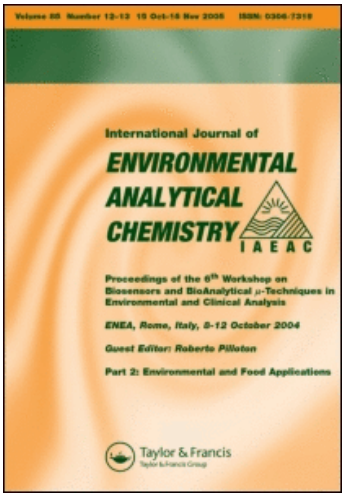

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Improvement of the Kjeldahl Method for Total Nitrogen Including Acid-Hydrolyzable Phosphorus Determinations in Freshwater Ecosystems

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Improvement of the Kjeldahl Method for Total Nitrogen Including Acid-Hydrolyzable Phosphorus Determinations in Freshwater Ecosystems

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A simple, faster and accurate alternative procedure for the traditional Kjeldahl method is reported. Besides total nitrogen determinations it also allows the determination of acid-hydrolyzable phosphorus. The procedure does not distinguish $N\text{-}NO_2$ and $N\text{-}NO_3$. The method is sensitive at the μg level in samples containing 10 to 200 $\mu g\, N\, l^{-1}$ and 10 to 200 $\mu g\, P\, l^{-1}$. Nitrogen and phosphorus are determined colorimetrically. Reproducibility is better than $\pm 10\%$. $P\text{-}PO_4$ and $N\text{-}NH_3$ in water, sediments, macrophytes and terrestrial plants collected in central Chile have been determined using the method reported here.

KEY WORDS: Freshwater, sediments, nutrients, Kjeldahl, nitrogen, phosphorus.

INTRODUCTION

Most of the organic N-compounds present in continental waters and sediments are digested by sulphuric acid. So far two methods have gained general acceptance for determination of total-N; the Kjeldahl method (1883),[10] which is essentially a wet oxidation procedure and the Dumas method (Kirsten, 1947),[9] which corresponds to a combustion (dry oxidation) procedure.

The conventional Kjeldahl procedure involves two steps:

a) Digestion of the sample to convert the nitrogen to ammonia. It is usually performed with H_2SO_4 and one or more catalysts.

b) Determination of the ammonia in the digest by titration of the ammonia liberated by distillation of the digest with alkali.

The regular macro; and semimicro-Kjeldahl methods have the disadvantage of being very intensive in labour and/or insufficiently precise.

Here we present an alternative procedure, faster and more accurate, for Kjeldahl-nitrogen determination, which also allows the determination of acid-hydrolyzable phosphorus. Kjeldahl-N is determined colorimetrically as ammonia by a variant of the indophenol blue complex method.[2,13] Acid-hydrolyzable phosphorus is determined colorimetrically as $P\text{-}PO_4$ by the phospho-molybdenum-blue method.[12] Measurements were made in a spectrophotometer Shimadzu model UV-Visible 150.

EXPERIMENTAL

The digestion is performed with H_2SO_4, facilitated by the presence of hydrogen peroxide (H_2O_2). The bound nitrogen and acid hydrolyzable phosphorus are converted to ammonium and orthophosphate ions respectively. The process does not distinguish between $N\text{-}NO_2$ and $N\text{-}NO_3$.

Range

This method is suitable for nitrogen compounds in samples with concentrations between 100 and 200 $\mu g\, N\, l^{-1}$. Phosphorus com-

pounds should be in the range of 10–200 $\mu g\, P\, l^{-1}$. Samples with higher concentrations require dilution.

