

**The Advantages of Measurement of Particulate Carbon, Nitrogen and Phosphorus by
Direct Analysis**

Chesapeake Bay Program

Monitoring Subcommittee

Analytical Methods and Quality Assurance Workgroup

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The Analytical Methods and Quality Assurance Workgroup (AMQAW) of the Monitoring Subcommittee of the Chesapeake Bay Program (CBP) seeks to recommend and implement analytical methodologies that are the most scientifically valid and consistent for ambient water quality monitoring sample collection and analysis. Currently, there is inconsistency between parts of the CBP in the sampling and analytical methodology for the determination of particulate concentrations. This document addresses the reasons for these inconsistencies and offers alternative sampling and analytical procedures to be implemented to eliminate these inconsistencies.

BACKGROUND

A comprehensive ambient water quality monitoring program of the Chesapeake Bay mainstem and tributaries was begun in 1984. This program has three goals (Chesapeake Bay Program, 1985):

1. to characterize "baseline" water quality conditions in the system.
2. to provide the highest quality data to allow managers to determine whether water quality conditions are changing.
3. to describe the system sufficiently that hypotheses concerning water quality processes can be articulated.

Since 1985, three laboratories (two in Virginia and one in Maryland) have been primarily responsible for the analysis of nutrient samples collected from the mainstem portion of the Chesapeake Bay. To help achieve the above goals, the AMQAW and participating laboratories within the CBP continue to seek methods and instrumentation to improve precision, accuracy and detection limits.

Sample analyses within the CBP Monitoring Program first began with all laboratories employing standard EPA protocols used for the analysis of freshwater and wastewater. These protocols included "whole water" and "dissolved" analyses of nitrogen, phosphorus and carbon. Particulate concentrations were calculated by subtracting the "dissolved" concentration from the "whole water" concentration. It was pointed out that these methods, although acceptable for "from the pipe" concentrations where measurements of the total loads discharged were the primary study objectives, were not sensitive enough for the low nutrient concentrations normally encountered in estuarine waters (D'Elia et al., 1987). Also, more direct methods for the determination of the particulate fractions of C, N, and P were available. Accurate measurement of this particulate fraction is important since it is a useful indicator of the biological component in the water column (Redfield, 1934).

For the period 1985-1986, measurements of the particulate and dissolved fractions by techniques appropriate for estuarine concentrations were already employed for some analytes by only one of the three laboratories analyzing the mainstem samples. CBP administrators were

concerned that appropriate methods should be used to gain the most accurate information possible for the Chesapeake Bay Program. They were also concerned that the three laboratories involved in analyzing these mainstem samples use similar techniques. A study was funded in 1986 which addressed four major concerns:

1) Should standard EPA techniques with their limits of detection be improved upon because they might be insufficient for many parameters, over much of the mainstem portion of the Bay, during much of the year? Detection limits of each method were compared.

2) Were standard EPA approaches to the determination of particulate carbon (PC), particulate nitrogen (PN) and particulate phosphorus (PHOSP) satisfactory? More useful information might be obtained by direct analysis of particulates and more accurate total C, N, and P concentrations might be obtained by adding the particulate concentrations to the dissolved concentrations, rather than calculating particulate concentrations by subtracting the dissolved concentrations from those of the "whole water". Side-by-side analyses of the "by difference" techniques to obtain particulate N and P concentrations were compared with the direct analysis methods.

3) What was the better method to obtain dissolved organic nitrogen data? Kjeldahl nitrogen, which measures the organic forms of nitrogen plus ammonium, was not well established as precise or accurate enough to provide reliable data for estuarine and marine samples. Alternative techniques, which have seen favor in oceanography, such as the total alkaline persulfate technique, although more precise, were not established as EPA standard methods. Side-by-side analyses of dissolved samples using Kjeldahl and alkaline persulfate techniques were performed.

4) Did the analytical costs associated with standard EPA approved protocols substantially exceed acceptable alternatives? A comparison of sample analysis costs of the various EPA approved and alternative techniques was made using one laboratory's pricing structure (D'Elia et al. 1987).

