Investigations into the impact of storage conditions and filtration on the analysis of natural organic matter in water

Boitumelo K. Nokeri[1], Savia S. Marais[2] and Patricia B.C. Forbes[1,*]

[1]*Department of Chemistry, University of Pretoria, Pretoria, South Africa*
[2]*Process Technology Department, Rand Water, Vereeniging, South Africa*

ABSTRACT

When treating water for drinking water purposes, it is crucial to consider the composition of natural organic matter (NOM). NOM is a complex mixture of organic compounds influencing water quality and treatment processes. To ensure accurate reporting of analytical results, suitable sample storage and preparation are essential for maintaining sample integrity. This study investigated the effects of different storage conditions on water samples collected from Africa's largest bulk water provider, which sources its water from the Upper Vaal Catchment area. Samples were stored for varying durations under different temperature and light conditions to assess their impact on dissolved organic carbon (DOC) and ultraviolet absorbance at 254 nm (UV_{254}). The results showed that storing water samples for 34 days in the dark, at room temperature or at 5 °C did not significantly alter the DOC and UV_{254} measurements compared to the initial sample measurements. The pre-washing of filters from different brands with ultrapure water indicated that there were retained UV-active contaminants in the filter materials, of which 81% to 91% were removed after washing with 25 mL of ultrapure water. Furthermore, the portable, battery-operated UV254 Go! analyser is a cost-effective tool for direct field measurements of NOM aromaticity.

KEYWORDS

natural organic matter; filter pre-treatment; storage conditions; dissolved organic carbon; water monitoring

Received 29 November 2024, revised 28 March 2025, accepted 6 May 2025

INTRODUCTION

Natural organic matter (NOM) is a complex mixture of organic compounds, such as humic and non-humic substances with a range of chemical properties, which are present in various environments due to the decaying of animal and plant matter.[1-3] NOM affects potable water quality by causing the undesirable yellow-brown colour of water from the presence of compounds called Gelbstoffe, causes unpleasant taste and odour of water, and acts as a carrier for metals and hydrophobic organic chemicals.[4] It tends to interfere with the removal of other contaminants during water treatment, contributes to membrane fouling, and promotes corrosion, whilst also serving as a source for bacterial growth in the water distribution systems. Additionally, NOM in water may react with disinfectants to produce potentially carcinogenic disinfection by-products (DBPs).[5-7] Therefore, there is a growing need for NOM research into combining conventional and advanced analytical techniques to provide a robust tool for its efficient characterization, thereby guiding drinking water treatment.

NOM is typically quantified by measuring the concentration of dissolved organic carbon (DOC) or total organic carbon (TOC).[8] Whilst both are measured as carbon (C) in mg/L, TOC is defined as the bulk organic carbon material contained in sediments in a water sample,[9] whilst DOC is defined as the organic carbon present in a water sample after filtering through a 0.45 µm pore size filter material.[9,10] Therefore, TOC includes both the dissolved and suspended organic carbon. Both parameters are measured using a TOC analyser by measuring the amount of carbon that can be oxidized to carbon dioxide which is then quantified by non-dispersive infrared absorption techniques or conductivity.[11,12] While a number of studies have been conducted on the storage of water samples from different ecosystems for NOM analysis,[13-18] the complexity of NOM makes it imperative to further look into sample storage conditions and preparation prior to analysis to enhance the accurate reporting of results. Furthermore, in addition to the complexity of NOM, the differences in storage conditions, types of waters analysed and locations of the water sources, make it difficult to employ one-size-fits-all approaches for sample storage and duration.

In a study conducted in Sweden, Norrman investigated the use of membrane filters for DOC filtration and observed no change in DOC concentration for surface water samples stored in polypropylene tubes for eight weeks at temperatures > 4 °C.[18] Another study investigated the effect of freezing and thawing on water samples. It was observed that the freezing of water samples did not decrease DOC concentrations but may have affected the composition of NOM in the samples. As a result, due to the inconsistent changes in UV-Vis absorbance, the freezing and thawing of water samples was not recommended.[13]

Similarly, in a study conducted on surface water from forest plots located in Germany, a decrease in DOC concentrations was observed in 89% of the water samples after storage for four weeks and freezing at 18 °C with subsequent thawing.[14] However, fast freezing the water samples with liquid nitrogen at -196 °C and storage for 42 days did not result in significant differences in DOC concentrations. Additionally, regardless of the freezing method, the subsequent thawing of the water samples resulted in changes in the spectroscopic and fluorescence properties of NOM, as also observed by Peacock et al.[13]

The storage bottles used were not investigated or even mentioned in most of the reported studies, except in Norrman.[18] Yoshimura investigated the use of plastic and glass bottle types from different manufacturers for storing seawater and surface water from Japan for the analysis of dissolved organic phosphorus (DOP) and DOC.[19] The water samples were stored in the dark at room temperature (25 °C) for a duration of up to 13 months. While the DOP concentrations remained relatively stable in multiple bottle types, DOC concentrations remained stable only in borosilicate glass and perfluoroalkoxy bottles. Additionally, borosilicate glass bottles resulted in less water loss through evaporation during storage, indicating effective sealing of the caps. As a result, borosilicate glass bottles were recommended for the storage of water samples for DOC analysis due to the least contamination and effective sealing. Lastly, although storage conditions were not investigated in their study on non-disinfected groundwater samples from Northen Italy, Gabrielli

*To whom correspondence should be addressed
Email: patricia.forbes@up.ac.za

ISSN 1996-840X Online / South African Chemical Institute / http://saci.co.za/journal

https://doi.org/10.17159/0379-4350/2025/v79a06

and co-workers stored water samples in a refrigerated container in the dark during transportation.[8] They subsequently stored the samples at 4 °C for 5 days before analysis for those stored in glass bottles, or they froze the samples stored in polyethylene bottles and analysed them within one month. It is also important to note that the glass bottles could break if they are too full, due to the expansion of water upon freezing.

In South African NOM research, it is typical for researchers to analyse water samples between 24 to 72 hours after sampling.[10,20,21] However, this may prove to be a challenge when the laboratory is far from the sampling site, if extensive sample preparation is required prior to analysis, or if there is not enough manpower or instrumentation to process the samples. Research thus needs to be done on water sample storage and duration under local ambient conditions.

Moreover, although the filtering of water samples is common practice in water analysis sample preparation to remove suspended particles, the leakage of analytes of interest present in the filter material is rarely considered.[22] Xie et al. investigated the impact of soaking filter membranes for up to seven days in ultrapure water prior to use.[23] They found that the organic matter leached from the polyvinyl pyrrolidone-polysulfone membrane decreased with increasing soak time from 60 min to five days. However, the leaching of organic matter depended on the solvent used. For example, when a solution of 7.1 mg/L chlorine was used, the concentration of leached organic matter increased from 0.5 to 2.0 mg/L after soaking for seven days. As a result, it was concluded that pre-soaking the membrane for five days with ultrapure water was sufficient as no significant increase in the TOC was observed thereafter.

Measuring bulk water characteristics, such as ultraviolet absorbance at 254 nm (UV_{254}), is important for understanding the changes in water quality, including NOM character and composition, in order to optimise water treatment and to inform the development of new treatment processes.[24] UV_{254} is commonly used as an indicator of NOM and its aromatic character, and is also recognised as a potential surrogate for DOC.[5,25] However, the acquisition of NOM data typically involves collecting samples and transporting them to the laboratory, and storing them prior to analysis using laboratory-based instrumentation that rely on electricity and desktop displays. With the growing field of technology and unstable power supply in developing countries, the option for instruments that are cost-effective and can be used in the field, is beneficial. As a result, new instruments, which are designed to operate without electricity, offer a promising solution. However, these instruments need to be evaluated against established benchtop laboratory instruments to ensure their accuracy and reliability.