The alu-block digestor

This kind of digestor is available commercially on the market but it is relatively expensive. With the simple device described here (Figure 1), a large number of samples with replicates can be digested at the

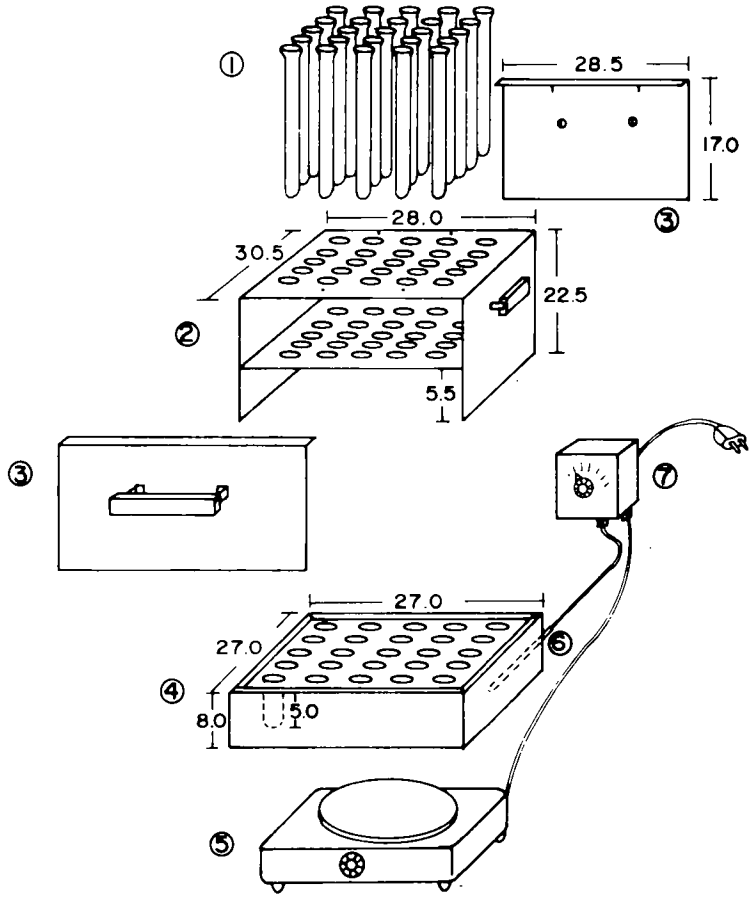

FIGURE 1 The Alu-block digestor. (1) Pyrex test tubes, 25.0 cm x 2.5 cm. (2) Test tube rack. (3) Cover. (4) Alu-block. (5) Electrical heater 1.5–2.0 kw. (6) Thermistor, Jumo K20300 T/C: FeKonst. (7) Electronic temperature controller, Jumo G21100 type Q50 w(t)-96/rell. Measures are given in cm.

same time. The device can be used in the analysis of Kjeldahl-N, total-P, and COD among others.

The calibration standard (without digestion) and Kjeldahl standard series

These were determined for both nitrogen and phosphorus. For nitrogen, a standard solution ($1\,\mu g\,ml^{-1}$ ammonium nitrogen) of ammonium sulphate was used. For phosphorus two standard solutions ($4\,\mu g\,ml^{-1}$ orthophosphate phosphorus and $0{,}4\,\mu g\,ml^{-1}$ orthophosphate phosphorus of potassium hydrogen phosphate (KH_2PO_4) were used. The Kjeldahl standard series were treated in the same way as the samples.

Samples

Freshwater samples from lakes (Rapel, Aculeo) rivers (Mapocho, Angostura), wells (Peñaflor), lake sediments (Aculeo) and biological samples (Macrophytes and terrestrial plants) were analysed for Kjeldahl-N and P by the Alu-block method. Samples were digested immediately after collection.

Digestion

a) *Water samples:* Exactly 25 ml of water samples (10 ml or 5 ml for samples with high nutrient concentration) are placed in a thick-walled pyrex tube (Figure 1). Exactly 0.2 ml concentrated sulphuric acid (p.a.) is added to each tube. The tubes are placed in the heating block and water in the samples evaporated at 105–110°C. At the end a dark, acidified, digested material should remain at the bottom of the test tube. Special care must be taken to keep the temperature constant within this range. Once the evaporation stage is completed (it may take up to 15 hrs) the samples are cooled and 2 drops (0,15 ml) of hydrogen peroxide (30%) (p.a.) are added.

