Water Analysis in South Africa: Inter-laboratory Comparison Studies. Part III: Nutrient Analysis

R. SMITH
[NATIONAL INSTITUTE FOR WATER RESEARCH OF THE COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH, P.O. BOX 395, PRETORIA, 0001]

Abstract

Part III of the programme of interlaboratory comparison studies involving South African laboratories engaged in water analysis is concerned with nutrient analysis. Evaluation of the results of the study showed that acceptable values were generally obtained for the six (Kjeldahl, ammonia, nitrate and nitrite nitrogen; and total and ortho-phosphate) constituents determined. In particular, nitrate and nitrite nitrogen results showed good accuracy and precision. Kjeldahl nitrogen and total phosphate analyses were characterized by the wide variety of procedures used for the digestion of the samples. Several different methods, both standard and non-standard, were also used for the determination of nitrate nitrogen. For purposes of standardization and comparison, the use of only current and recognized standard methods was recommended.

Introduction

In continuation of the programme of interlaboratory comparison studies involving South African laboratories engaged in water analysis (Smith, 1977, 1978), part III is concerned with nutrient analysis (i.e. Kjeldahl, ammonia, nitrate and nitrite nitrogen, and total and ortho-phosphate). The results obtained and the analytical methods used by the seventeen laboratories participating in the study are summarized and evaluated in this paper.

Sample Preparation

Each participating laboratory was supplied with two 500 m*l* samples, the procedure for the preparation of which was as follows:

Calculated amounts (based on a 100% purity value) of the following AR (or equivalent) grade chemicals were carefully weighed out and dissolved in known volumes of deionized distilled water: Glycine, ammonium chloride, potassium nitrate, sodium nitrite, potassium dihydrogen phosphate, and ß-glycerophosphoric acid disodium salt. After vigorous mixing to ensure complete homogeneity, the solutions were diluted to the required concentrations and 500 m*l* aliquots taken for each laboratory.

The samples were contained in 500 m*l* polythene bottles, which, prior to addition of the sample solutions, were treated, along with their plastic caps, as follows:

1. Soaking for 24 h in a 100 m*l* l^{-1} Contrad cleaning solution, followed by rinsing with deionized distilled water.

2. Soaking for 24 h in a 100 m*l* l^{-1} hydrochloric acid solution, followed by rinsing with deionized distilled water.

3. Rinsing with sample solution.

Both samples were preserved by the addition of 1 m*l* of a 10 g l^{-1} of AR grade mercuric chloride per litre of sample.

Analyses Requested

Samples 1 and 2: Kjeldahl nitrogen
Ammonia nitrogen
Nitrate nitrogen
Nitrite nitrogen
Total phosphate
Ortho-phosphate

Each laboratory was supplied with a table giving the concentration ranges of each constituent, and allowed complete freedom of choice as to the analytical procedures to be employed. It was also requested that references to standard methods, or copies of the methods used to carry out the various analyses, should be submitted with the results. A period of one

TABLE 2
SUMMARY OF RESULTS FROM ANALYSIS OF SAMPLE 2

Parameter	Units	Laboratory Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	True value (mg l^{-1})	Mean value (mg l^{-1})	Mean error (mg l^{-1})	Relative mean error (%)	Standard deviation (mg l^{-1})	Coefficient of variation (%)
Kjeldahl nitrogen	mg l^{-1} N	17,0	16,0	—	17,4	—	16,7	15,2	—	15,6	16,4	18,0	19,6	16,2	16,8	22,1*	—	—	17,5	16,8	0,7	3,9	1,2	7,2
Ammonia nitrogen		9,7	10,5	—	10,0	—	11,1	10,2	9,9	7,4	9,5	10,2	10,9	9,0	9,7	11,2	—	12,2	10,0	10,1	0,1	0,1	1,1	11,2
Nitrate nitrogen		7,4	9,7	—	7,1	7,6	9,5	7,9	8,4	9,0	7,5	7,8	8,1	7,1	7,9	7,9	7,1	7,0	8,0	7,9	0,1	1,2	0,8	10,5
Nitrite nitrogen		1,3	1,5	—	1,2	1,4	1,4	1,4	1,1	1,4	0,7	1,1	1,3	1,4	1,0	1,1	1,4	1,0	1,2	1,2	0	0	0,2	17,5
Total phosphate	mg l^{-1} P	5,2	7,4	—	6,0	—	6,3	6,3	—	5,6	—	—	—	6,2	4,8	—	—	—	6,0	6,0	0	0	0,8	13,3
Ortho-phosphate		5,2	7,4*	—	5,0	5,1	5,2	5,1	4,9	4,6	—	5,4	4,5	5,1	5,0	4,7	2,7*	4,0	5,0	4,9	0,1	2,0	0,4	7,6

