CIENCIA 14(1), 101 - 119, 2006
Maracaibo, Venezuela

Modified procedure for the simultaneous determination of nitrogen, phosphorus and sulphur by hydrogen peroxide microwave digestion and ion chromatography

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Recibido: 08-11-04 Aceptado: 27-03-06

Abstract

A modified method for the microwave oxidation with hydrogen peroxide of nitrogen, phosphorus and sulphur followed by the determination of the nitrate, phosphate and sulphate ion by ion chromatography is presented is this work. This method was modified in order to applied the methodology in more recalcitrant compounds where nitrogen, phosphorus and sulphur atoms were in ring system. The developed procedures were validated using pure compounds: sodium nitrite, sodium sulphite, L-cysteine, lysine, phosphonitrile chloride, saccharin, urea, and reference material prawn GBWO8572. Three steps procedure was necessary to digest compounds with a ring in the structure. The amount of recovery after each step in the modified method could be indicative of the nature of the compounds present in the sample. The maximum levels of phosphorus encountered in Lake Maracaibo (ca. 20.3 mg/L) correspond to a general level of productivity of a hyper-eutrophic lake.

Key words: Hydrogen peroxide; microwave digestion; nitrogen; phosphorus; sulphur.

Procedimiento modificado para la determinación simultánea de nitrógeno, fósforo y azufre por digestión con peróxido de hidrógeno usando microndas y cromatografía iónica

Resumen

Un método modificado por microondas usando peróxido de hidrógeno para la oxidación de nitrógeno, fósforo y azufre seguido de la determinación del nitrato, el fosfato y el ion de sulfato por la cromatografía de ion es presentado en este trabajo. Este método fue modificado para aplicación de la metodología previamente desarrollada en compuestos más recalcitrantes donde los átomos del nitrógeno, el fósforo y el azufre están en el anillo. El procedimiento fue validado usando compuestos puros: nitrato de sodio, sulfito de sodio, L-cisteína, lisina, cloruro de fosfo-

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nitrilo, sacarina, urea, y el material de referencia camarón GBWO8572. Tres etapas de digestión fueron necesarias para compuestos con los átomos de N,P y S en el anillo. La cantidad recuperada después de cada etapa usando el método modificado podría ser indicativa de la naturaleza de los compuestos en la muestra. Los niveles máximos de fósforo encontrados en el Lago Maracaibo (ca. 20,3 mg/L) corresponden a un nivel general de productividad de un lago hipereutrófico.

Palabras clave: Azufre; digestión microondas; fósforo; nitrógeno; peróxido de hidrógeno.

Introduction

Nitrogen occurs in numerous chemical compounds and in various environmental compartments (1). Nitrogen is present as free nitrogen (N_2) and as salts (NH_4^+, NO_3^-, NO_2^-) in soil and water (2-4). As an essential element for plant growth, it is a constituent of proteins, amino acids, vitamins, chlorophyll, enzymes, etc. Consequently, nitrogen availability may often be the limiting factor in plant growth and yield of agricultural crops. Phosphorus is a highly reactive element and forms compounds with various elements by direct bonding with or through oxygen. Phosphorus exhibits nine formal oxidation states from +5 to –3. Typical oxoacids such as orthophosphate (+5), hypophosphate (or di-phosphate) (+4); and hypophosphite, (+1) and their derivatives are well known. Sodium diphosphate is familiar as the phosphorylation agent for biological substances. Phosphorus oxo acids, phosphate and its polymers are important in nature and in industry, orthophosphoric acid is used as raw material in the manufacture of fertilizers, detergents, surfactants and flame retardants (5).

Phosphorus occurs in waters, either in dissolved or particulate forms and, as inorganic or organically bound species (6). Total phosphorus concentrations in water can vary from less than 0.01 mg/L in small, near pristine, mountain streams to over 1 mg/L in heavily polluted rivers (7).

Phosphorus entering a wetland or stream is typically present in both organic and inorganic forms. These forms are dissolved inorganic phosphorus, dissolved organic phosphorus, particulate inorganic and particulate organic phosphorus. Dissolved inorganic phosphorus is considered bioavailable, whereas organic and particulate phosphorus forms generally undergo transformations to inorganic forms before being considered bioavailable (7). Seawater contains various organic esters of phosphorus as well as orthophosphate.

Due to the pre-eminence of phosphorus in primary production in all kinds of aquatic environments, research has focused on the origin and fate of phosphorus in lakes and seas (8-11). In sediment from lakes, it has been demonstrated that there are many cases in which phosphorus has been the limiting nutrient (8). The classification of the trophic status of standing water bodies is still based on the total phosphorus concentrations suggested by Vollenweider and modified by Wetzel in 1983 (12). Some lakes in Central Europe have been regularly analysed during the last few years. Lakes such as Bodensee, Zurichsee and Greifensee have different degrees of eutrophication as a result of increases in phosphate concentration (13); for example, the phosphate loads in Lake Bodensee (Germany) have increased from 3 to 6 mg/m^3 per year (13).

Sulphur is the tenth most abundant element in the earth's crust (0.03-0.1% w/w) and it is found in both the elemental form and in metal sulphide ores The cycling of sulphur on the Earth's surface has been greatly increased since the start of the Industrial Revolution by the demand for fuel, metals and fertilizers. Despite a great deal of

study that the sulphur cycle has received in the past few years, there is still some uncertainity about many of the sources of the element. The sulphur and nitrogen cycles have a number of similarities, but one of the most important differences is that the major reservoir for nitrogen is the atmosphere, whereas the major available reservoir for sulphur is the earth's crust (14).

Dimethyl sulphide is a major biogenically produced sulphur compound, releasing about 20-40TgSa^{-1} from the oceans.The formation of hydrogen sulphide (H_2S) is a characteristic feature of anaerobic marine sediments due to the high levels of sulphate, as compared with nitrate, in the sea. The hydrogen sulphide that is produced may be released as a gas to the atmosphere, where it is oxidised or may undergo reaction with metal ions in the sediments or water column to form insoluble sulphides. The later transition metals and those metals which come after the transition metals in the periodic table are especially likely to form insoluble sulphides (15). Iron, because it is present in relatively large quantities, forms the major sulphides mineral reservoir such as triolite, FeS, and as iron pyrites FeS_2. The black colour of many sediments is partially due to the presence of iron sulphides as well as organic matter.

