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ORIGINAL ARTICLE

# In-Depth Assessment of the Effect of Sodium Azide on the Optical Properties of Dissolved Organic Matter

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## Abstract

Treatment and preservation of samples are critical issues in measuring the optical properties of dissolved organic matter (DOM) due to their high sensitivity to physical and chemical changes upon sample handling. In this study, we rigorously assessed the potential interferences of sodium azide ($NaN_3$) on DOM absorption and fluorescence. A wide range of different samples were poisoned with varying $NaN_3$ concentrations. Several commonly used optical parameters derived from absorbance and fluorescence spectroscopy were compared at different samples and conditions to assess the interfering effect of $NaN_3$. Our results showed that $NaN_3$ altered the original features of absorbance and fluorescence even at the lowest level of the addition. The absorption coefficients of $NaN_3$-treated samples increased up to 2608% at 254 nm and 66% at 280 nm relative to the untreated control. Fluorescence data revealed both a quenching effect and an enhancement in fluorescence. The effect of $NaN_3$ on fluorescence was highly variable and affected by the $NaN_3$ concentrations added, and the sources and the concentrations of DOM samples. None of these factors exhibited a clear linear behavior with $NaN_3$ levels, making it difficult to develop a correction method. It can be recommended from the findings not to use $NaN_3$ in preserving DOM samples for optical measurements.

**Keywords** Sodium azide · DOM · EEM · Absorption · PARAFAC · Quenching

## Introduction

Since the last decade, the optical properties (i.e., absorption and fluorescence) of dissolved organic matter (DOM) have been extensively used for DOM studies. They have been applied to many samples with the sources encompassing a wide array of environments such as seawater, freshwater, soil, sediments, and atmosphere [1–5]. In DOM pools, fluorescent DOM (FDOM) and chromophoric DOM (CDOM) properties have been frequently used to examine DOM distribution [6, 7], to track its sources [8–10], to study the photo- and biodegradation of DOM and its byproducts [11, 12] to characterize DOM, [13, 14], to calibrate the algorithms for CDOM retrieval from satellite [15, 16], as well as to monitor the DOM in the engineering systems including wastewater treatment facilities [17]. Their increasing use is also due to the implementation of various tools and indices to analyze and interpret CDOM and FDOM data.

The absorption data of DOM are commonly reported by using the absorption coefficients at different wavelengths and the related indices. The specific ultraviolet absorption at 254 nm ($SUVA_{254}$) gives information on the aromaticity of DOM molecules [18]. The spectral slope calculated between 275 and 295 nm ($S_{275–295}$), between 350 and 400 ($S_{350–400}$), and their ratio, Sr, are related to the molecular weight of CDOM molecules [19] and can give information on the percentage of terrestrial versus marine compounds [20]. Fluorescence is commonly measured by using tridimensional excitation and emission matrices (EEMs). The optimized drEEM toolbox [21] allows to pre-treat the raw EEMs and carry out the PARAllel FACtor Analysis (PARAFAC), which decomposes their complex signals into the simpler groups of dissimilar fluorophores (components). Fluorescence data are also utilized to calculate various indices that are related to the degree of humification (HIX) [22], the proportion of recently produced DOM (BIX) [23], and the source precursor (terrestrial or microbial) of DOM (FI) [24].

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Handling DOM samples requires a careful measure to avoid contamination and/or modification of the inherent optical properties, which is critical in DOM research areas. Light, pH, solution chemistry, and temperature are examples of environmental parameters which might affect (remove/produce/modify) the DOM properties. Therefore, the method of treating and preserving samples is paramount, particularly when absorption and fluorescence measurements are involved because of their high sensitivity to physical/chemical/biological modifications in samples [25]. A detailed review of sample treatment and preservation methods has been given by Spencer and Coble [26]. The effects of refrigeration and freezing of samples have been extensively studied and some guidelines have been also suggested [26, 27]. Likewise, the effect of sample acidification has been extensively explored and it is recognized to greatly affect the fluorescence properties [28, 29]. However, only a few studies reported the effect of poisoning using a chemical agent.