*Outlier

TABLE 3
ASSESSMENT OF RESULTS

Lab. No.	Kjeldahl N			Ammonia N			Nitrate N			Nitrite N			Total P			Ortho P			Totals		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
1	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	2	—	—	12	—	—
2	2	—	—	2	—	—	—	1	1	—	2	—	—	1	1	—	—	2	4	4	4
3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	2	—	—	2	—	—	1	1	—	2	—	—	2	—	—	2	—	—	11	1	—
5	—	—	—	—	—	—	2	—	—	1	1	—	—	—	—	2	—	—	5	1	—
6	2	—	—	2	—	—	—	2	—	2	—	—	2	—	—	2	—	—	10	2	—
7	—	2	—	2	—	—	2	—	—	1	1	—	2	—	—	1	1	—	8	2	—
8	—	—	—	2	—	—	2	—	—	2	—	—	—	—	—	2	—	—	8	—	—
9	1	1	—	—	—	2	1	1	—	2	—	—	2	—	—	2	—	—	8	2	2
10	2	—	—	2	—	—	2	—	—	—	1	1	—	—	—	—	—	—	6	1	1
11	2	—	—	2	—	—	2	—	—	2	—	—	—	—	—	—	2	—	8	2	—
12	—	1	1	2	—	—	2	—	—	2	—	—	—	—	—	1	1	—	7	2	1
13	2	—	—	2	—	—	1	1	—	2	—	—	2	—	—	2	—	—	11	1	—
14	2	—	—	2	—	—	2	—	—	1	1	—	—	2	—	2	—	—	9	3	—
15	—	—	2	2	—	—	2	—	—	2	—	—	—	—	—	2	—	—	8	—	2
16	—	—	—	—	—	—	2	—	—	2	—	—	—	—	—	—	—	2	4	—	2
17	—	—	—	—	1	1	—	2	—	1	1	—	—	—	—	—	2	—	1	6	1
Totals	17	4	3	24	1	3	23	8	1	24	7	1	12	3	1	20	6	4	120	29	13

A = Results between mean and ± 1 standard deviation;
B = Results between ± 1 and ± 2 standard deviations;
C = Results outside ± 2 standard deviations

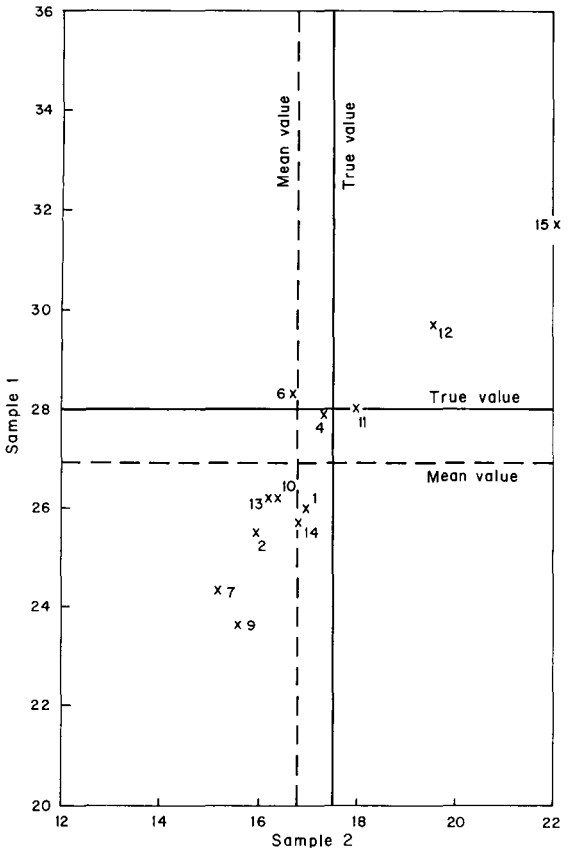

Figure 1
Kjeldahl nitrogen (mg/l N)