The tubes are then returned to the heating block. The temperature of the heating block is set to $250°C \pm 2°C$ and the water samples are digested for 3/4 hour. Care must be taken to allow air cooling of the upper part of the tubes during this step. After digestion the samples should be colourless. Otherwise, 2 drops (0.15 ml) more of hydrogen

peroxide are added and the digestion repeated. The tubes are cooled and about 5 ml distilled water are added to each. The tubes are reheated for 1 minute in order to dissolve any crystals formed during the digestion. The volume is made up to 50 ml with deionized water. 25 ml of the sample are transferred to a measuring cylinder. The procedure is repeated for each subsample (25 ml) for the determination of ammonia and orthophosphate respectively (see below).

b) *Sediment samples:* Exactly 10.0 mg of dried and finely ground (at least 100 mesh) sediment samples are weighed in an analytical balance and transferred into a thick-walled pyrex tube, made up to 25 ml W/V with deionized water and evaporated as for water samples. Sediment samples are digested at 250°C ± 2°C for at least 4.5 hours with 0.3–0.4 ml of H_2O_2. If necessary an extra 0.1 ml may be added, otherwise the procedure is as for water samples. Sediment digests do not clear (become colourless) as they generally contain a considerable amount of suspended material.[4]

Kjeldahl-ammonia nitrogen determination by the indo-phenol-blue method

Ammonia reacts with phenol and hypochlorite under alkaline condition to form indophenol blue complex, the colour intensity being proportional to ammonia concentration. Nitroprusside is used as a catalyst to facilitate colour development at room temperature.

Reagents: 1) Ammonia-free deionized water (AFW).

2) NaOH-EDTA solution. Dissolve 130 g NaOH (P.A.) in 1 l 0.1 M EDTA III (p.a.). This solution is used to prepare the hypochlorite solution. Stored in a polyethylene bottle, it is stable for a long time. This NaOH-EDTA solution must be checked in the following way: Add 0.2 ml conc. H_2SO_4 in 50 ml AFW and mix. Add 2.0 ml NaOH-EDTA solution and mix. The pH should be 10–12.

3) Hypochlorite solution: 120 mg dichlor sodium cyanurate (p.a.) is dissolved in 100 ml reagent 2. It is stable for 10 to 15 minutes.

4) Phenolic solution: dissolve 12 g Na-salicylate (p.a.) and 100 mg Na-nitroprusside (p.a.) in 100 ml AFW. It is stable for 10 to 15 minutes.

5) Concentrated sulphuric acid (s.g. 1.82) p.a.
6) Hydrogen peroxide (30%) p.a.

Experimental procedure: This step must be done in dim light. Add 2 ml phenolic solution to the sample and mix. Add 2 ml hypochlorite solution and mix. Place the samples in a dark place. After 90 minutes and within the next 48 h, measure the extintion at 685 nm.

Kjeldahl-orthophosphate determination

The same reagents as in Murphy and Riley, (1962) are used. Measurements are made at 720 nm.

Sources of error: Traces of hydrogen peroxide can interfere with the Kjeldahl-N determination with development of a blue-green-indophenol complex, yielding a yellow colour with low extintion values. The pH of sample solution after digestion is critical in both $N\text{-}NH_3$ (11–12) and $P\text{-}PO_4$ (1.5) determinations, otherwise colours will not develop.

RESULTS AND DISCUSSION

Results for the correlation between calibration standard series (without digestion) and Kjeldahl standard series for $N\text{-}NH_3$, $P\text{-}PO_4$ are presented on Figures 2a and 2b respectively. Each concentration value represents the mean of four determinations ± 1 standard deviation (vertical bars). In the concentration range 10 and 200 $\mu g\,l^{-1}$ no significant difference between calibration and Kjeldahl adsorbance means $(\bar{x}, \bar{y})$ values were observed. The method should not be applied to samples with more than 200 $\mu g\,l^{-1}$ P and/or N concentration. The minimum detectable concentration was determined by the analysis of standard samples of potassium dihydrogenphosphate, sodium glycerophosphate, urea and ammonium sulphate in the range 0.00 $\mu g\,l^{-1}$ to 20.00 $\mu g\,l^{-1}$ of N and P respectively. Sampling was carefully controlled. Triplicates of each concentration were assayed. Yields of standard samples ranged between 97% and 102% of a theoretical $100\% \pm 3\%$. Reproducibility of the Alu-block method for the water, macrophytes and sediment samples that were assayed