Sodium azide ($NaN_3$) is one of the most used chemical agents as a biocide (and thus as a preservative). It is highly toxic [30], inhibiting the cytochrome oxidase in bacteria and blocking their respiration. Kaplan [31] showed that the use of $NaN_3$ resulted in an initial organic carbon (OC) contamination and a decrease in its concentration within 3–4 weeks. However, $NaN_3$ is still used to preserve the samples and to inhibit microbial growth in photochemical experiments or in biodegradation experiments [32–37]. A summary of the previous studies using $NaN_3$ is listed in Table 1. Based on the literature, the effects of $NaN_3$ on absorption and fluorescence are yet contradictory. Astoreca et al. [39] and Ferrari et al. [38] reported no significant effect of $NaN_3$ on CDOM absorption above 300 nm. Patel-Sorrentino et al. [28] stated that the addition of $NaN_3$ did not affect the fluorescence intensity. None of these studies, however, presented any supporting results to prove the limited $NaN_3$ interferences. Meanwhile, Pisani et al. [41] and Parr et al. [42] mentioned no use of $NaN_3$ to avoid the concern over fluorescence alternation although no related data were shown. Recently, Park and Snyder [40] tested three biocides, including $NaN_3$, for DOM sample preservation, and they showed a notable decrease in fluorescence via the EEM spectra and an increase in absorption with the increase of the $NaN_3$ concentrations (30 and 60 mg/L). However, no quantitative data were presented in the study to support their observations.

To date, no clear conclusion has been reached on the effect of $NaN_3$ on FDOM or CDOM as no detailed study has been done yet. In this framework, the main goal of this study was to evaluate the effect of $NaN_3$ on DOM absorbance and fluorescence and if its effect changes according to the DOM source and/or concentration. For this purpose, a wide range of samples, from different sources (Standards, extracts and natural samples) and with varying dissolved organic carbon (DOC) concentrations (0.7 to 63 mg C $L^{-1}$), were poisoned with

**Table 1** Summary of the previous studies using sodium azide ($NaN_3$) for DOM optical properties

| Sample Type | Sample pre-treatment | $NaN_3$ concentration | DOC concentration | Purpose of $NaN_3$ addition | Sample preservation | $NaN_3$ effect | Reference |
|---|---|---|---|---|---|---|---|
| Stream | Filtration (GF/F) | 0.135 mM | 0.7–2.8 mg $L^{-1}$ | Test on samples preservation | Dark, room temperature | Yes (DOC) | [31] |
| Seawater + River | 0.2 μm filtration | 0.77 mM | 4.1–7.4 mg $L^{-1}$ | Sample preservation | 4 °C | Yes, for A < 300 nm | [38] |
| River | Fractionation | 0.4 mM | Not specified | Sample preservation | Dark, 4 °C | Not reported | [28] |
| River | Sieved 15 μm | 3.1 mM | 8.5–13.5 mg $L^{-1}$ | Inhibition of biodegradation during photochemical and biodegradation experiment | Incubation | Yes, for A < 370 | [32] |
| Soil + waste water treatment effluent | 0.45 μm filtration | 2 mM | 1.5–11.5 mg $L^{-1}$ | Inhibit aerobic microbial activity | 4 °C | Not tested | [33] |
| River and seawater | 0.2 μm filtration | 0.7 mM | Not measured | Long time samples preservation | 4 °C | Not tested | [39] |
| Wetland | Concentration and 0.2 μm filtration | 3 mM | 24.3–58.1 mg $L^{-1}$ | Abiotic controls on experiment | freeze | Not tested | [34] |
| Soil humic substances | 0.45 μm filtration | Not specified | 5 mg $L^{-1}$ | Prevent the effect of bacteria on humic substances | 4 °C | Not tested | [35] |
| Plant leachates | GF/F filtration | 0.7 mM | Not measured | Abiotic replicate to probe the effect of bacteria on CDOM generation | Irradiation experiment | Not tested | [36] |
| Waste water treatment effluent | 0.45 μm | 0.4 and 0.9 mM | No measured | Test as preservative for fluorescence measurements | 4 °C | Yes (EEMs) | [40] |
| Suwannee River Fulvic Acid + Algae | 0.2 μm filtration | 3 mM | 0.9–11 mg $L^{-1}$ | Inhibition of biodegradation during photochemical and biodegradation experiment | Incubation | Not tested | [37] |

different $NaN_3$ concentrations before they were analyzed by UV-vis and fluorescence spectroscopy. Treated DOM samples were compared to controlled samples (e.g., without $NaN_3$) at each different condition (e.g., sources, DOC concentrations, and $NaN_3$ concentrations) using common optical proxies for DOM studies (PARAFAC components, fluorescence indices, absorption indices) to provide recommendations to the community for future work.