Analytical determination of nutrients

The nitrogen, phosphorus and sulphur cycles are of particular significance in a number of biological and non-biological processes in the environment (16). Natural and anthropogenic effects can cause localised inter-related changes to the cycles. In order to assess the impact and extent of the changes, it is essential to develop analytical methods which allow the simultaneous determination of two or all three constituents in a wide variety of environmental samples.

Phosphorus determination involves two general steps, conversion of the phosphorus species to dissolved orthophosphate followed by determination of dissolved orthophosphate. Three digestion methods involving either perchloric acid, nitric-sulphuric acid mixture or persulphate solution are usually used. The phosphate generated is determined colorimetrically (17-19). Determination of phosphorus by inductively coupled argon plasma spectrometry is possible but requires that the instrument is adapted to work in the low ultraviolet region (20).

Several methods have been described in the literature for the determination of sulphur which include gravimetric, turbidimetric, ion selective methods, chemiluminescence and capillary gas chromatography (21-23). These methods are both time- and reactant-consuming. Recently ICP-AES has been used to determine sulphur with the disadvantage that the recoveries and interference show dependence on the wavelength used. Calcium and boron are considered spectral interferences, and potassium, magnesium and phosphorus cause inter-element interferences (24).

Historically, analysis of total nitrogen has been made by the Kjeldahl method (25). The sample is digested with a mixture of concentrated sulphuric acid and potassium sulphate and selenium or mercury is added as a catalyst. Using this method dissolved organic nitrogen and ammonia are measured, but the oxidized forms, nitrate and nitrite, must be determined separately. Another limiting factor in the nitrogen determination using the Kjeldahl method is the time required (ca. 12 hours). Other methods that have been used include photooxidation (26-27), generic combustion (28), pyrochemiluminescence (29) and peroxidisulphate oxidation (30-31).

A modified alkaline persulphate procedure has been developed for the simultaneous determination of nitrogen and phosphorus after oxidation to nitrate and phosphate, respectively (32). This digestion method has also been used, followed by ion

chromatography to determine the anions, nitrate and phosphate, but the method is subject to interference in the determination of phosphate due to a large sulphate peak (33-35).

In one of the first attempts at simultaneous determination, Ebina *et al.* (17) developed a method of oxidizing nitrogen and phosphorus to nitrate and phosphate, respectively using alkaline potassium peroxodisulphate. The composition of the oxidizing solution was carefully chosen so that its pH changed from basic to acidic during the oxidation step. The change in pH was necessary because oxidation with potassium peroxodisulphate of nitrogen and phosphorus occurs under basic and acidic conditions, respectively. The nitrate and phosphate ions were then determined colorimetrically.

In a different approach, Collins *et al.* (36) developed a method for the combined analysis of total phosphorus and Kjeldahl nitrogen in complex matrices using a pressure microwave digestion and final colorimetric determination of phosphorus. More recently, Matilainen and Tummavuori (24) investigated the application of ICP-AES to the determination of water soluble sulphur in fertilizers and reported on spectral and interelement effects. To be able to analyse both bound and water soluble fractions, samples have to be digested. However, existing digestion methods are not easily adapted to simultaneous determinations because the use of oxidants such as nitric and sulphuric acids and potassium peroxodisulphate precludes the determination of one or more of the analytes. The use of hydrogen peroxide as an oxidant has a number of benefits compared with some of the more traditional oxidants (37). These include long term storage stability, and when the oxidising power of the peroxide is spent, only water is left as the by-product, thus eliminating the need for expensive effluent disposal treatments. In addition, it is a relatively inexpensive reagent, etc. (38). Furthermore, samples digested with hydrogen peroxide can be used in analyses involving ion chromatography (39), potentiometry (40), colorimetry (41), UV-induced photoxidation (42), and other traditional techniques such as the cadmium reduction method (N)(43) and the ascorbic acid method (P) (44). The oxidation strength of hydrogen peroxide is much enhanced when it is activated by the presence of an alkali, acid, metal ions or UV light. Activation via peroxyacid formation is the most common industrial use of H_2O_2 (37, 45-46).

In this study, a modified simultaneous procedure to determine nitrogen, phosphorus and sulphur is described. Hydrogen peroxide, formic acid and a microwave digestion system were used to oxidise nitrogen to nitrate, phosphorus to phosphate and sulphur to sulphate which were determined by ion chromatography. The methodology was validated using a number of inorganic and organic N,P and S containing compounds and reference materials.

Materials and Methods

Apparatus

A Dionex QIC analyzer ion chromatograph equipped with a Dionex AG4A guard column, a Dionex AS4A anion separation column, and a Dionex AMMS-II suppressor and conductivity detector was used. The sample was injected into the chromatograph via a 100 µL sample loop, and eluted with a solution of 1.8 mM sodium carbonate /1.7 mM sodium bicarbonate at a flow rate of 1 mL/min. A chart speed of 0.5 cm/s, conductivity range setting of 30 µS, and conductivity suppressor solution of 12.5 mM H_2SO_4 were used throughout.

A Milestone model MLS-1200 Mega microwave system (24010 Sorisole, Italy) was used for the digestion of the samples.

The digestion programme was as follows:

Step	Power (W)	Time (min)
1	250	5
2	0	15
3	600	10
4	Ventilation	10

Reagents

The column eluent was prepared from reagent grade sodium carbonate and bicarbonate, and distilled deionized water (18 MΩ-cm, nanopure, Millipore Corporation, Massachusetts 01730, USA) The suppressor solution was prepared from 1.4 mL Aristar grade sulphuric acid (Merck, Poole, Dorset, UK) and made up to 2 L with distilled deionised water. The following analytical grade compounds were subjected to the digestion treatment: sodium nitrite, urea, L-cysteine and ammonium nitrate (all supplied by Merck, Poole, Dorset, UK), L-lysine and sodium pyrophosphate (both supplied by Aldrich, Gillingham, Dorset, UK), sodium sulphite (East Anglia Chemicals, UK). A 22% $^{v}/_{v}$ solution was prepared from Aristar grade 30% $^{v}/_{v}$ hydrogen peroxide. A reference material rain water LGC 6018 was used to test the ion chromatograph response.

Sample preparation

To test the efficiency of the oxidation procedure, solutions containing 50 µL of formic acid and 40-100 mg/L in nitrogen, phosphorus or sulphur were prepared.

Standard reference materials oyster tissue (NIST, SRM 1566a) and Buffalo River sediment (NIST SRM 2704) were used to validate the digestion procedure.