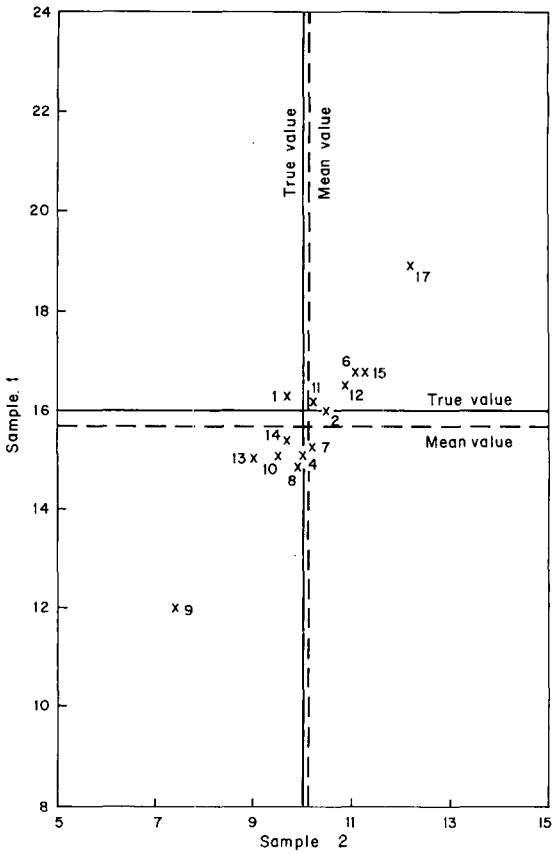

Figure 2
Ammonia nitrogen (mg/l N)

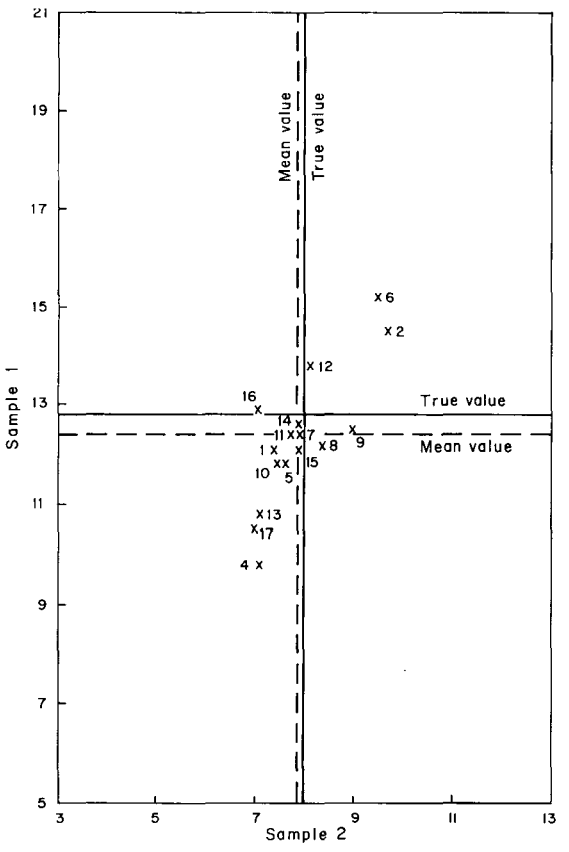

Figure 3
Nitrate nitrogen (mg/l N)