## Methods and Procedures

### Samples Preparation

Four standards from the International Humic Substances Society (IHSS, https://ihss.humicsubstances.org/) were selected: Pony Lake Fulvic Acid (PLFA, 1R109F), Nordic Reservoir NOM (1R108N), Suwannee River Humic Acid Standard III (SRHA, 3S101H), and Suwannee River Fulvic Acid Standard I (SRFA, 1S101F). Each standard was dissolved in Milli-Q and used as a stock solution.

Three environmentally relevant natural DOM samples were also used, which included freshwater, sea water, and sediment pore water. Freshwater was collected in Jung-Rang stream (Seoul, South Korea). Seawater was collected during NICE18A sampling expedition in Kongsfjorden, Svalbard Archipelago (78.985°N, 11.65°E) on April 2018. Both samples were filtered through pre-combusted and pre-washed GF/F filters (Whatman). The porewater samples were collected within 0.5 m below seafloor in the Beaufort Sea during ARA07C cruise in October 2017. They were slowly extracted from sediment cores by using acid-washed Rhizons (Rhizosphere Research Products). The samples were then immediately filtered through a 0.2 μm, acid-washed polytetrafluoroethylene filter.

In addition, two water extractable organic matter samples, from soil and algae, were used. Briefly, top soil (0-10 cm) sample was collected at Bukhansan National Park (37.727°N, 127.01°E) in South Korea. Commercial unicellular green algae (*Chlorella vulgaris*) was purchased from Aquanet Co., Ltd. (Gyeongsangnam-do, South Korea). The collected soil was homogenized, dried in an oven at 40 °C, and sieved (2.0 mm mesh), while the algae sample was freeze-dried and then ground. Both samples were soaked in Milli-Q water at a solid-to-liquid ratio of 1:20 and shaken for 24 h before centrifugation (5000 rpm for 15 min). The extracted solutions were filtered through a pre-combusted and pre-washed GF/F filter (Whatman).

A $NaN_3$ stock solution was prepared at 30 mM by diluting 0.4891 g of $NaN_3$ (Acros Organics, New Jersey, USA) in 250 ml of Milli-Q water. Each standard and extract was diluted with Milli-Q and amended with $NaN_3$ to obtain four working solutions at 10, 5, 2.5, and 1 mg C $L^{-1}$ with four different final $NaN_3$ concentrations of 0 (control), 0.03, 0.3 and 3 mM. The range of C concentrations was chosen to resemble a wide range of environmental samples, whereas the $NaN_3$ concentrations were chosen according to those used in previous studies (Table 1). The natural samples were used at their original concentrations and amended with $NaN_3$ to a final concentration of 0, 0.03, 0.3 and 3 mM. Experimental blanks were made by adding $NaN_3$ Milli-Q water to a final concentration of 0, 0.03, 0.3 and 3 mM. The experimental design is illustrated in Fig. S1 and details of the preparation of the solutions used for measurement are presented in Table S1.

### Analytical Measurements

All the measurements were conducted within the day from the preparation of the solutions.

To prepare solutions at known C concentration, the dissolved organic carbon (DOC) of the stock solutions was quantified by using a total organic carbon analyzer (Shimadzu TOC-VCPH) with an analytical reproducibility <2%. The concentration of each sample is reported in Table S1.

Absorption spectra were recorded between 200 and 800 nm at 0.5 nm intervals using a Shimadzu UV-1800 UV spectrophotometer. Absorption coefficient at 254 nm ($a_{254}$), 280 nm ($a_{280}$), and 350 nm ($a_{350}$) were calculated according to the following equation:

$$a_\lambda = \frac{A_\lambda \cdot 2.303}{l}$$

Where $A_\lambda$ represent the absorbance at a wavelength λ, and l, the pathlength (0.01 m). These three wavelengths were chosen based on the previous studies involving $NaN_3$ use [37, 43–45]. The spectral slope (S) in the 275–295 nm range [20] was calculated by non-linear fitting of the following equation:

$$a_\lambda = a_{\lambda 0} \cdot e^{-S(\lambda - \lambda 0)}$$

Fluorescence EEMs were measured with a Hitachi F-7000 fluorescence spectrophotometer. Excitation ranged 220 and 500 nm with a 5-nm interval, while emission ranged between 280 and 550 with a 1-nm interval. Slits were set at 5 nm, scan speed was 2400 nm/min, and the voltage was set at 650 V.

