



Total Dissolved Nitrogen and Phosphorus Determination in Coastal South Atlantic Water Based on UV Oxidation Method

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ABSTRACT: The UV oxidation is an efficient method to determine dissolved total nitrogen and phosphorus in seawater when compared with wet oxidation offering high reproduction and precision associated to easy sample preparation. In this process, dissolved organic compounds are oxidized to N and P inorganic forms (nitrate and phosphate) using a proper equipment (patent of the group) based on a potent UV lamp (1300W-2.000-2500Å). The use of this UV photo-oxidation method was performed in South Atlantic coastal waters showing concentrations until 5 times those obtained by traditional wet oxidation method. Some data from unpolluted seawaters presented followings average concentrations considering seasonal variations: TDN = 4.97 $\mu\text{mol N L}^{-1}$; DON = 3.44 $\mu\text{mol N L}^{-1}$; TDP = 0.73 $\mu\text{mol P L}^{-1}$; DOP = 0.50 $\mu\text{mol P L}^{-1}$ and in polluted seawaters, the follows averages were obtained: TDN = 70.77 $\mu\text{mol N L}^{-1}$; DON = 48.42 $\mu\text{mol N L}^{-1}$; TDP = 4.68 $\mu\text{mol P L}^{-1}$ and DOP = 1.58 $\mu\text{mol P L}^{-1}$. The distinct ranges obtained in two different scenarios (polluted and non-polluted waters) confirm that the UV oxidation constitutes an excellent method to analyze the waters considering total fraction of organic nitrogen and phosphorus. It is also recommended as pre-treatment to diminish the organic matter in water when necessary in analytical procedure of polluted seawater, reducing the organic interference during trace analysis in seawater.

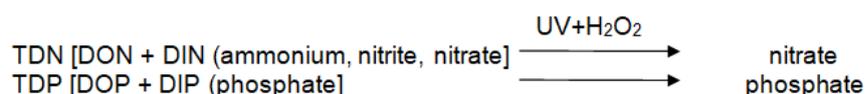
Keywords: photo-oxidation application, seawater, dissolved organic compounds, TDN and TDP in polluted waters, NOD and POD pristine values, environmental analytic technics.

I. INTRODUCTION

The availability of dissolved nitrogen and phosphorus in the organic forms in the surface seawater is remarkable in function of the metabolism of organisms, including the primary production and the food chain generation of organic matter and organic detritus. Besides it, there is a terrestrial and anthropogenic inputs. The regeneration process of inorganic forms occurs by oxidation of the organic matter during it sinking and also when it reaches the sediments. The inventory of dissolved N and P in the marine system includes organic and inorganic species of these elements and it is necessary to use the most adaptable methodologies to determine the total and organic forms in dissolved partition ($<0.7\mu\text{m}$) as a component to understand the biogeochemical cycling of these elements in the evaluation of the environmental impact of pollution. The UV oxidation is efficient to solve the analytical proposal, presenting facility and precision to be used in seawater samples based on photo-oxidation process. The photo-oxidation of organic compounds in solution to obtain orthophosphate was showed by MacLKelvie and Lyddy-Meaney (2005) and in seawater as showed by Aminot and Kerouel (2001). The process is also used to obtain nitrate from the oxidation of dissolved organic compounds (Armstrong et al. 1966; Armstrong and Tibbitts, 1968), but with rare evidence of the efficiency in function of the diversity of N compounds including a part of the most refractory forms. This work intends to evidence the advantage to use the photo-oxidation methods to determine total dissolved P and N in the seawater and its organic fraction focusing in environmental studies and also shown data from polluted and non-polluted coastal water in Southeastern Atlantic margin. The application of the UV-oxidation process to determine Total Dissolved Nitrogen (TDN) and Total Dissolved Phosphorus (TDP) is similar to the Armstrong et al., (1966) suggestion, but differing in the use of a proper apparatus for this analysis. In the determination of Dissolved Organic Nitrogen

(DON) there is some controversy due to the possibilities of the presence of organic refractory polymeric forms in the seawater (Suzuki et al., 1985; Williams and Druffel, 1988). The photo-oxidation of the organic matter in fresh water by ultra-violet radiation was observed by Beattie et al. 1961. Armstrong et al. (1966) found that the exposure of seawater to such a light source caused a decrease in the extinction coefficient at 2000-2500Å, characteristic of organic matter and it started investigations. Organic matter (C, N and P compounds) is generally destroyed by modification of methods requiring wet oxidation with perchloric acid or needing an autoclave treatment with persulfate and sulfuric acid. These methods are tedious and their precision is poor because of the difficulties of controlling contamination sources introduced by reagents used to destroy the organic matter and the contamination that occurs during the various transferring processes in the analysis.

