VARIATION AMONG AND WITHIN PROCEDURES FOR BIOAVAILABLE PHOSPHORUS

VARIATION AMONG AND WITHIN PROCEDURES FOR ESTIMATION OF BIOAVAILABLE PHOSPHORUS

by

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FORWARD

The Environmental Protection Agency was established to coordinate administration of the major Federal programs designed to protect the quality of our environment.

An important part of the Agency's effort involves the search for information about environmental problems, management techniques, and new technologies through which optimum use of the nation's land and water resources can be assured and the threat pollution poses to the welfare of the American people can be minimized.

The Great Lakes National Program Office (GLNPO) of the United States Environmental Protection Agency was established in Region V, Chicago, to focus attention on the significant and complex natural resource represented by the Great Lakes.

GLNPO implements a multi-media environmental management program drawing on a wide range of expertise represented by universities, private firms, State, Federal, and Canadian governmental agencies, and the International Joint Commission. The goal of the GLNPO program is to develop programs, practices and technology necessary for a better understanding of the Great Lakes Basin ecosystem and to eliminate or reduce to the maximum extent practicable the discharge of pollutants into the Great Lakes system. GLNPO also coordinates U.S. actions in fulfillment of the Great Lakes Water Quality Agreement of 1978 between Canada and the United States of America.

We hope that the information and data contained herein will help planners and managers of pollution control agencies make better decisions for carrying forward their pollution control responsibilities

Peter L. Wise
Director
Great Lakes National Program Office

ABSTRACT

One bioassay and five chemical extraction procedures for estimation of biologically available particulate phosphorus (BAPP) were applied to 12 samples of aquatic particulate matter from the lower Great Lakes region. The determinations were made to provide a basis for comparing or converting estimates among the procedures. Although the procedures extracted widely differing amounts of phosphorus (P), the results indicate that accurate comparisons or regression conversions may be made among procedures for most samples, but not for all samples. In fresh samples amounts of P extracted by the procedure of De Pinto were consistently closest in magnitude to the amounts taken up by algae during the bioassays. On both fresh and archived samples the procedure of Armstrong gave results that overestimated but correlated most closely with the bioassay results.

The effects of storage time (0 to 9 days) and temperature (4, 22, and 45 C) on concentrations of soluble reactive P (SRP) and BAPP were examined using unfiltered water samples from two rivers (Maumee and Huron) in the western basin of Lake Erie. The results showed significant changes in concentrations of the two forms of P for most combinations of time and temperature of holding, and the major changes occurred at 45 C for samples from both rivers. It was evident from the results that any period of sample storage could affect the reliability of SRP estimates in river samples held at 45 C. The results support the procedures recommended by USEPA and Standard Methods for the handling of water samples collected for P analysis. Observed changes in SRP during storage, however, were offset partially by inverse changes in BAPP, indicating that for storage times of no more than 9 days, the total bioavailable P (BAP) of water samples (SRP+BAPP) may be partially conserved.

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ACKNOWLEDGEMENTS

The cooperation and assistance of many persons were essential in completing this project. Special thanks are due to Dr. Bill Richardson, Dr. Mike Mullin, and other U.S. EPA personnel at the ERL-Duluth Large Lakes Research Station (Grosse Ile, MI) for the use of their facilities during the storage effects investigation; to Dr. Dave Baker for his willingness in supplying the manpower necessary to collect and send samples from the Ohio Rivers for use during the study of extraction methods; to Mr. Dave Payne of U.S. EPA Region V Quality Assurance Office for assistance in designing the analytical program; and to Ms. Marcella Gewirth, our Project Officer, from the Great Lakes National Program Office, for her interest in the project and guidance during its implementation.

SECTION 1

INTRODUCTION

BACKGROUND

For at least two decades, eutrophication of freshwater lakes and rivers has been an issue of national concern. A result of nutrient enrichment of water, eutrophication causes excessive production of algae and aquatic plants, depletion of dissolved oxygen, deterioration of fishery quality, and impairments to other beneficial uses of water resources. High nutrient inputs generally may be traced to intensified agriculture, urbanization, and direct discharges of a variety of wastes, including industrial wastewater, raw sewage, and municipal wastewater treatment plant effluents. Of the various nutrients that these sources provide to receiving lakes and rivers, phosphorus (P) generally has been determined as the key element giving rise to eutrophic conditions.

In response to the growing problem of eutrophication in the lower Great Lakes (Lakes Erie and Ontario), P discharges from point sources, such as wastewater treatment plants, have been reduced. Specifically, the International Joint Commission has recommended the reduction of total P concentrations in municipal wastewater effluents to 1.0 mg P/L and is considering further reductions (IJC 1970, 1972, 1978). Additionally, on the recommendation of the IJC (1978), detergent P levels in the lower Great Lakes basin have been reduced to help reduced P loads to the lakes. This has been accomplished by legislative actions of most, though not all, states and provinces in the basins of the lakes.

It is important to realize that the total, analytically-defineable quantity of P in a sample of natural water includes a variety of P forms, and these are not equally effective in stimulating growth of algae and other aquatic organisms. Orthophosphate, or soluble, monomeric inorganic P ($H_xPO_4^{x-3}$), is the principal form of P that is transported across cell membranes and, as such, is immediately available for direct uptake by algae and other primary producers (Wetzel 1983). As a consequence, elevated levels or availability of orthophosphate may be cited as the proximate cause of eutrophic conditions in most cases.

Much of the P contained in municipal treatment plant effluents, often over 70 percent, occurs as orthophosphate (analytically approximated as the soluble, molybdate-reactive fraction of total P, ie. SRP), and these sources have been shown (Young and De Pinto 1982) to contain relatively high levels of biologically available P (BAP). In contrast, however, only a small fraction of the P in unpolluted fresh waters, usually less than 10 percent, is present as orthophosphate (Wetzel 1983). Nonetheless, as shown by De Pinto *et al*. (1981) among other investigators, substantially more P than the orthophosphate fraction in natural waters is biologically available. Presumably, this can occur only through conversion of various soluble and insoluble particulate-bound forms to orthophosphate prior to biological uptake.

Recent studies have indicated that much of the P load entering the lower Great Lakes is from diffuse or non-point sources. In fact, it has been estimated that 50 percent and 40 percent of the total P loads to Lakes Erie and Ontario, respectively, are from diffuse sources (Chapra and Sonzogni 1979). Most of this P is contained in suspended particulate matter and has an unknown availablility. Yet, in any body of water, P availability is a function of several independent but partially intercorrelated variables which include current trophic status; abundance and diversity of biota; climate; thermal regime; morphometry; hydrology; mixing dynamics; orthophosphate concentration; concentration, size, shape, density, and distribution of forms of P associated with suspended solids (Logan et al. 1979).

In the face of such complexity, effective management of eutrophication through BAP load control requires some simplification of the problem. One approach to simplification has been to treat BAP as a state variable for mathematical simulation of water quality. This approach considers BAP as a quantity that may be measured and modeled much as any other state variable, such as dissolved oxygen or total P, and has given generally superior predictions of water quality for Lake Erie compared to a model that did not distinguish among forms of BAP (De Pinto et al. 1984). To calibrate and verify any such model, however, requires methods for analysis of BAP. Both steps would be necessary before a model involving BAP could be exploited for its potential management benefits.

STATEMENT OF THE PROBLEM

Making a conceptual distinction between total P and BAP is much easier than making an analytical one. This happens because measurement of truly bioavailable P is tied to quantification of a biological response. Thus, measurement of BAP requires the use of bioassay methods rather than chemical analysis. Unfortunately, the bioassays required for measurement of BAP generally require more time to perform and are less precise than the routine analytical procedures required for measurement of the constituents commonly used to characterize water quality. Simply stated, present knowledge concerning the forms and mobility of P in aquatic systems does not permit direct measurement of specific, discrete forms of P in water or sediments that can be equated with BAP.

For example, it is well accepted that soluble BAP depends principally on the orthophosphate fraction, which usually is analytically approximated by measurement of soluble (molybdate-)reactive phosphorus (SRP). However, data published by De Pinto et al. (1980) and Young et al. (1982) concerning bioavailability of wastewater P demonstrate that soluble BAP substantially exceeds that which may be measured as SRP. Moreover, the problem is compounded in the case of particulate BAP (BAPP) owing to the variety of physical and chemical phases that may be present. Awareness of the need for and the difficulties associated with bioassays, particularly with respect to estimation of BAPP, has led to a growing body of research directed toward development of empirical methods for quantification. For BAPP measurement, several investigators recently have used chemical extraction techniques.

In general BAPP extraction methods have been adapted from procedures developed by agronomists for assessment of soil fertility as related to crop production (Chang and Jackson 1957). Methods for BAPP analysis generally derive from the work of Williams and co-workers (Williams et al. 1971), who modified soil procedures for application to aquatic sediments. A common feature of most sediment BAPP measurement schemes is an extraction with strong

base, NaOH. Unfortunately, the specific procedural modifications that have been used in connection with aquatic sediments have tended to be unique to each investigation. This has resulted in the development of a variety of P extraction methods and, consequently, varying estimates of BAPP. Furthermore, comparisons between the various procedures has received little attention (Williams et al. 1980). In addition, few investigations have included bioassay determinations as a reference for comparisons between extraction procedures. When bioassays have been performed, other aspects of the methods have varied as much as the chemical extraction methods. This methodological variability has prevented any rational synthesis of the results of various investigations on a specific system. Thus, a need exists to examine the comparability of the various methods currently used to quantify BAPP.

Once methodological variances among procedures for estimation of BAPP are resolved and a procedure is selected, it becomes possible to implement management decisions that focus on controlling BAP. With respect to implementation, however, a matter of significant practical consequence concerns specification of appropriate procedures for handling samples in the quantities required for monitoring available P levels in the lower Great Lakes. Given the large number of samples that would be required, varying periods of time may elapse between sample collection and analysis. Such holding periods, and the conditions of holding, may affect the concentrations of all forms of P in the water, including available forms, through various chemical and microbial transformations. Examples of these transformations include microbial mineralization of organic P-containing compounds, immobilization of phosphate by microbial uptake, solubilization of inorganic phosphoric acid salts, formation of insoluble precipitates, and solid-solution partitioning phenomena involving the interfaces between sample solution and suspended solids or the container.

Despite an awareness of the problem, relatively little is known about the effects of storage on the BAP content of water samples. Examination of the effects of storage on orthophosphate (SRP) and BAPP can provide guidance on sample handling requirements for collection of accurate data in a field program that involves estimation of BAP forms on a large scale.

OBJECTIVES AND SCOPE

Stated in general terms, the objectives of this investigation focused on comparing a variety of procedures for estimation of BAPP in the lower Great Lakes region and on determining the rate and extent to which storage affects orthophosphate (SRP) and BAPP concentrations in natural water samples. Specifically, the objectives included the following elements:

1. Compare five chemical extraction procedures for estimation of BAPP.

2. Compare chemically defined estimates of BAPP to those obtained by bioassay measurements.

3. Determine the rate and extent to which concentrations of SRP are affected by time of storage, temperature during storage, sample source, and source related variables (initial pH, suspended solids, total P, conductivity).

For the first objective, a series of 12 sediment samples, collected from diverse aquatic systems around the lower Great Lakes, were analyzed using five

chemical extraction procedures for estimation of BAPP. The procedures that were compared are used currently by individuals engaged in research in the Great Lakes region. The chemical procedures for estimation of BAPP included those used by Dr. D.E. Armstrong et al. (1979) at the University of Wisconsin; Dr. D.B. Baker at the Water Quality Laboratory, Heidelberg College; Canada Center for Inland Waters (Mayer and Williams 1980); and two variants of the procedure of De Pinto et al. (1981) in use at Clarkson University.

Bioavailability bioassays using algae and sediments as the sole P supply for growth were performed on the same sediment samples as those analyzed to meets the requirements of the first objective. This was done to provide reference estimates of BAPP levels for inclusion in the analysis of comparability among the extraction procedures. The bioassays were performed using techniques that were developed and tested during previous investigations (De Pinto et al. 1981, De Pinto 1982, Young et al. 1982, Young and De Pinto 1982).

Meeting the third objective required a combined field and laboratory examination of the rate and extent to which storage causes changes in the concentrations of BAPP and immediately-available soluble P (SRP) in water samples that contain particulate matter. Experimental variables that were controlled included the sample source and time and temperature of holding; variables related to sample source were not controlled but were treated as covariates in the analysis of the fixed effects. Two different rivers were selected to provide a broad range of concentrations of P and suspended solids for incubation at three temperatures: 4, 22, and 45 C; and holding times up to nine days. The conditions at the upper limits (nine days, 45 C) were selected to simulate sample holding under extreme summer conditions as might be encountered during part of an extensive monitoring effort.

By evaluation of the relationship between several common methods for chemical measurement of BAPP and how they compare to bioassay measurements, the results of this investigation provide a basis for understanding the limit on accuracy that applies to efforts to integrate past and future research concerning determination of BAPP. Further, the results that pertain to the effects of storage will yield needed information on sample handling requirements for collection of accurate data on BAP.

Analysis of the results from the proposed study have not been directed toward support of a "best" procedure for chemically measuring BAPP. The term "best" when applied to BAPP methods can assume several connotations: accurate or unbiased, most consistent or precise, or, perhaps, easiest to implement, logistically. At best, "best" for a monitoring system may not be the "best" for surveillance, or the best for field- or laboratory-based research. Rather, the intent of the research has been to provide a systematic examination of the similarities and differences that exist between the results of the various procedures, including bioassay methods, for determination of BAPP. The results, therefore, constitute a guide for procedure selection that permits an informed choice insofar as that may be done by considering the potential analytical difficulties associated with each method, the degree of correlation to be expected between the selected procedure and other methods, and correlation of each method with truly available P as measured by bioassay.

This investigation has focused on characterization of BAPP in samples from fluvial systems in the lower Great Lakes region and the results so obtained are intended primarily to be used in that area. Nonetheless, the chemical extraction methods proposed for investigation are based on

fundamental aspects of the chemistry of P in soils and sediments. Furthermore, the variety of sources of particulate matter tested has provided generality for the conclusions of this study. Consequently, application of the results should not be overly constrained by unique geographical or geological features of the lower Great Lakes region.

SECTION 2

CONCLUSIONS

COMPARISONS AMONG EXTRACTION PROCEDURES

The major hypothesis involving the chemical extraction procedures for estimation of BAPP stated that the procedures did not differ with respect to amounts of P each would extract from samples of sediment. The results of this investigation, tested by analysis of variance, demonstrated significant and generally consistent differences in amounts of P extracted from 12 sediment samples by four of five procedures for estimation of BAPP in aquatic samples. Absolute differences in P extracted among the procedures were strongly dependent on individual sample characteristics. The wide variation in total P levels among the sediments was the main determinant of sample influence.

