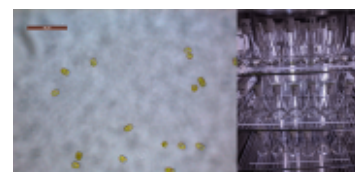


Toxicity of eight herbicides to the marine alga *Tetraselmis* sp.(Chlorophyta) (NESP TWQ 3.1.5, AIMS)



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| Title | Toxicity of eight herbicides to the marine alga <i>Tetraselmis</i> sp.(Chlorophyta) (NESP TWQ 3.1.5, AIMS) |
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Abstract

This dataset shows the effects of eight herbicides (detected in Great Barrier Reef catchments) on the specific growth rates (from cell density data) of the microalgae *Tetraselmis* sp. during laboratory experiments conducted in 2019.

The aim of this project was to apply standard ecotoxicology protocols to determine the effects of Photosystem II (PSII) and alternative herbicides on the growth of the marine microalgae *Tetraselmis* sp. Growth bioassays were performed over 3-day exposures using pesticides that have been detected in the Great Barrier Reef catchment area (O'Brien et al. 2016). These toxicity data will enable improved assessment of the risks posed by PSII and alternative herbicides to microalgae for both regulatory purposes and for comparison with other taxa.

Methods:

The chlorophyte *Tetraselmis* sp. (strain CS-317) was purchased from the Australian National Algae Supply Service, Hobart (CSIRO). Cultures of *Tetraselmis* sp. were established in EDTA-free Guillard's f/2 marine medium (Guillard and Ryther 1962) (1 ml L⁻¹ of f/2 medium in autoclaved natural seawater). Cultures were maintained in sterile 500 ml Erlenmeyer flasks as batch cultures in exponential growth phase with weekly aseptically transfers of 10 ml *Tetraselmis* sp. suspension to 300 ml f/2 medium. Culture were maintained at 28 ± 1°C, 33 ± 1.5 psu and under a 12:12 h light:dark cycle (80 – 100 μmol photons m⁻² s⁻¹).

Pesticide stock solutions were prepared using PESTANAL (Merck) analytical grade products (purity greater than or equal to 98%): diuron (CAS 330-54-1), metribuzin (CAS 21087-64-9), tebuthiuron (CAS 34014-18-1), bromacil (CAS 314-40-9), propazine (CAS 139-40-2), simazine (122-34-9), imazapic (CAS 104098-48-8) and haloxyfop-p-methyl (CAS 72619-32-0). The selection of pesticides was based on application rates and detection in coastal waters of the GBR (Grant et al. 2017, O'Brien et al. 2016). Pesticide stock solutions (100 – 1,000 mg L⁻¹) were prepared by dissolving aliquots of the pure compounds in ultrapure water using clean, acid-washed (5% nitric acid) glass screw-top containers. Simazine, tebuthiuron and haloxyfop-p-methyl were dissolved using the carrier dimethyl sulfoxide (DMSO) (less than or equal to 0.02 % (v/v) in exposure solutions). Diuron, imazapic, metribuzin, bromacil and propazine were dissolved in acetone (less than or equal to 0.01 % (v/v) in exposure). Stock solutions were stored refrigerated and in the dark.

Test protocols were based on previously published methods (Trenfield et al. 2015, OECD, 2011). Cultures of *Tetraselmis* sp were exposed to increasing concentrations of individual pesticides over a period of 72 h. Inoculum was taken from cultures in exponential growth phase (5-d old culture) and starting cell density assessed using a haemocytometer. For each treatment, a total volume of 250 mL test media was prepared in a clean, acid-washed 500 mL Schott bottle. Test media consisted of filtered (0.45 μm) seawater spiked with the respective pesticide stock, quarter strength EDTA-free f/2 media as nutrient source and *Tetraselmis* sp at a starting density of 2.5x10³ cells mL⁻¹. In each toxicity test, the response (specific growth

rate of the culture) of the treatments exposed to pesticide were assessed against a seawater control group (no herbicide).