To address the plethora of NOM research needs in the water sector, this study sought to investigate the effect of various water sample storage conditions, durations and preparation methods on NOM analysis, while also evaluating the performance and sensitivity of a portable battery-operated UV254 Go! analyser in comparison to conventional laboratory-based instruments. The ultimate goal was to ensure that the integrity of water samples is maintained from collection through to NOM measurements.

EXPERIMENTAL

Water sampling

This study focused on Africa's largest bulk water provider, which sources water from the Upper Vaal Catchment area. The two main drinking water treatment plants of the water utility, namely Plant A and Plant B, are located in the Gauteng Province, South Africa (SA). Inlet water samples (referred to as raw) were collected in addition to water samples after the sand filtration process (referred to as treated) from each WTP. The water samples were collected in labelled 1 L Schott amber glass bottles with unlined polypropylene caps and were filled to the brim to minimize contamination, volatilization, or oxidation reactions that could result from trapped air in the presence of a headspace. Subsequently, the water samples were stored in a cooler box during transportation to the laboratory.

Chemicals and materials

Ultrapure water was obtained from a Milli-Q system, 18 MΩ.cm at 25 °C (ELGA LabWater, United Kingdom). The investigated syringe filters were of the same material, namely nylon, and were obtained from two different brands, brand A and brand B. The diameter of the syringe filters was 25 mm with pore sizes of 0.45 µm. White gridded membrane filters – brand C - were of the same pore size with 47 mm diameter. An in-house built UV light box was used with a UV lamp (UVP UVGL-58, Analytik Jena, USA) to expose stored water samples to UV radiation during the sample storage condition experiments. Bulk water samples were taken in 1 L Schott borosilicate amber bottles with unlined screw caps (Borosil®, USA, supplied by Stargate Scientific, SA). Forty (40) mL amber and clear glass vials with silicone/polytetrafluoroethylene (PTFE) septa (ANPEL Laboratory Technologies, China) were used for sample storage investigations.

Pre-washing of filters

Fifty (50) mL of ultrapure water was filtered through each syringe and membrane filter respectively in 5 mL aliquots, which were then individually analysed for UV_{254} absorbance using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, USA) and a UV254 Go! analyser (Photonic Measurements, UK) (refer to the section on ultraviolet-visible analysis for additional information). Each experiment was replicated three times with a clean syringe or membrane filter and the average of the results was determined.

Sample storage

Two sets of raw and treated water samples were collected from similar sampling locations at both plants, after which the samples were filtered. The first set of samples, stored for 240 days, were collected on 26 April 2023. The second set of samples that were stored were collected on 14 December 2023, which were each divided into two aliquots for storage: (1) 34 days and (2) 98 days, respectively. Forty (40) mL of the filtered water samples were transferred into 40 mL vials with caps lined with silicone/PTFE septa and were subjected to various storage conditions. Figure 1 shows the schematic representation of the process followed for the sample storage investigation, from sample collection up to post-storage analyses.

The investigated storage conditions included storage in the dark, exposure to UV radiation in a UV light box at 365 nm, room temperature at 25 °C (RT) on the laboratory bench, exposure to direct sunlight at the Natural Sciences 1 building rooftop at the University of Pretoria, and refrigeration at 5 °C. Samples stored in the refrigerator and in the dark were stored in amber glass vials, while those stored under UV radiation, direct sunlight and at RT were stored in clear glass vials. The samples were analysed for UV_{254} absorbance and DOC concentration before and after storage.

Ultraviolet-visible analysis

Due to the diversity of NOM chromophores, UV at 254 nm (UV_{254}) is used as an indicator for NOM and its aromatic character. Additionally, UV_{254} is recognized as a potential surrogate for DOC. As such, a Cary 60 UV-Vis/ spectrophotometer (Agilent Technologies, USA) with a 10 mm path length quartz cuvette was used for all UV-Vis analyses. Ultrapure water was used to zero the instrument and absorbances at 254 nm were measured.

The portable, handheld UV254 Go! analyser (Photonic Measurements, UK), shown in Figure 2, utilises a 10 mm path length 2 mL quartz cuvette and stores the measured reference (ultrapure water in this study) for subsequent readings. It should be noted that the

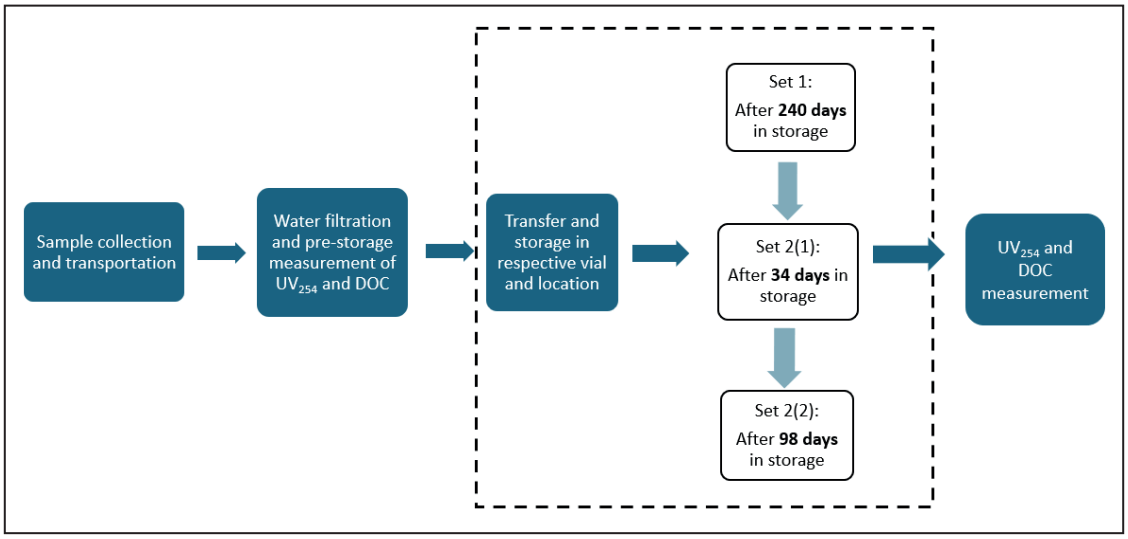

Figure 1: Schematic representation of the water sample collection, filtration and storage process (for 34, 98 or 240 days), with the inclusion of UV_{254} and DOC measurements before and after storage. Set 1 refers to the water samples collected in April and set 2 to the water samples collected in December where (1) and (2) represents the different storage times for this sample set.

Figure 2: The portable, handheld UV254 Go! analyser.

unit for UV_{254}, namely m^{-1}, indicates the absorption per m pathlength through water, which is the standard measurement in this context.