Stock standard solutions

Individual 1000 mg/L stock standard solutions of nitrate-N, phosphate-P, sulphate-S and nitrite-N were prepared from Aristar grade reagents (supplied by Merck) by dissolving 6.0679 g $NaNO_3$, 4.3937 g KH_2PO_4, 1.8145 g K_2SO_4 and 0.2020 g of $NaNO_2$, respectively, in one litre of distilled deionised water.

Mixed anion standard solutions of 1.0, 2.5, 5.0 and 10.0 mg/L, respectively, were used to calibrate the ion chromatograph.

Sample digestion

Ten mL of hydrogen peroxide solution were added to 5 mL of sample or 0.2 g of a reference material and 50 µL of formic acid added to the microwave sample vessel. The mixture was capped and the microwave programme initiated. At the end of the first run, the sample was allowed to cool to room temperature, a further 10 mL of the same strength hydrogen peroxide solution was added and then the same programme was repeated. After oxidation, the digest was cooled to room temperature, made up to 100 mL with distilled deionised water, and analysed on the ion chromatograph. Each compound was digested and analysed at least five times.

Sampling

The study area is located between Latitude 9°32' and 110' North and Longitude 71°01'-72°01' West (Figure 1). The area was represented by 17 sampling points where the samples were taken during November 1998 and March 1999. Three samples of 1 L of water (at one meter depth) were taken at 15 sites and 1 Kg of sediment sample was taken at 13 sampling sites.

All the *in situ* parameters were measured on the R/V Bergantin, the research vessel of ICLAM. Physicochemical parameters including temperature, pH, conductivity, salinity, dissolved oxygen and redox potential were measured with a Hydrolab Surveyor II at different depths of the lake. Before sampling all the plastic bottles were carefully acid washed and then rinsed with deionized water. The samples of water were taken using a diaphragm pump (JABSCO, PAR-MAX4, model 30620-00-12), the plas-

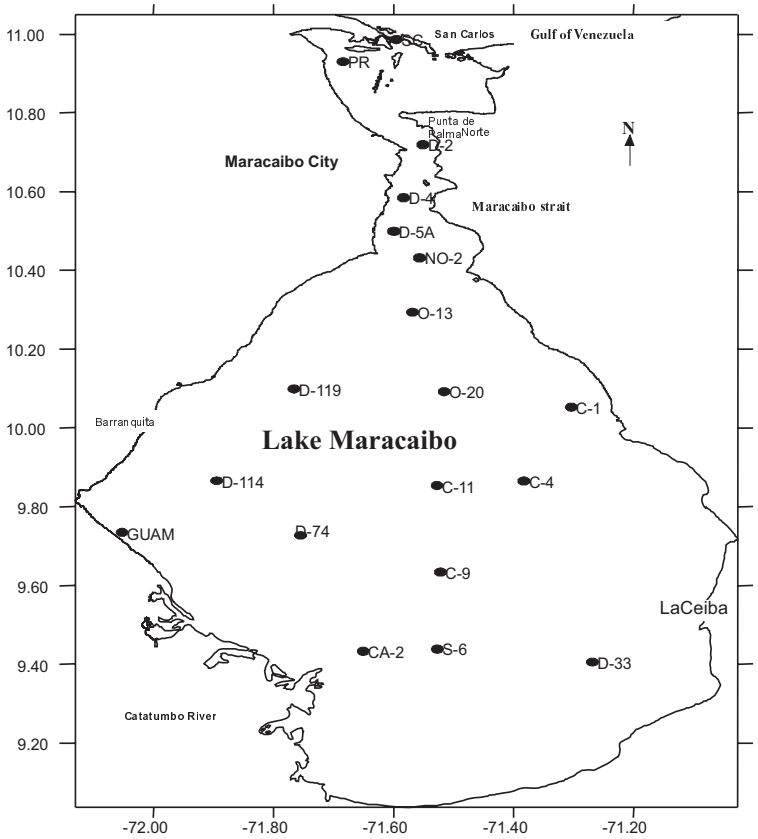


Figure 1. Lake Maracaibo and the sampling points.

tic bottles were rinsed with this water before the sample was taken. The samples were homogenized and kept at –4°C after sampling and during transportation to England.

The samples of sediment were taken using an Eckman dredge. The sediments were homogenized and kept in plastic bags at –4°C during sampling, after these were translated to a 50 mL plastic bottle and frozen to –20°C. The sediments were covered and mantained in the dark until analysis. Before lyophilization sediments were mantained frozen to avoid losses during defrosting.

The samples of sediment were lyophilised in a Hereaus Lyophilizer at –44°C for 12 h. After lyophilization, the sediments were homogenized with a mortar.

Results and Discussion

Concentration of the oxidising solution

Figure 2 shows the effect in percent recovery of varying hydrogen peroxide concentrations on the conversion of urea, sodium pyrophosphate and L-cysteine to nitrate, phosphate and sulphate, respectively.

It has been suggested that the oxidising power of hydrogen peroxide is enhanced when it is activated by either acid, metal ions or is exposed to UV light (21).

The extent of conversion of urea to nitrate was much improved (Figure 3) when a second 10 mL aliquot of the same concentration hydrogen peroxide solution was added and the sample subjected to the microwave programme for a second time. In subsequent experiments, 22% $^{v}/_{v}$ hydrogen

peroxide and the two stage digestion procedure were used to test the efficiency of the oxidation process on a variety of compounds.

Tables 1 and 2 summarise the extent of oxidation expressed as recoveries of total nitrogen, phosphorus and sulphur. Varying the amounts of urea, L-cysteine and sodium pyrophosphate did not affect the extent of oxidation (see Table 2). The very good recovery values indicate that the oxidation process is efficient at converting N, P and S from the form in which they occur in these compounds. The efficiency of the procedure in oxidizing compounds containing nitrogen-nitrogen bonds or amide groups, and condensed polyphosphates is currently being assessed. A comparison of the expected and found values for N, P, S (Table 1) using a paired-t test was found not to be statistically significant at the 95% confidence limits except for the L-cysteine for which high recov-