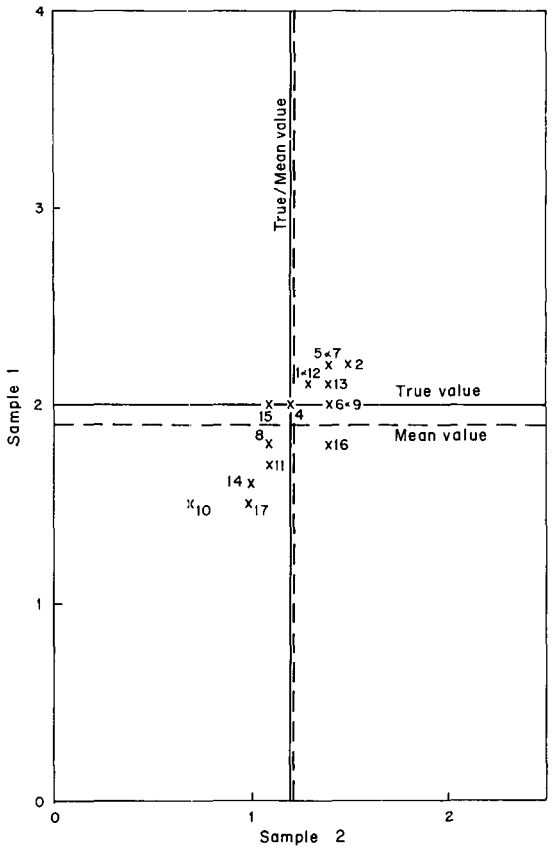

Figure 4
Nitrite nitrogen (mg/l N)

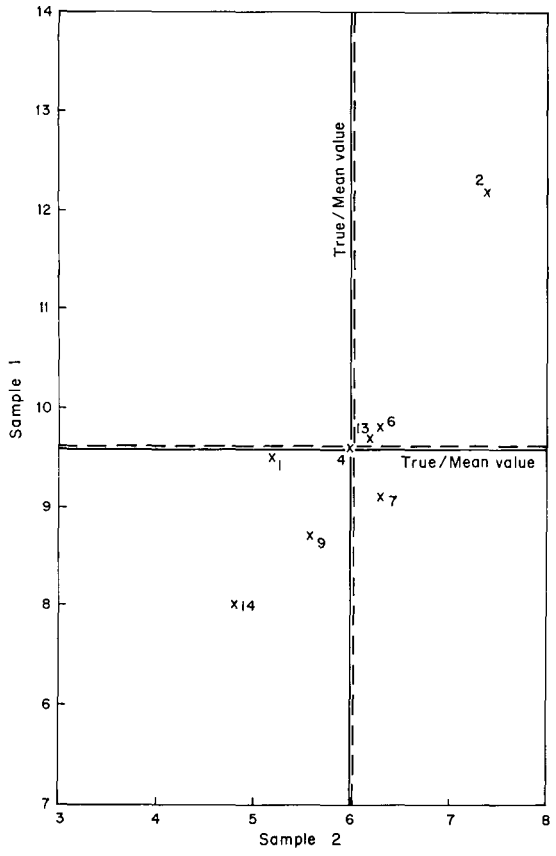

Figure 5
Total phosphate (mg/l P)

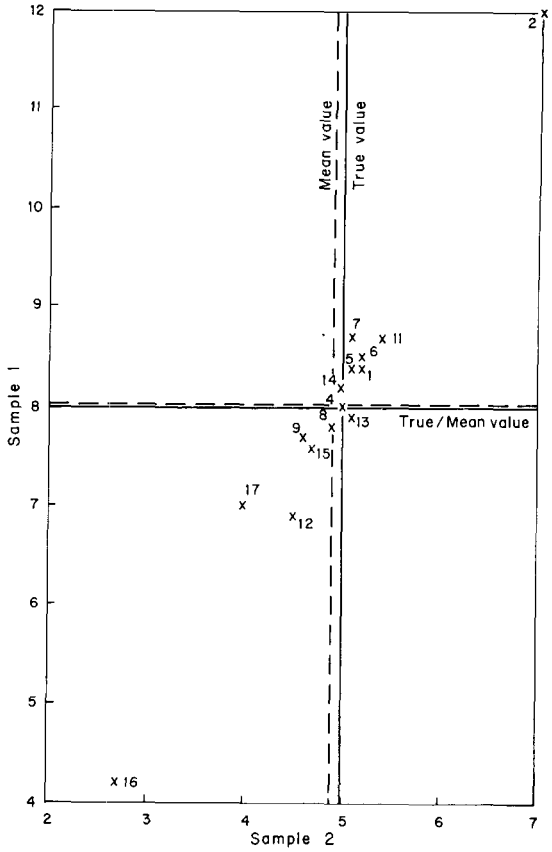

Figure 6
Ortho-phosphate (mg/l P)