Dissolved nitrogenous compounds in seawater are oxidized to nitrate in the presence of dissolved oxygen under action of the UV radiation energy. Ammonium can be photo-oxidized to nitrite and nitrate, because oxidative deamination occurs at an early stage in the photolysis of organic nitrogen compounds. Nitrite and nitrate are expected as end products of irradiation experiments. The first information about results of exhaustive irradiation of samples with small concentrations of organic matter in presence of excess oxygen was showed by Armstrong et al. (1966).



Dissolved organic phosphorus in the seawater by exposure to UV irradiation liberates phosphorus as inorganic phosphate from organic matter rapidly and without necessity of adding any reagents except a small quantity of hydrogen peroxide to provide an excess of oxygen in the water (Armstrong et al., 1966). To determine TDN and TDP, both processes are clean and require only addition of some drops of hydrogen peroxide (30%) in a sample volume, arranged in quartz tubes and then exposed to UV radiation for some hours. The final determinations consist of using the automatic or manual nitrate and phosphate determination as recommended for example, by Strickland and Parsons (1968); Tréguer and Le Corre (1975); Grasshoff et al., (1983); Aminot and Chaussepied (1983).

The oxidation of the dissolved organic matter present in the seawater using a light irradiation with a high watage UV lamp (>1000W).

The dissolved compounds in marine water include inorganic and organic fractions. The dissolved organic form is composed of different chemical organic groups, whose identification is associated with the development of chemical analytical and instrumental methods, since most of the components that make up the dissolved organic matter pool are found in low concentrations in seawater, which is a complex inorganic matrix (Tab. 1) for chemical analytical treatment. Given the trophic importance of the content of N and P in dissolved organic matter, considering recycling and synthesis processes of organic matter in biogeochemical cycles, regeneration process offering inorganic nutrients to the marine primary production, the facilities in determining the total content of dissolved N and P, as well as the ease in calculating dissolved organic fractions corresponding to an important tool in coastal and open sea oceanographic studies.

Table 1. Average composition of seawater with salinity ~ 34.4.

| Major inorganic components | ions (g kg ⁻¹) | (%) |
|--|----------------------------|-------|
| Ion chloride (Cl ⁻) | 18.980 | 55.04 |
| Sodium Ion (Na ⁺) | 10.556 | 30.61 |
| Sulfate Ion (SO ₄ ²⁻) | 2.649 | 7.68 |
| Magnesium Ion (Mn ²⁺) | 1.272 | 3.69 |
| Calcium Ion (Ca ²⁺) | 0.400 | 1.16 |
| Potassium Ion (K ⁺) | 0.380 | 1.10 |
| Bicarbonate ion (HCO ₃ ⁻) | 0.140 | 0.41 |
| Bromide Ion (Br) | 0.065 | 0.19 |
| Borate Ion (BO ₄ ³⁻) | 0.026 | 0.07 |
| Strontium Ion (Sr ²⁺) | 0.013 | 0.04 |
| Fluoride Ion (F ⁻) | 0.001 | 0.00 |
| Total | 34.482 | 99.99 |

