control trials: Stage 3, Phase One...
NIWA Client Report. www.biosecurity.nz
Outcome of Control Trials

- Downstream extent of dose 2-4 km (site specific)
- Elimination $\geq 42$ d if early stage infestation (NI incursion)
- Suppression of live cell density 3-4 km d/s in substantial mats
- Mats not degraded, need physical disturbance
- Colonization by other algal species
- Minimal effects invertebrates
- Minimal effects native galaxiids
- Risks to juvenile trout
  - Mitigation possible
- Cu accumulates in algal mats, rapid decrease
- Minimal accumulation in sediments
- Not a tool for long-term use
Outcome of Control Trials

- Elimination possible
  - if non-visible/early stage infestation
  - requires regular monitoring, early detection
  - early action, to increase probability of success
  - repeat treatments
  - multiple injection points
  - EARLY DETECTION IS PART OF TOOL

- Suppression possible
  - Trial on thicker mats >10mm
  - Long-term monitoring

- Risks to juvenile trout
  - Mitigation possible
  - Explore further for safety margins
Future Directions

• Gemex™ Refinement

• Screening other compounds

• Biological control of didymo

• Didymo physiology
Future Directions

- **Gemex™ Refinement**
  - Continued monitoring at Princhester Creek (ERMA)
  - Toxicity to fish
  - River trials
    - Early stage infestation, goal elimination
    - High biomass, thick mats, goal suppression
- Mechanism of toxicity of Gemex™
- Trickle dosing
- Neutralisation
Future Directions

• Screening other tools
  – Methods developed
  – Monowai facility still available

• Biological control of didymo
  – Screening algal samples

• Didymo physiology
  – Spring-fed creeks related work
  – Nutrient uptake mechanisms
Development of a Potential Didymo Control Tool

Kerry Bodmin
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Fish density -resident community

Rainbow trout
- Definite decrease at sites 5-6, still evident 77 d post-trt

Galaxiids
- Decreased density site 5, 20 d post-trt
- NSD 77 d post-trt

## Overall Effect of Gemex™ Treatment

<table>
<thead>
<tr>
<th>Distance (km)</th>
<th>Concn (mg/L)</th>
<th>Time (min)</th>
<th>Didymo INITIAL</th>
<th>Didymo RESPONSE</th>
<th>Inverts</th>
<th>Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4</td>
<td>15</td>
<td>50</td>
<td>Early, non-visible</td>
<td>“Elimination” 42 d</td>
<td>-</td>
<td>Minimal</td>
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<tr>
<td>0.9</td>
<td>11</td>
<td>60</td>
<td>Moderate</td>
<td>Suppression 21 d, partial 78 d</td>
<td>Negligible</td>
<td>Minimal</td>
</tr>
<tr>
<td>1.9</td>
<td>5</td>
<td>60</td>
<td>Advanced</td>
<td>Suppression 42 d</td>
<td>-</td>
<td>T –Marked G -Minimal</td>
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<tr>
<td>3.4</td>
<td>1</td>
<td>62</td>
<td>Advanced</td>
<td>Suppression 21 d</td>
<td>Minimal</td>
<td>T –Marked G -Minimal</td>
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<td>4.2</td>
<td>0.2</td>
<td>107</td>
<td>Moderate</td>
<td>Suppression 7 d</td>
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## Summary of Fish Community Response

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<thead>
<tr>
<th>Site</th>
<th>Dose</th>
<th>Impact Assessment</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
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<td>Control site</td>
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<tr>
<td>2</td>
<td>Target</td>
<td>Minimal</td>
</tr>
<tr>
<td>3</td>
<td>Target</td>
<td>Minimal</td>
</tr>
<tr>
<td>4</td>
<td>Target</td>
<td>Minimal</td>
</tr>
<tr>
<td>5</td>
<td>Off-target</td>
<td>Marked with rapid recovery for galaxiids but not trout</td>
</tr>
<tr>
<td>6</td>
<td>Off-target</td>
<td>Marked for trout but negligible for galaxids</td>
</tr>
<tr>
<td>7</td>
<td>Off-target</td>
<td>Minimal</td>
</tr>
<tr>
<td>8</td>
<td>Off-target</td>
<td>Minimal</td>
</tr>
</tbody>
</table>
Product Trials Summary

EDTA
• Not effective against didymo

Hydrothol®-191
• Minimally effective against didymo
• Fish Toxicity

Organic Interceptor™
• Effective against didymo
• Highly toxic to rainbow trout
• Multiple exposures?

Chelated Copper (Gemex™) MOST PROMISING BIOCIDE
• Relatively effective against didymo
• Minimal effect on fish
• Increase conc. to improve efficacy?

Jellyman, P.G. et al. (2006). *Didymosphenia geminata* experimental control trials: Stage One and Stage Two Phase One

Intensive trials of Gemex™

Tested
• Effective concentration (5, 10, 15, 20 mg Cu/L)
• Effect of water velocity
Intensive trials of Gemex™

Tested
• Effective concentration (5, 10, 15, **20** mg Cu/L)
• Effect of water velocity – **more effective in higher flows**
• Multiple applications (3 x 1 h applications, 24 h apart)
Intensive trials of Gemex™

Tested
• Effective concentration (5, 10, 15, 20 mg Cu/L)
• Effect of water velocity –more effective in higher flows
• Multiple applications (3 x 1 h applications, 24 h apart) –increase didymo mortality
• Long-term mat viability
Intensive trials of Gemex™

Tested
- Effective concentration (5, 10, 15, 20 mg Cu/L)
- Effect of water velocity –more effective in higher flows
- Multiple applications (3 x 1 h applications, 24 h apart) –increase didymo mortality
- Long-term mat viability -ongoing monitoring
Proceed to Field Trials

Trial
• Application of Gemex™ 20 mg Cu/L, 1 hour
• Develop application methods
• Precise dosing
• Examine effects on didymo
• Examine effects on non-target species
• Fate of chelated Cu in Gemex™
Fate of Gemex™-Water

**Total dissolved Cu concentration (mg Cu/L)**

<table>
<thead>
<tr>
<th>Day</th>
<th>Concentration (mg Cu/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day -6</td>
<td>0.000</td>
</tr>
<tr>
<td>Day 1</td>
<td>0.002</td>
</tr>
<tr>
<td>Day 4</td>
<td>0.004</td>
</tr>
<tr>
<td>Day 42</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* *Significant difference*