points should fall into each of the four quadrants. In practice, however, this rarely occurs; most graphs of this nature show the majority of points falling in the upper right or lower left quadrants, suggesting that systematic errors consistently produce high or low results. Each quadrant identifies different effects which influence a laboratory's results. In general, results in the upper right or lower left quadrants are indicative of the presence of systematic errors, for example poor instrument calibration, inaccurate standards, faulty technique etc. Results in the upper left or lower right quadrants are inconsistently affected, and may be due to human errors such as those caused by mistakes in the calculation, recording, or reporting of the results. (Greenberg *et al.*, 1969).

Figures 1—6 represent Youden graphs of the result pairs obtained by each laboratory for the six constituents studied. In all cases, most of the plotted points fell in the upper right or lower left quadrants, indicating a predominance of systematic errors.

For all constituents, good agreement was obtained between 'mean' and 'true' values, and the results in general, showed good accuracy and precision, particularly those for nitrate and nitrite nitrogen. (The 'true values' given in *Tables 1 and 2* and shown on *Figures 1 to 6* are based on the theoretical values calculated from the amounts of the reference chemicals added.)

Method Evaluation

Kjeldahl nitrogen

Recognised methods available for the determination of this constituent include:

Manual

After digestion with sulphuric acid, potassium sulphate, and mercuric sulphate, the sample residue is made alkaline with a sodium hydroxide — sodium thiosulphate solution. The ammonia produced is distilled into a boric acid solution and determined either colorimetrically, titrimetrically, or potentiometrically (APHA, 1975, EPA, 1974).

Automated

After digestion either as for "manual" or with a mixture of

sulphuric acid, perchloric acid, and selenium dioxide, the solution is treated with sodium hydroxide, phenol, sodium hypochlorite, and sodium nitroprusside, to form a blue colour, designated as indophenol blue (EPA, 1974).

The twelve laboratories who carried out this determination used either automated or manual methods similar to the above, with certain modifications, as shown in Table 4.

The analyses were characterized by the wide variety of digestion procedures employed, no less than eight different variations of reagent mixtures being described, in addition to three methods for the determination of the distilled ammonia. As stated earlier, current standard methods stipulate the use of either selenium dioxide or mercuric sulphate as the catalyst in the digestion process. Copper sulphate, mentioned in earlier editions of 'Standard Methods', has been replaced by mercuric sulphate, as better recovery of certain organic nitrogen compounds is obtained using the latter. Selenium dioxide, while giving high recoveries, cannot be used in combined automated total nitrogen and total phosphorus determinations, owing to precipitation of the selenium on addition of reducing agent to form molybdenum blue. Vanadium pentoxide, although not giving as high recoveries in some cases, has been recommended for these combined analyses (Gales and Booth, 1974).

Ammonia nitrogen

All six laboratories who carried out this determination by automated techniques used the standard phenate method, in which the sample is treated with sodium hydroxide, phenol, sodium hypochlorite, and sodium nitroprusside, to form a blue colour, designated as indophenol blue (APHA, 1975; ASTM, 1975; EPA, 1974).

One laboratory determined the ammonia nitrogen manually by means of the ammonia ion-selective electrode, but obtained relatively low results. The same laboratory also used the electrode technique to determine the ammonia in the distillate for the Kjeldahl nitrogen analysis, and again obtained low results. The possible presence of some significant determinate error is therefore indicated.

The remaining seven laboratories who carried out the determination manually, used distillation procedures involving the distillation of the ammonia from either an alkaline medium, or from an unbuffered solution, or, as stipulated in the 13th and earlier editions of "Standard Methods", from a phosphate buffered solution of pH 7,4 (APHA, 1971). None of the participants used the procedure given in the 14th and latest edition of "Standard Methods", in which the sample is buffered before distillation at pH 9 with a borate buffer, in order to decrease hydrolysis of cyanates and organic nitrogen compounds (APHA, 1975).