II. Material and methods

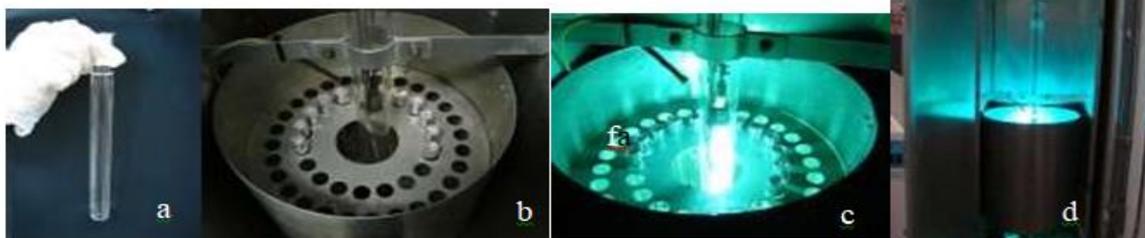
The sampled water was filtered in GF/F Whatman filter (0.7 μm of porosity). Dissolved Organic N and P present in seawater can be oxidized to nitrate and phosphate respectively using UV irradiation emitted by a mercury vapor lamp with a small amount of hydrogen peroxide (30%) being added to ensure the availability of oxygen to the sample oxidation process. The dissolved inorganic forms of nitrogen (nitrite and nitrate) initially and at the endpoint of the treatment were determined by the colorimetric method as described by Grasshoff et al., (1983) using AutoAnalyzer II, Bran-Luebbe) with a precision of $\pm 0.01\mu\text{M-N}$ for nitrite and $\pm 0.02\mu\text{M-N}$ for nitrate, while the N-ammonium is also analyzed, following the principle of Berthelot, 1859 as described by Grasshoff et al., 1983, presenting precision of $\pm 0.01\mu\text{M-N}$. The dissolved orthophosphate determination before and after treatment, was also determined by colorimetric methods described by the *op. cit.* author and measured in the spectrophotometer Zeiss PMQIII®. The later precision was $\pm 0.01\mu\text{M-P}$.

In the photo-oxidation, the seawater sample (about 60 mL) is placed in quartz tubes where 250 μL of 30% H_2O_2 is added, the sample is capped with a plastic bung with a micro-hole to ensure the balance of internal and external pressure. At the time of photo-oxidation, the plastic caps are protected with aluminum foil. Maximum oxidation efficiency is obtained with approximately 12h of exposure (Fig. 3) value obtained from tests with different concentrations of standard solution of bipyridine and β -glycerophosphate, forms of organic N and P respectively, with strong bonds, whose a break in 60-70% of N represents an efficient process to guarantee the oxidation of almost all organic molecules present in the marine environment in dissolved form, which hardly presents structures as resistant to oxidation as those used in the efficiency test (Saraiva, 2003).

In the process of exposure to irradiation, the plastic cover is protected with aluminum foil. After 12h, the N and P of the dissolved organic matter is transformed into nitrate and phosphate and a new analysis of the concentration of these salts using the colorimetric methods described is performed. The sum of inorganic forms of nitrogen (N-ammonium + nitrite + nitrate) compose the Dissolved Inorganic Nitrogen (DIN) determined initially. The photo-oxidized samples reveal the values of Total Dissolved Inorganic Nitrogen (DTN) and the values of Dissolved Organic Nitrogen is obtained by the difference between DTN and DIN, in the case of phosphorus, the same reasoning is applied, that is, knowing the DIP (Dissolved Inorganic Phosphorus) and DTP (Total Dissolved Phosphorus) by difference, POD (Dissolved Organic Phosphorus) is obtained. The oxidation of organic N and P is performed simultaneously from 60 mL of sample. For organic phosphorus, the method efficiency is measured by the oxidation of β -glycerophosphate and the efficiency reaches 100% of recuperation. For the determination of nitrate and nitrite and phosphate, standard solutions are prepared with inorganic salts as mentioned by the recommended Grasshoff et al. (1983) methods. To evaluate the oxidation of organic-N compounds, solutions with known concentration of urea and EDTA are often placed to test the oxidation efficiency accompanying Bipyridine solution that represent a strong refractory source of N as recommended (Fig. 4). Urea and EDTA solutions present a high efficiency in the photo-oxidation to form nitrate in relation to bipyridine answer. The precision of method follows the discriminated cited before. The saline matrix ($> 30\text{g L}^{-1}$) does not present significant interference in the analyses.

III. DISCUSSION

A photo-oxidation equipment, patented by the author group, basically constituted by a source (lamp) with a UV emission 2000-2500 \AA and a power of about 1200W (Heraeus-Hanau®), as described by Braga & Braga (1999) (Fig. 1).



Fotos: Braga E. S.