Extraction results for the two variants of the De Pinto procedure differed by an average of less than 7 ug P/g for the 12 sediments, the total P of which averaged 1277 ug P/g. This difference was not significant ($p>0.05$). Thus, use of filtration rather than high-speed centrifugation for solid-liquid separation prior to color development did not appear affect the results of the analysis to an appreciable extent.

Ranked according to the average fraction of total sediment P extracted by each, the procedures and their approximate proportions, in parentheses, would be ordered:

De Pinto/Filtr. ~	De Pinto/Centrif. <	Baker <	Armstrong <	CCIW <	Total P
(1.0)	(1.0)	(2.0)	(2.8)	(3.6)	(6.4)

Analysis of the extraction data from all samples indicated that the the amount of sediment P extracted by any given procedure was not, in all cases, a simple proportion of that extracted by any of the other procedures. Rather, the results obtained from a given procedure depended, to some degree, on factors specific to individual samples. In the most severe case, results that were reproducible but quite anomalous were obtained when the De Pinto-based procedures were applied to sediments that had been held in storage for several years. The other extraction procedures, however, gave characteristic results. In general, therefore, it may be concluded that a simple regression equation may serve to convert estimates of BAPP by one procedure into equivalent estimates by another procedure as long as the original estimates are for freshly collected samples.

A high degree of intercorrelation existed among the extraction results obtained by the methods of Baker, Armstrong, and CCIW for all the samples in this study. The results obtained by these procedures also correlated significantly with the total P levels of the sediment samples. This suggests that the three procedures may extract P from approximately the same physicochemically bound fraction or fractions of total P, though with different efficiencies. Since these procedures were strongly intercorrelated

for all samples, regression equations could be used to convert BAPP estimates between each of the three procedures with acceptable accuracy for both fresh and stored samples.

None of the procedures investigated during this project was obviously superior to the others as an estimator of algal bioassay-determined BAPP. Amounts of P extracted from freshly collected sediments by the De Pinto procedures were consistently closest in magnitude to the amounts taken up by algae during the bioassays and could, therefore, be considered the most accurate predictors of BAPP among the methods tested. On the other hand, while the Baker, Armstrong, and CCIW procedures gave extractable P levels that generally overestimated the bioassay results, they correlated most closely with the bioassay results for both fresh and stored samples. As a consequence, these three procedures were the most precise for prediction of BAPP using regression equations. Among the various procedures, that of Baker was the simplest to perform and easily could be adapted for use in most moderately-equipped analytical laboratories; however, the extraction methods of De Pinto were the most reproducible.

THE EFFECTS OF SAMPLE STORAGE ON AVAILABLE PHOSPHORUS

Water samples from two rivers held under controlled conditions of temperature for varying periods showed significant changes in sample concentrations of SRP, BAPP by the method of Baker (1983), and BAPP per unit of suspended solids. The changes depended simultaneously on all the experimental factors: sample source, temperature of holding, and time of holding. This means that SRP concentrations in samples from the Huron River changed over time and as a function of temperature in a manner that did not parallel that observed in samples from the Maumee River. Changes in SRP concentrations for temperatures of 4 and 22 C were small for samples from both rivers for all holding periods, and, with the exception of the 9 day holding period at 4 C for the Huron River samples, none of the differences were significant for samples held at these temperatures. Samples held at 45 C, however, demonstrated significant changes within the first 0.5 day period of incubation in samples from both rivers.

The direction of the change in SRP concentrations was similar for samples from both rivers when incubated at 45 C; in both cases the concentration of SRP decreased to very low levels. At 4 and 22 C, however, the direction of change in SRP was dissimilar between the rivers; in the Huron River samples SRP increased, while in the Raisin River samples, SRP did not change enough to establish a reliable trend.

The data on effects of storage on BAPP and BAPP per unit weight of suspended solids were analyzed to test hypotheses similar to those for SRP. The results indicated significant changes in BAPP concentrations occurred during storage and that the changes were dependent simultaneously on all of the main effect variables: sample source, temperature of holding, and time of holding. Normalization of the BAPP for suspended solids concentration did not alter these conclusions.

Incremental changes in concentrations of SRP and BAPP during sample storage were tested to determine whether changes in one "available" fraction would be predictable from changes in another. The results showed a significant negative correlation between the two quantities, which indicates that losses of SRP during storage were balanced to an extent by increases in BAPP. Based on a regression analysis of the incremental changes,

approximately 90 percent of SRP concentration losses and gains were reflected in reciprocal gains and losses of BAPP in the water samples. Since, as noted above, different procedures for extraction of BAPP from sediments gave different results, it is possible that a value other than 90 percent would have resulted had these samples been analyzed by one of the other procedures. Nonetheless, it appears that changes in SRP during storage may be accompanied by simultaneous and opposite changes in BAPP.

Changes in SRP during storage were evaluated by an analysis of covariance to determine whether initial pH, conductivity, and concentrations of total suspended solids, total P, and SRP in field filtered subsamples were related to changes in SRP during each storage period. Among these variables, only the concentration of SRP in samples immediately after collection was a consistent factor relating to changes in SRP during storage. Samples with high initial concentrations of SRP tended to be those that lost more SRP during storage; this was characteristic of the samples from the Maumee River, on the average. Effects attributable to initial SRP, however, were not significant for more than two days after storage was begun.

SECTION 3

RECOMMENDATIONS

COMPARISONS AMONG EXTRACTION PROCEDURES

This investigation has shown that it is feasible to use regression relationships to transform BAPP estimates among several methods when freshly collected sediments have been analyzed. This practice, however, is not recommended for sediments that have been held in storage for long periods.

The anomalous results observed for stored samples underscore a need for additional research to improve knowledge of factors that give rise to varying estimates of BAPP by different chemical and biological analytical methods. It is recommended that additional research be undertaken that focuses on fundamental properties of BAPP and development of appropriate methods for its quantitation.

Inasmuch as the concept of BAPP has assumed a significant role in development of Great Lakes water quality management strategies, it is recommended that a single procedure for BAPP measurement be adopted for common use within the Great Lakes community. To do otherwise would confine BAPP to research functions only and would preclude its application and acceptance for monitoring and surveillance activities, even though it has been largely out of concern for the latter that the concept has developed. Since estimates of BAPP depend on the procedures selected, any successful program that contains surveillance or monitoring activities must employ a single, standardized method to allow the data to have application beyond the components of the specific investigation.

Since the concept of fractioning total P into available and unavailable portions has been demonstrated to be realistic in the laboratory, the next step should be a field scale test of the concept. It is recommended that a monitoring and research program be undertaken to demonstrate the feasibility, potential value, and logistical problems of using BAP as a management tool for improvement of water quality. The program should focus on one of the Great Lakes, or a significant segment thereof, and include collection of data on BAP inputs, outputs, and changes in storage within the system. It is emphasized that a field-oriented program like this would mandate use of a single procedure for analysis of BAP to insure transfer of compatible data for mutual use among program participants.

THE EFFECTS OF SAMPLE STORAGE ON AVAILABLE PHOSPHORUS

The data presented on the effect of sample storage on concentrations of BAP suggest that for temperatures in the range of 4 to 45 C, no holding time is truly satisfactory for all samples if they are to be analyzed for SRP and BAPP. Thus, it is recommended that water samples collected for analysis of

BAP forms be refrigerated and the analysis be performed as soon as possible (24-48 hours). These recommended storage conditions do not differ significantly from those given in Standard Methods (APHA, 1981) and by the USEPA (1976).

If samples for BAP determination cannot be analyzed immediately or refrigerated, some method of preservation should be selected. Otherwise, concentrations of the quantities of interest are subject to redistribution between solution and solid phases in the samples. It is interesting to note, however, that the redistribution of fractions appears to be somewhat conservative on the average, since loss from the SRP fraction may be picked up partially during analysis as a gain in the BAPP fraction. Thus, an approximation to the total quantity of BAP (particulate and soluble) in a water sample may be estimable from a stored sample if both SRP and BAPP are determined and summed, and if the storage period is not longer than a few days. Additional research on the nature and extent of BAP redistribution during sample storage could be helpful in establishing sound alternatives for preservation or immediate analysis.

SECTION 4

METHODS AND MATERIALS

COMPARISONS AMONG EXTRACTION PROCEDURES

Study Design

Replicate analyses on a series of sediment samples with a range of P biological availability provided the basis for comparisons among BAPP extraction procedures. The procedures compared during this investigation are described in a detailed, stepwise manner in Appendix A and include those of Armstrong et al. (1979), Baker (1983), Canada Center for Inland Waters - CCIW (Mayer and Williams 1982), and De Pinto et al. (1981); the procedure of De Pinto et al. (1981) was studied as two procedures differing only by use of centrifugation or filtration of the extractant solution from the sediments immediately prior to neutralization for color development. The procedures all differ in some respects but they have in common a period of contact between sediments and NaOH. Table 1 is a summary of the procedures and unique points about each.

In addition to these procedures, algal bioassays were performed to provide estimates of BAPP as a control against which each of the chemical procedures could be compared and for use as a covariate in comparing the procedures among themselves. The bioassay procedure involved use of the DCDA (Dual Culture Diffusion Apparatus) of De Pinto (1982), and a detailed description of the DCDA bioassay procedure is given in Appendix A.

Sampling

For BAPP comparisons, river water and bottom sediment samples were collected from seven major tributaries to the Lower Great Lakes. These included the Cuyahoga, Maumee, and Sandusky Rivers of Ohio; Cattaraugus Creek, and the Genesee and Oswego Rivers of New York; and the Raisin River in southeastern lower Michigan. The location of the sampling sites is shown in Figure 1.

Sampling of the Ohio and Michigan rivers began mid-March 1984 and continued through mid-April of that year. Samples were collected by personnel from the Water Quality Laboratory at Heidelberg College, (Tiffin, Ohio). Collections were made as surface grab samples near the center of the main channel during major storm runoff events. Bottom sediment samples were taken during early May 1984 from each of the three New York rivers. Also included in the sample set were two archived bottom sediment samples collected during the fall 1984 from the southwestern shore of Lake Erie, near Monroe, Michigan. To complete the set of 12 samples, a standard river sediment sample from the National Bureau of Standards (SRM 1645 River Sediment) was included.

TABLE 1. METHODS FOR DETERMINATION OF BIOAVAILABLE PARTICULATE PHOSPHORUS IN USE IN THE GREAT LAKES REGION.

Procedure	Extractant	Extractant:Solid Ratio	Comment**
Armstrong	0.1 N NaOH+ 1.0 N NaCl	2000:1	Approximates NAIP
Baker	0.1 N NaOH+ 1.0 N NaCl	1250:1	Solids on filters during extraction
CCIW	CDB(reductant) then 0.1 N NaOH	500:1	Sequential; Sum = NAIP
De Pinto*	0.1 N NaOH	600:1	Correlations with BAPP as determined by algal bioassay

* The method of De Pinto includes both a filtration and a centrifugation separation of solids and extractant.

** NAIP = Non-apatite Inorganic Phosphorus
BAP = Bioavailable Phosphorus

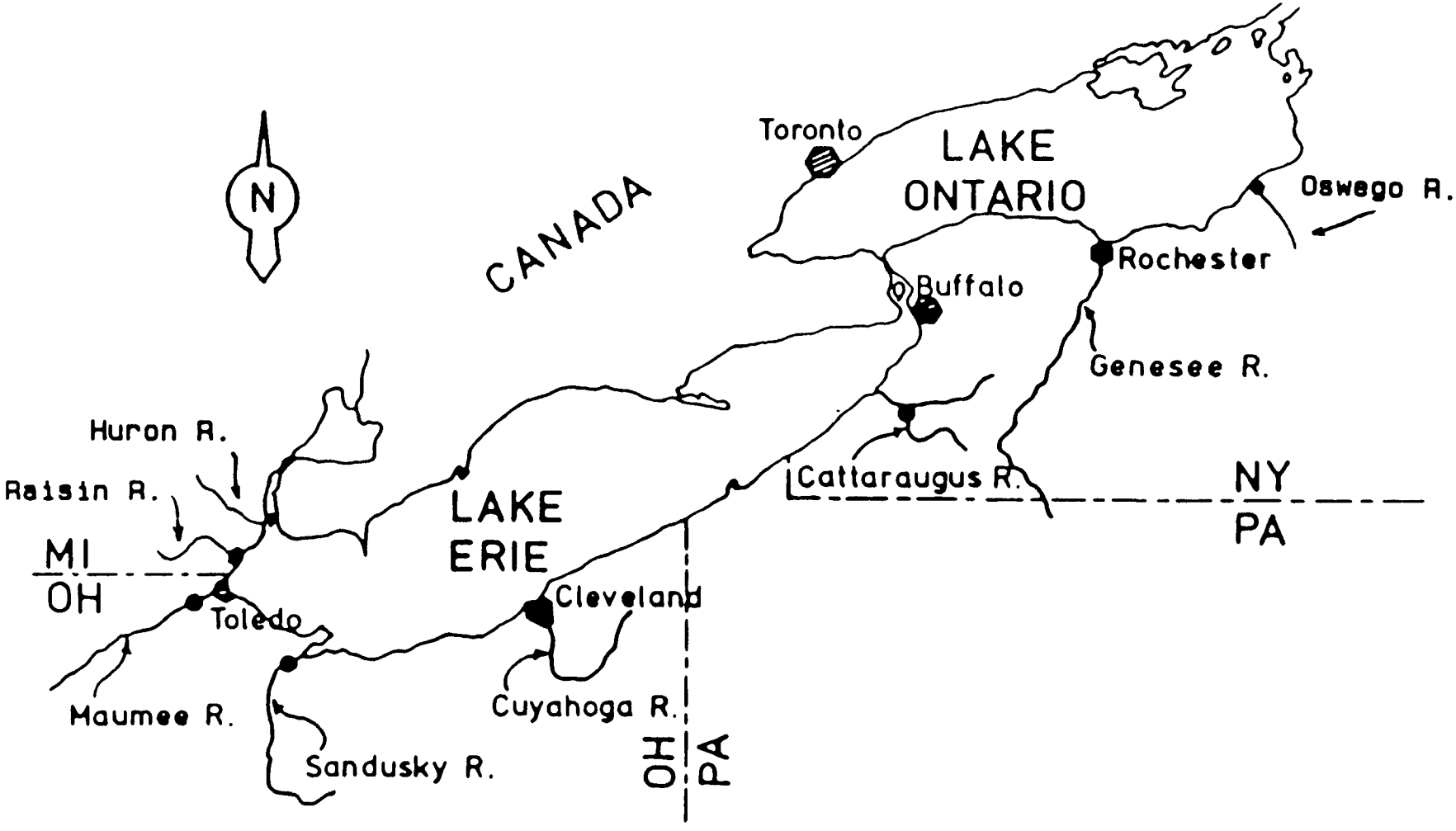

Figure 1. Location of sampled systems in the lower Great Lakes region.