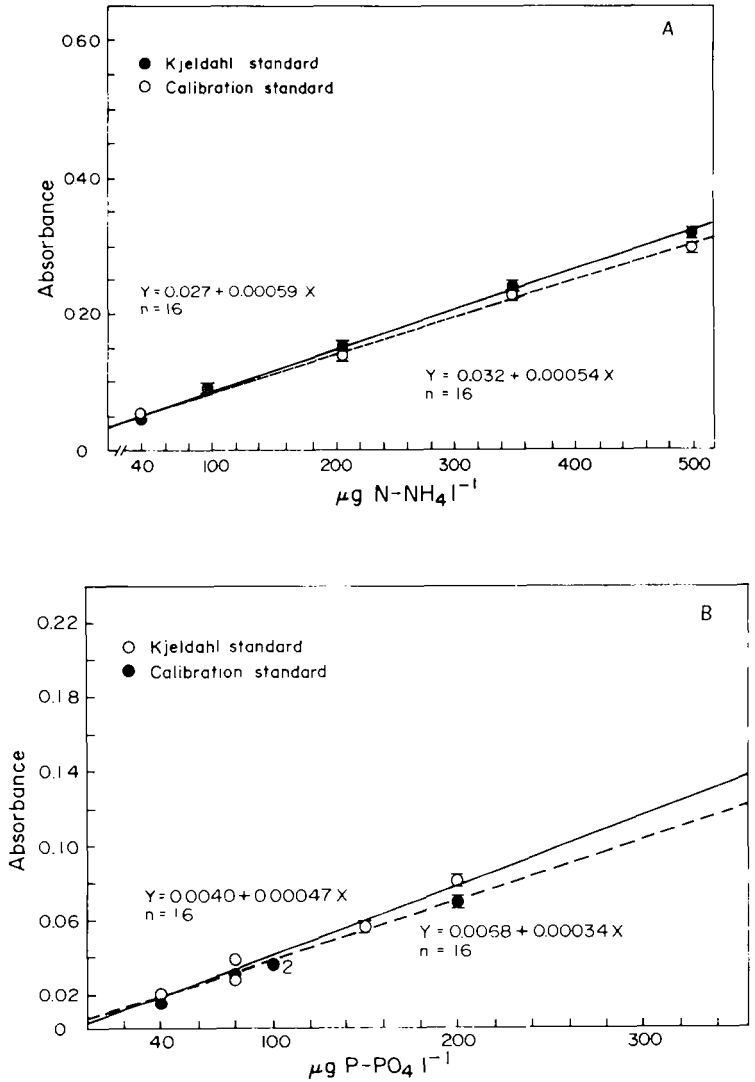

FIGURE 2 Correlation between calibration standard series (without digestion) and Kjeldahl standard series for $N\text{-}NH_3$(A) and $P\text{-}PO_4$ (B) by the Alu-block method. Blank absorbances for $N\text{-}NH_3$ calibration standard (0.0085) and Kjeldahl standard (0.01) were measured. Blank absorbances for $P\text{-}PO_4$ standards fluctuated between 0.00 and 0.002.

(Table I). Results showed an average deviation better than 10% in repeat determinations of $N\text{-}NH_3$ and $P\text{-}PO_4$. Nitrogen and phosphorus concentration levels were within the expected ranges described by Margalef[11] for macrophytes and Bolin and Cook for sediments.[3]

Experiments were designed for testing the method in sediment samples for the effect of temperature, digestion time, sample size, amount of catalyst and operator errors.

Bremner[4] has shown that the most important factor in Kjeldahl digestion of soils is the temperature of the treatment with H_2SO_4. Loss of N occurs when the temperature of digestion exceeds about 400°C.[5,7] Our experience with the Alu-block shows that a temperature of 250°C was satisfactory for total N-analysis. Higher temperatures (up to 300°C) did not increase nitrogen or phosphorus values significantly.