Figure 1. Photo-oxidation apparatus: quartz tube with toggle and valve (a) quartz tube with sample and protected cover on the drum (c), cabinet with source, ventilation, exhaust fan and timer, drum, lamp and temperature sensor; (d) Equipment developed and patented by Braga et al. (1999).

A thermostat controlled the internal temperature a (~80°C) and a stronger exhaust fan in upper part of the cabinet ensure the forced ventilation. A timer control the exposition time, that considering the maximum oxidation of ~75% of bipyridine (organic macromolecule with strong N links) in 12h of exposition (Fig. 1).

The photo-oxidation of organic matter in seawater with UV irradiation vs. chemical oxidation

Considering the amounts of nitrate and phosphate standards as reference in a curve of evaluation of the organic phosphorus oxidation process after 12h exposure showed 100% efficiency β-glycerophosphate oxidation based on phosphate curves, while bipyridine its oxidation was 75% and urea oxidized 85% (Fig. 2).

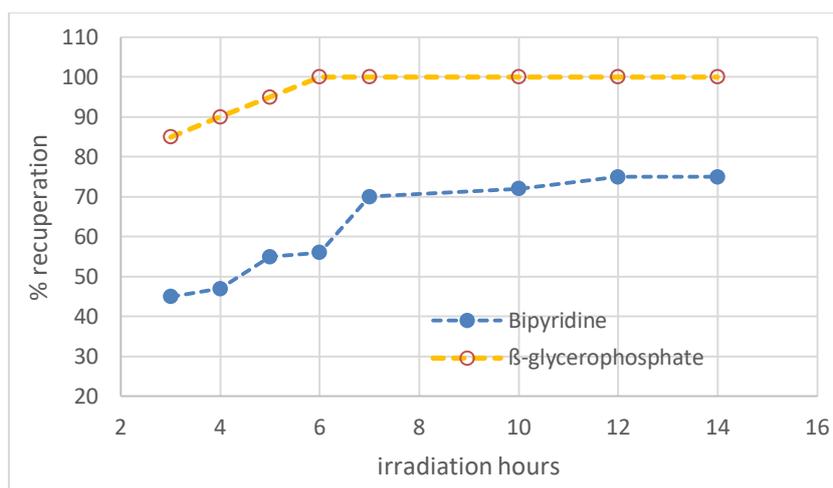


Figure 2. Efficiency of photo-oxidation during the hours of irradiation exposition of bipyridine (organic molecule with strong N link) and β-glycerophosphate (organic molecule with strong P link).

The oxidation of these two organic forms of nitrogen shows that there is a 20% deficiency in oxidation, therefore the values obtained by photo-oxidation, even being 70% higher than those obtained by oxidation with persulfate, should be corrected by 20%, especially in cases where N-ammonium shows values between 10 and 20 μM, where the high values of organic nitrogen must be very representative, including a greater participation of refractory forms, and it is also recommended to dilute the samples in this situation, where eutrophication will already being implanted.

Suzuki et al. (1985) obtained values of DON by photooxidation two times bigger than by with persulfate oxidation recommended by Grasshoff et al., here we observed almost 5 times more.

Table 2. Values of TDN and TDP in natural seawater samples determined using photo-oxidation and wet chemical oxidation methods (n=5).

| Samples | TDN _{photo} | TDN _{wet} | %TDN* | TDP _{photo} | TDP _{wet} | %TDP* |
|---------|----------------------|--------------------|-------|----------------------|--------------------|-------|
| 1 | 8.05±0.80 | 1.71±0.30 | 21 | 0.76±0.13 | 0.61±0.18 | 80 |
| 2 | 4.75±0.41 | 0.81±0.22 | 17 | 0.90±0.12 | 0.76±0.15 | 84 |
| 3 | 3.21±0.35 | 0.72±0.18 | 22 | 0.63±0.12 | 0.51±0.14 | 81 |
| 4 | 4.66±0.48 | 0.81±0.21 | 25 | 0.53±0.16 | 0.45±0.21 | 85 |