Nitrate nitrogen

Eight laboratories carried out this determination by automated techniques. Of these, three used the cadmium reduction method, in which the sample is passed through a granulated copper-cadmium column to reduce nitrate to nitrite, which, along with the nitrite originally present, is determined by diazotising with sulphanilic acid and coupling with N-(1-naphthyl)-ethylene diamine dihydrochloride to form a red colour suitable for photometric measurement. The original nitrite value is obtained separately by omitting the reduction step. Nitrate is then obtained by difference (APHA, 1975; EPA, 1974).

Another three laboratories used a similar method except that nitrate reduction was effected by hydrazine sulphate. The other two laboratories employed a method evolved by Holz and

TABLE 4
EVALUATION OF METHODS USED FOR KJELDAHL-NITROGEN DETERMINATION

Lab. No.	Manual or automated	Digestion procedure	Ammonia determination after distillation
1	Automated	H_2SO_4, $HC1O_4$, SeO_2	Colorimetric (Indophenol)
2	Manual	H_2SO_4, K_2SO_4, $HgSO_4$	Titration
4	Automated	H_2SO_4, $HC1O_4$	Colorimetric (Indophenol)
6	Automated	H_2SO_4, $HClO_4$, V_2O_5	Colorimetric (Indophenol)
7	Automated	H_2SO_4, K_2SO_4, HgO	Colorimetric (Indophenol)
9	Manual	H_2SO_4, K_2SO_4, $HgSO_4$	NH_3 selective ion electrode
10	Manual	H_2SO_4	Titration
11	Manual	H_2SO_4, K_2SO_4, $HgSO_4$	Titration
12	Manual	H_2SO_4, K_2SO_4, $CuSO_4$	Titration
13	Manual	H_2SO_4, K_2SO_4, $CuSO_4$	Titration
14	Manual	H_2SO_4, $CuSO_4$	Titration
15	Manual	H_2SO_4, $CuSO_4$	Nesslerization/spectrophotometer

TABLE 5
EVALUATION OF METHODS USED FOR TOTAL PHOSPHATE DETERMINATION

Lab. No.	Manual or automated	Digestion procedure	Orthophosphate determination
1	Automated	H_2SO_4 (manual)	Ascorbic acid reduction method
2	Automated	$HC10_4$, HNO_3 (manual)	Ascorbic acid reduction method
4	Automated	H_2SO_4, $K_2S_2O_8$ (manual)	Ascorbic acid reduction method
6	Automated	H_2SO_4, $HC10_4$, V_2O_5	Ascorbic acid reduction method
7	Automated	H_2SO_4, K_2SO_4, HgO	Ascorbic acid reduction method
9	Manual	H_2SO_4, $(NH_4)_2$ S_2O_8	Ascorbic acid reduction method; spectrophotometer
13	Manual	H_2SO_4	Ascorbic acid reduction method; spectrophotometer
14	Automated	H_2SO_4, HCl (manual)	Ascorbic acid reduction method

Kremers (1970), wherein a rhenium-nitrate complex is formed in the reaction between potassium perrhenate, stannous chloride, diacetyl monoxime, and the nitrate in the sample. Unreacted rhenium forms a reddish-brown Re-damo couple, which can be measured photometrically.

The cadmium reduction method is considered to be superior to the other two methods, as well as being the only one of the three described in recent recognized standard procedures. It has been found that, for optimum results, a concentration of 13 to 15 mg l^{-1} of hydrazine sulphate must be used, otherwise low nitrate values may be obtained, while use of potassium perrhenate rapidly results in the formation of deposits on the walls of the flow cell, with resulting reduction in light transmittance (Siebert, 1977).