The conclusions from a workshop held at Chesapeake Biological Laboratory in 1987, which dealt with nutrient analytical techniques for estuarine samples, and from a study (D'Elia, et al., 1987) on the same topic, found that the methods which were thought to be more appropriate for low level estuarine concentrations included not only the direct determination of particulate C, N, and P but also more sensitive analytical techniques for total dissolved nitrogen. A comparison of EPA approved methods versus those adapted for the Chesapeake Bay Mainstem Monitoring Program is found in Figure 1.

Direct measurement of particulate fractions offers many advantages over "by difference" determinations. Elemental analysis is extremely precise and a representative, concentrated sample can always be obtained by filtering enough water through an inert filter. Direct analysis provides information that would otherwise be lost if only a whole water sample were analyzed (D'Elia, 1987). The "by-difference" technique sometimes leads to negative values (where the dissolved portion is greater than the whole water portion), particularly in waters where the particulate fraction is a small contributor to the overall concentrations at certain times of the year. These

negative results translate into a "no value" in the data base thereby making differentiation of the true temporal and spatial variability for a certain portion of the biological component very difficult to determine (Head, 1985).

This point is illustrated in the following example. Samples from three stations of the Patuxent River tributary monitoring program are routinely split between two laboratories: one laboratory using "by difference" and the other "direct" techniques to determine particulate concentrations. All particulate carbon samples analyzed "directly" resulted in measureable concentrations. At two stations (one in the estuarine zone and one in the transition zone between estuarine and tidal freshwater), the 1992 data calculated "by difference" for particulate carbon concentrations averaged 7% negative values. At the tidal freshwater station, the percent of particulate values which were negative was considerably greater--38.9%. Overall, 19% of the particulate carbon data calculated "by difference" were negative. These results are presented in Table 1.

Table 1. Patuxent River particulate carbon split sample data calculated "by difference": 1992. Stations XDE2792 (estuarine), XED4892 (transition) and PXT0603 (tidal fresh). Concentrations in mg C/L.

	XDE2792	XED4892	PXT0603
Observations	28	29	36
No. Negative	2	2	14
% Negative	7.1	6.9	38.9

SUMMARY OF EXPERIMENTAL METHODS AND RESULTS

A study, conducted from June - September 1986 (1) compared Kjeldahl analysis for dissolved N with results obtained from the persulfate oxidation technique, (2) compared determination of particulate "by difference" N and P with direct methods and (3) compared dissolved P concentrations from "acid" persulfate digestion with dissolved P concentrations from the "alkaline" persulfate digestion. Samples collected as part of the Maryland portion of the Chesapeake Bay Mainstem Monitoring Program were analyzed for this comparison. Whole water samples were collected in accord with the established protocols. For the separation of the particulate and dissolved fractions, samples were filtered in the field immediately following collection by filtering a known volume of sample through a precombusted glass fiber filter. The volume filtered varied depending on the location, depth and season. Particulate C and N were analyzed directly utilizing an elemental analyzer. The elemental analyzer combusted the sample, yielding nitrogen, carbon dioxide and water vapor. Particulate P was determined utilizing a high temperature combustion and acidic extraction technique (Aspila, et al., 1976). The alkaline persulfate digestion method for the analysis of total dissolved N and P was employed to break down the organic and inorganic components to nitrate and phosphate in an alkaline medium at the start of the digestion. The medium became acidic as the digestion proceeded, completing the breakdown of organic phosphorus compounds to phosphate (D'Elia, et al., 1977; Valderrama, 1981).

Results of this study (D'Elia, et al, 1987) demonstrated that detection limits were much lower using the direct methods of particulate analysis. The detection limit for the persulfate digestion technique for N was lower than that for the Kjeldahl analysis. Results also demonstrated that the direct measurement of particulate N is more precise than the "by-difference" technique. It also concluded that the persulfate N technique was more precise, less costly and generated considerably less hazardous waste than the Kjeldahl analysis. Field replicate data also indicated that the direct measurement of particulate P is more precise than the "by-difference" technique. The methods recommended as a result of this study also showed a 33.3% cost saving to the granting institution.