Dissolved organic carbon analysis

The DOC measurements for the original filtered water samples were obtained using a Shimadzu TOC-L analyser (Shimadzu, Japan). The instrument was calibrated using potassium hydrogen phthalate (KHP) at concentrations of 1, 5, 10, 20 and 30 mg/L. DOC analysis of the samples after the storage experiments were conducted at an accredited SANAS testing laboratory (Waterlab (Pty) Ltd, accreditation no: T0391, Pretoria, South Africa). A Sievers M9 TOC analyser (Sievers, USA supplied by Chemetrix, SA) was used to determine the concentration of DOC in the filtered water samples. The filtering of water samples allows for the measurement of DOC by the TOC analyser. A single-point calibration using 50 ppm KHP was conducted for the Sievers M9 TOC analyser prior to sample analysis. A volume of 40 mL per water sample was analysed in both cases, the measurements were replicated three times and the average of the results were taken.

Statistical analysis

Excel was used for general statistical analysis, such as the calculation of means and standard deviations (SD), as well as performing F-tests, t-tests and ANOVA. These analyses were performed on UV_{254} measurements related to the pre-washing of filter membrane types prior to sample filtration and UV_{254} measurements for sample storage investigations. Additionally, statistical comparisons were made between the conventional UV-Vis spectrophotometer and UV254 Go! analyser.

RESULTS AND DISCUSSION

Pre-washing of filters

The results showed a decrease in UV_{254} absorbance with an increase in the pre-wash volume of ultrapure water (Figure 3). Specifically, passing 20 mL of ultrapure water through each filter type was enough to reduce UV-active contaminants by approximately 80%, 91% and 89% for brands A, B and C, respectively. After pre-washing with 30 mL of ultrapure water, the UV_{254} measurement increased particularly for syringe filter brand A, but then decreased again after 45 mL of water washing. This is likely due to the elution of additional UV active components which were more strongly retained by the filter material. It is evident that the error bars for the conventional instrument were larger than for the UV254 Go! instrument, especially for the elevated absorbance readings, which may indicate instrument instability or inadequate blank correction. In their study, Ou et al. observed DOC leaching from syringe filter membranes made from nine different materials: cellulose acetate, composite cellulose esters, mixed cellulose esters, polyether sulfone, regenerated cellulose esters, modified polypropylene, nylon, polytetrafluoroethylene and polyvinylidene fluoride.[22] Similarly, for all materials, DOC leaching decreased with an increase in the pre-wash volume of a 6% sodium hypochlorite buffer solution. However, although the nylon syringe filters had a higher DOC leakage, the leaching decreased by 94.4% after pre-washing with 50 mL of the buffer solution, highlighting the necessity to pre-wash filters to remove contaminants prior to filtering water samples.

Based on the average absorbance of the first washing, brand A syringe filters had less UV-active contaminants than brand B.

However, after one wash (5 mL), more UV-active contaminants were washed off from brand B than from brand A, indicating that brand B's UV-active contaminants were less strongly retained. This is also seen from the larger error bars and the increase in absorbance for brand A (Figure 3a), indicating the removal of strongly retained contaminants only after 35 mL of washing. Furthermore, the standard errors of the absorbance measurements were much smaller for brand B, indicating that the three syringe filters tested were more similar in composition in this case and were of a more consistent quality.

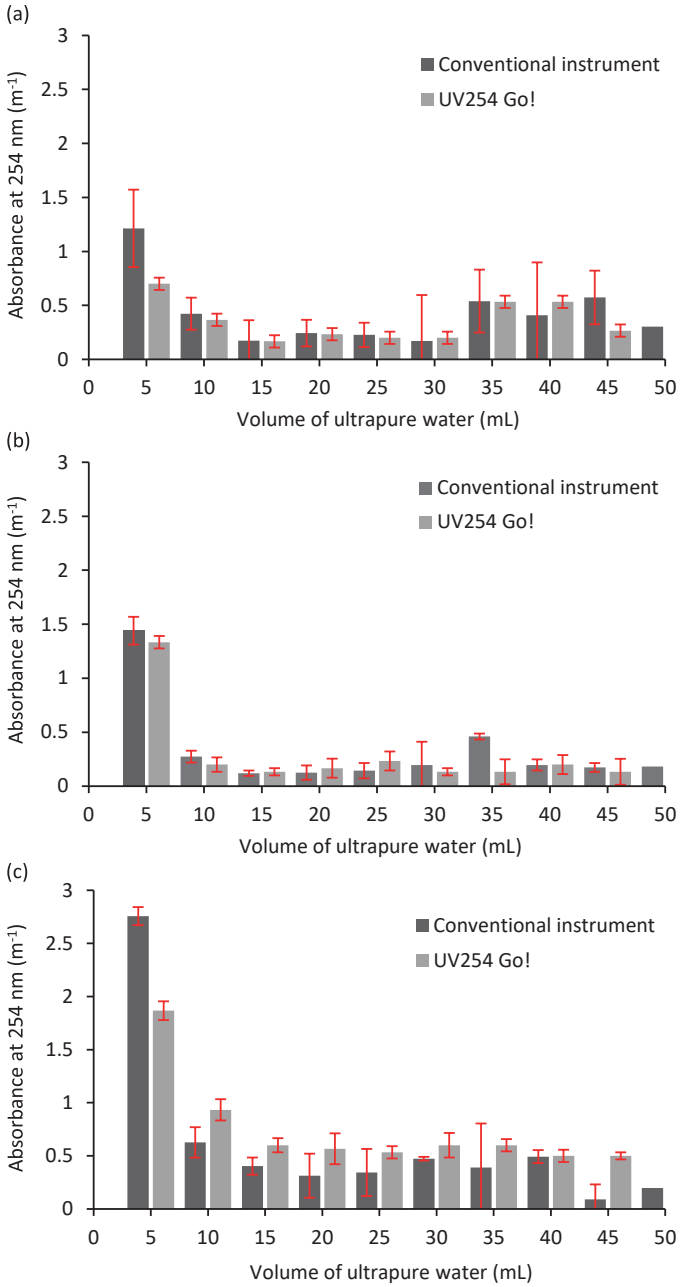

Figure 3: The impact of pre-wash volumes on UV_{254} absorbance of ultrapure water for nylon material syringe filters (**a**) brand A, (**b**) brand B and a mixed cellulose ester membrane filter (**c**) brand C. The error bars indicate the standard error of the average measurements (N=3) of each filter washing.

To compare how well UV-active contaminants could be removed from each filter brand, average initial UV_{254} measurements, percentage relative standard deviation (% RSD) and the percentage of the reductions in UV_{254} active contaminants (% reduction) after 25 mL of washing, were calculated using results from the conventional UV-Vis spectrophotometer and the portable UV254 Go! analyser (Table 1).

Using results obtained from the conventional UV-Vis spectrophotometer, brand A recorded less % reduction after 25 mL washing compared to brands B and C. This is also an indication that the brand A filters contained more strongly retained UV-active contaminants. As a result, brand A was excluded as a filter option to be considered. Both brands B and C had higher UV_{254} percentage reductions after 25 mL washing: brand B syringe filters obtained an average of 90% reduction and brand C membrane filters obtained an 88% reduction. Similar percentage reductions were obtained using the UV254 Go!.

Brand B syringe filters had a higher reduction percentage overall and thus UV-active contaminants were more easily removed by pre-washing with ultrapure water than for membrane filters. However, syringe filters are more expensive (~R550/100 pk), tedious to use and generated more waste. Conversely, the membrane filters were cheaper (~R570/150 pk) and were easier and more efficient to use because large volumes of sample could be filtered in one go without the filter clogging, unlike the syringe filters that could only handle a few mL (less than 10 mL) of water depending on the presence of suspended particles in the sample being filtered. As a result, membrane filters were chosen for filtering of water samples after pre-washing with 25 mL ultrapure water.