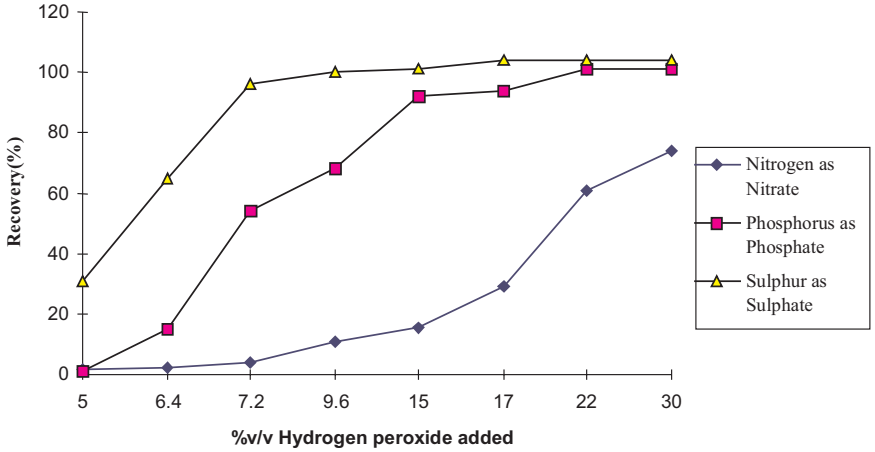


Figure 2. Effect of varying hydrogen peroxide concentration on the recovery of nitrogen, phosphorus and sulphur from urea, sodium pyrophosphate, and L-cysteine, respectively after the first digestion.

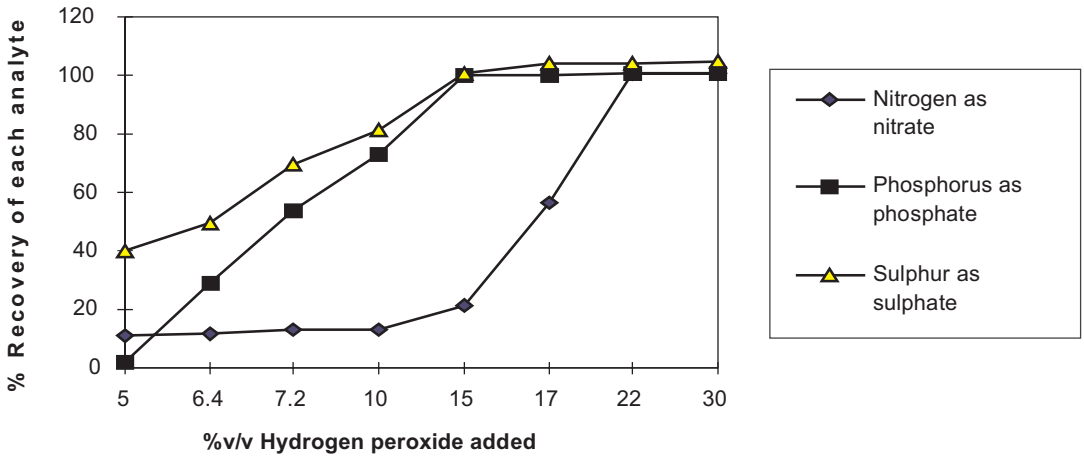


Figure 3. Effect of varying hydrogen peroxide concentration on the recovery of nitrogen, phosphorus and sulphur from urea, sodium pyrophosphate, and L-cysteine, respectively after the second digestion.

Table 1
Recoveries of nitrogen, phosphorus and sulphur as nitrate, phosphate and sulphate ions from different concentrations (mg/L) of pure compounds after digestion with 22% $^{v}/_{v}$ hydrogen peroxide (n = 5)

Compound	$N\text{-}NO_3^-$ Expected	$N\text{-}NO_3^-$ Found	$P\text{-}PO_4^{3-}$ expected	$P\text{-}PO_4^{3-}$ found	$S\text{-}SO_4^{2-}$ Expected	$S\text{-}SO_4^{2-}$ found
Urea	9.93	9.96 ± 0.62				
L-Lysine	4.00	4.01 ± 0.04				
Ammoniun nitrate	6.49	6.68 ± 0.06				
Sodium nitrite	10.0	10.02 ± 0.08				
L-Cysteine	2.26	2.12 ± 0.01			5.17	6.10 ± 0.01
Sodium Pyrophosphate			9.78	9.80 ± 0.13		
Sodium sulphite					5.38	5.35 ± 0.04
Mix l-Cysteine and sodium pyrophosphate	2.26	2.12 ± 0.01	9.78	9.65 ± 0.26	5.17	6.11 ± 0.02

Table 2
Recoveries of nitrogen, phosphorus and sulphur using different concentrations of analyte and 22% v/v hydrogen peroxide

Compounds	Concentration expected (mg/L)	Concentration found(mg/L)	% Recovery
Urea ($N\text{-}NO_3^-$)	5.00	4.85	97.0
	9.93	10.40	104.7
	6.00	5.42	90.3
	6.24	5.50	88.1
	8.00	7.45	93.1
L-Cysteine($S\text{-}SO_4^{2-}$)–	23.10	22.59	97.7
	11.48	12.11	105.4
	15.11	14.44	95.5
	10.00	10.50	105.0
	5.17	6.10	117.0
Sodium Pyrophosphate ($P\text{-}PO_4^{3-}$)	10.00	9.56	95.6
	20.50	22.19	108.2
	6.49	6.72	103.5
	31.8	30.13	94.7
	9.78	9.70	99.2

eries were obtained. The difference in the results could be due to the poorer sensitivity for the determination of sulphate ions at low concentrations.

Analytical performance

A chromatogram of a mixture of L-cysteine and sodium pyrophosphate after oxidation is shown in Figure 4. The mean ± sd retention times for nitrate, phosphate and sulphate ions were: 4.11 ± 0.14, 6.60 ± 0.05 and 8.65 ± 0.24 min, respectively. The three peaks are very well resolved and as a result samples containing widely different proportions of the analytes can be analyzed without interferences.

Calibration graphs obtained from mixed anion standards gave the following highly linear best-fit equations:

Nitrate: $y = 1.18 \times 10^7 x - 7.18 \times 10^6$
($r^2 = 0.9970$)

Phosphate: $y = 4.71 \times 10^7 x - 3.78 \times 10^6$
($r^2 = 0.9886$)

Sulphate: $y = 4.37 \times 10^6 x - 3.76 \times 10^6$
($r^2 = 0.9865$)

y = Peak area (arbitrary units)

x = Anion concentration (mg/L)

Detection limits were calculated from the calibration graphs using the method of Miller and Miller (8). The results were 0.123 mg/L N-nitrate, 0.251 mg/L P-phosphate and 0.850 mg/L S-sulphate. The detection limits based on 0.2 g of sediment were 0.006% w/w N, 0.012% w/w P and 0.042% w/w S.