Sample Handling and Preparation

For each of the Ohio and Michigan rivers, approximately 10 liters of river water were collected and placed in acid-cleaned polyethylene cubitainers. Upon return to the Water Quality Laboratory at Heidelberg College, the cubitainers were stored at 4 C. Within a few days of collection, the samples were placed in insulated shipping containers (Trans-Temp) with frozen ice-packs and shipped to Clarkson University by Greyhound Bus.

Upon receipt at Clarkson, the cubitainers were mixed thoroughly and the contents filtered under positive pressure through 0.4 micron pore-diameter polycarbonate membranes (Nuclepore). The non-filterable residue retained on the filter was removed using a flexible spatula and resuspended in sufficient P-free synthetic medium (Martin 1983) to yield a solids concentration of approximately 10 mg/ml. The resuspended "concentrates" of non-filterable residue were refrigerated at 4 C until analysis. Martin (1983) observed that such residue concentrates stored at 4 C lost an average of less than two percent of total sediment P to the resuspension medium over a period of several months.

Sediments from the Genesee and Oswego Rivers, Lake Erie, and Cattaraugus Creek were collected as bottom grab samples. The entire contents of the sediment grabs were mixed to homogeneity and an aliquot of each was transferred into an acid-cleaned polyethylene jar. The jars then were placed on ice in a cooler and transported by automobile to Clarkson University. Upon arrival at the Clarkson laboratory, the bottom sediment samples were resuspended in the same P-free medium as the non-filterable residue samples described previously to solids concentrations of approximately 10 mg/ml. To improve analytical precision in a few of the more heterogeneous samples (Cattaraugus and Genesee), pebbles, sticks, coarse and fine sand fractions were removed from the sediment concentrates by sedimentation prior to placement into storage at 4 C.

Analytical Program

Prior to performing the BAPP extractions it was necessary to determine the suspended solids concentration of each of the sediment concentrates. This was done using standard methods (APHA 1981), and involved vacuum-assisted filtration of a known volume of sample through a dried and tared, 0.45 micron pore-diameter nitrocellulose membrane for collection of the non-filterable residue. After drying to constant weight at 103 C, the residue was determined by weight change of the filter plus residue on a Mettler Model A30 analytical balance.

Measurement of P in water was performed colorimetrically, using the method of Murphy and Riley (1962) as described in Standard Methods (APHA 1981). Sample pretreatments included filtration through 0.45 micron pore-diameter nitrocellulose membranes to separate soluble fractions from the total sample for determinations of SRP and sulfuric acid-peroxydisulfate digestions for conversion of combined P to orthophosphate for determinations of total P. Both of these steps were performed, when required, prior to color development.

Data Analysis

The data on BAPP were analyzed by factorial analyses of variance and covariance with the extraction procedures and the samples as main effect variables in the analysis. The factorial design was selected rather than blocking on individual samples since it was felt that the procedures might not yield proportionately similar amounts of extractable P among samples but that sample-procedure interaction could be significant and should be tested. Total P and algal bioassay determined BAPP initially were used as covariates, but they were found to be redundant with each other and could not improve the efficiency of tests for differences among procedures and subsequently were dropped from the analysis. Additionally, all-pairwise correlation analyses and regression analyses were performed to clarify the relationships between the procedures.

EFFECTS OF SAMPLE STORAGE ON AVAILABLE PHOSPHORUS

Study Design

Investigation of the effects of short term storage on the concentration of orthophosphate and BAPP in river water was conducted over a period of three weeks beginning mid-March 1984. The work was performed at a temporary laboratory site established at the U.S. Environmental Protection Agency Large Lakes Research Station at Grosse Ile, Michigan (ERL-Duluth).

Over a period of nine days, water samples were collected daily from the study rivers and transported to the temporary laboratory. Subsamples were filtered immediately upon collection in the field for determination of initial SRP concentrations. Upon arrival at the laboratory, each sample was split into 15 subsamples for incubation at temperatures of 4, 22, and 45 C for periods of 0.5, 1.0, 2.0, 4.0, and 9.0 days. At initiation and at termination of each incubation period, subsamples were sacrificed for determination of SRP and BAPP by the method of Baker (1983).

River Selection

The rivers selected for the storage effects study were the Huron and Maumee Rivers. They were chosen after review of data from the Corps of Engineers on suspended sediment and orthophosphate concentrations in tributaries to the lower Great Lakes. The review was undertaken with the intent of selecting two rivers with widely differing concentrations of both materials to maximize the possibility that the investigation would include samples of both low (<0.05 mg P/L) and high (>0.10 mg P/L) concentrations of P and suspended solids. Other considerations for selection were: accessibility from either Grosse Ile, MI or Tiffin, OH (either was a potential site for location of a temporary laboratory) and contribution of significant flow to the lake.

Because of its very large flow and high concentrations of SRP and suspended solids, the Maumee River was an obvious choice. The other stream, therefore, had to be one that generally contained low levels of these materials. The Huron River (MI) and Honey Creek (OH) appeared to be the best alternatives, based on the above criteria. Due to lower levels of soluble reactive P in the Huron River, it ultimately was selected as the second

river. The data that led to this selection is summarized in the following:

For the period 3 March 1977 through 27 June 1977, the mean daily maximum concentration of SRP and suspended solids (standard deviations in parentheses) for the three rivers named above were:

Maumee River:	0.112 mg P/L (0.029 mg P/L) and	213.4 mg SS/L (316.7 mg SS/L)
Honey Creek:	0.063 mg P/L (0.029 mg P/L) and	30.8 mg SS/L (76.3 mg SS/L)
Huron River:	0.025 mg P/L (0.019 mg P/L) and	24.7 mg SS/L (10.7 mg SS/L)

Sampling

On three days during the period 21-29 March 1984, ten samples of approximately ten liters each were collected from both the Maumee and Huron Rivers. Upon collection the water was placed in acid-cleaned polyethylene cubitainers. Each cubitainer was immediately subsampled and the aliquot was filtered through a 0.4 micron pore-diameter polycarbonate membrane filter. A hand pump was used to provide vacuum during filtration. The filtrate was transferred to a clean 125 ml polyethylene bottle for later analysis of SRP, and the bottle was placed on ice in a cooler. The remainder of the sample also was placed in the dark and cooled for transport to the temporary laboratory.

Upon arrival at the laboratory, the contents of each cubitainer were mixed thoroughly and 100 ml aliquot subsamples were transferred to a series of 15 new, acid-cleaned 125 ml polyethylene bottles for incubation at temperatures of 4, 22, and 45 C and a second series of four similar bottles for determinations of initial concentrations of total and soluble reactive P and suspended solids. Incubation of the subsamples was done in darkened, thermostatted enclosures and temperature was monitored by frequent observations with a mercury thermometer.

Determinations also were made of initial pH and conductivity. The pH was determined using an Orion Model 501 ion analyzer with a glass-AgCl combination pH electrode. Conductivity was measured using a YSI Model 31 conductivity bridge.

During the storage effects study, all orthophosphate concentrations were determined colorimetrically using a Baush and Lomb Spectronic 710 spectrophotometer. Color development was performed using the method of Murphy and Riley (1962) as described in Standard Methods (APHA 1981) and was done after filtration of the samples through 0.4 micron pore-diameter polycarbonate membranes (Nuclepore). Preliminary tests on standard orthophosphate solutions and river water showed the laboratory filtration apparatus and the field filtration units gave nearly identical results (Appendix F). Initially a set of standard solutions ranging from 0 to 200 ug P/L were analyzed with each group of samples to provide a standard curve against which to read sample concentrations.

All orthophosphate concentrations throughout the study were calculated from this initial standard curve. Subsequent analyses were performed using the Youden "A-B" technique for calibration control. Included for implementation of the technique were two deionized water blanks, a 100 ug P/L orthophosphate standard, an "A" solution with an orthophosphate concentration of approximately 20 ug P/L, and a "B" solution with an orthophosphate

concentration of approximately 160 ug P/L.

At elapsed time intervals of 0.5, 1.0, 2.0, 4.0, and 9.0 days from the beginning of incubation, one subsample (in 125 mL bottle) from each was removed from each incubator and filtered under vacuum through a 0.4 micron pore-diameter polycarbonate filter. The filtrate was analyzed immediately for SRP. The non-filterable residue was retained on its filter and placed in a clean 125 ml polyethylene bottle for storage by refrigeration until later chemical extraction of BAPP.

The BAPP extractions and analyses of total P were performed upon return to Clarkson University from the temporary laboratory at Grosse Ile, Michigan. The extraction method used was similar to the NaOH/NaCl procedure of Baker (Table 1, p.15; and Appendix A) modified by substituting a 0.4 micron pore-diameter polycarbonate membrane for the 0.6 micron pore-diameter polyvinyl chloride membrane recommended by Baker (1983). The latter no longer are manufactured and preliminary testing showed the polycarbonate membranes would withstand extraction by 0.1 N NaOH for the required period of shaking.

Data Analysis

Effects of storage conditions on concentrations of SRP and BAPP were tested by analyses of variance and covariance. The experimental arrangement permitted analysis as a double split-plot design since the samples were split with respect to treatments (temperature of incubation) and split within treatments for repeat measurement (time of incubation). Other designs, such as latin squares or randomized complete blocks also could have been used effectively for analysis of the experiment; however, the double split-plot model required fewer assumptions about the nature of the processes affecting the samples and was conservative with respect to the partitioning of variance. Covariates used to improve the efficiency of the analysis included total P, total suspended solids, pH, conductivity, and field filtered SRP.

SECTION 4

RESULTS AND DISCUSSION

EFFECTS OF SAMPLE STORAGE ON AVAILABLE PHOSPHORUS

The results of total and SRP analyses, as well as suspended solids, pH, and conductivity measurements from both Huron and Maumee river samples are presented in Table 2. These data indicate distinct differences between samples from the two rivers. For example, the 10 Huron River samples were characterized by relatively low levels of total P (67.6-82.7 ug P/L), SRP (4.2-9.5 ug P/L), and suspended solids (10-26 mg/L). In contrast, the levels of these parameters were considerably higher for samples from the Maumee River. With respect to pH and conductivity, however, the Maumee values were lower than those from the Huron River.

The response of both Huron and Maumee river samples during storage for as long as nine days at three different temperatures (4, 22, and 45 C) is shown graphically in Figures 2 and 3. Each data point represented on these figures is the average response of 10 samples collected during the study. A listing of SRP measurements for all treatment combinations is given in Appendix B.

Examination of the Maumee River plot shows essentially no change in SRP concentration for samples stored at 4 and 22 C. In contrast, Huron River samples stored at these same temperatures decreased in concentration for the first two days of storage, then increased somewhat throughout the remainder of the study. Johnson et al. (1975) found SRP levels increased rapidly but subsequently decreased in stream samples stored at 5 C for 30 days. Others have also observed increases in the soluble inorganic P content of river water stored at 4 and 23 C for 12 weeks (Klingaman and Nelson 1976). Heron (1962) found that lake water samples stored at room temperature exhibited an increase in P concentration during the first 10 hours of storage. This increase was followed by decreasing concentrations for the remainder of the study, a sequence of concentration changes similar to those observed during the present study on samples from the Huron River. Additionally, Murphy and Riley (1956) reported that the SRP of two seawater samples stored for one month at 20 C had increased from 13.5 to 15.7 and 25.7 to 27.2 ug P/L, respectively.

After a substantial initial increase in SRP concentration, within 12 hours of storage at 45 C, samples from the Huron River rapidly decreased to extremely low levels. Similarly, the Maumee River samples lost a significant amount of SRP during the first 4 days of storage, but unlike the Huron samples increased slightly between 4 and 9 days. It is apparent from these data that the kinetics of SRP transformations did not follow simple rate laws over the incubation periods. The treatment combination giving rise to the most consistent response was that for the Maumee samples incubated at 45 C. For this group of samples the half-life of SRP was approximately 1 d over the first 4 days of incubation. This is equivalent to a first order loss rate of

TABLE 2. CHEMICAL CHARACTERISTICS OF WATER SAMPLES COLLECTED FOR STORAGE EFFECTS STUDY.