Furthermore, statistical analysis of the UV_{254} measurements from the pre-washing of each filter was performed using results from the conventional UV-Vis spectrophotometer as this is more widely employed than the UV254 Go! and is the accepted standard technique. F-tests were performed to compare the variance in the UV_{254} measurements obtained for brand A and B; and brand B and brand C, respectively. Table 2 shows the results of each analysis performed, where the F-statistic (F-stat) is the ratio of the variation between groups and within groups, the p-value measures the probability of obtaining values below F-stat, and F-critical (F-crit) is the threshold value used to decide whether to reject or accept the null hypothesis,[26,27] which in this case is that absorbances between brands A and B; and B and C, respectively, are statistically similar after washing with 25 mL of ultrapure water.

From the F-tests (Table 2), the calculated F-stat and p-values were higher than the significant level value of 0.05. This indicated that there were no statistically significant differences in the UV_{254} measurements of the 25 mL pre-wash volume between brands A and B, and B and C. This was also evident as the F-crit values were higher than the F-stat values, indicating that the null hypothesis was rejected and the 25 mL wash volume absorbances between brand A and B; and B and C, were statistically similar.

Additionally, ANOVA analysis was performed to compare the means between and within all three brands (Table 3), where the sum of squares (SS) measures the variance in the UV_{254} measurements, and the mean square (MS) is a calculation of the average between and within the different brands.

Table 1: The average initial UV_{254} measurements, standard deviations (SD), % RSDs and % reductions in UV_{254} absorbances resulting from 25 mL ultrapure water washing of the syringe and membrane filters as measured using both the conventional UV-Vis spectrophotometer and the UV254 Go!.

Filter brand	Average of initial UV_{254} absorbance measurements	SD	% RSD	% reduction
A	1.21 (0.70)	0.21 (0.10)	94 (50)	81 (71)
B	1.44 (1.33)	0.12 (0.15)	80 (65)	90 (83)
C	2.76 (1.87)	0.36 (0.25)	105 (47)	88 (77)

The values outside the brackets are for the conventional UV-Vis spectrophotometer and those inside the brackets are for the UV254 Go!

Likewise, ANOVA analysis yielded F-stat and p-values greater than 0.05 and F-crit larger than F-stat, indicating that there were no statistically significant differences in the UV_{254} measurements of the 25 mL wash volume across all three brands. Therefore, statistically, the different brand syringe filters and the membrane filters performed similarly after 25 mL washing in terms of the release of UV-active components from the filter material. This implied that regardless of the chosen filter, the obtained results were statistically similar. As a result, the deciding factors in the choice of filter to employ were the amount of UV-active contaminants present in each filter as determined by UV_{254} absorbance in the first washing, the cost of the filters, their ease of use and the amount of plastic/consumable waste generated. It was thus determined that pre-washed membrane filters (brand C) were to be used in further experiments to characterize NOM in the water samples. The same conclusions could be made with the results obtained with the portable analyser.

Table 2: F-test analysis of the conventional UV-Vis spectrophotometer UV_{254} results obtained from the pre-washing of (a) brand A and brand B, and (b) brand B and brand C filters.

(a) Comparison between brand A and brand B

	Brand A	Brand B
Mean	0.23	0.15
Variance	0.05	0.01
F-stat	4.68	
p-value	0.18	
F-crit	19	

(b) Comparison between brand B and brand C

	Brand B	Brand C
Mean	0.15	0.34
Variance	0.01	0.13
F-stat	0.08	
p-value	0.07	
F-crit	0.05	

Effects of sample storage conditions on NOM content

The DOC concentrations and UV_{254} measurements for each sample and storage condition were analysed and the results obtained with the TOC analyser are presented in Table 4. Note that a different sample was used for the 240 day storage experiment (data italicized), which had a higher initial DOC concentration.

The results suggested that changes in DOC concentrations differed per storage condition. While each storage condition generally showed some decrease in DOC concentrations over time, the extent of this decline varied among the storage conditions and samples. Note that the treated sample from Plant A stored for 98 days in direct sunlight, assigned an asterisk (*), was lost due adverse weather conditions, thus no data was collected for this sample. Additionally, the Plant A treated sample stored at 5 °C appeared to be an outlier and was therefore statistically tested using the Z-score method.

Table 3: Single factor ANOVA analysis of the conventional UV-Vis spectrophotometer UV_{254} results obtained from the pre-washing of all filter types and brands

Groups	Count	Sum	Average	Variance	
Brand A	3	0.68	0.23	0.05	
Brand B	3	0.44	0.15	0.01	
Brand C	3	1.03	0.34	0.13	
Source of Variation	SS	MS	F-stat	p-value	F-crit
Between Groups	0.06	0.03	0.47	0.64	5.14
Within Groups	0.37	0.06			
Total	0.43				

Table 4: DOC concentrations (mg/L) of water samples analysed on the day of sampling and stored under different storage conditions with analysis after 34, 98 and 240 days.

	Initial	Dark	UV radiation	RT	Direct sunlight	5 °C
			Plant A raw			
34 days	**5.7**	5.7	5.3	5.7	4.2	5.8
98 days	**5.7**	5.0	4.5	4.9	2.3	5.3
240 days	***6.4***	*6.5*	*5.3*	*6.5*	*4.4*	*7.0*
			Plant A treated			
34 days	**4.8**	5.2	4.7	4.9	3.5	5.1
98 days	**4.8**	4.2	3.8	4.2	*	17
240 days	***6.0***	*7.8*	*5.0*	*5.7*	*4.0*	*6.1*
			Plant B raw			
34 days	**5.6**	5.8	5.4	5.9	5.0	6.0
98 days	**5.6**	4.8	4.0	5.0	5.4	5.3
240 days	***6.9***	*6.5*	*5.2*	*6.2*	*5.5*	*7.1*
			Plant B treated			
34 days	**5.3**	4.8	4.7	4.9	4.2	5.9
98 days	**5.3**	4.0	3.6	4.2	4.4	4.4
240 days	***5.1***	*5.6*	*4.9*	*5.6*	*4.2*	5.8

*Sample lost during storage due to bad weather. Italicized values represent different samples used.

Z-score is the calculation of $Z = \frac{X - \mu}{\sigma}$, where X is the suspected outlier, μ is the mean of the data and σ is the standard deviation. A Z-score > $|3|$ indicates that the point is statistically an outlier.[27,28] A Z-score of 30.92 was calculated, implying that the Plant A treated sample stored at 5 °C (17 mg/L) was statistically an outlier, suggesting potential sample contamination. Consequently, this data point was excluded to ensure a more accurate assessment of the relationship between the initial DOC concentrations and changes across different storage conditions over time.

For DOC analysis, concentrations deviating by more than 0.5 mg/L from the initial measurement were considered large differences, while those differing by less than 0.5 mg/L were deemed small differences. Based on the comparison of DOC concentrations across various storage conditions, it was evident that samples stored at RT and in the dark exhibited concentrations closest to the initial measurements for up to 34 days in storage. Conversely, samples exposed to direct sunlight showed a decrease, likely due to photolytic reactions, resulting in lower averages compared to the initial DOC concentrations. These findings suggest that sample temperature during transportation may not significantly affect sample integrity with respect to overall DOC content. However, to avoid potential degradation, the use of amber bottles is recommended to shield samples from direct sunlight or radiation exposure.