Based largely on these results, EPA Region III accepted the direct determination of the particulate fractions of C, N and P and the analysis of dissolved N and P via persulfate digestion

of filtered water samples for the entire Mainstem Monitoring Program. Thus, the three laboratories involved in these sample analyses adopted similar methods and instrumentation.

The precision of the three laboratories using direct particulate analysis of C, N and P compared to "by difference" data is well documented in the CBP's Coordinated Split Sample Program; 1990-1991 (AMQAW, 1993). Coefficients of variation of replicated samples analyzed by direct measurement were less than 7% while "by difference" coefficients of variation were greater than 25%. Also, the incidence of negative particulate concentrations which some p501 times occur using the "by difference" method as documented in AMQAW (1993), is not a factor when direct measurement techniques are employed.

EPA's awareness that there are no approved methods for estuarine/coastal nutrient analyses, has resulted in funding during the past few years to study, develop and validate methods for estuarine/coastal and low level analyses. As part of the validation for the direct analysis of particulate carbon and nitrogen, the comparability of results between laboratories nationwide has also been investigated. Identical samples were shipped to laboratories for elemental analysis. The results from the thirteen instruments (from three manufacturers) were comparable for all five different samples concentrated on glass fiber filters and also for five sediment samples. The statistical comparisons to determine single analyst precision and pooled Method Detection Limit are underway (U.S. EPA, Environmental Monitoring Systems Laboratory, Cincinnati).

MEETING THE REQUIREMENTS OF THE DATA USERS--PRESENT AND FUTURE

The data produced by the direct particulate analysis of C, N and P are important components to enable the modeling and managing of water quality and living resources of the Chesapeake Bay and its tributaries. The ratios of particulate carbon to particulate nitrogen and of particulate carbon to particulate phosphorus are employed to quantify the contribution to the water column by living material. Only the direct determinations of particulate carbon, nitrogen and phosphorus concentrations in the mainstem water column samples have yielded data that were useful for this important quantification of carbon, nitrogen and phosphorus stoichiometry (Cercó and Cole, 1992). Elsewhere in the data analysis to characterize the Chesapeake and its drainage basin, some experiments on the splitting of the labile and refractory components of point and nonpoint-source loads were rendered invalid by low precision in particulate analysis when these concentrations were determined by difference (Cercó, personal communication).

With management and scientific interest shifting to the Bay's tributaries, it is appropriate to consider whether the most suitable methods are being used to analyze samples from these areas. The same sort of clear definition of processes in the mainstem resulting from direct particulate methods should be provided for the tributaries as well. No information will be lost and considerable information will be gained.

For those managers whose interest is in only the magnitude of the total fraction concentrations (e.g., ambient surface water quality monitoring), then the addition of particulate and total dissolved concentrations will yield the total concentration of concern. If this is presently not an

accepted (Federal Register) method for determining total N, P and C, then it should be. In a major comparability study ($n = > 700$), results showed that total phosphorus (TP) determined directly by the acid persulfate digestion on a whole water sample and TP obtained by summing the direct determination of the particulate fraction with dissolved phosphorus determined with either alkaline or acid persulfate methods were nearly identical. The slopes of the regressions were 1.151 and 0.988, respectively, where a slope of 1 would indicate complete agreement (D'Elia, et al., 1987).

Another comparison for nitrogen, using 1992 data from the Patuxent River, also illustrates the similarity of the data obtained from the two methods to determine total N concentrations. Total N values determined by the traditional summing of whole water Kjeldahl plus nitrite+nitrate were quite similar to total N values obtained by adding particulate N results to the total dissolved nitrogen results (slope = 0.92, $r^2 = 0.89$; Figure 2). Similarly, Total C data comparisons obtained by summing DOC with PC data with TOC data yielded a slope of 1.17 with an r^2 value of 0.62.

SUMMARY AND CONCLUSIONS

Immediate partitioning of dissolved and particulate fractions of a water sample followed by the immediate analysis of those various fractions is the recommended, most accurate procedure for analyzing surface waters for the various nitrogen, phosphorus and carbon components. It is also acknowledged that immediate shipboard analyses of particulate/dissolved samples and their components is highly impractical as well as cost prohibitive.