Source and Sample Number	Date of Collection	Total Phosphorus (ug P/L)	Soluble Reactive Phosphorus (ug P/L)	Suspended Solids (mg/L)	pH	Conductivity (umho/cm)
Huron #1	21Mar84	81.1	4.9	24	8.15	540
Huron #2	21Mar84	79.5	4.2	21	8.17	500
Huron #3	21Mar84	67.6	4.6	26	8.16	520
Huron #4	26Mar84	77.2	9.5	11	8.09	610
Huron #5	26Mar84	79.6	9.4	17	8.09	580
Huron #6	26Mar84	70.8	9.4	18	8.11	600
Huron #7	26Mar84	80.4	8.7	16	8.10	600
Huron #8	29Mar84	69.2	9.5	10	8.07	570
Huron #9	29Mar84	78.8	8.8	11	8.07	560
Huron #10	29Mar84	82.7	8.9	13	8.07	560
Maumee #1	21Mar84	467.7	92.2	189	7.77	330
Maumee #2	21Mar84	392.7	95.5	199	7.77	320
Maumee #3	21Mar84	409.2	93.5	170	7.77	330
Maumee #4	26Mar84	308.6	94.4	127	7.81	355
Maumee #5	26Mar84	319.2	73.3	133	7.81	340
Maumee #6	26Mar84	317.6	73.1	130	7.82	345
Maumee #7	26Mar84	331.2	73.5	139	7.81	350
Maumee #8	29Mar84	412.2	71.6	198	7.86	345
Maumee #9	29Mar84	425.7	70.5	204	7.86	350
Maumee #10	29Mar84	425.7	76.6	215	7.86	340

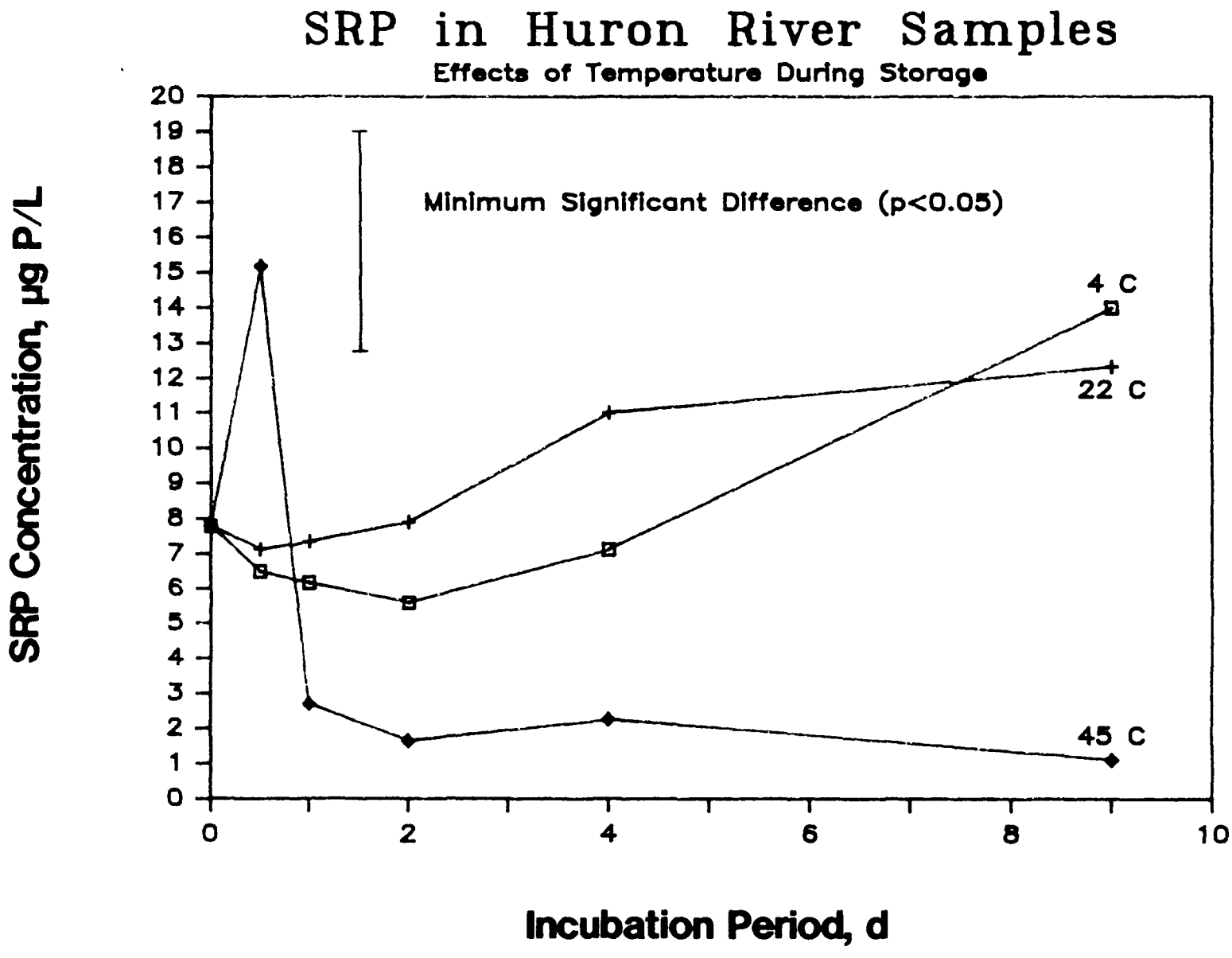

Figure 2. Concentration of SRP in Huron River samples as a function of temperature and holding time during storage effects study.

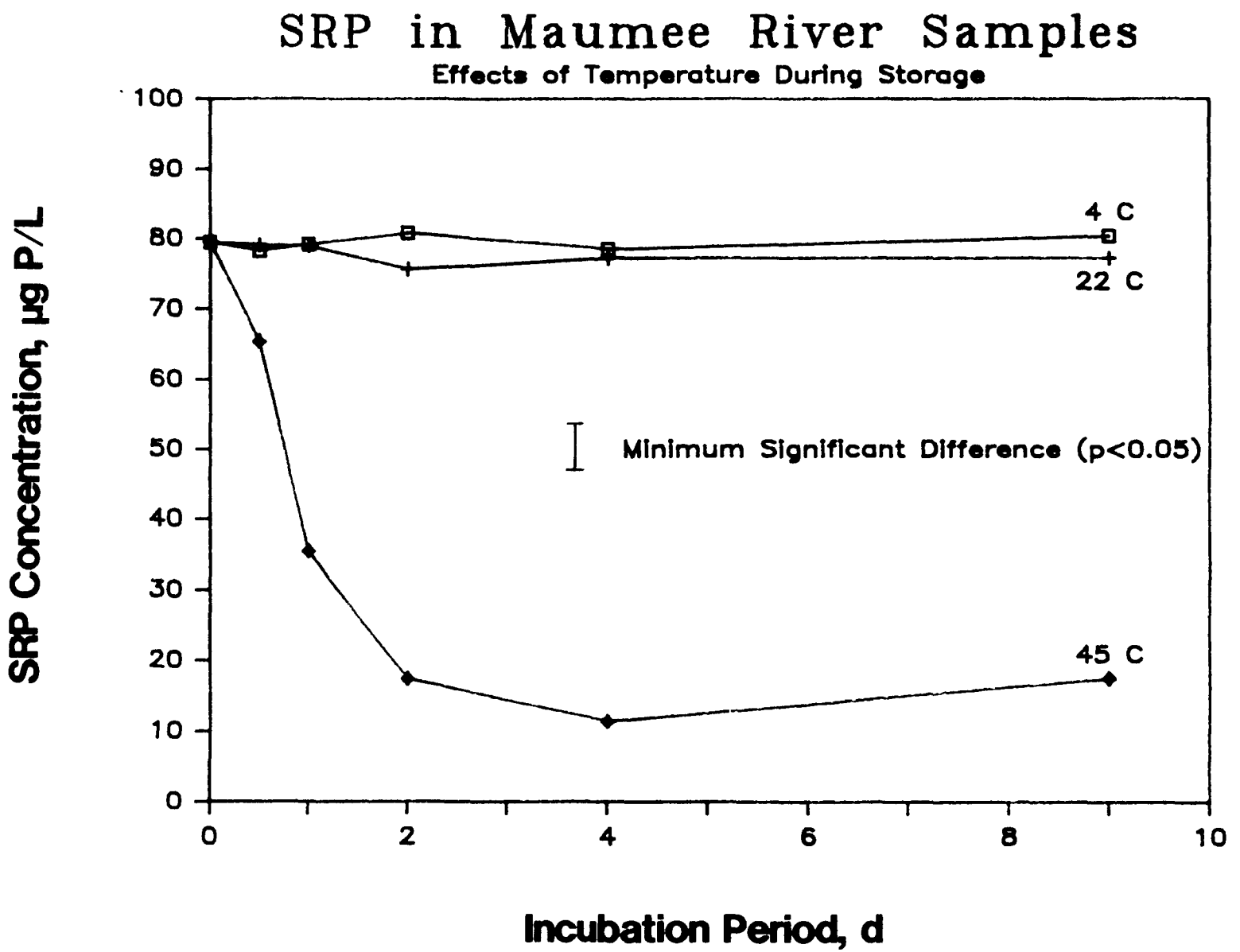

Figure 3. Concentration of SRP in Maumee River samples as a function of temperature and holding time during storage effects study.

approximately 0.7/d. The variability of the transformation, however, precluded a systematic analysis of reaction rate or order for the stored samples.

Although Figures 2 and 3 illustrate the general trends of SRP concentration during storage, statistical analysis of the data provides a more objective interpretation of storage effects. Analysis of variance (Appendix E.1) indicated that significant changes in SRP concentration occurred during storage, and that the changes depended simultaneously on three variables: sample source (river), temperature during storage, and time of storage. This means that changes in SRP concentrations in Huron River samples, caused by varying time and temperature of storage, did not parallel the changes observed in samples from the Maumee River under similar conditions of holding. For instance, changes in SRP concentration for temperatures of 4 and 22 C were small for samples from both rivers for all storage periods, and with the exception of the 9 day storage period at 4 C for the Huron River samples, none of the differences were significant ($p>0.05$) for samples held at these temperatures. However, samples from both rivers stored at 45 C demonstrated significant changes within the first 12 hours of incubation.

The direction of net change in SRP concentration was similar for samples from both rivers when incubated at 45 C for periods longer than 12 hours. In both cases the concentration of SRP decreased to very low levels. At 4 and 22 C, however, the direction of change was dissimilar between the rivers. In the Huron River samples, SRP decreased initially then increased, and the amount of change overall was small though significant ($p<0.05$). In the Maumee River samples, on the other hand, SRP did not change enough to establish a reliable trend at these temperatures.

It has been widely accepted that increases in SRP concentration during storage are likely due to bacterial or enzymatic decomposition of organic P compounds (Murphy and Riley 1956; Gilmartin 1967; Thayer 1970; Johnson et al. 1975; Klingaman and Nelson 1976). In fact, Heron (1962) demonstrated that a viable bacterial population is essential to bring about changes in the P concentration of lake water samples low in orthophosphate (2.6 ug P/L). Decreases in the levels of orthophosphate, on the other hand, are thought to be the result of either utilization of P by a developing bacterial population (Gilmartin 1967), or sorption reactions (Johnson et al. 1975). No data were collected on microbial populations during the storage effects study reported here, but no unusual features would be expected for the study systems.

Concentrations of BAPP in the stored water samples are given in Appendix C. These data were analyzed statistically to test the effects of storage on BAPP (Appendix E.2) and BAPP per unit weight of suspended solids (Appendix E.3). The results of the analysis of variance were similar to those derived from the SRP data and indicate that significant changes in BAPP concentration took place during storage. As was true for changes in SRP, the changes in BAPP depended simultaneously on three variables: sample source (river), temperature during storage, and time of storage. Normalization of the BAPP data for suspended solids concentration did not alter these conclusions.

Incremental changes in concentrations of SRP and BAPP during sample storage were tested to determine whether changes in one bioavailable fraction, SRP, would be predictable from changes in another, BAPP. The results showed a

significant negative correlation between the two quantities ($r=-0.562$ for $N=238$), which indicates that a loss of SRP during storage was balanced to an extent by an increase in BAPP (Figure 4). Based on a regression analysis of the incremental changes, approximately 90 percent of SRP concentration losses and gains were reflected by reciprocal gains and losses in BAPP. It is possible, however, that a value other than 90 percent would have resulted if the samples had been analyzed by a procedure other than Baker's (1983). Nevertheless, it appears that changes in SRP during storage may be accompanied by concomitant and opposite changes in BAPP. To an extent, therefore, the sum of the concentrations of SRP and BAPP may be conservative in water samples that are stored for brief periods, possibly up to nine days. Thus, it appears that an approximation of the total quantity of BAP (particulate and soluble) in a water sample may be estimated from a stored sample if both SRP and BAPP are determined and summed.

Additional statistical analyses were performed to determine the extent that initial pH, conductivity, and concentrations of total suspended solids, total P, and SRP in field filtered samples were related to changes in SRP during storage. An analysis of covariance (Appendix E.4) indicated that the only factor tested that related significantly ($p<0.05$) to changes in SRP during storage was the concentration of SRP in field filtered samples. As might be expected intuitively, samples that lost more SRP during storage tended to be those with high initial concentrations. This was particularly characteristic of samples from the Maumee River stored at 45 C. However, the analysis demonstrated that effects attributable to intitial SRP were not significant for more than two days after storage was begun.

From this investigation it can be concluded that for temperatures in the range of 4-45 C, no storage time was wholly satisfactory for Huron River samples analyzed for SRP and BAPP. Similarly, storage of Maumee River samples at 45 C permitted significant changes to occur in SRP and BAPP levels. However, storage at 4 and 22 C appeared effective for inhibition of these changes in the Maumee samples. Nonetheless, in the absence of direct evidence that samples from a given system do not require special storage precautions, it is recommended that water samples collected for analysis of BAP be stored under refrigeration and that the analysis be performed as soon as possible. This procedure is equivalent to that recommended by USEPA (1976) and Standard Methods (APHA 1981). If the samples cannot be analyzed immediately or placed in cold storage, then effective methods of preservation should be sought and implemented.

COMPARISON OF BIOAVAILABLE PARTICULATE PHOSPHORUS PROCEDURES

Chemical Extraction Methods

The results of the BAPP extractions on 12 sediment samples for all procedures are summarized in Table 3. The data presented are mean values expressed as ug of P extracted per gram of sediment. Results of the total P analyses are also included in the table. A complete listing of replicate measurements for all samples is given in Appendix D.