Similarly, for UV_{254} analysis, measurements deviating by more than 1.5 m^{-1} from the initial measurement were considered large differences, while those differing by less than 1.5 m^{-1} were deemed small differences. Based on the results obtained with the conventional UV-Vis spectrophotometer, as shown in Table 5, samples stored in the dark and at RT recorded UV_{254} absorbances closest to the initial measurements for storage for up to 34 days. Similarly, Peacock et al. noted that DOC concentrations significantly decreased in peatland surface and pore waters from the United Kingdom (UK) when stored in the dark at 4 °C for 138 to 1082 days, suggesting that biological processes occur slowly in the dark.[13] Similar results were also observed in a study conducted on UK, Canadian and USA surface waters where Carter et al. reported a 5% decrease in DOC concentrations after storage in the dark at 5 °C for 50 to 120 days.[29] However, as seen with the DOC concentrations obtained in this study, samples stored at 5 °C generally exhibited slightly higher values compared to the initial values. This may be attributed to the unfortunate recurrent power disruptions that were experienced at the department during the experiment, causing unintended fluctuations in refrigerator temperature and uncertainty in these results. However, in a study comparing the storage of runoff water samples after refrigeration at 4 °C and freezing at -18 °C, it was found that storage in the refrigerator for 21 and 44 days resulted in a decline of both DOC and TOC concentrations.[15] Additionally, Nachimuthu et al. found that regardless of the storage duration, DOC and TOC measurements were higher for the refrigerated samples than the frozen samples.[15] Storage in the refrigerator recorded decreases of up to 55% and 35% in TOC and DOC measurements, respectively, indicating sample degradation during storage at 4 °C. As a result, the study recommended filtering samples for DOC analysis and using unfiltered samples for TOC analysis prior to storing them in the freezer at -18 °C immediately after collection.

The changes in DOC and UV_{254} measurements between the sample storage durations differed in magnitude between samples and storage conditions. As a result, the percentage decrease for both DOC and UV_{254} was calculated for each storage condition under the different storage durations (Tables 6 and 7).

From these results, it is evident that samples should be analysed within a month to minimise variations from the initial composition. While Yoshimura found that the type of storage bottle used, plastic or glass, played a significant role in DOC stability of seawater samples,[19] this was not a concern in this study as glass bottle type were used for all samples. A subsequent study found that approximately 20% loss of DOC occurred after storing surface and pore water samples from peatlands for three years in the dark at 4 °C, indicating that cold temperatures reduced the rate of sample degradation.[13] Although water samples in this study were stored for a shorter duration (34 to 240 days), a 2 – 17% decrease in DOC concentrations (8% on average) was observed when stored at 5 °C in amber glass bottles, compared to a 0 – 30% decrease (11% on average) for storage in amber glass bottles kept in the dark. This confirms that cold temperatures do reduce the rate of sample degradation with respect to DOC. Similarly, Carter et al., reported only a 5% decrease in DOC concentrations for UK, Canada and USA surface water samples stored at 5 °C for 50 to 120 days.[29] Kothawala, et al. reported up to a 45% decrease in DOC concentrations for Swedish boreal lake water samples that were incubated in 20 °C water baths in the dark for 3.5 years.[30] The variation in these reported results indicates that different water samples

Table 5: UV_{254} measurements (m^{-1}) of water samples analysed on the day of sampling and stored under different storage conditions with analysis after 34, 98 and 240 days.

	Initial	Dark	UV radiation	RT	Direct sunlight	5 °C
			Plant A raw			
34 days	**24.8**	23.5	20.9	23.7	12.9	29.1
98 days	**24.8**	23.2	18.9	22.9	8.50	27.6
240 days	***32.7***	*28.2*	*23.7*	*30.2*	*12.2*	*32.8*
			Plant A treated			
34 days	**13.9**	14.3	12.6	14.1	5.50	14.3
98 days	**13.9**	13.6	11.3	13.7	*	14.4
240 days	***17.6***	*18.8*	*12.9*	*17.2*	*7.20*	*18.5*
			Plant B raw			
34 days	**24.6**	24.0	21.4	24.0	13.6	34.3
98 days	**24.6**	20.7	17.3	2.7	12.8	24.5
240 days	***34.0***	*31.0*	*22.6*	*26.0*	*20.4*	*33.2*
			Plant B treated			
34 days	**14.4**	13.6	12.1	13.7	6.40	14.0
98 days	**14.4**	12.9	10.8	13.3	7.49	13.9
240 days	***16.7***	*16.9*	*12.6*	*16.9*	*7.40*	*20.1*

*Sample lost during storage due to bad weather. Italicized values represent different samples used.

behave differently during storage, thus highlighting the importance of conducting storage stability experiments for the water samples of interest in a specific study.

On average, a high percentage decrease in DOC concentrations was observed after storage under direct sunlight and a low decrease was observed after storage at 5 °C and at RT. Similarly, the average for each storage duration across the various samples was calculated and found to be lower after storage for 34 days, which increased significantly after storage for 98 days and decreased again after storage for 240 days (Table 8).

Conversely, UV_{254} was observed to generally be more stable in the first 34 to 98 days after storage in the dark, at RT and at 5 °C, with Plant B raw 5 °C being an exception. This was consistent with what was reported by Peacock et al.[13] Samples stored under exposure to UV radiation and direct sunlight recorded higher % declines, suggesting a change in NOM character due to possible photodegradation from the light sources. However, it should be noted that while the samples recorded low % decreases for UV_{254}, their DOC chemical compositions may have differed significantly.[13,31] Comparably, high percentage decreases in absorbance were observed after storage under direct sunlight and UV radiation and a low decline was observed after storage at RT, in the dark and at 5 °C. Similarly, the average for each storage duration across the various samples was recorded be lower after storage for 34 days for storage at RT and in the dark (Table 9).

Since the water samples stored for 240 days were not collected on the same day as those stored for 34 and 98 days, DOC concentration

Table 6: DOC concentration percentage decrease per sample under various storage conditions. Positive values represent an increase in the DOC concentration while negative values represent a decrease in the concentration.

	Dark	UV radiation	RT	Direct sunlight	5 °C
			Plant A raw		
34 days	0	-7	0	-26	2
98 days	-12	-23	-14	-60	-7
240 days	2	-17	2	-31	9
			Plant A treated		
34 days	8	-2	2	-27	6
98 days	-13	-21	-13	*	-
240 days	30	-17	-5	-33	2
			Plant B raw		
34 days	4	-4	5	-11	7
98 days	-14	-29	-11	-4	-5
240 days	-6	-25	-10	-20	3
			Plant B treated		
34 days	-9	-11	-8	-21	11
98 days	-25	-32	-21	-17	-17
240 days	-0	-4	10	-18	14

*Sample lost during storage due to bad weather. Plant A treated sample stored for 98 days at 5 °C (–) was determined to be an outlier for DOC concentrations and was thus excluded.

Table 7: UV_{254} percentage decrease per sample under various storage conditions. Positive values represent an increase in the UV_{254} absorbance while negative values represent a decrease in the absorbance.