The drEEM toolbox [21] was used to perform EEMs correction (i.e., blank subtraction, inner filter correction, and Raman normalization) and PARAFAC analysis. The EEMs were subtracted by the experimental blanks (i.e. Milli-Q + $NaN_3$) having the same $NaN_3$ concentration as the sample. The validation of PARAFAC model was made by split half analysis and percentage of explained variance (98.3%).

By using corrected EEMs, classical fluorescence indices were calculated. Humification Index (HIX) was calculated as the ratio of the areas under the emission spectra over 435–480 nm to 300–345 nm at an excitation wavelength of

255 nm [22]. Index of recent autochthonous contribution (BIX) was calculated as the ratio of the fluorescence intensity at the emission wavelength of 380 nm to 430 nm at an excitation wavelength of 310 nm [23]. Fluorescence Index (FI) was calculated as the ratio of the emission intensity at 450 nm to that at 500 nm at excitation 370 nm [24].

## Results

In this study, the most commonly used parameters such as PARAFAC components, fluorescence indices, absorption coefficients, and spectral slope, were used to assess the effect of $NaN_3$ on the spectroscopic properties of DOM. The differences ($\Delta$) in the parameters between the $NaN_3$–treated samples and the control equivalent were calculated to quantify the effect of $NaN_3$ on CDOM/FDOM. This difference was reported as follows.

$$\Delta\% = \frac{X_{NaN3} - X_{control}}{X_{control}} \cdot 100$$

Where, for each parameter, $X_{NaN3}$ is the value of the $NaN_3$-treated sample, and $X_{control}$ is the value of the untreated control sample (Tables S2 to S4). The following paragraphs present the effect of $NaN_3$ according to: i) its concentration; ii) the source of DOM; and iii) the DOM concentration.

### PARAFAC Components

The PARAFAC analysis was carried out using the entire dataset (112 EEMs) and separate datasets according to $NaN_3$ concentrations, each containing 28 EEMs (the dataset groups without $NaN_3$, with 0.03 mM of $NaN_3$, with 0.3 mM of $NaN_3$, and with 3 mM of $NaN_3$), in which excitation below 250 nm and emissions over 500 nm were removed.

A 3-component model was validated by using all the five datasets. The excitation and emission spectra of the components were identical (Fig. 1). The data presented hereinafter refer to the model, which included all the samples.

The spectra of the components were compared with previous literature by using the OpenFluor database [46]. 51 matches were found with a similarity score of >90% (Table S5). The spectral characteristics of component 1 (C1) are similar to those previously reported for terrestrial humic-like substances (Ex/Em, <250–320/478). The excitation and emission maxima of component 2 (C2) are typical of protein-like (tryptophan-like) compounds (275 and 341 nm, respectively). Component 3 (C3) has the spectral characteristics (Ex/Em, <250–310/399) similar to humic-like substances. The low emission maxima, with respect to the terrestrial humic-like component, suggests the attribution of C3 to the so-called marine or microbial humic-like substances.

### Effect of $NaN_3$ Concentrations

The $\Delta\%$ calculated for each sample at the three different $NaN_3$ concentrations (i.e. 0.03 $NaN_3$–0 $NaN_3$, 0. 3 $NaN_3$–0 $NaN_3$, and 3 $NaN_3$–0 $NaN_3$) are shown in Tables S2, S3 and S4.

The effect of NaN3 concentration on absorption and fluorescence was initially evaluated on the experimental blanks to verify the possible contamination due to the addition of $NaN_3$. The absorption coefficients at 254 and 280 nm showed positive $\Delta\%$ increasing with the increase of $NaN_3$ addition (Table S2). The absorption at 350 nm showed very little $\Delta\%$ compared to the other two wavelengths, with a negative value (−0.5%) for the addition of 0.3 mM $NaN_3$ (Table S2). Similarly, a substantial, positive $\Delta\%$ (32 to 209%, Table S3) was observed for the three PARAFAC components, with the exception of C2 and C3 at 0.3 $NaN_3$ addition which showed small negative values (−6 and − 8%, respectively, Table S3). The $NaN_3$ concentration showed an almost linear relationship with its effect on absorption, whereas there was no linearity with its effect on fluorescence.

