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Toxicity of dissolved and precipitated aluminium to marine diatoms

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ABSTRACT

Localised aluminium contamination can lead to high concentrations in coastal waters, which have the potential for adverse effects on aquatic organisms. This research investigated the toxicity of 72-h exposures of aluminium to three marine diatoms (*Ceratoneis closterium* (formerly *Nitzschia closterium*), *Minutocellus polymorphus* and *Phaeodactylum tricornutum*) by measuring population growth rate inhibition and cell membrane damage (SYTOX Green) as endpoints. Toxicity was correlated to the time-averaged concentrations of different aluminium size-fractions, operationally defined as <0.025 μm filtered, <0.45 μm filtered (dissolved) and unfiltered (total) present in solution over the 72-h bioassay. The chronic population growth rate inhibition after aluminium exposure varied between diatom species. *C. closterium* was the most sensitive species (10% inhibition of growth rate (72-h IC10) of 80 (55–100) μg Al/L (95% confidence limits)) while *M. polymorphus* (540 (460–600) μg Al/L) and *P. tricornutum* (2100 (2000–2200) μg Al/L) were less sensitive (based on measured total aluminium). Dissolved aluminium was the primary contributor to toxicity in *C. closterium*, while a combination of dissolved and precipitated aluminium forms contributed to toxicity in *M. polymorphus*. In contrast, aluminium toxicity to the most tolerant diatom *P. tricornutum* was due predominantly to precipitated aluminium. Preliminary investigations revealed the sensitivity of *C. closterium* and *M. polymorphus* to aluminium was influenced by initial cell density with aluminium toxicity significantly ($p < 0.05$) increasing with initial cell density from 10^3 to 10^5 cells/mL. No effects on plasma membrane permeability were observed for any of the three diatoms suggesting that mechanisms of aluminium toxicity to diatoms do not involve compromising the plasma membrane. These results indicate that marine diatoms have a broad range in sensitivity to aluminium with toxic mechanisms related to both dissolved and precipitated aluminium.

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1. Introduction

Over the past few decades, localised inputs of aluminium to marine ecosystems have increased as a consequence of anthropogenic activities, such as coastal mining and dredging operations, discharges associated with alumina production, the disturbance or drainage of acid sulphate soils for coastal development and the use of aluminium in sacrificial anodes for the protection of offshore assets. These activities can increase aluminium concentrations above the natural background concentrations of coastal waters. Open ocean dissolved aluminium concentrations are typically <0.7 μg/L (Kramer et al., 2004; Measures et al., 2005; Middag et al., 2011), while in coastal waters they range from 0.1 to 16.7 μg/L (Angel et al., 2016) and can be as high as 83 μg/L in heavily industrialised harbours such as Port Curtis, QLD, Australia (Angel et al., 2012).

An important distinction between aluminium speciation in seawater compared to freshwater is the absence of cationic species (Al^{3+}, $AlOH^{2+}$ and $Al(OH)_2^+$) which dominate under acidic pH conditions (Wilson, 2011). In the marine environment (pH 8.0–8.3, 35 PSU), aluminium speciation is dominated by the aluminate anion ($Al(OH)_4^-$) and to a lesser extent neutral aluminium hydroxide ($Al(OH)_3^\circ$) (Millero et al., 2009) with insignificant amounts of colloidal aluminium (Angel et al., 2016; Moran and Moore, 1989). At high total aluminium concentrations (above approximately 500 μg/L) precipitation of dissolved aluminium, mostly as $Al(OH)_3$ and to a lesser extent as hydrotalcite ($Mg_6Al_2CO_3(OH)_{16}.4H_2O$), increases and dominates speciation (Angel et al., 2016).

The change in aluminium speciation with pH, time and concentration is an important consideration when performing toxicity tests as bioavailability and hence toxicity is directly related to metal

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speciation. It is therefore particularly important that investigations into understanding the mechanisms of toxicity be linked to metal speciation. At present, there are no analytical methods to measure the ionic and neutral dissolved forms of aluminium in seawater. However, size fractionation may be used to obtain information on the relative concentrations of dissolved, colloidal and precipitated forms of aluminium.

Robust data on the chronic toxicity of aluminium to marine organisms was recently expanded and collated to derive a high-reliability ANZECC (Australian and New Zealand Environment Conservation Council) water quality guideline value for the protection of marine organisms from aluminium (Golding et al., 2015). In toxicity tests with marine algae, the aluminium concentrations reported to cause 10% inhibition (IC10) range over 3 orders of magnitude, from 14 to 6800 μg Al/L, highlighting the interspecies variability in aluminium sensitivity between marine algae (Golding et al., 2015). Amongst the more sensitive species of marine algae to aluminium are the golden-brown flagellate, *Isochrysis galbana* (72-h IC10 of 420 μg/L at 24 °C) (Trenfield et al., 2015) and the two diatoms, *Minutocellus polymorphus* (72-h IC10 of 690 (580–800) μg/L at 21 °C) (Golding et al., 2015) and *Ceratoneis closterium* (72-h IC10 of 18 (11–26) μg/L at 21 °C for the temperate strain (Golding et al., 2015) and 14 (3–25) μg/L at 32 °C for the tropical strain (Harford et al., 2011)). The high sensitivity of *C. closterium* to aluminium heavily influenced the recently derived high-reliability ANZECC guideline value for aluminium in marine waters of 24 μg total Al/L providing protection for 95% of species, highlighting a need to examine the factors influencing aluminium toxicity to marine diatoms in more detail (Golding et al., 2015).

