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# The bioavailability of different dissolved organic nitrogen compounds for the freshwater algae *Raphidocelis subcapitata*

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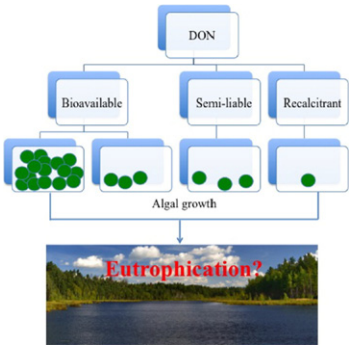
## HIGHLIGHTS

- DON algal bioavailability varied greatly from one DON compound to another.
- DON compounds were classified as "bioavailable", "semi-liable" and "recalcitrant".
- Different bioavailable DON compound showed different uptake rate.
- The contribution to algal cell yield varied from one DON compound to another.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Understanding which factors affect the algal bioavailability of dissolved organic nitrogen (DON) compounds in natural surface waters is important for our understanding of nutrient biogeochemistry and water quality management. We used nitrogen uptake kinetics and algal cell yield to characterize the algal bioavailability of 22 dissolved DON compounds that are commonly found in natural surface waters and wastewater treatment plant effluents, including urea, amino acids, amino sugars, nucleotides, pyrimidines, oraganonitriles, polyacrylamide, EDTA, caffeine, phenolic compounds and humic acids. Twelve of these compounds were highly bioavailable, including urea, dissolved free amino acids, bovine serum albumin, DNA, RNA, ATP, AMP, acetonitrile and caffeine. Four compounds had intermediate bioavailability including two humic acids (Elliott Soil and Pahokee Peat), glycylglycine, RNA and uracil. The remaining six compounds were classified as recalcitrant, i.e., EDTA, 2,3-Dinitrophenol, aminobenzoic acid, polyacrylamide and Aldrich humic acid. For many of the compounds tested, the algal cell yield was only 60–80% of expected relative to DON uptake. These results help explain why some DON compounds are more likely to persist in natural systems, and why the DON pool is often recalcitrant in surface waters.


## 1. Introduction

Nitrogen (N) is a critical limiting nutrient for primary production in many lakes, rivers, estuaries, and coastal marine ecosystems (Conley et al., 2009). Elevated nitrogen inputs can promote eutrophication, and lead to increased frequencies and magnitudes of phytoplankton blooms, including harmful taxa (e.g., toxic, hypoxia inducing, and food-web-altering) (Paerl, 1988; Hallegraeff, 1993; Paerl, 1997). Nitrogen concentrations and especially the forms of nitrogen available can strongly influence the species composition of phytoplankton communities (Anderson et al., 2002). The dissolved nitrogen in waters is

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composed of dissolved inorganic nitrogen (DIN; $NO_3^-$/$NO_2^-$ and $NH_4^+$/$NH_3$) and a very heterogeneous matrix of dissolved organic nitrogen (DON) compounds. Recently, increased attention has been focused on the role that DON may play in structuring aquatic ecosystems (Kang and Mitchell, 2013; Pisani et al., 2017; Shen et al., 2015; Zhai et al., 2016). This is due to the increasing DON inputs in surface waters and the complex bioavailability of the DON pool in natural systems (Bronk et al., 2010).

DON is the dominant nitrogen pool in many marine and freshwater systems (Antia et al., 1991; Berman and Bronk, 2003). Surface water DON can originate from natural and anthropogenic sources throughout the water- and airshed (Seitzinger et al., 2002), and anthropogenic nitrogen sources are often the major DON inputs to surface waters (Seitzinger and Sanders, 1997). Anthropogenic nitrogen sources include municipal point source discharges, and nonpoint sources, such as agricultural runoff, atmospheric deposition of fossil fuel derived $NO_x$ and other combustion products, agricultural emissions, and N-enriched groundwater (Paerl, 1997). Relative to non-point sources, point sources are easier to quantify and regulate. Therefore, in order to control eutrophication, strict N discharge limits are often set for wastewater treatment plant (WWTP) effluents. With the installation of biological nutrient removal systems in more WWTPs and efficient DIN removal, DON is often a large fraction of the N pool in point source discharges and in some cases accounts for up to 85% of the total nitrogen (TN) discharged from municipal and industrial WWTPs (Pehlivanoglu and Sedlak, 2004).

It is well established that the DIN pool is readily utilized by phytoplankton and strongly contributes to eutrophication (D'Elia et al., 1986; Ryther and Dunstan, 1971). However, the effect of DON is more complex because some DON compounds may be recalcitrant (Urgun-Demirtas et al., 2008; Neff et al., 2003). The algal bioavailable DON (ABDON) fraction in natural waters can range from 10 to over 80% (Seitzinger and Sanders, 1997; Stepanauskas et al., 1999a; Wiegner and Seitzinger, 2001; Stepanauskas et al., 2000). Our previous work showed that in the effluents of advanced N removal WWTPs 30 to 100% of the DON pool was recalcitrant, and the DON fraction varied considerably from one WWTP to another (Li et al., 2015; Fan et al., 2017). With increasing DON contributions to natural waters and wastewater effluents, it is especially important to understand how the bioavailability of different DON species varies (Bronk et al., 2007).