Therefore, in practical terms, the following procedure after collection of a water sample is recommended:

- A. Immediate field filtration of the water sample to separate the particulate from the dissolved fraction.
- B. Chilling/freezing these samples as an acceptable alternative preservation technique (Please refer to preservation position paper).
- C. Direct analysis of particulate fractions.

These analytical techniques have been in use since 1987 throughout the mainstem of the Chesapeake Bay, and have also been used in other EPA sponsored estuarine monitoring programs such as the Long Island Sound Study (1988 to present). While these analytical techniques have proven superior to other, less sensitive methods, they have only become acceptable for programs which are applicable to pollution research under Section 106 of the Clean Water Act. The analytical methodology for the ambient monitoring of both the freshwater and saline portions of the tributaries has not, for the most part, been modified to include these alternative analytical methods. The question is why? If the major reason is that some techniques are documented by the Federal Register while others are not, then comparable, acceptable alternative techniques

need to be included in the Federal Register. The state laboratories involved in ambient surface water nutrient analysis realize the value and importance of the analytical methods presently being used in the Mainstem Chesapeake Bay Monitoring Program. These alternative techniques should be made available to them.

This willingness to depart from Section 306 (Clean Water Act) methods which are mandated for effluent monitoring to the more flexible Section 106 requirements applicable to pollution research for a relatively small percentage of their sample load is an indication of the willingness of these laboratories to provide pertinent, quality data. They should be allowed to use the same analytical techniques appropriate for the analyses of non-compliance surface ambient water quality monitoring samples as the university laboratories involved in the Mainstem Chesapeake Bay Program.

Certainly, tributary nutrient concentrations exhibit many of the same characteristics as those from the Chesapeake Bay. These include (1) salinity effects that must be accounted for when analyzing for phosphate, (2) salinity gradients in every set of samples and (3) low concentrations of many dissolved and particulate species at the mouth of the tributary with generally increasing concentrations as one moves inland. The importance of taking the next logical step and applying those techniques presently used in the analyses for the Mainstem Chesapeake Bay to its surrounding tributaries is gaining increasing support, as it well should. If these tributary monitoring efforts are not mandated under compliance monitoring to meet legal requirements, but rather fall under the same category (Section 106) of the Clean Water Act as Chesapeake Bay Mainstem Monitoring, then the most appropriate methods of analysis can and should be used.

In conclusion, the reasons for allowing alternative analytical methods are clear:

1. Concentrations associated with tributary monitoring, although in many cases higher than found in the Chesapeake Bay, still do not constitute "from-the-pipe" discharge concentrations.

2. With recent emphasis placed on the role of tributaries (amendments to the Bay agreement), the most accurate and precise data need to be collected. Direct analysis of particulates and persulfate digestion of dissolved nitrogen and phosphorus are more precise and accurate than by difference and Kjeldahl analyses.

3. Comparability of data from the tributaries with the mainstem is crucial. In order to best achieve the CBP goals, it is critical that trends observed in each tributary be related as closely as practicable to those in the mainstem. This has already been attained for comparisons of Patuxent River and mainstem data since the methods are identical. Differences in the environmental matrix and practical considerations will limit the extent to which inconsistencies can be eliminated; however, scientific credibility and programmatic changes demand taking every possible step available to unify the field and laboratory methodology.