Method validation

The N, P and S contents for NIST SRM 1566a oyster tissue and NIST SRM 2704 Buffalo river sediment samples digested with 22% $^{v}/_{v}$ hydrogen peroxide are given in Table 3. Satisfactory agreement with the certified values was obtained. The presence of a sample matrix did not have an adverse effect on the recoveries.

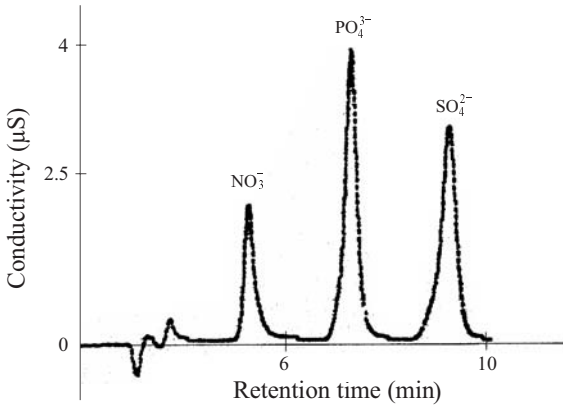


Figure 4. Chromatogram of a sample containing L-cysteine and sodium pyrophosphate after oxidation to nitrate (1), phosphate (2) and sulphate(3).

The proposed method for the oxidation of N, P and S followed by the determination of the nitrate, phosphate and sulphate ion by ion chromatography gave satisfactory results for the compounds tested. The effectiveness of this procedure is demonstrated by the good recoveries obtained for the two SRMs, oyster tissue and Buffalo river sediment. However, this work was focused on the application of the method to more recalcitrant compounds where the N, P and S atoms are in ring systems.

Chemical speciation of nitrogen, phosphorus and sulphur

This previously reported method (39) was modified in order to extend the range of compounds that can be analysed for total nitrogen, phosphorus and sulphur. Parameters affecting the extent of oxidation such as microwave power, hydrogen peroxide concentration and microwave program sequence were optimised. By altering the amount of hydrogen peroxide added to the sample, and the stepwise use of the micro-

Table 3
Comparison of the quantities of nitrogen phosphorus and sulphur found using the proposed method and the reported values for the standard reference materials (n = 3)

Element		Oyster Tissue (%w/w ± 95% confidence limit)	Buffalo River (%w/w ± 95% confidence limit)
N	Found	6.62 ± 0.28	
	Reference value	6.81	
P	Found	0.62 ± 0.02	0.09 ± 0.01
	Certified	0.62 ± 0.02	0.10 ± 0.01
S	Found	0.87 ± 0.01	0.43 ± 0.05
	Certified	0.86 ± 0.02	0.40 ± 0.01

wave programme, it was possible, depending on the nature of the compound, to control the extent of the oxidation. Anions formed after oxidation of the samples were separated and determined by ion chromatography with conductivity detection. The developed procedures were validated using pure compounds: sodium nitrite, sodium sulphite, L-cysteine, lysine, phosphonitrile chloride, saccharin, urea, and reference material prawn GBWO8572.

Modified method

The instrumental settings for the microwave digestion were modified so that stepwise oxidation of the following compounds could be achieved. Parameters such as hydrogen peroxide added to an organic acid, the power of the microwave, and time of digestion were studied in order to control the oxidation to nitrate, phosphate and sulphate respectively.

The modified microwave programme was as follows in Table 4.

Sample digestion

Ten mL 30% v/v hydrogen peroxide were added to 1 mL of sample or 0.2 g of a reference material followed by 50 µL of formic acid in the microwave vessel. The mixture was capped and the microwave programme initiated. For organic nitrogen and sulphur compounds, at the end of the first run, the sample was allowed to cool to room temperature, a further 5 mL of the same strength of hydrogen peroxide solution was added and then the same programme was repeated. For cyclic compounds and the reference material, an additional step was included after addition of 5 mL hydrogen peroxide. After oxidation, the digest was cooled to room temperature, made up to 25 mL with distilled deionised water, and analysed on the ion chromatograph.

Table 4
Microwave conditions in each step used with the modified method

Step	Power (W)	Time (min)
1	250	5
2	0	15
3	450	10
4	0	10
5	650	10
6	Ventilation	15

Inorganic and linear organic compounds

The Figure 5 (a, and b) shows variation of the conversion of urea to nitrate when dif-

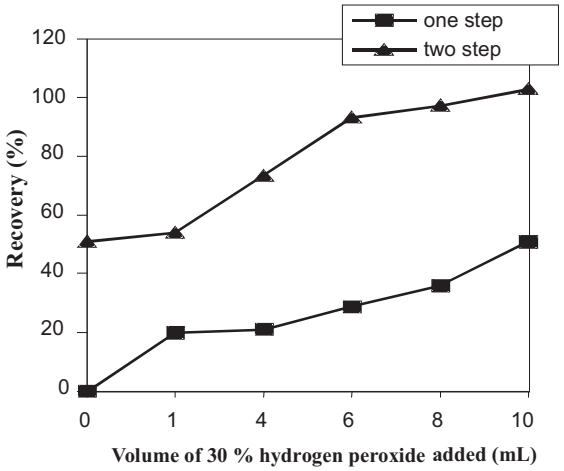


Figure 5 (a). Recoveries of nitrogen from a solution of 9.72 mg/L of urea when different microwave program steps are used.

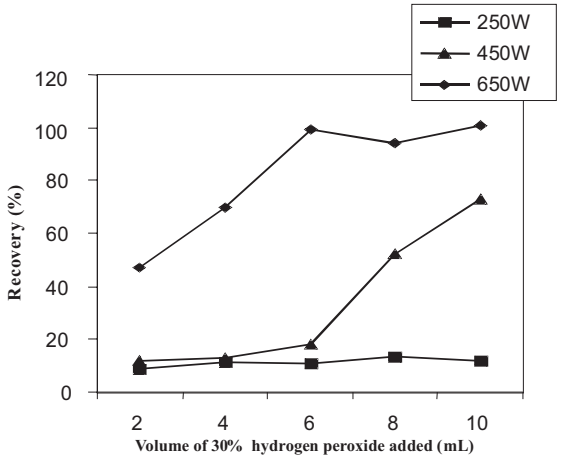


Figure 5 (b). Recoveries of nitrogen using different power.

ferent powers of the microwave, and one or two oxidation steps were used. An appreciable increase in the recovery is seen with the additional step. The nitrite (Figure 6, a and b) and ammonium ions (Figure 7, a and b) are converted to nitrate in only one step.