As for the blanks, the effect of $NaN_3$ concentration on the different DOM samples absorption parameters exhibited a rather linear behavior. The data reported in Table 2 show an increase in $|\Delta\%|$ with the increase of $NaN_3$ concentration at all the wavelengths and in $S_{275-295}$. The most pronounced effect of $NaN_3$ can be observed at 254 nm with a decreasing tendency at longer wavelength. This can be seen also by the slope of the relationship between the absorption parameters and $NaN_3$ concentrations, which is higher than 10 $mM^{-1}$ $m^{-1}$ for $a_{254}$ and shows a decreasing trend with increasing wavelengths (Table S2). Substantial effect of $NaN_3$ was observed at 280 nm and 350 nm even with a low $NaN_3$ concentration (Table 2).

The $\Delta\%$ calculated for the components (Table S3) point out the marked variability of $NaN_3$ effect showing both quenching (negative values) and enhancement (positive values) of fluorescence irrespectively of the $NaN_3$ concentration. For instance, in the NOM sample at 1 mg C $L^{-1}$, C2 fluorescence decreased to −100% with the addition of 0.03 mM $NaN_3$, while it increased by ~6% with the addition of 3 mM $NaN_3$. Similarly, the fluorescence indices $\Delta\%$ showed both increase and decrease in their values.

To quantify the effect of $NaN_3$ concentration on all selected optical parameters, the averages of the $\Delta\%$ absolute value ($|\Delta\%|$) are reported over the whole set of different DOM samples with varying DOC concentrations. (Table 2; for each $NaN_3$ concentration the average of the $|\Delta\%|$ was calculated considering all samples poisoned with such amount of $NaN_3$ irrespective of the source or DOC concentration).

Similarly, an increasing effect with the increase of $NaN_3$ concentration was observed for the fluorescence

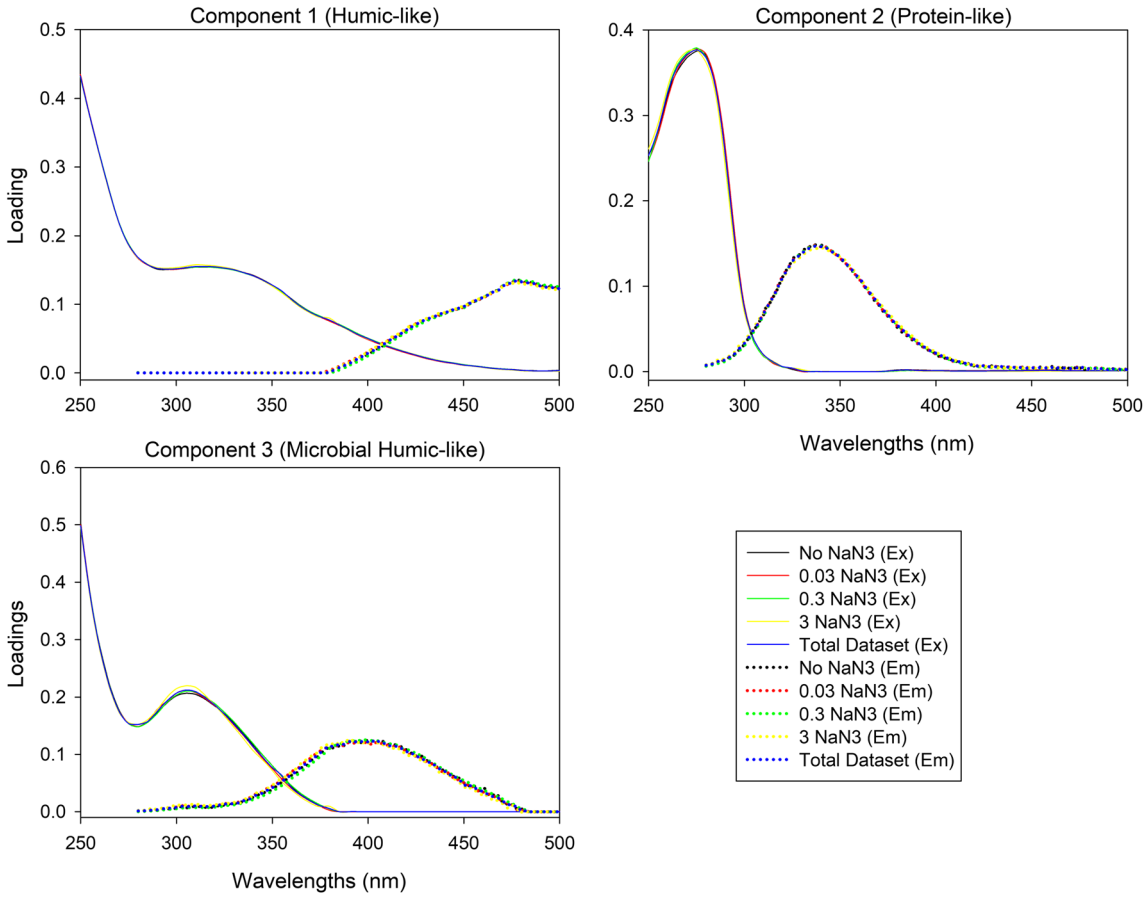


**Fig. 1** Excitation and emission spectra of the PARAFAC components for the 5 different datasets used. The lines are fully overlapped with each other