For each test, 2 – 3 replicate 125 mL Erlenmeyer flasks (50 mL test volume) were assessed. Flasks were incubated at 27 – 29.0°C under a 12:12 h light:dark cycle (80 – 100 µmol photons m⁻² s⁻¹). After 72h, sub-samples (7 ml) were taken from each flask and cell densities measured using a flow cytometer (BD Accuri C6, BD Biosciences, CA, USA). Specific growth rates (SGR) were expressed as the logarithmic increase in cell density from day i (ti) to day j (tj) as per equation (1), where SGR_{i-j} is the specific growth rate from time i to j; X_j is the cell density at day j and X_i is the cell density at day i.

$$\text{SGR}_{i-j} = [(\ln X_j - \ln X_i) / (t_j - t_i)] (\text{day}^{-1}) \quad (1) \quad (1)$$

Mean SGR for a pesticide treatment, relative to the mean control SGR was used to derive chronic effect values for growth inhibition. A test was considered valid, if the SGR of control replicates was greater than or equal to 0.92 day⁻¹. Physical and chemical characteristics of each treatment were measured at 0 h and 72 h including pH, salinity, electrical conductivity and dissolved oxygen. Temperature was logged in 10-min intervals over the duration of the test. Sub-samples for chemical analysis were taken at 0 h and 72 h from each treatment.

Format:

The dataset is summarised in one file named 'Tetraselmis sp pesticide toxicity data_eAtlas.xlsx'

Data Dictionary:

The excel spreadsheet has one tab for each pesticide which include specific growth rate (SGR) data. The last tab of the dataset shows the measured (start and end of test) water quality (WQ) parameters (pH, salinity, dissolved oxygen (DO), and temperature) of each pesticide test.

For each 'pesticide'_SGR tab:

SGR = specific growth rate - the logarithmic increase from day 0 to day 3

Nominal (µg/L) = nominal herbicide concentrations used in the bioassays

Measured (µg/L) = measured concentrations analysed by The University of Queensland

Rep = replicate notation is 1-3

Average T3_CellsPer_ml = cell density at day 3

Average ln(day3) = natural logarithm of cell density at day 3

T0_CellsPer_ml = average cell density at day 0

ln(day0) = natural logarithm of cell density at day 0

References:

O'Brien, D. et al. Spatial and temporal variability in pesticide exposure downstream of a heavily irrigated cropping area: application of different monitoring techniques. *J. Agric. Food Chem.* 64, 3975-3989 (2016).

Grant, S. et al. Marine Monitoring Program: Annual Report for inshore pesticide monitoring 2015-2016. Report for the Great Barrier Reef Marine Park Authority, Great Barrier Reef Marine Park Authority, Townsville, Australia. 128 pp, <http://dSPACE-prod.gbrmpa.gov.au/jspui/handle/11017/13325> (2017).

Trenfield, M. A. et al. Aluminium, gallium, and molybdenum toxicity to the tropical marine microalga *Isochrysis galbana*. *Environ. Toxicol. Chem.* 34, 1833-1840, doi:10.1002/etc.2996 (2015).

OECD. Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test. (OECD Publishing, 2011).

Data Location:

This dataset is filed in the eAtlas enduring data repository at: data\nescp3\3.1.5_Pesticide-guidelines-GBR

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| Metadata language | eng |
| Character set | UTF8 |
| Hierarchy level | Dataset |

OnLine resource

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| Linkage | https://nesptropical.edu.au/index.php/round-3-projects/project-3-1-5/ |
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| Protocol | WWW:LINK-1.0-http--related |
| Linkage | https://eatlas.org.au/nesp-twq-3/pesticide-guidelines-gbr-3-1-5 |
| Protocol | WWW:LINK-1.0-http--related |
| Linkage | https://eatlas.org.au/pydio/public/au-nesp-twq-3-1-5-aims-pesticide-guidelines-gbr-tetraselmis-sp-2020-02-26 |
| Protocol | WWW:LINK-1.0-http--downloaddata |

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| Role | Point of contact |
| Topic category | Biota |

Extent

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|-------------|-------------------------------|
| Description | Great Barrier Reef, Australia |
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Metadata author

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| Organisation name | Australian Institute of Marine Science (AIMS) |
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