The Figure 6 (b) shows the chromatogram following oxidation of the nitrite so-

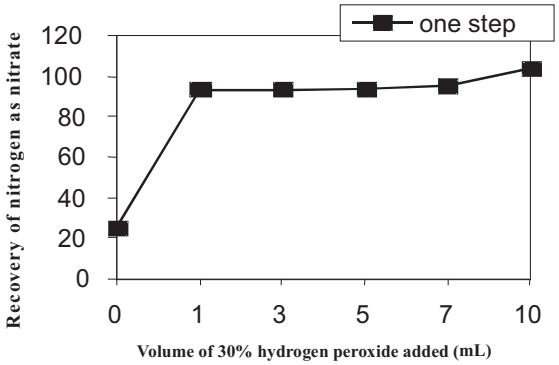


Figure 6 (a). Recoveries of nitrogen from a solution of 40 mg/L of sodium nitrite when a one step program is applied.

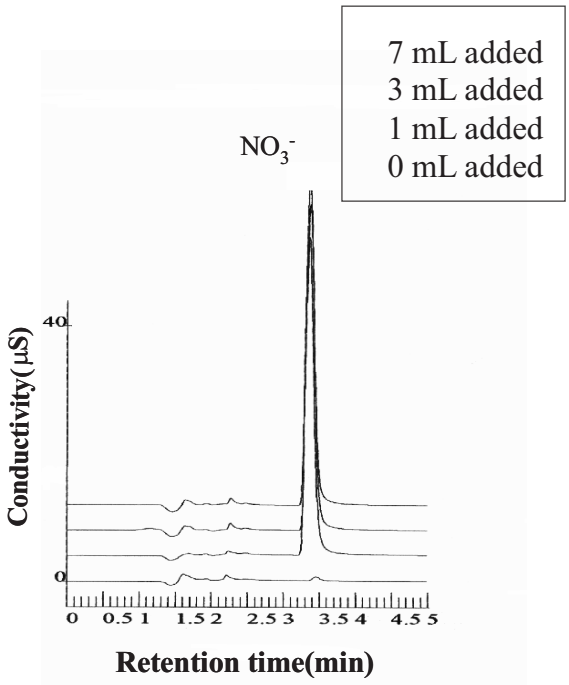


Figure 6 (b). Chromatogram of the 40 mg/L solution of nitrite after oxidation with hydrogen peroxide.

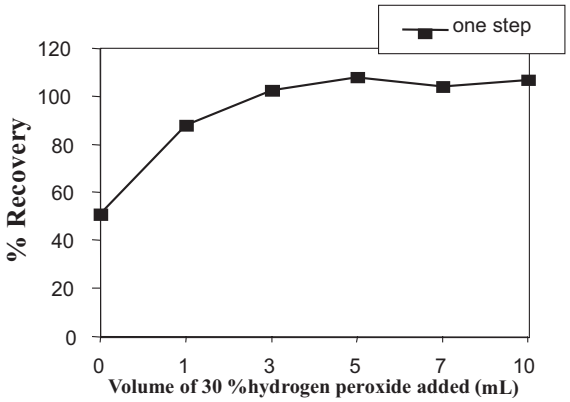


Figure 7 (a). Recoveries of nitrogen from ammonium chloride when a one step program is applied to the sample.

lution with different concentrations of hydrogen peroxide; the nitrate peak increases in size with the addition of hydrogen peroxide until total conversion to nitrate is achieved.

Organic compounds such as urea, L-Lysine and L-cysteine require a two step programme in order to convert to them to nitrate and sulphate, respectively (Table 5).

Cyclic organic compounds

Experiments were performed with the cyclic compounds saccharin (FW = 183.19) and phosphonitrile chloride (FW = 347.66).

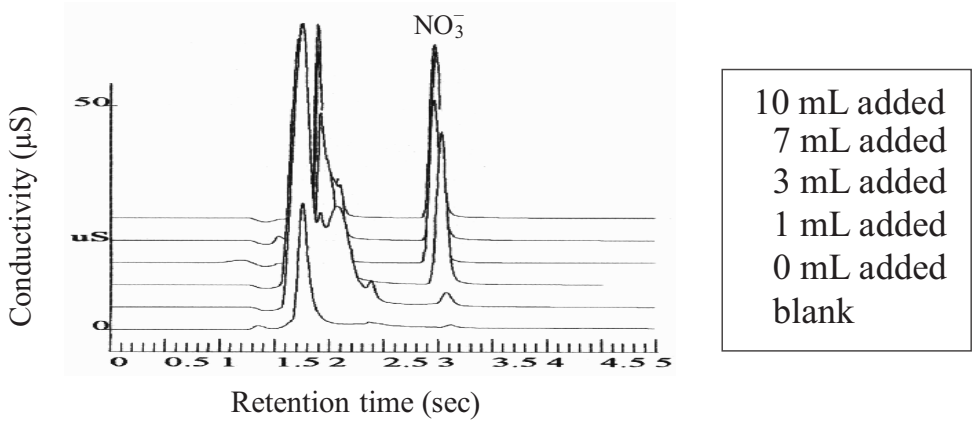
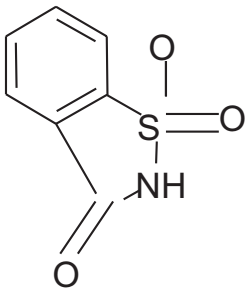


Figure 7 (b). Chromatogram showing the variation of the nitrate peak from ammonium chloride when different volumes of hydrogen peroxide are added to the sample.