	Dark	UV radiation	RT	Direct sunlight	5 °C
			Plant A raw		
34 days	-5	-16	-4	-48	17
98 days	-6	-24	-8	-66	11
240 days	-14	-28	-8	-63	0
			Plant A treated		
34 days	3	-9	1	-60	3
98 days	-2	-19	-1	*	4
240 days	7	-27	-2	-59	5
			Plant B raw		
34 days	-2	-13	-2	-45	-9
98 days	-16	-30	-89	-48	0
240 days	-9	-34	-24	-40	-2
			Plant B treated		
34 days	-6	-16	-5	-56	-3
98 days	-10	-25	-8	-56	-3
240 days	1	-25	1	-56	20

*Sample lost during storage due to bad weather.

Table 8: Average DOC concentration decreases for water samples after storage in the dark, under UV radiation, at RT, under direct sunlight and at 5 °C for 34, 98 and 240 days. Positive values represent an increase in the DOC concentration while negative values represent a decrease in the concentration.

Storage condition	Dark	UV radiation	RT	Direct sunlight	5 °C
34 days	1%	-6%	0%	-21%	7%
98 days	-16%	-26%	-15%	-27%	-10%
240 days	9%	-16%	-1%	-26%	7%
Average DOC decrease	-2%	-16%	-5%	-25%	1%

Table 9: Average UV_{254} absorbance decreases for water samples after storage in the dark, under UV radiation, at RT, under direct sunlight and at 5 °C for 34, 98 and 240 days. Positive values represent an increase in the UV_{254} absorbance while negative values represent a decrease in the absorbance.

Storage condition	Dark	UV radiation	RT	Direct sunlight	5 °C
34 days	-3%	-14%	-3%	-52%	14%
98 days	-5%	-25%	-26%	-57%	5%
240 days	-4%	-29%	-8%	-55%	6%
Average DOC decrease	-4%	-23%	-12%	-55%	8%

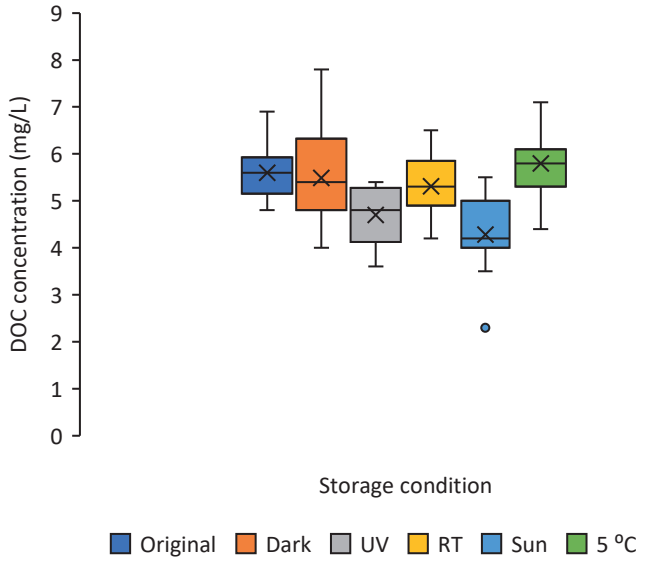

Figure 4: DOC concentration decreases for water samples after storage in the dark, under UV radiation, at RT, under direct sunlight and at 5 °C. All storage durations were plotted for each storage condition.

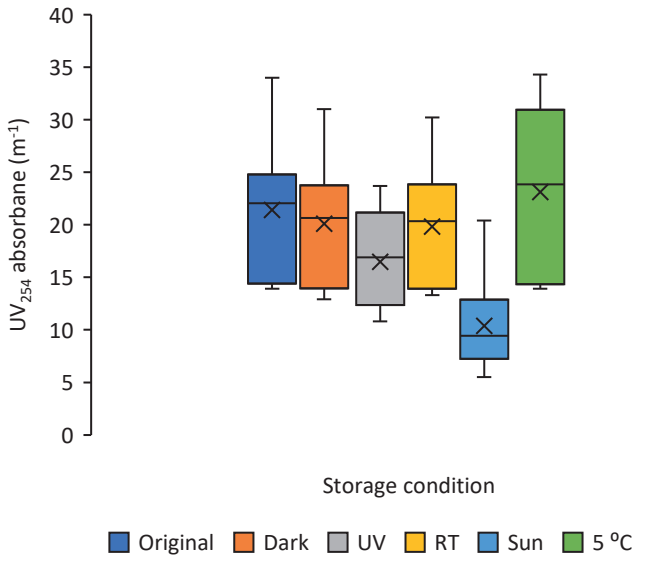

Figure 5: Decrease in UV_{254} absorbance for water samples after storage in the dark, under UV radiation, at RT, under direct sunlight and at 5 °C. All storage durations were plotted for each storage condition.

decreases were plotted to evaluate how the various storage conditions influenced the DOC concentration in the samples. Figure 4 shows a box and whisker plot of the decreases in DOC concentrations for water samples after storage under the different conditions. The top and bottom lines indicate the maximum and minimum decreases in concentrations, excluding the outliers which are points outside these limits.[32] Visually, it was observed that the closest DOC concentration to the original was obtained after storage at RT and at 5 °C, and possibly in the dark although storage under this condition showed a higher variation in values.

Similarly, the box and whisker plot for the UV_{254} measurements showed that the closest UV_{254} values to the initial measurements were after storage in the dark, at RT and at 5 °C (Figure 5). However, samples stored at 5 °C recorded higher variation in measurements, likely as a consequence of the unstable temperature of the refrigerator arising from power outages. Differences in the average DOC concentrations and UV_{254} levels showed that the composition of NOM in the samples varied under the different storage conditions and durations.

On the account that water samples stored for 34 days had lower UV_{254} absorbance and DOC concentration decreases, t-test statistical analysis of the storage data were performed to compare the initial DOC concentrations and UV_{254} absorbance measurements to the post storage values for each storage condition. Table 10 shows the results for each analysis performed, where t-statistic (t-stat) is the ratio of the means between the initial measurement and after storage for each condition, and the p-value is the probability of obtaining values below t-stat and t-critical (t-crit).

It was determined that storing samples in the dark, at RT and at 5 °C did not have a statistically significant effect on the DOC and UV_{254} measurements. The mean and standard deviation (SD) of these parameters remained closer to the initial measurements. Furthermore, this conclusion was supported by the p-value, where $p < 0.05$ in all cases, indicated that the data was not significantly different. However, storing samples under direct sunlight or UV radiation had a statistically significant effect on both parameters. As a result, for transportation or storage of water samples prior to analysis, it is recommended that samples be stored at 5 °C in amber storage containers for a maximum of 34 days to maintain sample integrity and minimise degradation. Importantly, brief periods at RT (such as during sample preparation) should not impact on the results significantly.

Portable UV254 Go! analyser

For the UV_{254} parameter of the portable analyser, comparative measurements were taken during the impact of filter pre-washing experiments, as well as from samples collected during at different times.

Table 10: Statistical data of the DOC and UV_{254} measurements before and after sample storage for 34 days under different storage conditions. N = 12 for each storage condition (excluding direct sunlight and 5 °C where N =11). SD = standard deviation.