The results of this investigation, tested by analysis of variance (Appendix E.5), demonstrated significant, consistent differences in amounts of P extracted from various sediment samples by four of the five chemical

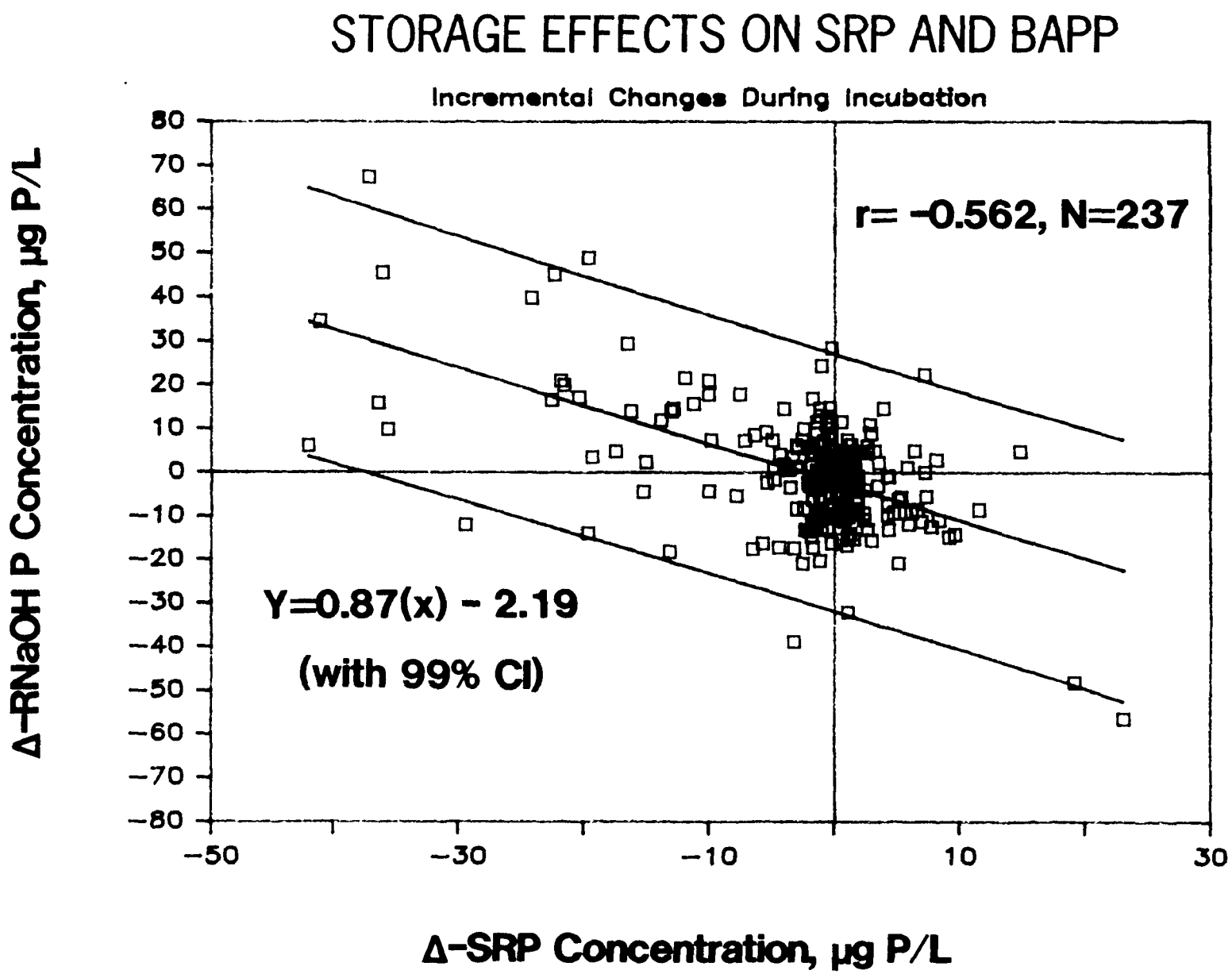

Figure 4. Regression of incremental changes in BAPP on SRP during storage effects study.

TABLE 3. SUMMARY OF BIOAVAILABLE PHOSPHORUS ANALYSES, ug P/g.

SOURCE	SAMPLE	TOTAL P	CCTCENT	CCTFILT	BAKER	ARMSTRONG	CCIW	BIOASSAY
MAUMEE R.	1	112.3	228.7	225.0	424.2	368.7	580.6	309.0
CUYAHOGA R.	2	1124.3	397.4	385.1	492.3	509.2	691.1	399.5
SANDUSKY R.	3	987.9	296.0	289.2	323.3	344.3	592.7	227.7
RAISIN R.	4	1261.5	137.8	134.9	429.2	454.6	635.8	---
MAUMEE R.	5	836.8	189.1	183.1	221.4	222.8	411.4	233.0
SANDUSKY R.	6	900.0	263.6	255.9	260.9	276.3	525.2	183.5
L. ERIE	7	2209.9	415.1	396.5	1197.0	1559.4	1929.7	1526.7
L. ERIE	8	2472.9	264.8	250.1	1295.8	1790.2	2230.4	2040.3
OSWEGO R.	9	1007.2	223.9	219.2	226.1	231.8	334.3	106.0
GENESEE R.	10	607.5	91.3	85.6	124.8	128.9	223.9	56.7
CATTARAUGUS CREEK	11	423.9	30.2	29.7	33.7	33.8	79.9	2.7
NBS STANDARD SEDIMENT	12	985.6	38.9	43.2	108.9	115.9	291.4	---

extraction procedures. Extraction results for the two variants of the De Pinto procedure (CCTCENT, CCTFILT on Figure 5) differed by an average of less than 7 ug P/g for the 12 sediments, which was not significant ($p>0.05$). Thus, the use of filtration rather than high-speed centrifugation for solid-liquid separation prior to color development gave no appreciable bias to the results of the analysis.

Among the samples, total P ranged from 418 to 2482 ug P/g, with the highest values measured in the samples from Lake Erie and the lowest measured in samples from Cattaraugus Creek. Ranked according to the average fraction of total sediment P extracted by each, the procedures would be ordered:

De Pinto/Filtr. ~	De Pinto/Centrif. <	Baker <	Armstrong <	CCIW <	Total P
(1.0)	(1.0)	(2.0)	(2.8)	(3.6)	(6.4)

This relationship is shown graphically in Figure 5.

The amount of P extracted by the various procedures depended interactively on sample-specific factors. This means the P extracted from sediments by one or more of the procedures was not a simple, consistent proportion of that extracted by each of the other procedures. From this finding the conservative conclusion may be drawn that it is not always possible to apply simple factors such as regression coefficients to convert estimates of BAPP by one procedure into estimates by another procedure. Doing so could result in some biased BAPP estimates. This conclusion is too simplistic, however, as the following more detailed consideration of the results will show.

This procedure-sample dependency may be illustrated by examining the extent of correlation between the BAPP procedures. A complete table of correlation coefficients for all possible procedure pairings is presented in Appendix E.6.

The results obtained by the methods of Baker, Armstrong, and CCIW showed a high degree of intercorrelation ($r>0.992$ for $N=12$). Further, the procedures correlated strongly ($r>0.975$ for $N=12$) with the total P levels of the sediment samples (Figure 6). This suggests the procedures of Baker, Armstrong, and CCIW may extract P from the same physicochemically bound fraction or fractions of total P, but do so with varying efficiencies.

The extraction results obtained by the two modifications of the De Pinto method also showed significant positive correlation ($p\leq 0.05$) with each of the other extraction procedures and total P, but the magnitude of the correlation was less ($0.566<r<0.647$ for $N=12$). As will be explained, the lower correlation was caused by anomalous results from the two Lake Erie bottom samples.

In Figure 7 BAPP estimates by the procedures of Baker, Armstrong, and CCIW procedures are compared with those obtained by the De Pinto centrifuge procedure (CCTCENT in Figure 7) for the study sediments. With the exception of the results for the Lake Erie samples, it appears that a relatively linear relationship exists between BAPP extracted from the sediments by the various methods. The extractable P values determined by the De Pinto procedure for the Lake Erie samples seem anomalous and an obvious cause of much of the

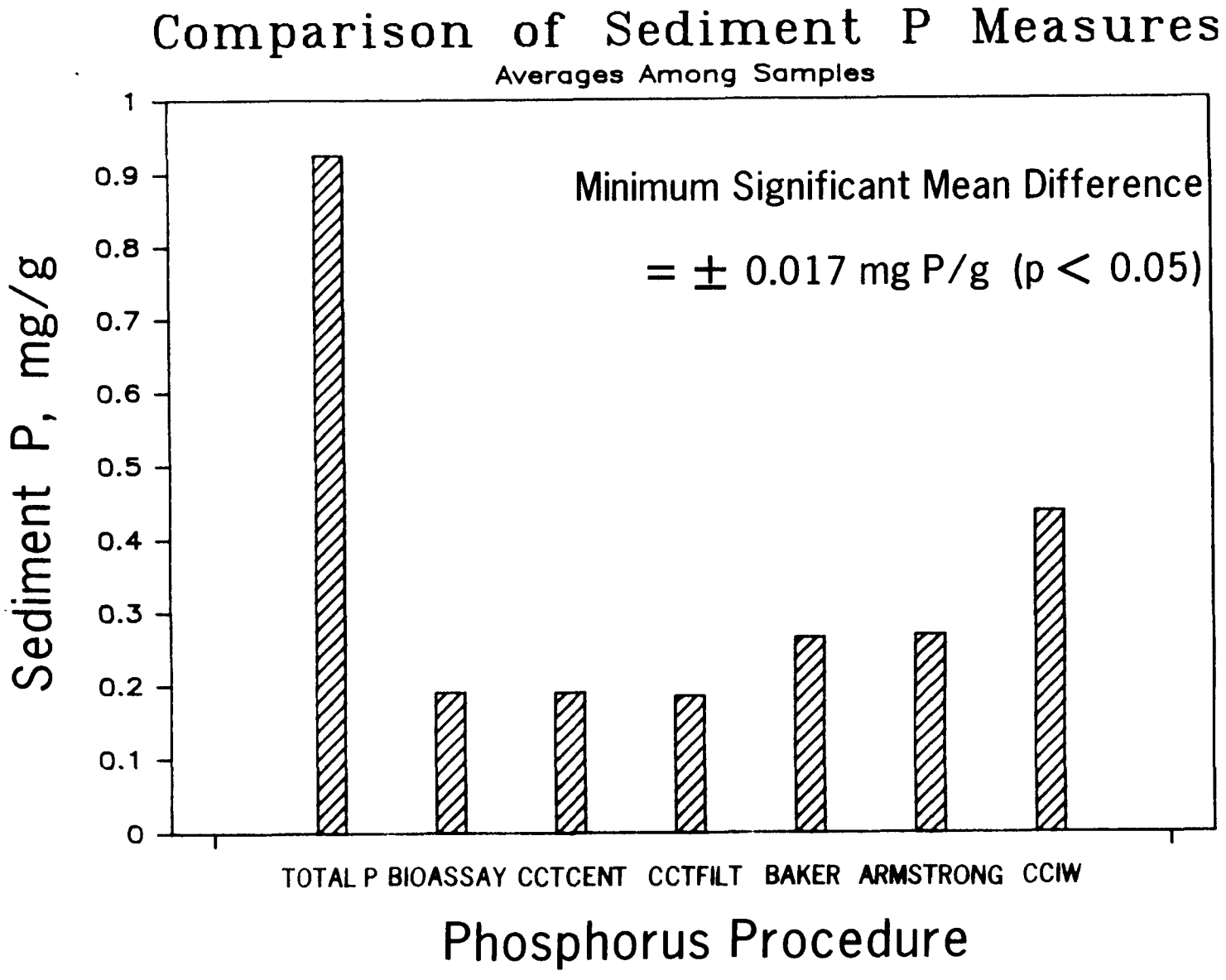

Figure 5. Amounts of P measured by each procedure, averaged over all sediment samples.

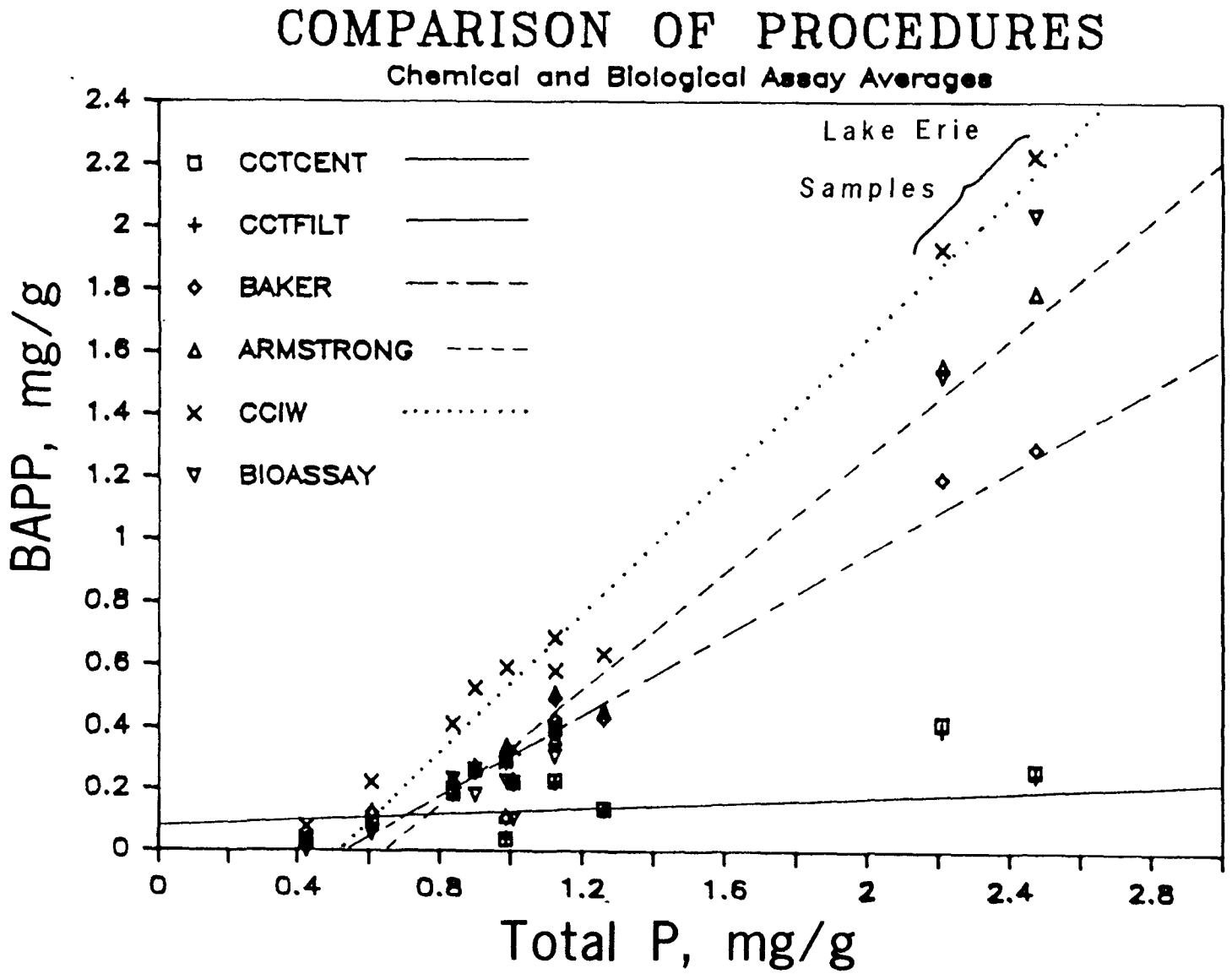

Figure 6. Amounts of P measured on each sample, averaged over all analytical procedures.