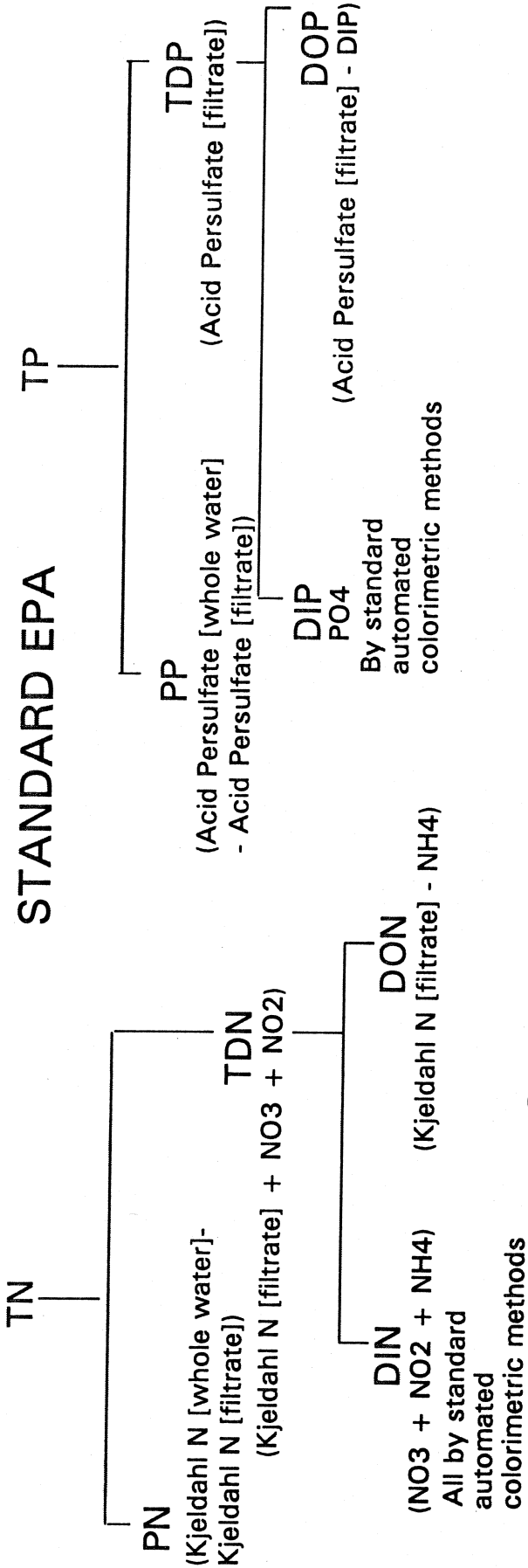
4. The continuing growing awareness that has developed among state laboratories of the important role they play in ambient water quality monitoring necessitates that they utilize

techniques which are appropriate for the data quality objectives of the program. Along with this awareness, there should be a commitment by state and federal agencies to help these laboratories procure state of the art instrumentation.

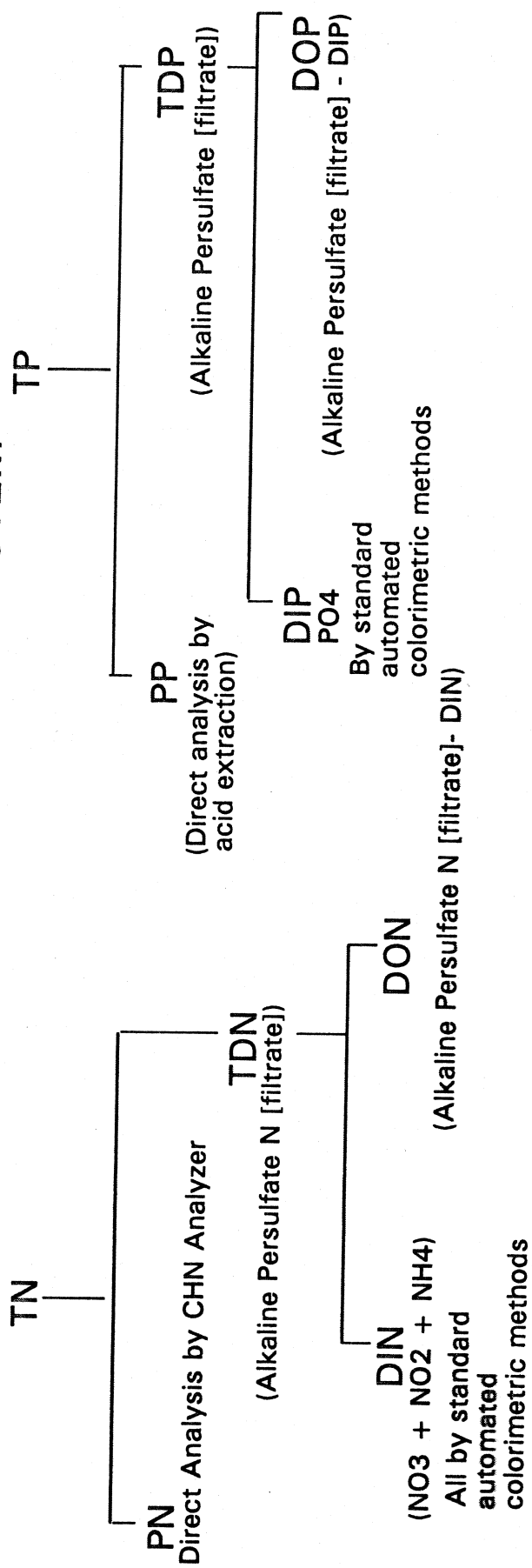
5. Hazardous waste generation and the cost of its removal must be considered. Kjeldahl analysis generates a tremendous amount of hazardous waste (concentrated sulfuric acid, concentrated sodium hydroxide and mercuric sulfate). Persulfate analysis of dissolved nitrogen and phosphorus generates no hazardous waste.