Antia et al. (1991) showed the DON compounds that have been detected in natural waters worldwide include high molecular weight DON compounds, e.g., humic bound nitrogen, and low molecular weight DON compounds including ureides, amideines, amino acids, amino sugars, purines, pyrimidines, nucleotides, pteridines and flavins. The DON composition of different municipal wastewater effluents also varies considerably but current methods are only able to identify ≈10% of the DON in these discharges (Pehlivanoglu-Mantas and Sedlak, 2008), with dissolved free and combined amino acids accounting for the majority of the identifiable wastewater-derived DON (Pehlivanoglu-Mantas and Sedlak, 2008).

Previous research has shown low molecular weight DON compounds like urea, dissolved free amino acids and nucleic acids can be readily assimilated by bacteria and phytoplankton (Czerwionka, 2016; Wheeler and Kirchman, 1986; Tranvik, 1993). Compounds such as proteins, dissolved combined amino acids and amino polysaccharides are believed to be semi-liable (Carlson and Ducklow, 1995). Conversely, humic bound nitrogen substances have been reported to be recalcitrant (Parkin and McCarty, 1987). However, to date, the bioavailability of a wide variety of organic nitrogen compounds has not been systematically characterized.

The goal of this study was to investigate the algal bioavailability of different DON compounds and how much these compounds actually support algal growth. The various factors that control DON bioavailability are still poorly understood and obtaining a more complete understanding of the characteristics of DON compounds represents an important challenge in eutrophication management. The bioavailability of 22 pure DON compounds that are commonly detected in natural waters and WWTP effluents were tested using phytoplankton based nutrient uptake and cell yield bioassays, widely used methods for ABDON quantification (Pehlivanoglu and Sedlak, 2004; Urgun-Demirtas et al., 2008; Liu et al., 2011; Qin et al., 2015). Based on the bioassay results, a general framework to characterize the bioavailability of DON compounds was developed and the potential contributions of different DON compounds to eutrophication were discussed within the context of water residence time.

## 2. Methods

### 2.1. Dissolved organic nitrogen compounds

Information on the DON compounds used for this study and their final nitrogen concentration in the bioassays is listed in Table 1. The compounds tested were prepared as stock solutions and combined with a synthetic N-free algal growth medium (Miller and Greene, 1978), with fresh solutions prepared prior to each bioassay experiment.

### 2.2. Nitrogen uptake bioassay

The freshwater alga *Raphidocelis subcapitata* (formerly knew as *Selenastrum capricornutum*) was used for these bioassays because it has been used as a standard test organism for eutrophication studies for over 35 years (Miller and Greene, 1978; Pehlivanoglu and Sedlak, 2004). Stock cultures were maintained using the nutrient medium described by Miller and Greene (1978). Seven to ten days prior to the bioassays, algae were centrifuged and resuspended into N-free medium to induce N-stress (Ellis and Stanford, 1988). A 300 mL stock solution with a synthetic N-free nutrient medium was prepared in triplicate for an inorganic nitrogen control sample (ca. 1.5 mg/L $NH_4^+$-N and 1.5 mg/L $NO_3^-$-N) and each DON species. N-starved algae were added to the samples at a starting concentration of $2 * 10^5$ cell $mL^{-1}$ to initialize the experiments. Samples were incubated at 18 ± 1 °C under continuous fluorescent lighting of 4300 ± 430 lm in a horizontal shaker at 110 rpm for 21 days. The 21 day incubation period was based upon the maximum growth potential for the study algae in laboratory conditions. On days 0, 0.33, 1, 2, 3, 7, 14 and 21, twenty mL samples were collected in replicate, and filtered for TDN analyses and algal cell density determination. The ABDON fraction (%) was calculated as the percentage of N remaining in the solution by the end of the incubation relative to the initial N concentration.

The three humic acids used for these experiments were also tested for potential physical-chemical interactions between the humic matter associated DON and the growth media during the 21 day experiment. This was done because the high ionic content of the growth media could cause the humic acids to flocculate, which would alias as DON uptake. The same stock solution preparation and incubation conditions were used except these solutions were incubated without algae.

The algal cell density was determined using a Coulter Multisizer III particle size analyzer by passing the samples through a 100 μm aperture, with every sample read three times. Prior to each reading, background particle concentrations were estimated by testing parallel samples that were not inoculated with algae. For the D-glucosamine treatment, chlorophyll-a was also determined using a fluorometer and its algal cell density was further calculated using a standard curve between Chl-a and cell density ($r^2 = 0.9996$, see corresponding equation in SI). This was done because D-glucosamine caused the algal cells to form aggregates.

### 2.3. Chemical analyses

The DON (measured as TDN) concentration for each solution on the above mentioned sampling days were analyzed according to Standard