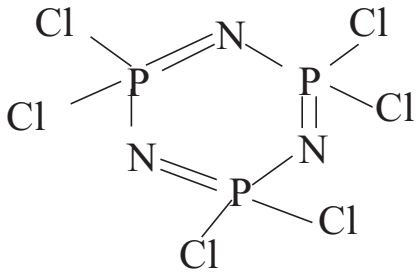
Table 5
Recoveries of nitrogen, phosphorus and sulphur obtained using different compounds and the modified programme

Compound	Added (mg/L)	Found (mg/L)	Recovery (%)
Sodium sulphite(S)	25.8	26.0 ± 0.2	100.7
Sodium pyrophosphate (P)	30.9	30.9 ± 0.1	100.1
Sodium nitrite (N)	8.1	8.6 ± 0.6	106.0
Ammonium chloride(N)	40.0	42.5 ± 2.1	106.0
L-Lysine (N)	7.1	7.2 ± 0.2	101.4
Urea (N)	17.7	17.0 ± 1.2	96.0
Saccharin (N)	2.3	2.2 ± 0.1	95.6

Saccharin



Phosphosnitrile Chloride



In Figure 8 (a and b), the recoveries of saccharin in response to varying amounts of hydrogen peroxide added and the number of steps used, and the resulting chromatograms, are shown. All of the nitrogen is converted to nitrate in the third step. Sulphate is also formed in this step.

Figure 9 shows a graph of percentage of conversion of N and P from a solution of 51.4 mg/L digested in one, two and three steps. Three steps are required to convert all the phosphorus in phosphonitrile chloride to phosphate.

The Table 6 shows the recovery of nitrogen and sulphur from a mixture of nitrite, urea and saccharin and a mixture of L-lysine, saccharin and cysteine.

The Figure 10 shows the presence of a sulphite peak in the first and the second steps, before the sulphate peak.

As shown in Table 7 most of the nitrogen is converted to nitrate in the third step from the prawn reference material (Prawn GBW08572). An increased recovery of 70% from the second to the third steps indicates

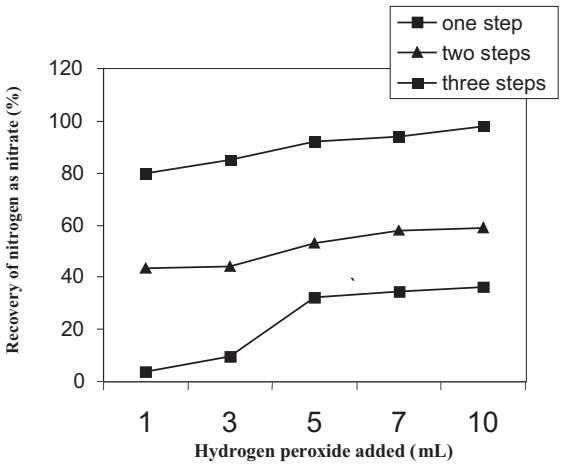


Figure 8 (a). Variation of the recoveries of nitrogen from saccharin when a different step program is used.

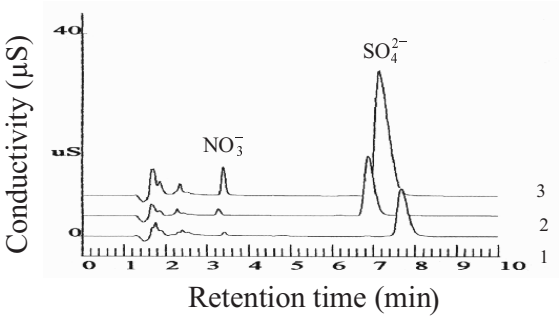


Figure 8 (b). Chromatogram of the saccharin solution after oxidation.

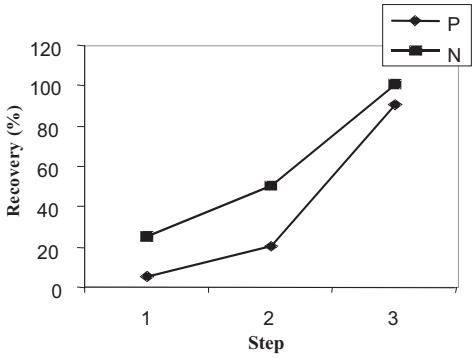


Figure 9. Recoveries of phosphorus and nitrogen from a solution of phosphonitrile chloride using a three step program method.

Table 6
Recoveries of nitrogen obtained from two mixtures: Mixture 1: containing 12.71 mg-N/L as nitrite, 9.72 mg-N/L urea and 2.96 mg-N/L saccharin; Mixture 2: 4.05 mg-N/L as nitrite, 7.12 mg-N/L L-lysine and 2.96 mg-N/L saccharin respectively

Element	One step	Two steps	Three steps	Added (mg/L)	Found (mg/L)
Mix 1 (N) (mg-N/L)	18.5	22.8	25.0	25.4	25.6
Recovery (%)	72.8	89.6	100.7		
Mix 2 (N) (mg-N/L)	7.7	11.4	15.0	14.3	15.0
Recovery (%)	54.1	79.8	104.8		

Table 7
Recoveries of nitrogen and phosphorus obtained from a reference material (Prawn GBW08572) using the modified programme

Element	One step(%)	Two steps(%)	Three steps(%)	Added (%w/w)	Found (%w/w)
N	21.5	25.0	95.9	14.1	13.6
P	34.8	93.6	108	0.85	0.91

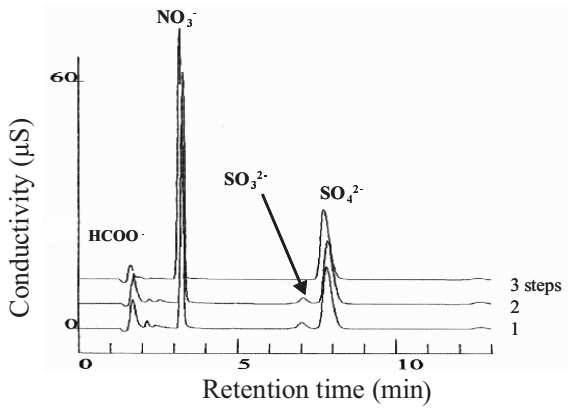


Figure 10. Chromatogram of a digestion of a mix of saccharin, nitrite and L-lysine after the three steps runs method.

that the nitrogen is found probably in a cyclic compounds, whereas most of the phosphorus is present as phosphate or related inorganic form.

In order to ensure that there are not cyclic compounds present, if is advisable to carry out the three step digestion programme so that all nitrogen is converted to nitrate. The varying amount of recovery after each step is maybe indicative of the nature of the compounds present in the sample. On the basis of this difference in behaviour it maybe possible to obtain speciation information from the samples.

Environmental results

The method for nitrogen, phosphorus and sulphur total content was applied to the samples of water and lyophilised sediment from Lake Maracaibo, and the ion chromatographic determinations were validated with two reference materials for nitrate, phosphate and sulphate in rain water and in river water, giving a good agreement. The results are shown in Table 8.