6. Cost savings for the analyses are basically the same as those reported by D'Elia et al (1987), but the cost of hazardous waste removal has sky rocketed since 1987.

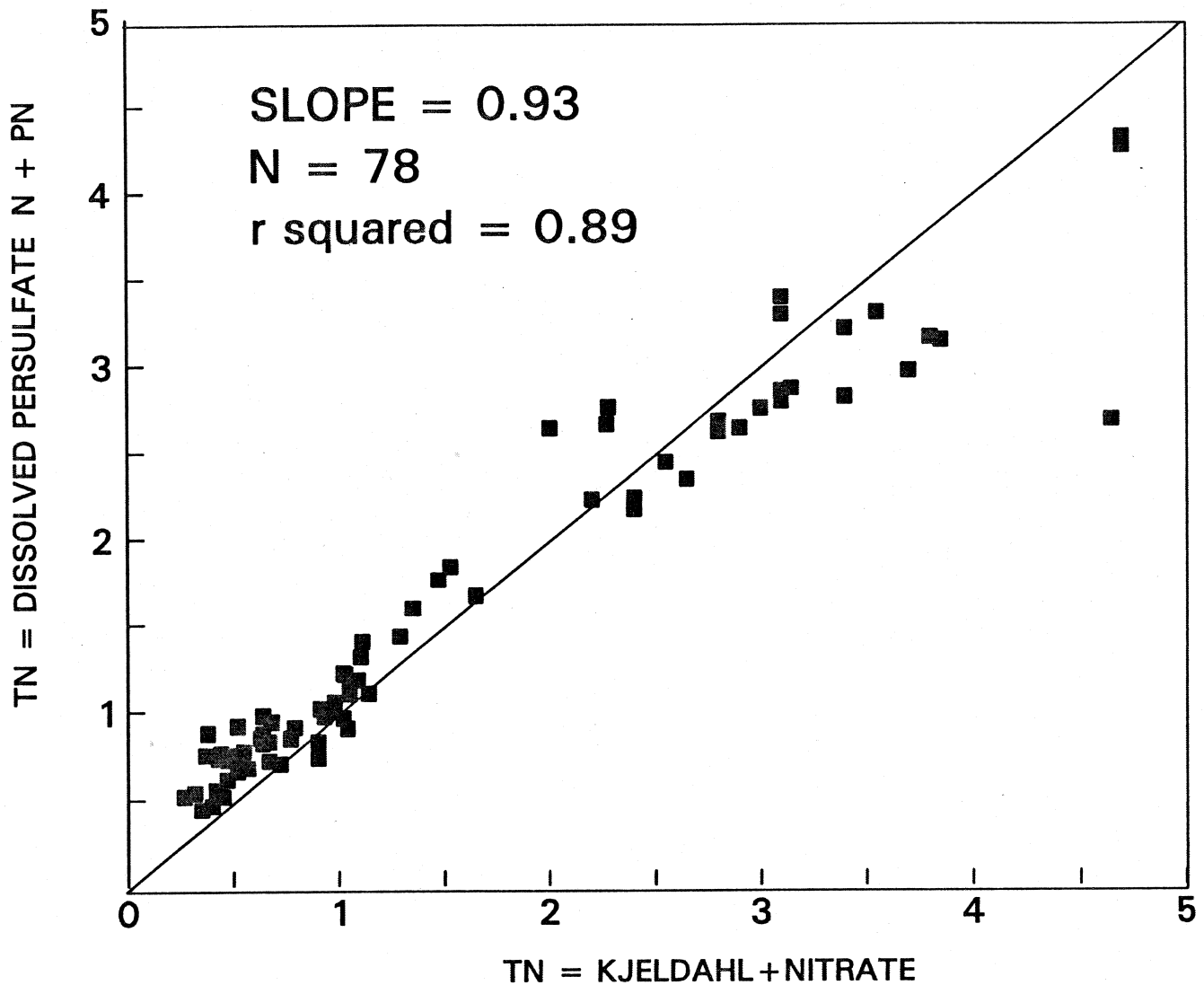
7. Determination of carbon and nitrogen in sediments and particulates of estuarine/coastal waters using elemental analysis (Method 440.0) has been recently validated by the United States Environmental Protection Agency (1992).