| | | | | | | |
|--|-----------|-----------|----|-----------|-----------|----|
| 5 | 5.85±0.40 | 0,95±0.30 | 16 | 0.75±0.17 | 0.64±0.26 | 85 |
| 6 | 6.60±0,45 | 0,99±0.32 | 15 | 0.57±0.14 | 0.49±0.19 | 86 |
| 7 | 7.48±0.49 | 1,80±0.26 | 24 | 0.86±0.20 | 0.74±0.16 | 86 |
| 8 | 9.90±0.85 | 1.80±0.27 | 18 | 0.82±0.21 | 0.70±0.18 | 85 |
| 9 | 5.76±0.42 | 0.99±0.28 | 17 | 0.77±0.18 | 0.65±1.23 | 84 |
| <ul style="list-style-type: none"> • % of the value obtained by wet chemical oxidation in relation to photo-oxidation method. | | | | | | |

Considerations on the photo-oxidation method of organic matter dissolved in sea water

Organic matter (C, N, P) is usually destroyed by wet oxidation using perchloric acid or using a solution of persulfate and sulfuric acid and subjecting the sample to autoclaving. This method is laborious and its accuracy is low, in addition to having a high risk of contamination due to the various steps to be followed (Armstrong et al., 1966 *op. cit.*). The nitrogenous compounds present in seawater are oxidized to nitrate in the presence of oxygen and under the action of UV radiation. Ammonium can be oxidized to nitrite and nitrate due to the oxidative deamination that occurs in the early stages of photolysis of organic nitrogen compounds. The first information showing the results of UV irradiation of seawater samples with small concentrations of organic matter, ensuring excess oxygen assuring the organic nitrogen oxidation to nitrate and organic phosphorus compounds in orthophosphate.

The environmental importance of to know the Dissolved organic matter and its N and P content is reference in the evaluation of the organic charge and eutrophication level in coastal systems mainly influenced by anthropogenic inputs as by effluents and domestic sewage (Braga et al. 2000) contributing to the excess of inorganic nutrients as ammonium, nitrite, nitrate and phosphate, evidencing the unbalanced biogeochemical cycling of the organic matter.

Importance of these determination in the environmental study

The total organic matter dissolved in the seawater has low natural concentrations in the ecosystem balance and, the human action, that is, the accentuated interferences of anthropic origin, also known as pollution, are responsible for the introduction of a accentuated load of organic matter both in particulate form and in dissolved form in aquatic systems. The coastal zone deserves special attention regarding the disposal of domestic sewage rich in organic matter as well as in relation to many other dumps of different origins and composition. Submarine sewage outbreaks have been used more and more as a way of solving the problems of sewage disposal in cities located in coastal areas. This has corresponded to one of the biggest pollution problems in the world at the moment, given that many capitals are located in the coastal zone. The treatment of sewage when captured by the sanitation network, in the case of Brazil, is precarious, and often only the primary treatment of the effluent captured is carried out, which does not decrease the load of organic matter, which is high, especially in domestic sewers.

Environmental studies are concerned with the load of dissolved organic matter, however the range of components that integrate the so-called organic matter in the marine environment is wide, being mentioned: free amino acids, sugars, lipids, nucleic acids, carboxylic acids, terpenes, sterols, hydrocarbons among others. The knowledge of the total amount of organic matter, in addition to contributing to the assessment of the trophic characteristics of a water, also signals the degree of anthropic interference that can lead to irrecoverable cases of pollution. For example, high values of Total Dissolved Nitrogen (NTD) and Total Dissolved Phosphorus (PTD) are found in places with high domestic and industrial pollution, such as Baixada Santista, where 312 $\mu\text{mol L}^{-1}$ of NTD was observed inside the Canal from Cubatão (Saraiva, 2003).

The dissolved organic N represent the most complex step to understand the biogeochemical cycle of N in the marine ecosystem and also the less studied (Paul, 1983). The organic matter seasonal studies are not abundant and the big part of the studies evidence one from these two important forms (DON and DOP). One pioneer to determine DOC, DON and DOP was Duursma (1961). DON always caused discussion in function of the refractory polymeric forms (Suzuki et al., 1985; William & Druffel, 1988). To contribute to the dimension of the TDN, DON, TDP and DOP in different coastal ecosystems, we present ranges obtained in Southeastern São Paulo state, in Brazil determined using the photo-oxidation method and considering two pristine areas and one impacted by contaminant from diverse

source and composition. The seasonal observations are presented in table 3.