parameters, with the exception of C2. Within the components, C2 was the most affected, while HIX was found to be the most affected fluorescence index.

## Effect of $NaN_3$ on Different DOM Sources

To investigate the effect of $NaN_3$ according to the DOM origin, for each source (soil extract, algae, extract, SRHA, SRFA, NOM, PLFA, and natural samples), the average of the $|\Delta\%|$ was calculated irrespective of the DOC or the $NaN_3$ concentration (e.g., for each parameter in PLFA the $|\Delta\%|$ at 1, 2.5, 5, 10 mg C $L^{-1}$ with 0.03, 0.3 and 3 mM of $NaN_3$ were averaged together). The minima, maxima, and averages of $|\Delta\%|$ values for all the parameters are shown in Table S6. The parameters showing the highest $|\Delta\%|$ for each DOM source are reported in Table 3.

These results clearly show that the protein-like fluorescent component (C2), and HIX index are the most affected optical parameters by $NaN_3$ regardless the DOM sources. The only exceptions are the natural samples which are mostly affected in C3 (microbial humic-like). Regarding the fluorescence indices, FI and BIX are the most affected in the natural samples and soil extract, respectively. A deeper look at the rest of the parameters (Table S6) shows a DOM source-dependent effect. These parameters are, indeed, affected differently according to the DOM source. For instance, the humic-like components (C1 and C3) are alternating as the second most affected component.

In general, the humic-like component (C1) and the FI and BIX indices were found to be the least affected parameters (Table S6).

## Effect of $NaN_3$ at Different DOM Concentrations

Four examples, representative of all the dataset, are displayed in Fig. 2 to show the effect of $NaN_3$ on fluorescence parameters at different DOM concentrations. There were no consistent and linear trends of the $NaN_3$ effects on the optical properties with increasing DOM concentrations. In some cases, there were even alternations of positive and negative values with varying DOM concentrations.

The slopes of the relationship between $\Delta\%$ and the DOM concentration were calculated for each DOM sample and each parameter at four different $NaN_3$ concentrations (Table S7). The presence of both positive and negative slopes, even within the same source samples, confirm the absence of a consistent trend of $NaN_3$ effects on fluorescence properties with varying DOM concentrations.

**Table 2** Minimum and maximum of Δ% (see the text for its calculation), and average of Δ% absolute value (| Δ% |) calculated for all the parameters at different sodium azide (NaN3) concentrations. Δ% > | 10| are highlighted in bold

| | 0.03 mM $NaN_3$ | | | 0.3 mM $NaN_3$ | | | 3 mM $NaN_3$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | Δ% Min | Δ% Max | \| Δ% \| average | Δ% Min | Δ% Max | \| Δ% \| average | Δ% Min | Δ% Max | \| Δ% \| average |
| C1 | **−18.9** | **14.3** | 3.0 | −7.8 | 9.9 | 3.4 | **−10.4** | **15.1** | 5.2 |
| C2 | **−100** | **17.49** | **25.1** | **−52.1** | **90.5** | **18.6** | **−100** | **34.1** | **20.5** |
| C3 | **−20.8** | 2.9 | 3.5 | −8.6 | 9.8 | 3.5 | **−14.4** | **10.8** | 5.6 |
| HIX | −4.9 | **12.8** | 2.7 | **−19.2** | **22.4** | 5.4 | **−31.6** | **23.6** | 6.3 |
| FI | **−14.2** | 3.7 | 2.3 | **−19.6** | **12.9** | 3.8 | **−10.7** | **12.2** | 3.1 |
| BIX | −9.3 | 1.9 | 2.4 | **−14.9** | 4.1 | 3.4 | **−28.7** | 8.2 | 5.8 |
| $a_{254}$ | −0.3 | **28.4** | 4.3 | 3.0 | **280.2** | **35.8** | **24.1** | **2608.0** | **342.3** |
| $a_{280}$ | −6.0 | 7.6 | 1.9 | −3.6 | **11.6** | 2.8 | −7.2 | **65.5** | 9.6 |
| $a_{350}$ | **−25.0** | **17.9** | 3.6 | **−13.9** | **18.2** | 4.1 | **−25.6** | **53.0** | 7.5 |
| $S_{275–295}$ | −5.4 | 4.3 | 1.1 | −0.3 | **18.3** | 4.3 | **1.8** | **111.7** | **29.9** |