The results for different digestion times used for sediment samples indicate that the higher values of $N\text{-}NH_3$ and $P\text{-}PO_4$ were obtained with a digestion time of 270 minutes. Further digestion failed to produce a significant increase in the amounts of nitrogen or phosphorus. Our results are consistent with digestion times recommended by Bremner[5] for soil samples treated by a conventional macro-Kjeldahl. Goltermann *et al.*,[7] recommend a digestion time of one hour for water samples. A digestion time of 45 minutes for water samples and one hour for biological samples gave satisfactory results with the Alu-block method.

Conventional macro-Kjeldahl methods use soil samples in the range of 0.25 g to 5.0 g.[7] Our, results show that for the Alu-block method satisfactory values of $N\text{-}NH_3$ and $P\text{-}PO_4$ were obtained with 0.005 g and 0.01 g of solid samples. After ANOVA no significant difference exists between samples. These sample quantities are also recommended by Burek.[6] Lower values of nitrogen and phosphorus were obtained in samples with over 0.01 g. Undetermined interferences caused failures of the development of colour for N and P analysis. Results varying the amount to hydrogen peroxide indicate that 0.4 ml for 10 mg solid samples and 0.15 ml for 25 ml water samples give satisfactory values, free from interference, in the determination of N and P. Burek used hydrogen peroxide in amounts of about 0.15 and 0.30 ml for water and solid samples.[6]

The Alu-block method was tested with two different operators.

TABLE I

Reproducibility of the Alu-block method in the analysis of samples.

Origin of sample	Kjeldahl-P individual values	$\mu g l^{-1}$ ($mg g^{-1}$)[a] mean values ± 1 S.D.	Kjeldahl-N individual values	$\mu g l^{-1}$ ($mg g^{-1}$)[a] mean values ± 1 S.D.
Embalse Rapel	56.0		83.6	
(superficial water)	56.0	58.6 ± 4.6	83.6	78.8 ± 4.8
	64.0		69.6	
Laguna Aculeo	48.0		222.9	
(superficial water)	48.0	50.6 ± 4.6	222.9	250.8 ± 48.2
	56.0		606.6	
Peñaflor	61.9		212.4	
(well-water)	61.9	61.6 ± 0.2	216.4	214.0 ± 1.6
	61.4		213.6	
	61.4		213.7	
Río Angostura	196.6		69.1	
(polluted water)	195.2	193.2 ± 50.0	72.1	73.6 ± 6.7
	195.2		69.6	
	185.7		83.6	
Río Mapocho	557.1		215.5	
(polluted water)				
Puente Manuel	538.1	543.1 ± 9.3	233.5	220.6 ± 11.2
Rodriguez	538.1		212.9	
	539.1			
Laguna Aculeo	0.9		1.5	
(sediment)	0.9	0.9 ± 0.008	1.5	1.6 ± 0.01
	0.9		1.6	
			1.5	
Macrophytes:				
Elodea canadensis	0.7		4.8	
	0.6	0.6 ± 0.04	4.1	4.5 ± 0.3
	0.6		4.6	
Miryophillum	0.2		3.8	
verticillatum	0.2	0.2 ± 0.02	3.9	3.8 ± 0.1
	0.2		3.7	
Callitriche	0.4		4.3	
autumnalis	0.4	0.4 ± 0.002	4.9	4.5 ± 0.2
	0.4		4.5	
	0.4		4.3	

[a]Unit for sediment and macrophytes samples.

For a t-test with 25 d.f. no significant differences ($p \leqq 0.001$) were found. For F-test no significance was found for the upper 2.5% points.