	Initial	Dark	UV radiation	Room temperature	Direct sunlight	5 °C
			DOC			
Mean (SD)	5.35 (0.40)	5.38 (0.46)	5.03 (0.38)	5.35 (0.53)	4.23 (0.61)	5.70 (0.41)
t-stat		-0.13	2.93	$-3.00x10^{-15}$	5.83	-3.36
p-value		0.45	0.03	0.31	$5.04x10^{-3}$	0.02
			UV_{254}			
Mean (SD)	19.43	18.85 (5.67)	16.75 (5.09)	18.88 (5.75)	12.35 (8.51)	22.93 (10.35)
t-stat		1.61	4.75	2.02	3.27	-1.52
p-value		0.10	$8.86x10^{-3}$	0.07	0.02	0.11

Table 11: Paired t-test analysis for the comparison of the UV254 Go! analyser and conventional UV-Vis spectrophotometer UV_{254} measurements obtained from both the pre-washing of syringe and membrane filters and water samples collected from two treatment plants across different seasons.

	UV254 Go!	UV-Vis spectrophotometer
Average	8.22	8.27
Variance	134.13	147.20
% RSD	141	147
Observations	46	46
t-stat	-0.17	
p-value	0.43	
t-crit	1.68	

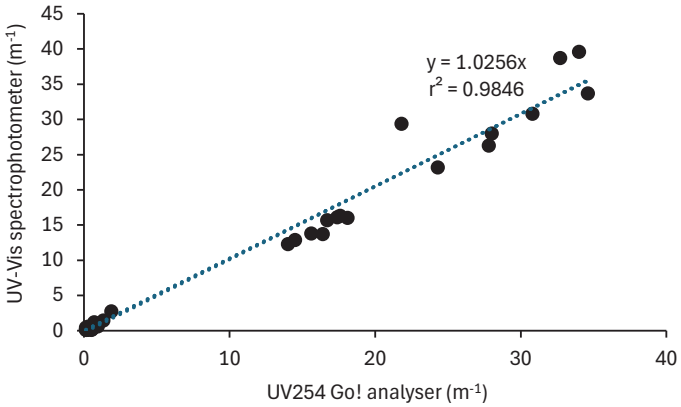

Figure 6: Scatter plot showing the relationship between the UV_{254} measurements obtained with the UV254 Go! analyser and the conventional UV-Vis spectrophotometer.

A scatter plot of the results obtained with the conventional UV-Vis spectrophotometer versus the UV254 Go! analyser was drawn to assess the relationship between the UV_{254} measurements of the same samples obtained from the two instruments. Figure 6 shows the scatter plot with the data points displaying a straight-line trend with a correlation coefficient (r^2) value of > 0.98 and a gradient very close to one. This shows that there was a strong correlation between the measurements and the two instruments produced similar results for the same set of samples.

A paired t-test of the UV_{254} measurements was performed, and the results are shown in Table 11. The measurements from the two instruments had similar averages, differing by 0.05. This small difference suggested that the UV254 Go! typically recorded the same UV_{254} values compared to the conventional UV-Vis spectrophotometer. The calculated %RSDs were high for both the UV254 Go! and conventional spectrophotometer, due to the wide range of values. A low t-stat value (-0.17, Table 11) suggested that there was a small difference between the UV_{254} results from the two instruments. This was also seen with the p-values (0.43 and 0.86) and the t-crit values greater than the significance level of 0.05,[27] indicating that the differences between the UV_{254} measurements from the UV254 Go! and the UV-Vis spectrophotometer were not statistically significant.

Although the error bars for the conventional instrument were larger than for the portable instrument during the investigation of the pre-washing of filters (Figure 3), there was no statistically significant difference between the UV_{254} measurements obtained with the UV254 Go! and the UV-Vis spectrophotometer. As a result, it was concluded that the UV254 Go! is a convenient and cost-effective means to determine NOM aromaticity in the field and at the raw water source, enabling a fast response by water treatment plant operators to changes in composition.

CONCLUSION

The effects of various sample storage conditions on NOM content were investigated to optimize water sample transportation, storage method and storage duration to enhance the accuracy of analytical results. The DOC concentrations and UV_{254} absorbance measurements differed per storage condition. Particularly, samples stored at RT and in the dark maintained measurements closest to the initial measurements for up to 34 days in storage. This suggested that moderate sample temperature variations that do not exceed typical RT during transportation and sample preparation may not impact sample integrity with respect to overall DOC content and UV_{254} absorbance. It was concluded that samples should be analysed within a month to minimise variations from the initial composition, which was also supported by statistical tests. Moreover, it was recommended that samples should be stored in amber bottles to shield them from direct sunlight or UV radiation exposure.

The pre-washing of 0.45 µm syringe and membrane filters was investigated for the presence of UV-active residual contaminants that may leach into the water samples and influence reported results. All brands showed a decrease in UV_{254} absorbance as the pre-wash volume of ultrapure water increased. Notably, passing 20 mL of ultrapure water through each filter was enough to reduce the UV-active contaminants by up to 91%. Contaminants from brand B were more easily removed with ultrapure water than brand C. However, upon further statistical analysis, it was found that the three filters performed statistically similar with regards to the release of UV-active components after washing with ultrapure water and were of a consistent quality. As a result, membrane filters were the preferred filter option due to their ease of use as well as environmental and cost implications.

The quantification of NOM using the portable UV254 Go! analyser for field based analysis showed a strong correlation between the UV_{254} measurements obtained to that from a conventional spectrophotometer, indicating that this is a convenient and cost-effective tool for the quick determination of NOM aromaticity in the field.

The need to transport water samples from the abstraction point to the laboratory for analysis, along with the requirement for extensive sample preparation and the number of analyses to be performed, are

significant challenges in the characterization of NOM. As a result, these findings provide important insights into sample handling and preparation and offer practical solutions for NOM monitoring in water.

ACKNOWLEDGEMENTS

Funding from Rand Water through the Professorial Research Chair of Patricia Forbes and the National Research Foundation is gratefully acknowledged. Resources provided by the Department of Chemistry in the Faculty of Natural and Agricultural Sciences at the University of Pretoria are also acknowledged.

AUTHOR CONTRIBUTIONS

Boitumelo K. Nokeri: Conceptualization, data curation, formal analysis, investigation, methodology, visualization and writing – original draft preparation. Savia S. Marais: Methodology, resources, supervision and writing – review & editing. Patricia B.C Forbes: Conceptualization, formal analysis, funding acquisition, methodology, project administration, resources and writing – review & editing.

DECLARATION OF INTERESTS

There are no competing or financial interests to declare.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES

Not applicable.

ORCID IDS

Boitumelo K. Nokeri: https://orcid.org/0000-0002-7353-3823
Savia S. Marais: https://orcid.org/0000-0003-1683-0917
Patricia B.C. Forbes: https://orcid.org/0000-0003-3453-9162