CHESAPEAKE BAY MAINSTEM



**Figure 2. Comparison of total nitrogen methods: Patuxent River 1992.
Whole water Kjeldahl plus Nitrate VS TDN + PN.**



TDN = Total Dissolved Nitrogen (persulfate)

**PN = Particulate Nitrogen
Concentration in mg N/L.**

REFERENCES

- Aspila, K.I., H. Agemian, and A.S.Y. Chau. 1976. A semi-automated method for the determination of inorganic, organic, and total phosphate in sediments. *Analyst* 101: 187-197.
- AMQAW. 1993. Chesapeake Bay Coordinated Split Sample Program Annual Report, 1990-1991. CBP/TRS 76/93, Chesapeake Bay Program, Annapolis, MD 85 pp.
- Cerco, C. and T. Cole. 1992. Application of the Three-Dimensional Eutrophication Model CE-QUAL-ICM to Chesapeake Bay, May 1992 Draft, U.S. Army Engineers Waterways Experiment Station, Vicksburg, MS.
- Cerco, C. Environmental Laboratory, Environmental Processes and Effects Division, U. S. Army Engineers Waterways Experiment Station, 3909 Halls Ferry Rd., Vicksburg, MS 39180.
- D'Elia, C.F., R.E. Magnien, C.F. Zimmermann, P.A. Vaas, N.L. Kaumeyer, C.W. Keefe, D.V. Shaw, and K.V. Wood. 1987. Nitrogen and phosphorus determinations in estuarine waters: A comparison of methods used in Chesapeake Bay monitoring. CBP/TRS 7/87, Chesapeake Bay Program, Annapolis, MD 26pp.
- D'Elia, C.F., P.A. Steudler and N. Corwin. 1977. Determination of total nitrogen in aqueous samples using persulfate digestion. *Limnol. Oceanogr.* 22: 760-764.
- Head, P.C. 1985. Salinity, dissolved oxygen and nutrients, p.94-114. In: P.C. Head [ed.], *Practical Estuarine Chemistry*. Cambridge University Press. Cambridge.
- Monitoring 1984: A first report of the Chesapeake Bay Program Monitoring Subcommittee. CBP, Annapolis, Md.
- Redfield, A.C. 1934. On the proportions of organic derivatives in seawater and their relation to the composition of plankton. *James Johnstone Memorial Volume, Liverpool*, p. 177-192.
- United States Environmental Protection Agency. 1992. *Methods for the Determination of Chemical Substances in Marine and Estuarine Environmental Samples*. Environmental Monitoring Systems Laboratory, Cincinnati, OH. 45268.
- Valderrama, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry* 10: 109-122.