## Discussion

### Effects of $NaN_3$ on Absorbance

In an aqueous solution, sodium azide is dissociated into sodium cation (i.e., $Na^+$) and azide ion (i.e., $N_3^-$), a linear triatomic molecule. The UV-vis absorption spectra of azide ion is characterized by two maximum at 190 nm and 230 nm, representing the low-intensity transition and the higher-intensity transition, respectively [47]. Absorbance at 254 nm and $SUVA_{254}$ index are common proxies used to characterize the organic matter. $N_3^-$ absorbs at this wavelength (i.e., 254 nm) as shown by McDonald et al. [47] and confirmed in this study. Our experimental results showed that $a_{254}$ increases linearly with the increase of $NaN_3$, in both Milli-Q blanks and DOM samples. The maximum increase of $a_{254}$ rose up to 2608% of the control sample (at 3 mM of $NaN_3$, Table 2). To circumvent this issue, some authors used the absorbance at 280 nm or even at 350 nm and their associated SUVA (i.e., $SUVA_{280}$ and $SUVA_{350}$) [37, 43–45]. However, the results of this study demonstrated that, even at 280 nm and 350 nm, there is an increase or decrease in the absorption with addition of sodium azide (Table 2). A deeper look showed that the negative values at 280 nm corresponded to the algae extract samples (Table S2). This can be explained by the typical shoulder (260–280 nm) associated by the presence of tryptophan and tyrosine [48–50], which can be higher than the $NaN_3$ absorption. Meanwhile, the spectra of the $NaN_3$-treated and the control samples may become indistinguishable and noisier at 350 nm, which make it difficult to discriminate between the effect of $NaN_3$ and the error due to the sensitivity of the instrument. The spectral slope ($S_{275–295}$) is also a parameter calculated on the range of wavelengths related to the absorption shoulders of $NaN_3$. Its variation for the $NaN_3$-treated samples relative to the control sample ranged from −5.4% to a maximum of 112% (Table 2). The large variability between the minima and the maxima Δ% suggests the absorbance parameters are more sensitive to the $NaN_3$ concentration than fluorescence parameters.

**Table 3** Summary of the optical parameters exhibiting the highest interferences from $NaN_3$ addition (highest absolute value of Δ%) for each DOM source

| | Component (\| Δ% \|) | Index (\| Δ% \|) |
|---|---|---|
| Pony Lake Fulvic Acid | C2 (5.5) | HIX (3.5) |
| Nordic Reservoir NOM | C2 (**34.5**) | HIX (4.6) |
| Suwanee River Humic Acid | C2 (**20.7**) | HIX/BIX (2.8) |
| Suwanee River Fulvic Acid | C2 (**49.4**) | HIX (5.4) |
| Soil Extract | C2 (**24.7**) | BIX (**10.0**) |
| Algae Extract | C2 (7.9) | HIX (8.3) |
| Natural Samples | C3 (4.5) | FI (5.0) |

The average of | Δ% | (see the text for its calculation) are reported in parenthesis

### Effects of $NaN_3$ on Fluorescence

As presented the above, the use of sodium azide highly affects the fluorescence of DOM, both quenching and enhancing the original fluorescence of DOM samples. The combination of both quenching and enhancement effects has already been reported in other studies [51, 52]. The effects are also variable depending on the samples. A variety of factors may be involved in the fluorescence quenching. The related processes, which can occur during the excited state lifetime, may include excited state reactions,