The remaining eight laboratories carried out manual determinations of this constituent. Of these, one used the Devarda's alloy reduction method, in which nitrate and nitrite are reduced to ammonia under hot alkaline conditions in the presence of Devarda's alloy. The ammonia formed is trapped in a receiving flask containing boric acid solution and is determined by nesslerization or titration. (APHA, 1975; Government Gazette, 1969). Another laboratory carried out a direct determination using the nitrate ion-selective electrode (APHA, 1975). A third laboratory used a method involving the photometric measurement of the yellow colour produced in the reaction between nitrate and phenol disulphonic acid. (This procedure appeared in the 13th edition of "Standard Methods", but is excluded from the latest edition). The other five laboratories employed the "sodium salicylate method" in which the yellow colouration obtained by the addition of sodium hydroxide to the nitrososalicylate formed in the reaction between nitrate and salicylic and sulphuric acids is measured photometrically at 410 nm (Carow and Raquet, 1936; Muller and Widemann, 1955). Again, despite the popularity of this method, it is not mentioned in current recognized standard procedures.

Nitrite nitrogen

The seven laboratories that determined this constituent by automated techniques and the nine carrying out the determination manually all used basically the sulphanilic acid method. This method involves the photometric measurement of the intensity of the reddish purple colour produced at a pH of 2 to 2,5 by the coupling of diazotised sulphanilic acid with N-(1-naphthyl)-ethylene diamine dihydrochloride. Measurement is made at 520 nm (ASTM 1975; Government Gazette, 1969) or 540 nm (EPA, 1974) or 543 nm (APHA, 1975).

Total phosphate

Only eight laboratories (six by automated and two by manual methods) carried out the determination of this constituent. As in the Kjeldahl nitrogen determination, a wide variety of digestion procedures was employed, the eight laboratories between them using seven different digestion mixtures (Table 5).

However, all eight laboratories used the same procedure for the determination of the ortho-phosphate after digestion, namely the ascorbic acid reduction method, which involves treating the digested sample with an acid mixture of ammonium molybdate and potassium antimonyl tartrate. Phosphomolybdic acid is formed which is reduced to intensely coloured molybdenum blue by ascorbic acid (APHA, 1975; ASTM, 1975; EPA, 1974).

The current 14th edition of "Standard Methods" stipulates that, depending on the severity of oxidation required, the sample should be digested using either perchloric-nitric acid, sulphuric-nitric acid, or ammonium persulphate-sulphuric acid digestion mixtures, in order to convert the phosphorus present in the sample in various forms to soluble ortho-phosphate (APHA, 1975).

Ortho-phosphate

Of the fifteen laboratories determining this constituent (nine by automated and six by manual methods), thirteen employed the standard ascorbic acid reduction method previously described. One laboratory used hydroquinone instead of ascorbic acid to effect the reduction of the phosphomolybdic acid. The remain-

ing laboratory used the sulphonic acid method, wherein the sample is treated with an acid mixture of ammonium molybdate, 1-amino 2-naphthol -4 sulphonic acid, sodium metabisulphite, and sodium sulphite. The blue colour produced is measured photometrically at 690 nm (Government Gazette, 1969).

No significant differences were found in the precision or accuracy between manual and automated methods for any of the six constituents studied.

Conclusions

Only 8% of the results submitted could be regarded as unacceptable. The results, in general, exhibited a good degree of precision and accuracy.

No less than eight different digestion procedures, as well as three methods for the determination of the distilled ammonia, were used for the Kjeldahl nitrogen analysis.

In the ammonia nitrogen analysis, most of the laboratories that carried out the determination manually by a distillation process, either distilled the ammonia from an unbuffered solution, or from a phosphate-buffered solution of pH 7,4. Recent standard texts stipulate distillation from a borate-buffered solution of pH 9.

A wide variety of methods, both standard and non-standard, were employed for the nitrate-nitrogen determination. The cadmium reduction method is preferred for automated analysis of nitrate. In the case of nitrite-nitrogen, however, all of the participants used basically the same standard sulphanilic acid procedure.

A wide variety of digestion procedures was also employed for the determination of total phosphate, although all eight laboratories participating in this analysis used the standard ascorbic acid reduction method for the determinations of the ortho-phosphate after digestion. This method was also used by all but two of the fifteen laboratories that performed the ortho-phosphate analysis.