REFERENCES

1. Marais SS, Ncube EJ, Msagati TAM, Mamba BB, Nkambule TI. Investigation of natural organic matter (NOM) character and its removal in a chlorinated and chloraminated system at Rand Water, South Africa. Water Sci. Technol: Water Supply. 2017;17(5):1287–1297. https://doi.org/10.2166/ws.2017.028.
2. Chen W, Yu HQ. Advances in the characterization and monitoring of natural organic matter using spectroscopic approaches. Water Res. 2021;190,116759. https://doi.org/10.1016/j.watres.2020.116759.
3. Feng H, Liang YN, Hu X. Natural organic matter (NOM), an underexplored resource for environmental conservation and remediation. Mater. Today Sustainability. 2022;19:100159. https://doi.org/10.1016/j.mtsust.2022.100159.
4. Manahan SE. Environmental Chemistry. 7th ed. United States of America: Lewis Publishers; 2000. 55–93.
5. Matilainen A, Gjessing E, Lahtinen T, Hed L, Bhatnagar A, Sillanpää M. An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. Chemosphere. 2011;83:1431–1442. https://doi.org/10.1016/j.chemosphere.2011.01.018.
6. Matilainen A, Vepsäläinen M, Sillanpää M. Natural organic matter removal by coagulation during drinking water treatment: A review. Adv. Colloid Interface Sci. 2010;159(2):189–197. https://doi.org/10.1016/j.cis.2010.06.007.
7. Anderson LE, Demont I, Dunnington DD, Bjorndahl P, Redden DJ, Brophy MJ, Gagnon GA. A review of long-term change in surface water natural organic matter concentration in the northern hemisphere and the implications for drinking water treatment. Sci. Total Environ. 2023;858(1):159699. https://doi.org/10.1016/j.scitotenv.2022.159699.
8. Gabrielli M, Pulcini F, Barbesti G, Antonelli M. Source to tap investigation of natural organic matter in non-disinfected drinking water distribution systems. Environ. Sci. Water Res. Technol. 2023;10(1):128–143. https://doi.org/10.1039/d3ew00280b.
9. McNichol A, Osborne E, Gagnon A, Fry B, Jones G. TIC, TOC, DIC, DOC, PIC, POC- unique aspects in the preparation of oceanographic samples for 14C-AMS. Nucl. Instrum. and Method Phys. Res. B. 1994;92:162–165. https://doi.org/10.1016/0168-583X(94)95998-6.
10. Haarhoff J, Kubare M, Mamba B, Krause R, Nkambule T, Matsebula B, Menge J. NOM characterization and removal at six Southern African water treatment plants. Drink. Water Eng. Sci. 2010;3:53–61. https://doi.org/10.5194/dwes-3-53-2010.
11. Volk C, Wood L, Johnson B, Robinson J, Zhu H, Kaplan L. Monitoring dissolved organic carbon in surface and drinking waters. J. Environ. Monit. 2002;4(1):43–47. https://doi.org/10.1039/B107768F.
12. Ibrahim N, Aziz H. Trends on natural organic matter in drinking water sources and its treatment. Int. J. Sci. Res. Environ. Sci. 2014;2(3):94–106. https://doi.org/10.12983/ijsres-2014-p0094-0106.
13. Peacock M, Freeman C, Gauci V, Lebron I, Evans C. Investigations of freezing and cold storage for the analysis of peatland dissolved organic carbon (DOC) and absorbance properties. Environ. Sci: Process Impacts. 2015;17(7):12900–1301. https://doi.org/10.1039/C5EM00126A.
14. Thieme L, Graeber D, Kaupenjohann M, Siemens J. Fast-freezing with liquid nitrogen preserves bulk dissolved organic matter concentrations, but not its composition. Biogeosciences. 2016;13:4697–4705. https://doi.org/10.5194/bg-13-4697-2016.
15. Nachimuthu G, Watkins M, Hulugalle N, Finlay L. Storage and initial processing of water samples for organic carbon analysis in runoff. MethodsX. 2020;7:101012. https://doi.org/10.1016/j.mex.2020.101012.
16. Fonvielle J, Felgate S, Tanentzap A, Hawkes J. Assessment of sample freezing as a preservation technique for analysing the molecular composition of dissolved organic matter in aquatic systems. RSC Adv. 2023;13:24594. https://doi.org/10.1039/D3RA01349A.
17. Fonvielle JA, Giling DP, Dittmar T, Berger SA, Nejstgaard JC, Lyche Solheim A, Gessner MO, Grossart H-P, Singer G. Exploring the suitability of ecosystem metabolomes to assess imprints of brownification and nutrient enrichment on lakes. J. Geophys. Res.: Biogeosci. 2021;126(5):e2020JG005903. https://doi.org/10.1029/2020JG005903.
18. Norrman B. Filtration of water samples for DOC studies. Mar. Chem. 1993;41(1-3):239–242. https://doi.org/10.1016/0304-4203(93)90125-8.
19. Yoshimura T. Appropriate bottles for storing seawater samples for dissolved organic phosphorus (DOP) analysis: A step toward the development of DOP reference materials. Limnol oceanogr-Meth. 2013;11:239–246. https://doi.org/10.4319/lom.2013.11.239.
20. Nkambule T, Krause R, Haarhoff J, Mamba B. Treatability and characterization of natural organic matter (NOM) in South African waters using newly developed methods. Phys Chem Earth Parts ABC. 2011;36(14-15):1159–1166. https://doi.org/10.1016/j.pce.2011.07.006.
21. Moyo W, Chaukura N, Motsa M, Msagati T, Mamba B, Heijman S, Nkambule, T. Monitoring the characteristics and removal of natural organic matter fractions in selected South African water treatment plants. Water Pract Technol. 2020;15(4):932–946. https://doi.org/10.2166/wpt.2020.075.
22. Ou T, Fang C, Ding S, Zhao T, Liu S, Yu Z, Chu W. Has the formation of disinfection by-products been overestimated? Membrane leakage from syringe filter heads serves as unexpected precursors. Chemosphere. 2020;258:127278. https://doi.org/10.1016/j.chemosphere.2020.127278.
23. Xie P, de Lannoy C-F, Ma J, Wiesner M. Chlorination of polyvinyl pyrrolidone–polysulfone membranes: Organic compound release, byproduct formation, and changes in membrane properties. J Membr Sci. 2015;489:28–35. https://doi.org/10.1016/j.memsci.2015.03.058.
24. Nkambule T, Krause R, Haarhoff J, Mamba B. Natural organic matter (NOM) in South African waters: NOM characterisation using combined assessment techniques. Water SA. 2012;38(5):697–706. https://dx.doi.org/10.4314/wsa.v38i5.7.
25. Chatterjee S, Simonoff J. Handbook of regression analysis. New Jersey: John Wiley & Sons, Inc. 2013.
26. Skoog D, Holler F, Crouch S. Principles of Instrumental Analysis, 6th ed. Belmont: Thomson Brooks/Cole. 2007.
27. Schwertman NC, de Silva R. Identifying outliers with sequential fences. Comput. Stat. Data Anal. 2007;51(8):3800–3810. https://doi.org/10.1016/j.csda.2006.01.019
28. Merza E, AL-Anber N. Fast ways to detect outliers. J. Tech. 2021;3(1):66–73. https://doi.org/ 10.51173/jt.v3i1.287.
29. Carter H, Tipping E, Koprivnjak J, Miller M, Cookson B, Hamilton-Taylor J. Freshwater DOM quantity and quality from a two-component model of UV absorbance. Water Res. 2012;46(14):4532–4542. https://doi.org/10.1016/j.watres.2012.05.021.

30. Kothawala D, von Wachenfeldt E, Koehler B, Tranvik L. Selective loss and preservation of lake water dissolved organic matter fluorescence during long-term dark incubations. Sci. Total Environ. 2012;433:238–246. https://doi.org/10.1016/j.scitotenv.2012.06.029.
31. Weishaar JL, Aiken GR, Bergamaschi BA, Fram MS, Fujii R, Mopper K. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environ. Sci. Technol. 2003;37(20):4702–4708. https://doi.org/10.1021/es030360x.
32. Ferreira JE, Miranda RM, Figueiredo AF, Barbosa JP, Brasil EM. Box-and-whisker plots applied to food chemistry. J. Chem. Educ. 2016;93(12):2026–2032. https://doi.org/10.1021/acs.jchemed.6b00300.