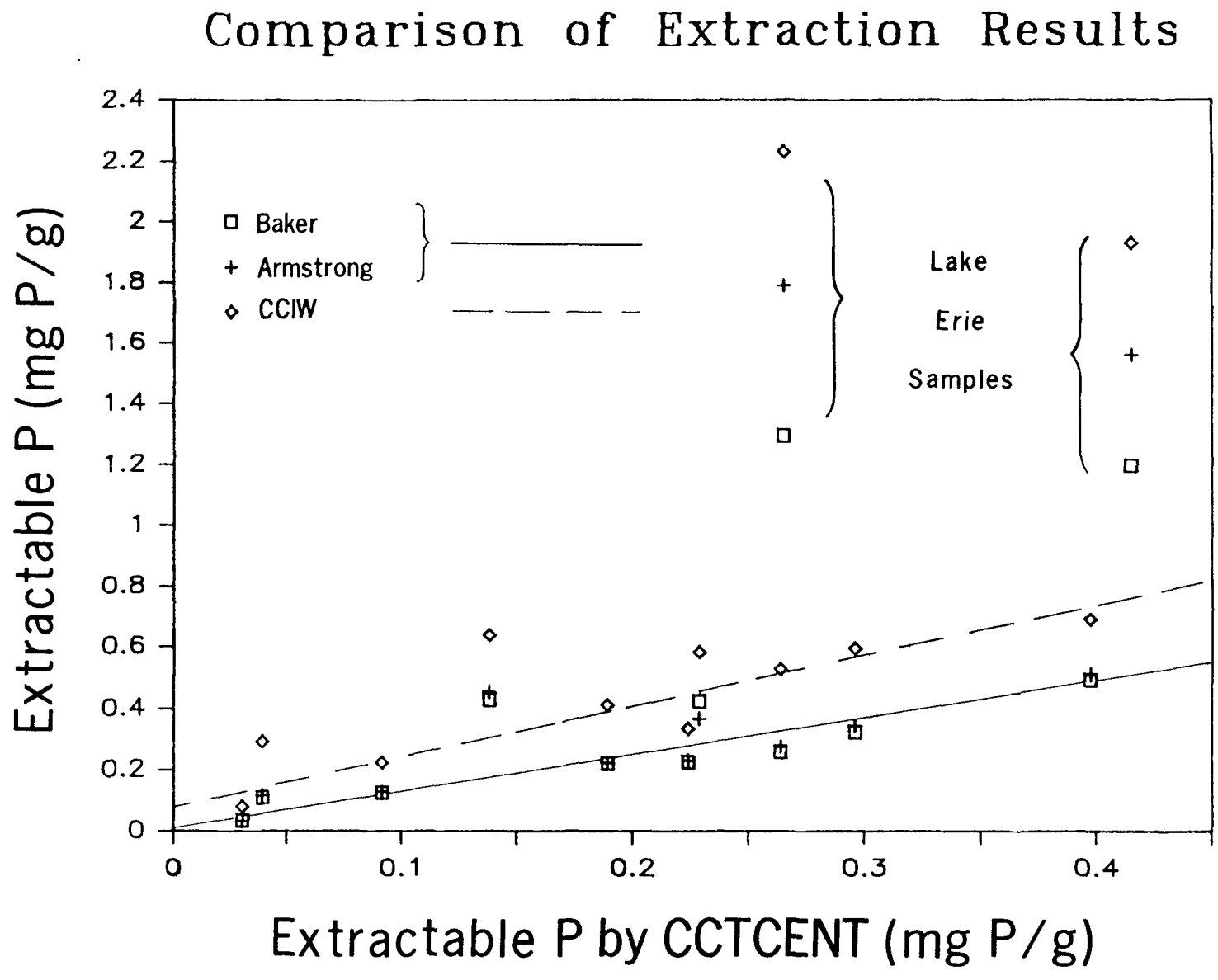

Figure 7. Comparison of extractable P among all sediment samples.

procedure-sample interaction noted in the analysis of variance. Still, deletion of the Lake Erie samples from the data set reduced the magnitude but did not eliminate the statistical significance of the interaction between samples and procedures ($p<0.0001$). This means conversion of BAPP estimates by a linear regression procedure would incur statistically significant bias, though possibly inconsequential for management purposes, for some sample-procedure combinations, even if the Lake Erie samples were unique and could be disregarded as outliers.

Concern over the irregular extraction results obtained for the Lake Erie sediments by the De Pinto procedures led to futher tests of the sediments using the procedures. These tests revealed that the Lake Erie samples were extremely sensitive to solution:sediment ratio used for the extraction. For example, when Lake Erie #2 (Sample #8) was re-extracted using the reagents of the De Pinto method, but employing a solution to sediment ratio comparable to the Baker and Armstrong procedures (approximately 2000:1), 1300 ug P/g was extracted compared to 250 ug P/g originally extracted with the lower ratio (500:1). The higher amount, 1300 ug P/g, is virtually identical to the BAPP estimate obtained by the Baker procedure for that sample (Table 3 and Appendix D). Clearly the estimate of BAPP from the stored Lake Erie sediments was sensitive to the solid:solution ratio used during the extraction.

Comparison With Bioassays

The results of 14 day algal bioassays for 10 sediment samples are summarized in Table 4. The results shown are mean values of triplicate bioassay measurements performed on each sample. The total P concentrations of the sediments placed in the DCDA's are also presented. Bioassays were not performed, however, using either the Raisin River or the National Bureau of Standards samples. It was believed that the high levels of heavy metals (Cr=29,600 ug/g, Zn=1720 ug/g, Pb=714 ug/g) in the NBS sample would adversely affect algal growth and bias the results; lack of sufficient sample volume precluded analysis of the Raisin River sediment sample.

All of the samples, except those from Lake Erie, released essentially all BAPP (algal-available P) within 5.0 days. Release of P from the Cattaraugus Creek and Genesee River sediments was extremely rapid and was virtually complete within 2.0 days. The Lake Erie samples, on the other hand, continued to release considerable amounts of P throughout the two week bioassay analysis. The amount of P determined by bioassay to be bioavailable ranged from 2.6 to 2100 ug P/g or from 0.6 to 84.9 percent of total sediment P. As an illustration of the nature of the BAPP bioassays, cumulative P release is plotted in Figure 8 as a function of time for bioassays performed on Samples #1 and 7 (Cuyahoga River and Lake Erie #1).

A comparison of bioassay results with those obtained by Martin et al. (1983), who used the same bioassay procedure as reported here on some of the same rivers, is presented in Table 5. The results shown are mean values of triplicate bioassay measurements performed on several samples from each river. Martin et al. (1983) calculated ultimate BAPP values from data on cumulative P uptake by algae over time. The calculation assumed the rate of uptake depended only on the rate of release of BAPP from the sediment particles and the amount of BAPP initially present on the particles at the start of the bioassay. In this investigation, calculation of ultimate BAPP

TABLE 4. AVAILABLE PHOSPHORUS RELEASED BY SEDIMENTS DURING BIOASSAY EXPERIMENTS, ug P/g.

SAMPLE	TOTAL P	DAY OF ALGAE HARVEST				CUMULATIVE P UPTAKE	
		2.0	5.0	9.0	14.0	ULTIMATE	% TOTAL P
CUYAHOGA	1124.3	335.4	45.7	--	7.4	388.5	34.5
CATTARAUGUS	423.9	2.6	--	--	--	2.6	0.6
L. ERIE #1	2209.9	696.3	467.2	245.5	117.6	1550.0	70.1
L. ERIE #2	2472.9	1054.9	601.9	281.0	153.6	2100.0	84.9
GENESEE	607.5	55.8	0.6	--	--	56.4	9.3
MAUMEE #1	1123.3	255.8	53.0	--	--	308.8	27.5
MAUMEE #2	836.8	207.6	25.7	--	--	233.4	27.9
OSWEGO	1007.2	96.5	8.2	--	1.2	105.9	10.5
SANDUSKY #1	987.9	195.4	32.4	--	--	227.8	23.2
SANDUSKY #2	900.0	125.3	8.2	--	--	183.5	20.4

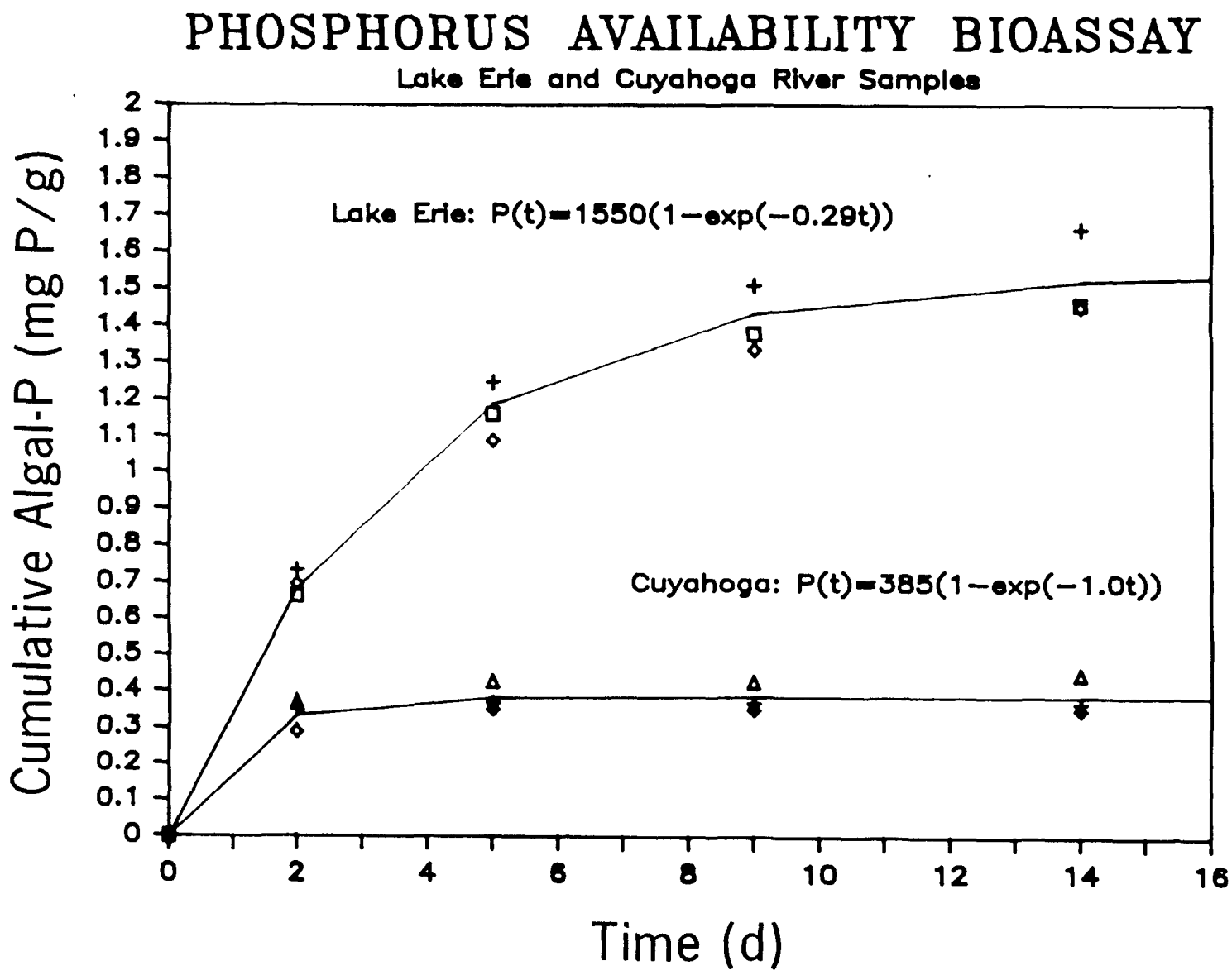

Figure 8. Illustration of bioassay results for determination of algal-available P.

TABLE 5. AVERAGE AVAILABLE PHOSPHORUS RELEASED BY SEDIMENTS DURING BIOASSAY EXPERIMENTS, ug P/g (FROM MARTIN ET AL., 1983).

SOURCE	NUMBER OF SAMPLES	TOTAL P	CUMULATIVE P UPTAKE	
			ULTIMATE	% TOTAL P
CUYAHOGA	4	1314.0	449.2	33.9
CATTARAUGUS	5	559.0	38.8	7.7
L. ERIE #1	1	2656.0	1435.0	54.0
L. ERIE #2	1	3044.0	1482.4	48.7
GENESEE	1	900.0	173.8	19.3
MAUMEE	11	1308.0	337.3	25.0
SANDUSKY	17	1145.0	247.1	21.4

release was done only for the samples assayed from Lake Erie, since they continued to release P throughout the 14 day procedure. Calculation of ultimate BAPP for the other samples was not necessary since they had released essentially all BAPP by the end of 5.0 days. For these samples, the amounts of P reported as ultimately available in Table 4 correspond to the total measured uptake by the algae during the bioassays.

Comparison of the bioassay results from this investigation with those obtained by Martin et al. (1983) revealed highly similar results for the three Ohio rivers (Cuyahoga, Maumee, Sandusky). However, the Cattaraugus and Genesee river sediments released somewhat less P than that reported by Martin et al. (1983). This discrepancy may be due to the fact that Martin et al. analyzed water column suspended sediments, while in this study measurements were performed on bottom sediments collected from the two New York rivers.

Comparison of the two samples from Lake Erie revealed a pronounced dissimilarity in the findings from each investigation. Martin et al. (1983) found that 48.7 and 54.0 percent of the total sediment P was released from these same samples. On the other hand, results from the present investigation showed that the samples contained amounts of ultimate BAPP corresponding to 70.1 and 84.9 percent of total P, respectively (actual measured levels were 69.1 and 84.6 percent, respectively). The reasons for the wide differences in results between the two investigations are unknown; however, the duration of storage between the analyses of Martin et al. (1983) and those done for this investigation (approximately 4 years) may have permitted changes in the P-retaining properties and BAPP levels of the sediments. This will be discussed subsequently.

In order to assess how well the chemical extraction procedures compared with BAPP determined by the algal bioassays, correlation coefficients were calculated between the bioassay measured BAPP and that estimated from chemical measurements (Appendix E.6). The results of the comparisons are shown graphically in Figure 9. The methods of Baker, Armstrong, and CCIW all showed a high degree of correlation ($r>0.981$ for $N=10$) with the bioassay data, implying that these procedures could be used for accurate prediction of BAPP. Comparisons involving algal-determined BAPP and the two variations of the De Pinto method were strongly biased by the results for the Lake Erie samples and showed poor correlations as a consequence.

As noted in comparing the chemical procedures for BAPP, the Lake Erie samples gave atypical results, at least by comparison with the results for the other samples as analyzed by the various procedures. This anomaly may have been a consequence of the age of the Lake Erie sediment samples, which had been held approximately four years in refrigeration storage. Although the ageing mechanisms were not investigated, two processes may have been involved. The first would include microbial oxidation of relatively unavailable organic P to relatively bioavailable, inorganic P during storage. Accompanying this process would be a second one: slow oxidation and higher ordering of Fe oxides which would increase levels of occluded non-apatite inorganic P. This latter fraction is not generally considered to be extractable by the procedure used by De Pinto, but is extractable by the procedure of CCIW, and apparently is by that of Armstrong (Armstrong et al. 1979) and possibly Baker (1983).