Although acceptable results were obtained in most cases, it should be emphasized that synthetic samples were used for the study. It is very possible that some of the methods employed may not give as good results with natural water and wastewater samples, particularly those containing organic nitrogen and phosphorus compounds.

For increased reliability of results, therefore, as well as for purposes of standardization and comparison, consideration should be given to the use of only recognized and updated standard methods of analysis.

The results obtained from this study should assist each participating laboratory to assess the effectiveness of their analytical procedures and the comparative reliability of the results obtained therefrom.

Acknowledgements

The following laboratories participated in the study:
National Institute for Water Research, Pretoria:
1 Water Quality Division
2 Physico-chemical Technology Division
National Institute for Water Research, Natal Regional Laboratory, Durban.
National Institute for Water Research, Cape Regional Laboratory, Bellville.
National Institute for Water Research, SWA Regional Laboratory, Windhoek.
South African Bureau of Standards (Water Division), Pretoria.
Hydrological Research Institute, Department of Water Affairs, Pretoria.
Health Chemical Services, Department of Health, Pretoria.
Rand Water Board, Vereeniging.
City Health Department, Laboratory and Technical Services Board, Johannesburg.
Department of Water Affairs, Windhoek.
Municipal Laboratory, Pretoria.
Municipal Laboratory, Durban.
Municipal Laboratory, Cape Town.
Municipal Laboratory, Port Elizabeth.
Municipal Laboratory, Kempton Park.
Municipal Laboratory, Windhoek.

The assistance of the staff of these laboratories who carried out the analyses is gratefully acknowledged. This paper is published with the approval of the Director of the National Institute for Water Research.

References

AMERICAN PUBLIC HEALTH ASSOCIATION – AMERICAN WATER WORKS ASSOCIATION – WATER POLLUTION CONTROL FEDERATION (1971) *Standard methods for the examination of water and wastewater.* 13th Edition, New York.

AMERICAN PUBLIC HEALTH ASSOCIATION – AMERICAN WATER WORKS ASSOCIATION – WATER POLLUTION CONTROL FEDERATION (1975) *Standard methods for the examination of water and wastewater.* 14th Edition, New York.

AMERICAN SOCIETY FOR TESTING AND MATERIALS (1975) *Annual book of ASTM Standards. Part 31. Water.* Philadelphia, Pennsylvania.

CAROW, H. and RAQUET, D. (1936) Colorimetric determination of nitrates in water in presence of chlorides. *J. Pharm. Chim. Paris.* **23** 446–447.

ENVIRONMENTAL PROTECTION AGENCY (1974) *Methods for chemical analysis of waters and wastewaters.* Cincinnati, Ohio.

GALES, M. and BOOTH, R.L. (1974) *Simultaneous and automated determination of total phosphorus and total Kjeldahl nitrogen.* EPA 670/4-74-002. Environmental Protection Agency, Cincinnati, Ohio.

GOVERNMENT GAZETTE (1969) *R.3208: Regional Standards for industrial effluents.* Pretoria.

GREENBERG, A.E., MOSKOWITZ, N., TAMPLIN, B.R., and THOMAS, J. (1969) Chemical reference samples in water laboratories, *J. Am. Wat. Wks. Ass.* **61** 599–602.

HOLZ, F. and KREMERS, H. (1970) Automatische bestimmung von nitrat in pflanzensubstanz – Landwirtschaftl. *Forsch.* **23**(1) 23.

MULLER, R. and WIDEMANN, O. (1955) Bestimmung des nitrations in Wasser. *Vom Wasser* **22** 247.

SIEBERT, M.L. (1977) Private communication.

SMITH, R. (1977) Water analysis in South Africa: Interlaboratory comparison studies. Part I: Introductory study. *Water S.A.* **3**(2) 66–71.

SMITH, R. (1978) Water analysis in South Africa: Interlaboratory comparison studies. Part II: COD, pH and electrical conductivity analyses. *Water S.A.* 4(1) 4–9.

YOUDEN, W.J. (1960) The sample, the procedure, and the laboratory. *Analyt. Chem.* **32**(13) 23A–37A.