The Alu-block method was compared with the conventional macro-Kjeldahl method,[14,4] analyzing the nitrogen content in water, sediment and biological samples (Table II). For biological samples the macro-Kjeldahl method gave slightly greater percentages of nitrogen as compared to the Alu-block digestor. Nevertheless deionized water blanks treated as samples gave following results; $245 \pm 49.49\,\mu g\,l^{-1}$ for the macro-Kjeldahl method and $196.5 \pm 14.84\,\mu g\,l^{-1}$ for the Alu-block method. Two sources of difference between methods arise from the macro-Kjeldahl experiments: lack of accuracy in $N\text{-}NH_3$ titration, and nitrogen contamination along the Kjeldahl-apparatus. For lake water and sediment samples the Alu-block method gave larger percentages of nitrogen. For macro-Kjeldahl calculations we assumed that 1 ml 0.01 N H_2SO_4 used in titration of samples equals approximately 0.14 mg $N\text{-}NH_4$.[5]

The Alu-block method for acid-hydrolyzable phosphorus was compared with the autoclave method of Jeffries *et al.*[8] Results for a calibration standard series are shown in Figure 3. Each value

TABLE II

A comparison of macro-Kjeldahl and Alu-block methods for nitrogen analysis. Mean values of two samples.

Type of sample	Macro Kjeldahl		Alu-block	
	% nitrogen	±1 S.D.	% nitrogen	± 1 S.D.
Biological (green leaves) Boldo, *Peumus boldus*	1.3	0.002	1.05	0.004
Biological (green leaves) Boldo, *Peumus boldus*	1.2	0.007	1.1	0.002
Biological (weathered leaves) Peumo: *Cryptocarya alba*	0.5	0.005	0.4	0.002
Biological (weathered leaves) Peumo: *Cryptocarya alba*	0.4	0.002	0.4	0.000
Biological (weathered leaves) Boldo: *Peumus boldus*	0.4	0.005	1.2	0.002
Surface water (Aculeo lake)	0.001	0.000	0.002	0.000
Surface sediment (Aculeo lake)	2.3	0.000	3.1	0.000

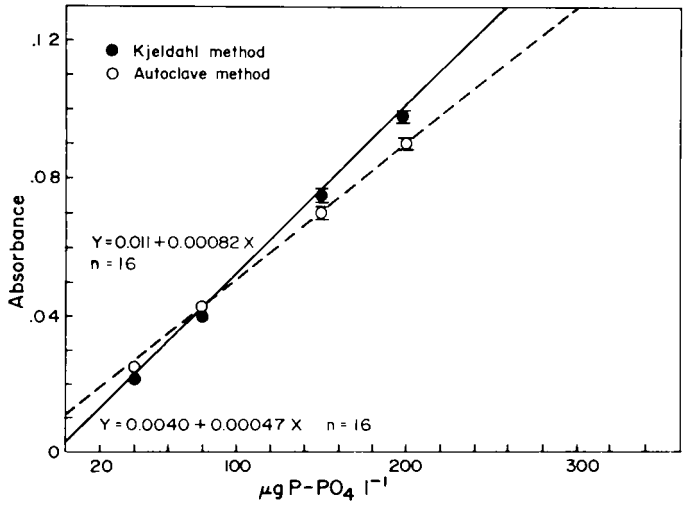

FIGURE 3 Comparison of the Alu-block method with the autoclave method[8] in determination of total phosphorus.

represents the mean of four measurements ± 1 Standard deviation (vertical bars). Similar, well correlated values were obtained by both methods. Absorbance mean values $(\bar{x}, \bar{y})$ were not significantly different.

So far several Kjeldahl methods have been proposed for determination of total-N,[4,5,1] and little reasonable doubt is left that the Kjeldahl method is satisfactory for total-N analyis of most types of samples. Goltermann *et al.*,[7] state that polyphosphates and some organic phosphorous compounds are hydrolysed by H_2SO_4 to orthophosphate. The addition of H_2O_2 completes the destruction of organic material. Nevertheless phosphorus recovery limitations of the subject procedure should be taken in account if samples with large amounts of complexed phosphorous (chitin for example) will be analysed.

However the alternative procedure presented here improves the technique compared with conventional types of Kjeldahl in two ways: It allows the handling of a great number of samples in a short period of time and, by measuring $N\text{-}NH_3$ and $P\text{-}PO_4$ by a colourimetric instead of a titration method, precision and accuracy are improved.

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