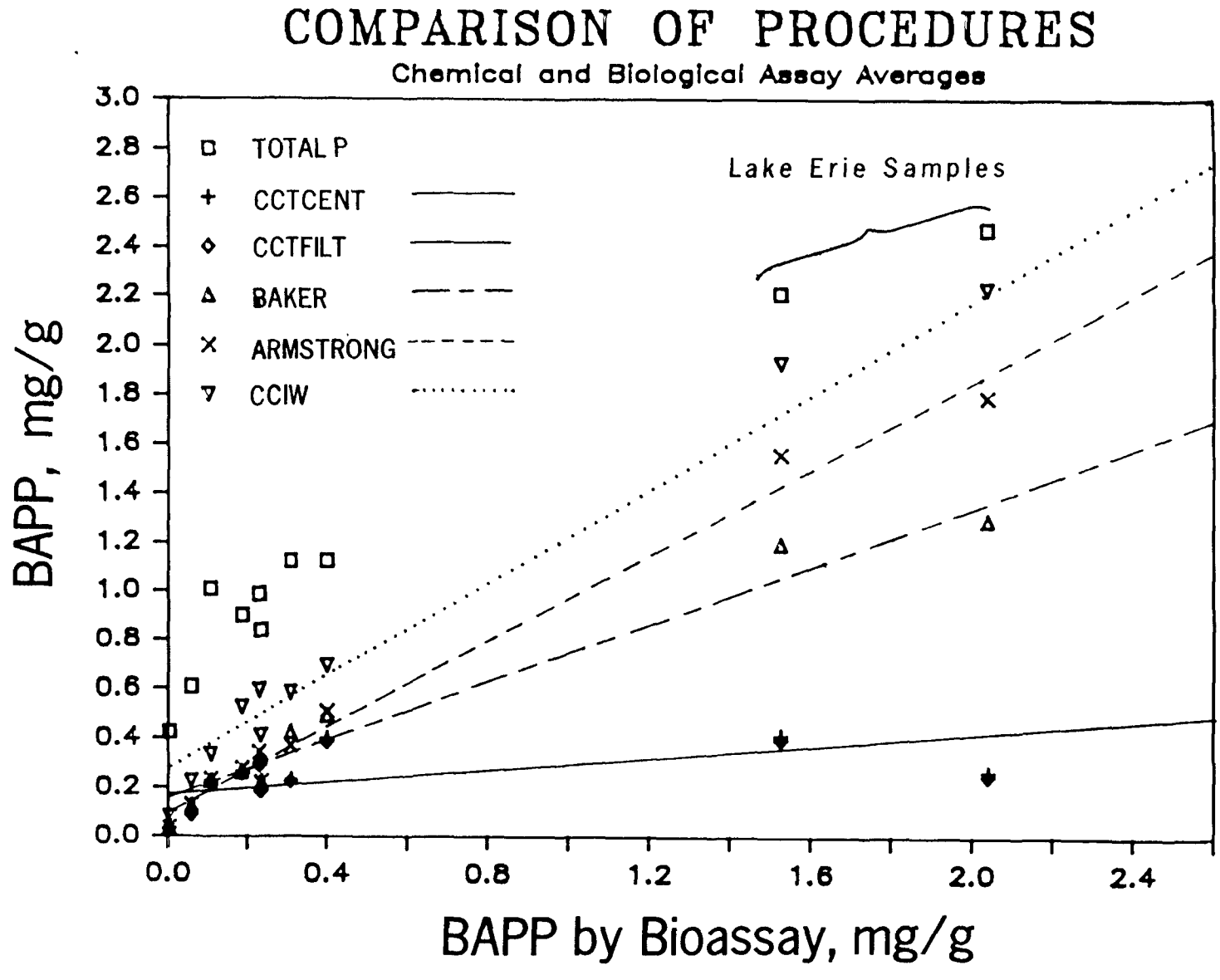

Figure 9. Comparison of chemical and biological measurements of BAPP for all samples.

Interconversion of BAPP Estimates

In view of the solution:sediment sensitivity of the stored Lake Erie samples and the possibly related anomalous extraction results, the analysis of correlation between the extraction procedures was recast with a focus on freshly collected samples. This analysis is summarized in Table 6. The tabulated data show strong intercorrelation ($r \geq 0.85$, $p \leq 0.01$) among all the BAPP procedures, including chemical and biological estimates, plus total P. As stated earlier, this suggests that these BAPP estimation methods quantitate similar physicochemically-bound fractions of total P, but do so with different efficiencies. The strength of the intercorrelations indicates that interconversion of BAPP estimates is possible with good accuracy and precision as long as fresh sediment samples are used for initial BAPP estimates. Also, as shown in Figure 10, all the procedures correlate well with the algal bioassay data, which means transformation of a chemical BAPP estimate to a biological one is feasible for all methods, again with the caveat of using fresh sediments for analysis.

Regression equation slopes and intercepts, developed for interconversion of BAPP estimates using the data acquired and procedures tested in this study, also are given in Table 6. With respect to prediction of algal-available P, the procedures of De Pinto gave results that were closest in magnitude to the values determined by bioassay. This is indicated in Table 6 by the values of the slopes relating bioassay estimates as a dependent variable (BIOASSY as a Row Header) to either of the De Pinto procedures (CCTCENT or CCTFILT as a Column Header); each slope is essentially 1.0. Thus, these procedures could be considered to be the most accurate predictors of algal BAPP among those tested. For these same data, however, the procedures of Baker, Armstrong, and CCIW were the most precise predictors of algal BAPP, since they correlated most highly with the bioassay results.

Using a similar all-pairwise approach but considering both fresh samples and the stored ones from Lake Erie (Appendix E.6), the procedure of Armstrong is shown by its excellent correlation ($r=0.994$, $N=10$) to be the most precise predictor of the bioassay results. However, the strong intercorrelation between the Armstrong, Baker, and CCIW (and algal BAPP) estimates means any of these chemical methods for BAPP estimation would be precise for both fresh and stored samples. Thus, it is recommended that either the Baker, Armstrong, or CCIW procedures be selected when samples that have been stored must be chemically analyzed for BAPP. When freshly collected bottom or suspended sediments are to be analyzed, however, it appears that any of the five extraction procedures may be used, and the results interconverted between each procedure with sufficient accuracy to suit a wide range of purposes.

TABLE 6. ALL PAIRWISE COMPARISON OF EXTRACTION PROCEDURES.*

EXTRACTION PROCEDURE	EXTRACTION PROCEDURE**						
	TP	CCTCENT	CCTFILT	BAKER	ARMSTRNG	CCIW	BIOASSY
TP		0.8902 0.0030 1.9210 463.3	0.8947 0.0027 1.9799 462.4	0.9266 0.0009 1.5357 471.7	0.9143 0.0015 1.5416 468.7	0.9005 0.0023 1.0778 413.0	0.8452 0.0082 1.5938 573.9
CCTCENT	0.8902 0.0030 0.4125 -146.5		0.9999 0.0001 1.0253 0.6375	0.9044 0.0020 0.6946 32.11	0.9561 0.0002 0.7470 17.45	0.9425 0.0005 0.5228 -9.699	0.8666 0.0054 0.7572 71.34
CCTFILT	0.8947 0.0027 0.4043 -145.3	0.9999 0.0001 0.9751 -0.5670		0.9064 0.0019 0.6789 30.33	0.9567 0.0002 0.7290 16.31	0.9437 0.0004 0.5104 -10.34	0.8669 0.0053 0.7387 68.93
BAKER	0.9266 0.0009 0.5591 -226.6	0.9044 0.0020 1.1777 10.11	0.9064 0.0019 1.2103 10.263		0.98691 0.0001 1.0040 -2.208	0.9516 0.0003 0.6873 -32.11	0.9581 0.0002 1.0900 56.49
ARMSTRNG	0.9143 0.0015 0.5423 -210.7	0.9561 0.0002 1.2237 1.348	0.9567 0.0002 1.2556 1.926	0.9869 0.0001 0.9701 9.020		0.9657 0.0001 0.6856 -30.24	0.9500 0.0003 1.0624 62.87
CCIW	0.9005 0.0023 0.7523 -229.4	0.9425 0.0005 1.6992 64.52	0.9437 0.0004 1.7446 65.09	0.9516 0.0003 1.3176 82.92	0.9657 0.0001 1.3603 70.11		0.9340 0.0007 1.4713 150.7
BIOASSY	0.8452 0.0082 0.4483 -203.1	0.8666 0.0054 0.9918 -23.49	0.8669 0.0053 1.0174 -22.97	0.9581 0.0002 0.8421 -32.00	0.9500 0.0003 0.8495 -34.91	0.9340 0.0007 0.5929 -65.11	

* Results averaged within samples by procedure prior to analysis; data not included from Lake Erie bottom sediment, Raisin River, and NBS SRM. For all statistical significance tests, N = 8 and $|r| > 0.707$ for $p < 0.05$. All correlations significant at $p < 0.01$.

** Format of Tabulated Data:
Coefficient of Correlation (r)
Probability of Zero Correlation (Ho: r=0)
Slope of Bivariate Regression Lines (b1), [(ug/g)/(ug/g)]:
Column headers are independent variables
Row headers are dependent variables
Intercept of Bivariate Regression Line (bo), [ug/g]

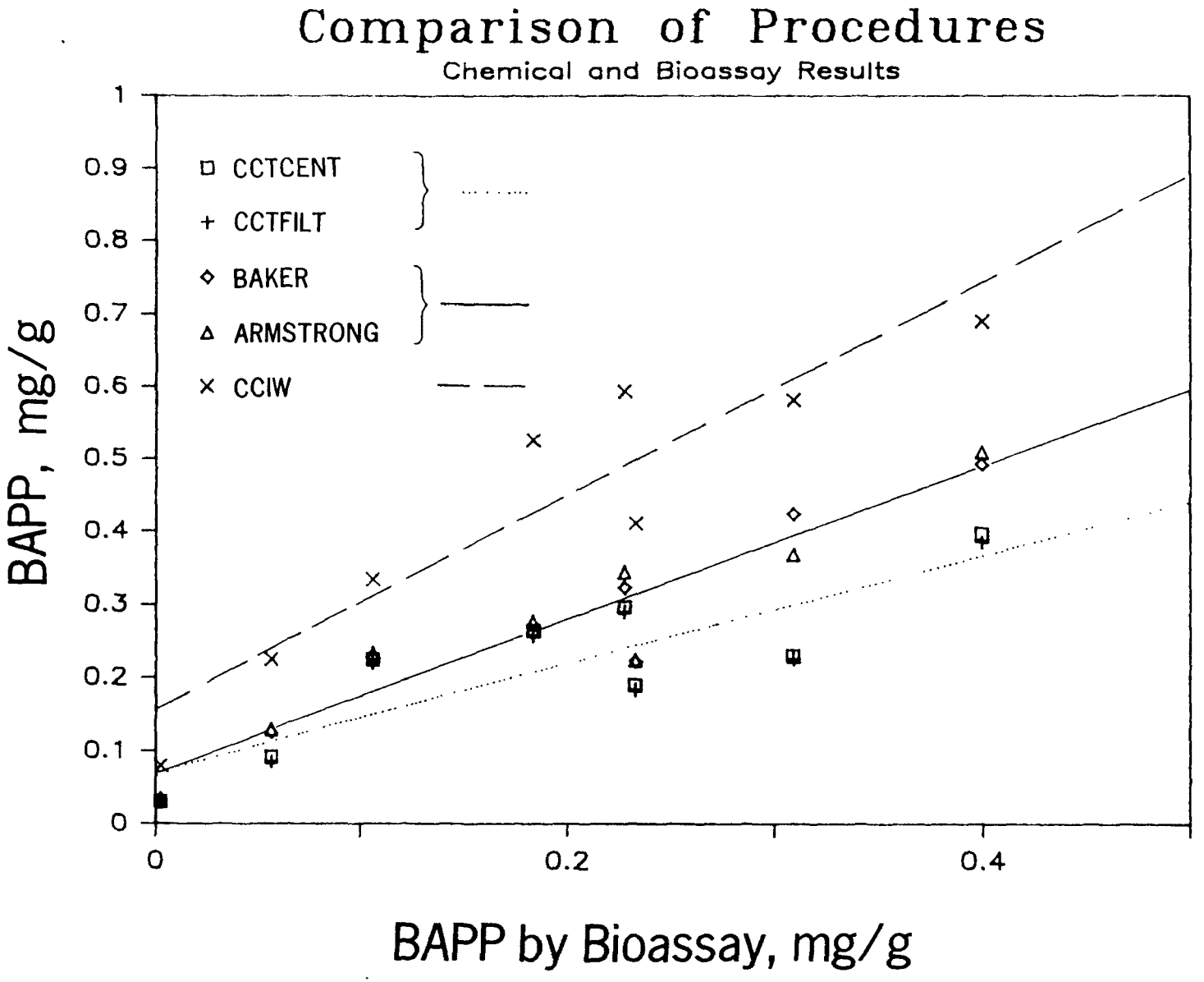

Figure 10. Comparison of chemical and biological measurements of BAPP.

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APPENDICES

APPENDIX A

DETAILED DESCRIPTIONS OF SEDIMENT ANALYTICAL METHODS

APPENDIX A. Detailed Descriptions of Sediment Analytical Methods

THE NAOH EXTRACTION METHOD OF DE PINTO (1982)

Introduction

Given in the following is a specific description of a procedure for the measurement of a fraction of the particulate phosphorus in a water sample that shows close correlation with the quantity of the particulate phosphorus which is biologically available. In general, the procedure requires collection of a relatively large volume of raw water which contains particulate matter. The particles are then collected by filtration onto a membrane filter and resuspended in a phosphorus-free medium to form a sediment concentrate. Aliquots of the sediment concentrate are put in contact with approximately 0.1 N NaOH for a specified period. Then, the particles are separated from solution by filtration and the filtrate is analyzed for orthophosphate by standard colorimetric methods. The concentration of orthophosphate in the NaOH extract, divided by the concentration of solids during the extraction, yields the estimated quantity of available particulate phosphorus per unit dry weight of solids. Multiplication of this value by the suspended solids concentration of the original water sample equals the concentration of available particulate phosphorus in the original sample. The analysis should be performed in duplicate.