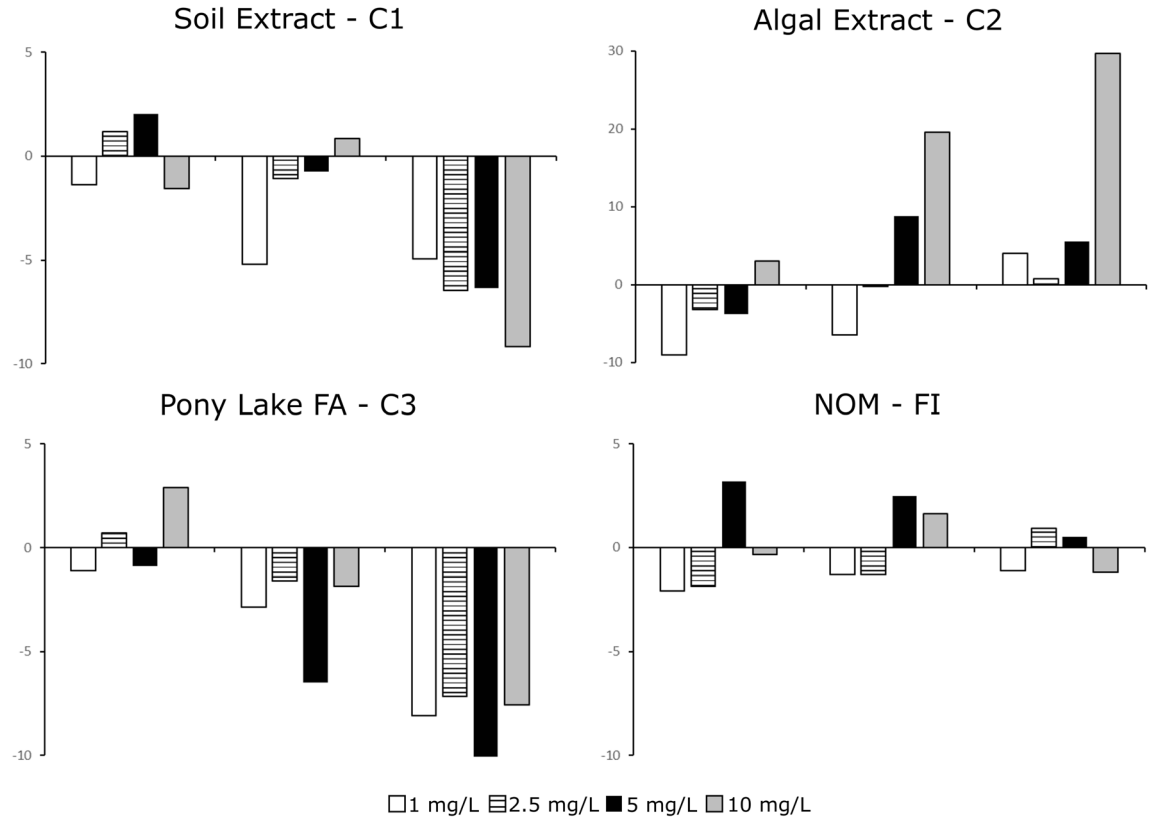


**Fig. 2** Histograms showing the effect of sodium azide on the selected fluorescence parameters at different DOM concentrations. The calculation of Δ% is described in the text

molecular rearrangement, energy transfer, ground-state complex-formation, and collisional quenching [53]. Several studies suggested that many organic compounds are quenched by the addition of inorganic salts (i.e., inorganic anion) including sodium azide [54–56]. One of the commonly acceptable mechanisms to explain the quenching process for this present case would be a transfer of an electron from the azide anion to the electronically excited molecules (e.g., preferentially aromatic compounds), followed by a reverse electron-transfer reaction leading to the original unexcited state of the system [54].

The quenching effects were more pronounced at lower wavelengths (e.g. C2). The humic-like component (C1) and BIX or FI indices were instead the parameters that were affected the least, presumably due to their locations at longer wavelengths. Although the effect is lower at higher wavelengths, it cannot be overlooked.

## Conclusions

In the attempt to clarify the contrasting results reported in the literature, this study presents for the first time an in-depth investigation of $NaN_3$ effects on absorption and fluorescence of DOM. The comparative results based on nine different DOM samples confirmed its interferences on DOM absorption and fluorescence. The high variability of the effect can be ascribed to the combination of several factors, including the concentration of $NaN_3$ itself, DOM source-dependent interferences, and the concentration of DOM samples. Still, most of these factors showed a non-linear behavior, making the effect of $NaN_3$ addition unpredictable. For these reasons, it is highly recommended not to use $NaN_3$ for the studies requiring absorption and fluorescence measurements. The best practical choice to secure the original optical properties of DOM samples should be to test the effect of minimal invasive preservation methods (e.g., refrigeration and freezing) on the specific samples under examination. A detailed review on the studies reporting interference of alternative preservation methods with some recommendations can be found in Spencer and Coble [26].

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## Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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