The forms of greatest interest in waters are nitrate, nitrite, ammonia and organic nitrogen, and all can be oxidised by the hydrogen peroxide in the proposed method for total nitrogen.

Nitrogen levels in aquatic system, as with phosphorus, are intimately linked with excessive algal growth, as are seen in Lake

Maracaibo. Total nitrogen levels in waters can vary from as low as 0.1 mg/L to in excess of 10 mg/L in heavily polluted aquatic systems (47). These total nitrogen levels are exceeded in most of the sampling points in Lake Maracaibo, for which total nitrogen varies in the range 1.6-135.0 mg/L (Table 9).

Apart from the natural input of nitrogen from rainfall, the main inputs of nitrogenous matter into freshwater is from agricul-

Table 8
Results of the reference materials (Rain water LGC 6018) for ion chromatography

Element	Rain Water (found) (mg/L)	Rain Water (RM) (mg/L)
N	1.1 ± 0.1	1.0 ± 0.3
S	4.8 ± 0.4	5.3 ± 0.2

*SD: Standard deviation.

Table 9
Results of the total (mg/L) nitrogen, phosphorus and sulphur in water samples from Lake Maracaibo determined by the three steps program

Sampling points	N (mg/L)	SD	P (mg/L)	SD	S(mg/L)	SD
PR	2.2	0.2	< 1.0		24.7	0.8
SC	1.6	0.0	< 1.0		22.6	1.4
D-2	9.5	0.4	< 1.0		18.4	0.7
D-4	76.8	0.9	4.7	0.1	54.3	0.5
D5a	11.6	0.6	6.3	5.9	53.0	1.4
NO2	135.0	7.8	1.2	0.1	270.3	9.6
O-13	75.7	1.1	< 1.0		76.3	3.1
O-20	101.4	0.4	< 1.0		733.0	122.3
C-1	19.8	1.3	< 1.0		187.6	15.5
C-11	60.5	6.0	10.8	1.7	250.5	22.1
C-9	63.7	4.4	6.5	0.6	942.4	37.7
CA-2	109.0	2.9	8.1	2.2	239.0	19.4
D-33	64.2	0.8	10.6	1.4	140.0	1.3
D-119	126.1	1.9	< 1.0		575.7	99.7
D114	97.4	0.1	< 1.0		1009.0	10.9
S-6	124.0	1.9	< 1.0		128.1	12.5
Guam	75.6	0.5	20.3	0.1	1227.6	121.9
D-74	5.40	0.5	1.2	0.0	1.06	0.0

tural land (47), via wastewater point discharges or diffuse runoff. Lake Maracaibo also receives wastewater discharges from the cities of Maracaibo, Cabimas and Santa Rita, without any pre-treatment. Most of the area around the Lake is covered by farms that also can contribute to the nitrogen input to the lake.

Total phosphorus in waters can vary from less than 0.01 mg/L in uncontaminated waters to over 1 mg/L in heavily polluted rivers (48-49). Nitrogen may be a limiting nutrient in some situations (50) but phosphorus is generally regarded as the limiting nutrient for primary production (51), as shown by the results determined in Lake Maracaibo. Excessive loading of phosphorus in its various physico-chemical forms is known to be causal factor in the eutrophication of waters. Furthermore, classification of the trophic status of standing water bodies is still largely based on the total phosphorus concentration (52). The maximum levels of phosphorus encountered in Lake Maracaibo (ca. 20.3 mg/L) correspond to a general level of productivity of a hyper-eutrophic lake, in terms of the Vollenweider classification, modified by Wetzel (53), as shown in study of this lake during 1998 (54).

The values for total nitrogen content in water and sediments (Table 10), are very high if they are compared with a subtropical bay in Oahu, Hawaii, for example, where Stimson and Larned determined the nitrogen efflux from the sediments (55). The maximum concentrations of the dissolved nitrogen in positions close to the sediments were in the range of 0.38-0.72 µM. The concentration in water one meter in depth in Lake Maracaibo exceeds this range, but the concentration of total phosphorus was

Table 10
Total nitrogen, phosphorus and sulphur (µmol / g) found in sediments during the sampling of Lake Maracaibo and determined by the three step program

Sampling points	N	SD	P	SD	S	SD
PR	3.9	0.3	< 0.03		320.5	12.0
SC	0.9	0.4	< 0.03		33.0	3.0
D-2	3.2	0.3	< 0.03		287.8	39.1
D-4	4.8	0.3	< 0.03		770.3	29.0
D5a	1.7	0.1	1.2	0.1	11.7	1.20
NO2	5.2	0.3	1.3	0.1	470.9	11.6
O-13	2.6	0.1	9.5	0.1	1719.2	35.4
O-20	10.6	1.1	40.6	3.9	1947.3	40.5
C-1	4.2	0.4	31.1	0.3	132.8	13.4
C-11	9.6	1.9	12.9	1.2	1248.0	27.6
C-9	8.1	1.5	15.0	1.0	1176.9	69.5
CA2	0.1	0.1	27.1	1.0	1412.3	30.6
D-33	1.0	0.1	10.4	1.0	1975.6	91.1

lower (except O-20) than those found in other lakes such as Lannngjon, Flaten and Gommaren in Sweeden where the range of total phosphorus is [36.2-62.6 µmol/g](56).

Sediments can accumulate sulphur in the range of 132.8 (µmol/g) where pyrite is the most common mineral form of sulphur (5). In Lake Maracaibo, the concentration of sulphur is in the range 11.6-1975 µmol/g and these concentrations could be associated with the intrusion of salt waters from the Caribbean Sea to the lake. It is also characteristic of depletion of dissolved oxygen, with concentrations of oxygen around zero mg/L in the centre. In this zone, sulphur occurs as a reducible form of mostly HS^-, and it can result in the precipitation of metals such as Hg, Pb and Se.

Conclusions

The modified method for the microwave oxidation with hydrogen peroxide of nitrogen, phosphorus and sulphur followed by the determination of the nitrate, phosphate and sulphate ion by ion chromatography gave satisfactory results for the compounds tested. This method was modified in order to applied the methodology in more recalcitrant compounds where nitrogen, phosphorus and sulphur atoms were in ring system. The amount of recovery after each step in the modified method could be indicative of the nature of the compounds present in the sample. The results of Lake Maracaibo showed high concentrations of the three elements (N, P and S) in the samples of water and sediment. Lake Maracaibo can be classified as a hyper-eutrophic lake because the high concentrations of phosphorus.

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