Biotic factors such as initial cell density have been found to influence metal toxicity, with increased cell density reducing the sensitivity of microalgae to copper, zinc and cadmium (Franklin et al., 2002; Moreno-Garrido et al., 2000; Vasseur et al., 1988). These authors have suggested that a decrease in sensitivity at higher initial cell densities may be due to lower metal accumulation rates (Moreno-Garrido et al., 2000) or less metal bound per algal cell at a higher cell density resulting in overall greater metal adsorption by the greater algal biomass thereby causing a depletion of the equilibrium concentration of dissolved metal in solution (Franklin et al., 2002). It is not known whether diatoms influence aluminium speciation and toxicity in marine waters, but it is hypothesized that toxicity would decrease with increasing initial cell density. The most sensitive diatom to aluminium (*C. closterium*) has so far only been tested at an initial cell density of 10^4 cells/mL (Golding et al., 2015; Harford et al., 2011). Therefore, this study utilised a lower, more environmentally realistic initial cell density of 10^3 cells/mL and investigated the influence of initial cell density on toxicity and speciation of aluminium to marine diatoms.

Few studies have examined the mechanisms of aluminium toxicity to marine organisms and at present we have limited mechanistic understanding of why some organisms are sensitive to aluminium and others are tolerant (Xie et al., 2015). An understanding of the mechanisms of toxicity is of practical importance as it provides a basis for interpretation of toxicity data. Diatoms play a key role in the biogeochemical cycling of silicon and aluminium in marine systems and evidence exists that the presence of aluminium in diatoms reduces the dissolution of silica from the cell wall (Dixit et al., 2001; Lewin, 1961; Van Bennekom et al., 1991). Therefore it is hypothesized that diatoms with different silica cell wall compositions will have different sensitivities to aluminium. Hence, the three species of marine diatoms chosen for investigation were: *C. closterium*—a pennate diatom with a siliceous cell wall; *M. polymorphus*—a centric diatom with a siliceous cell wall; and *Phaeodactylum tricornutum*—a pennate diatom with a weakly siliceous cell wall.

The plasma membrane can be a critical target for the action of metals since it is a surface where interactions with the extra-cellular environment occur. Metal exposures increase cell membrane permeability through mechanisms such as oxidative stress (Mallick and Rai, 2002). Plasma membrane permeability can be evaluated with the use of a nucleic acid dye such as SYTOX Green, allowing additional insight into the mechanism of toxicity (Haugland, 2005).

The aim of the present study was to relate aluminium sensitivity of three marine diatoms to aluminium size-fractionated species (dissolved, colloidal and precipitated forms) and investigate possible mechanisms of aluminium toxicity. Specifically, this study examined: (i) total and dissolved aluminium concentrations over the duration of the bioassays; (ii) the chronic toxicity of aluminium to three marine diatoms with differing cell wall/shape characteristics; (iii) the effect of aluminium on membrane permeability and hence membrane damage; and (iv) the influence of initial cell density on the toxicity of aluminium.

2. Methods

2.1. General analytical

Unless otherwise stated, all plasticware was acid-washed before use by soaking in 10% v/v nitric acid (Tracepur, Merck, Darmstadt, Germany) for 24 h, then rinsed 10 times with deionized water (18 MΩ/cm, Milli-Q, Millipore). Algal culture media and aluminium bioassay solutions were prepared with natural seawater collected from a rock platform at Cronulla, NSW, Australia (34°04′13.35″ S, 151°09′25.69″ E). The seawater was collected using acid-washed high-density polyethylene carboys (5 and 10 L), filtered (0.45 μm Sartobran P MidiCaps, Sartorius) within 3 h and stored at 4 °C in the dark until use. Measurements of pH used a Thermo Orion pH metre with an epoxy body Iodine/Iodide probe (meter model 420, probe model ROSS 815600, Thermo Fisher Scientific, USA) which was calibrated daily against pH 4.00, 7.00 and 10.00 buffers (Orion Pacific, Sydney, NSW, Australia).

2.2. Diatom cultures

Marine diatoms were obtained from the CSIRO Collection of Living Microalgae, Marine and Atmospheric Research, Hobart (Tasmania, Australia). *C. closterium* (formerly *Nitzschia closterium* (Ehrenb.) W. Smith (strain CS-5, temperate)) was cultured in natural seawater f medium with the trace metal concentrations halved (Guillard and Ryther, 1962). *M. polymorphus* (Hargraves and Guillard) Hasle, Von Stosch and Syvertsen (strain CS-3) and *P. tricornutum* Bohlin (strain CS-29/4) were cultured in f/2 growth medium (half strength f medium, Guillard and Ryther (1962)). All cultures were maintained in a temperature-controlled room at 21 °C on a 12:12 h light:dark cycle (Sylvania F20W/154-RS daylight fluorescent tubes; 70 μmol photons/m^2/s) and transferred into fresh media weekly. Cultures of *P. tricornutum* were axenic (based on a lack of bacterial growth on PYEA plate growth) while *C. closterium* and *M. polymorphus* were not.

2.3. Preparation of aluminium test solutions

Aluminium stock solutions (1 and 10 g Al/L) were prepared on the day of toxicity test initiation by the addition of aluminium chloride to sodium hydroxide solution, to counteract the pH decrease in spiked test solutions caused by aluminium hydrolysis (Angel et al., 2016), according to Golding et al. (2015). Aluminium test solutions (10–10,000 μg Al/L nominal) were prepared in acid-washed 500 mL polycarbonate containers by adding appropriate aliquots (<1% of final volume to maintain seawater pH and salinity) of the

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