Table 3. Mean values and standard deviation of TDN, DON, TDP and DOP of some oceanographic stations in the region of Cananéia and Santos- São Paulo - Brazil.

| | TDN | DON | TDP | DOP |
|--|------------------|-----------------|---------------|---------------|
| μM | | | | |
| Ubatuba ~23°32'S - 45°04'W (n = 40) – Non polluted coastal system | | | | |
| Winter - 1991 | (4.09 ± 0.93) | (3.75 ± 0.97) | (0.37 ± 0.13) | (0.22 ± 0.13) |
| Spring - 1991 | (7.29 ± 4.33) | (4.00 ± 1.86) | (0.58 ± 0.32) | (0.22 ± 0.07) |
| Summer - 1992 | (5.23 ± 2.79) | (4.73 ± 2.83) | (0.30 ± 0.10) | (0.26 ± 0.20) |
| Fall - 1992 | (5.36 ± 1.92) | (4.31 ± 2.03) | (1.21 ± 0.44) | (0.90 ± 0.44) |
| Winter - 1992 | (4.62 ± 2.38) | (3.33 ± 2.41) | (2.17 ± 0.55) | (1.83 ± 1.13) |
| Spring - 1992 | (2.46 ± 2.53) | (2.04 ± 2.52) | (0.49 ± 0.12) | (0.21 ± 0.11) |
| Cananéia ~25°07'S - 47°56'W (n = 16) - Non polluted estuarine system | | | | |
| Summer -1992 | (5.77 ± 2.33) | (1.52 ± 0.96) | (0.41 ± 0.06) | (0.25 ± 0.04) |
| Winter - 1992 | (4.92 ± 2.16) | (3.86 ± 2.10) | (0.31 ± 0.13) | (0.12 ± 0.10) |
| Santos-São Vicente ~24°02'S - 46°24' W (n=33) – Polluted coastal system | | | | |
| Summer -1999 | (30.56 ± 12.54) | (10.29 ± 5.32) | (4.79 ± 1.54) | (0.58 ± 0.15) |
| Winter - 1999 | (75.16 ± 25.10) | (51.84 ± 9.58) | (5.80 ± 1.54) | (2.58 ± 1.53) |
| Spring - 1999 | (106.60 ± 34.20) | (83.12 ± 23.12) | (3.44 ± 1.05) | (1.58 ± 1.02) |

Table 3 shows very high values obtained in all seasonal periods in the Santos-São Vicente region, where there is an important industrial hub, two important cities in the Baixada Santista and the largest port in Latin America. The regions of Ubatuba and Cananéia, present natural values of total and organic forms of N and P. The differences in seasonal concentrations in the N and P pools show differences in the oxidation processes of organic matter and take into account different processes in the middle and end of the seasonal period, as well as annual differences, this leads to the possibility of evaluating climate change together with the study of the biogeochemical cycles of nutrients (N and P).

IV. CONCLUSION

Eutrophication is a process caused by the excess of nitrogenous and phosphate components in the marine environment, triggering an algal “bloom” that generates negative effects on the ecosystem, contributing to the decrease in water quality, low light penetration for organisms below algae layer, difficulty in gas exchange with the atmosphere, high production of debris with the senescence of the algal population with the death of other species, high biological oxygen demand, generation and accumulation of organic matter in the bottom forming putrid bottoms, decreased biodiversity among other problems. Thus, the balance between the organic forms present in a system, the adequate availability of inorganic N and P ensure a healthy primary production, these being some factors that lead a system to maintain the balance in its biogeochemical cycles, contributing to the appropriate biotransformation processes of organic matter and thus, maintaining the sustainability of the ecosystem, without implying excessive energy losses and export of matter, or excessive enrichment processes causing undesirable effects on the balance of the ecosystem. The balance between organic and inorganic forms of two main nutrients that control primary marine production is an important sensor of environmental “health”. Facilitating chemical analytical procedures for determining these environmental compartments is undoubtedly an important tool in the study and preservation of the coastal and marine environment.

V. ACKNOWLEDGMENT

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