Sample Collection

The volume of sample that should be collected is dependent on the suspended solids concentration of the water being sampled. In all cases collect a volume of sample that will yield a minimum of 0.50 ug of sediment. With careful handling of the sample, 0.50 ug of sediment will be adequate to perform suspended solids measurements on both the raw sample and the sediment concentrate that will be prepared, and to perform duplicate NaOH extractions on the sediment. Low solids concentrations, for example, 20 to 100 mg/1, will naturally require a larger volume of sample than will a solids concentration of 200 to 1,000 mg/1. Regardless of the solids concentration it is wise to collect more sample than is needed. Samples should be stored in polyethylene bottles or carboys, transported in cooled containers, and stored at 4 C. Samples should be held for processing no longer than 48 hrs.

Cleansing of Glassware and Apparatus

Wash all glassware, storage containers, and other apparatus that will come in contact with the sediments and solutions with 1+1 HCl and rinse thoroughly with deionized water. Avoid the use of detergents containing phosphate. Wash the membrane filters to be used to separate the NaOH extract from the sediments by either soaking the membranes in deionized water for 24 hrs (50 membranes/2 liters), or soaking the membranes in deionized water (50 membranes/2 liters) for 3-1 hour periods, changing the deionized water after each period (Standard Methods, 15th Edition, Section 424A, p. 412).

Apparatus

a. Van Dorn sampler or equivalent

b. Polyethylene bottles or carboys.

c. Vacuum filtration unit and vacuum source; side-arm flasks, 125 ml and 1,000 ml or larger.

d. 0.4 micron pore diameter polycarbonate membranes.

e. Sediment Pipets: Prepare these by enlarging the tip openings of graduated Mohr pipets or volumetric transfer pipets depending on the type needed. Generally, a 5 to 10 ml graduated Mohr type will be needed. Use a sediment pipet whenever pipeting a suspended solids sample.

f. Centrifuge tubes with screw-cap tops calibrated to 50.0 ml. Alternatively, any plastic or glass container with a water-tight cap may be used, provided that it has been calibrated to contain 50.0 ml and can accommodate vigorous shaking without leaking for 17 hours.

g. Drying oven with thermostatic control capable of maintaining 103 C.

h. Desiccator

i. Analytical Balance, capable of weighing to 0.1 mg.

j. Rotary shaker table or equivalent, with adjustable speed control.

k. Spectrophotometer with infrared phototube for use at 880 nm.

l. Magnetic stir table and stir bars.

Reagents

a. 1+1 HCl acid wash solution.

b. 0.125 N NaOH extractant solution: Dissolves 5.0 g of NaOHin 1,000 ml of deionized water.

c. Stock phosphate solution 1.00 ml 50.0 ug PO_4-P: Dissolve 219.5 mg of anhydrous potassium dihydrogen phosphate (KH_2PO_4) in 1,000 ml of deionized water.

d. Standard phosphate solution, 1.00 ml 1.00 ug PO_4-P: Dilute 10.00 ml of the 1.00 ml 50.0 ug PO_4-P stock phosphate solution to 500 ml.

e. Resuspension Medium: 0.1 N NaCl: Dissolve 5.8 NaCl in 1,000 ml of deionized water.

f. Phenolphthalein indicator solution, aqueous.

g. H_2SO_4 Neutralization acid, 5 N: Use the 5 N H_2SO_4 solution

prepared for the combined reagent.

h. Solutions for combined reagent:

   Sulfuric Acid, 5 N
   Potassium antimonyl tartrate
   Ammonium molybdate
   Ascorbic acid, 0.1 M

   Prepare these solutions as outlined in Standard Methods, 15th Edition, 1980, Section 424F., p. 420: Ascorbic Acid Method.

i. Combined reagent: Combine the reagents above as outlined in Standard Methods, ibid.

Preparation of Sediment Concentrate

Initially, it is necessary to determine the suspended solids concentration of the raw sample using the method outlined in Standard Methods (15th Edition, 1980, Section 209G, p. 97). An exception to the Standard Methods procedure is that 0.4 micron pore diameter polycarbonate membranes should be substituted for glass fiber filters. Always ensure that the sample is well mixed before removing aliquots for solids measurement, and take for analysis a volume of sample that will yield 25 to 50 mg of dry nonfiltrable residue on the membrane.

From the solids measurement, calculate the volume of raw sample equivalent to at least 0.40 g of dry sediment and filter this volume of raw sample through a 0.4 micron pore diameter polycarbonate membrane. More than 1 membrane may be required due to pore blockage as the sediment accumulates on the membrane. The filtration time and the number of membranes necessary will be a function of the suspended solids concentration of the raw sample and the grain size distribution of the sediment particles.

After filtering the required volume of raw sample, transfer the sediment from the membranes to a 125 ml Erlenmeyer flask by scraping the bulk of the sediment from the membrane with a spatula. Rinse any sediment sticking to the spatula and remaining on the membranes into the concentrate flask with the medium for resuspension, 0.1 N NaCl dispensed through a polyethylene squirt bottle. Also, recover any sediment that sticks to the filtration apparatus on the edge that comes in contact with the membrane. When rinsing the spatula and membranes take care not to exceed the volume of suspension medium needed to approximate a 10 mg/ml suspended solids concentration. After the collected solids have been transferred to the erlenmeyer flask, add additional 0.1 N NaCl to yield a final suspended solids concentration of approx. 10 mg/ml. For example, if enough raw sample is filtered to yield 0.40 g of sediment, the final volume of suspension media should be 40 ml. Likewise, if enough raw sample is filtered to yield 0.50 g of sediment, the final volume of suspension media would be 50 ml.

When the concentrate is prepared, add a magnetic stir bar, stopper or seal the flask with parafilm, and mix the concentrate to homogeneity on a magnetic stir table. The sediment concentrate then may be further processed or stored at 4 C up to 60 days.

Suspended Solids Determination on Sediment Concentrate

Determine the solids concentration of the concentrate by the method used for the raw sample. For an estimated solids concentration of 10 mg/ml, filter 3 to 4 ml of the concentrate. Remove aliquots by sediment pipet from the concentrate while it is actively being mixed on a magnetic stir table.

NaOH Extraction

From the suspended solids value of the concentrate, calculate the volume of concentrate equivalent to 0.1 g of dry sediment. This volume should not be less than 6.0 ml or exceed 14.0 ml.

With a sediment pipet transfer the volume of concentrate equivalent to 0.1 g dry solids to a 50 ml centrifuge tube. Fill the tube to the 50 ml mark with 0.125 N NaOH. Cap the tube and mix its contents continuously for 17 hrs at room temperature with a mechanical shaking device such as a rotary shaker table. Adjust the rate of mixing so that it is just fast enough to keep the sediments well mixed within the tube. The use of 0.1 g of sediment in a 50 ml centrifuge tube is not mandatory. However, the solution:solid ratio during the extraction should be kept within the range of 500 to 600:1. Other sediment aliquots and extraction volumes may be employed at the analyst's discretion if the solution:solid ratio is maintained within this range.

Following the 17 hrs of mixing, divide the NaOH extract into approximately equal portions and separate the NaOH extract from the sediments of one portion using vacuum filtration through a pre-washed 0.4 micron pore diameter polycarbonate membrane and into a dry 125 ml side-arm flask. On the other portion, separate the sediments and extract by high-speed centrifugation (~32,000xg) for 30 minutes.

Measurement of Reactive NaOH-Extractable Phosporus

The orthophosphate concentration of the NaOH extract generally falls in the range: 200-800 ug P/L. Make dilutions of the extract as determined by its expected phosphorus concentration and the light path length used with the spectrophotometr. A table containing light path lengths and the range of phosphorus concentrations that can be measured at each light path length is presented in Standard Methods, 15th Edition, 1980, Section 424 F, p. 420. Pipet the appropriate volume of filtered extract into a 50 ml volumetric flask.

To the volumetric flask containing the required aliquot of extract add 1 drop of phenolphthalein indicator. The extract should turn red. Add 5 N H_2SO_4 dropwise (swirling the flask between drops) until the red color is discharged. Fill the volumetric to the 50 ml mark with deionized water and transfer the contents of the volumetric to a dry 125 ml Erlenmeyer flask. Add 8.0 ml of the combined reagent to the Erlenmeyer and mix thoroughly by swirling the flask. Allow at least 10 minutes for color development and measure the absorbance of the sample at 880 nm within 30 minutes of the combined reagent addition (Standard Methods, 15th Edition, 1980, Section 424 F, p. 420). Use a deionized water-combined reagent blank as the reference solution.

Preparation of Standard Curve

Prepare a series of 5 phosphorus standards that will bracket the expected phosphorus concentration in the diluted extract. Prepare these standards by making dilutions of the 1 ug P/ml standard phosphorus solution in 50 ml volumetric flasks. Also prepare 2-50 ml deionized water blanks. Once the dilutions have been made transfer the contents of the volumetrics to dry 125 ml Erlenmeyer flasks, add 8.0 ml of the combined reagent to each standard and blank, and mix thoroughly by swirling. Allow 10 minutes for color development and measure the absorbance of the samples against the reagent blanks at 880 nm within 30 minutes of the mixed reagent addition.

THE NAOH EXTRACTION METHOD OF BAKER (1983)

a. Filter 50 ml of a well-mixed sample through a Millipore BD (polyvinyl chloride) 0.6 micron pore-diameter membrane. (Millipore BDWP 04700). Keep filtrate and filter.

   For this step, the volume filtered should be adjusted to permit collection of between 2 and 20 mg of sediment on the filter. For this reason, suspended sediment analysis should preceed setting up this test.

b. Place the filter in a French Square bottle. A French Square may be replaced with any glass screw cap bottle which will hold 25 ml, have enough room for agitation, and fit on a shaker.

c. Add 25 ml of 0.1 N NaOH in 1.0 N NaCl to the French Square.

d. Cap the French Square and place it on a shaker bath for 18 hours.

e. At the end of the 18 hour period, filter the extract through a Millipore AP prefilter (Millipore AP25 04700) and into a 125 ml Erlenmeyer flask.

f. Add 25 ml of 0.1 N sulfuric acid to neutralize the extract, and mix well.

g. Determine the soluble reactive phosphorus content of the neutralized extract by the modified single-reagent method (APHA, 1980).

THE NAOH EXTRACTION METHOD OF ARMSTRONG ET AL. (1979)

a. By high-speed centrifugation (13,000 rpm, fixed-angle rotor with 50 ml bottles), of a suitable volume of water sample, or previously concentrated sample (see Method A, above), collect approximately 15 mg suspended sediment (dry weight) into a 50 ml (nominal volume) polypropylene centrifuge bottle.

   To determine the exact weight of suspended solids collected in the tube, analysis of suspended solids on the sample prior to centrifugation, would be required to calculate the liquid volume needed for centrifugation.

b. Add 30 ml of 0.1 N NaOH in 1.0 N NaCl to the sediments in the centrifuge bottle. Cap the bottle and place on a shaker table for 18 hours.

c. At the end of the 18 hour period of extraction, separate the sediments from the extract, first by high-speed centrifugation (as above), then filter the extract through a 0.4 micron pore-diameter membrane (polycarbonate).

d. Neutralize the filtered extract with 2 N sulfuric acid, using phenolphthalein as an indicator.

e. Determine the soluble reactive phosphorus content of the neutralized extract by the modified single-reagent method (APHA, 1980).

THE NON-APATITE EXTRACTION METHOD OF WILLIAMS AND MAYER (1980)

CDB Extractable Inorganic Phosphorus

a. Weigh 0.1 g of freeze-dried particulate matter into a 100 ml centrifuge bottle.

b. Add 50 ml of 0.22 M Na-citrate/0.11 M Na-bicarbonate reagent.

c. Immerse tubes in 85 C water bath. After 15 minutes add 1.0 g Na-dithionite. Maintain samples in water bath at 85 C for a further 15 minutes stirring frequently with a glass rod.

d. Centrifuge extract for 15 minutes at 2000 rpm.

e. Transfer supernatant quantitatively into 100 mL volumetric flasks, leaving residue undisturbed in the tubes.

f. Add 25 mL of 1.0 M NaCl solution to each tube. Wash residue well by vortex mixing.

g. Centrifuge the extract at 2000 rpm for 10 minutes.

h. Transfer extract to volumetric flask as in (e). Save residue for NaOH extraction.

i. Add 1 mL $FeCl_3$ to each flask.

j. Allow flasks to stand exposed to atmosphere for 2-3 days (covered with filter paper or paper towels) until development of yellowish-brown color indicates that the oxidation of the dithionite is complete.

k. Filter sample, adjust pH to neutral. Make up to 100 mL and analyze for ortho-P.

l. Standard of 500 ug of P and distilled water blank also carried through the procedure.

NaOH Extractable Inorganic Phosphorus

a. Add 50 ml of 1 N NaOH to the residue from the procedure for extraction of CDB-P (D.1. above).

b. Stopper, shake well and place tubes on moon walker apparatus over night.

c. Centrifuge for 15 minutes at 2000 rpm.

d. Transfer 20 mL aliquot of the supernatant solution to another centrifuge tube.

e. Add 10 mL of 3 N HCl to centrifuge tube containing 20 mL aliquot.

f. Mix with vortex mixer and centrifuge for 15 minutes at 2000 rpm.

g. Transfer a 10 mL aliquot of the clarified supernatant to a 50 mL volumetric flask.

h. Adjust pH to neutral with 0.1 N HCl and make up to 50 mL.

i. Analyze for ortho-P.

j. Discard remainder of NaOH extract.

k. Standard of 50 ug of P and distilled water blank also carried through the procedure.

NOTE: Non-Apatite P NaOH-Extractable P + CDB Extractable P

THE BIOASSAY METHOD OF DE PINTO ET AL.(1981) AND DE PINTO (1982)

Using the two chambered, continuously-stirred, Dual Culture Diffusion Apparatus (DCDA) of De Pinto (1982), sediments for bioassay analysis of available phosphorus are introduced into a darkened, "phosphorus-release" vessel and phosphorus- starved algae are placed in a transparent "algal-assay" vessel. The two vessels are clamped together such that the vessel contents are separated only by a 0.4 micron pore-diameter membrane filter. The membrane allows diffusion of soluble substances, such as phosphorus, from one vessel to the other, but it keeps the algae and sediments in their respective vessels. The rate of diffusion across the membrane does not limit the rate of movement of phosphorus from the sediments to the algae, since it has been found to be large relative to typical release rates for natural sediments.

The algae to be used for the bioassays will be harvested from phosphorus-starved, seven day cultures of Selenastrum capricornutum. The algal medium to be used for all bioassay work will be one modified from that of Guilliard and Lorenzen (1972), with the only modification being that involving phosphorus additions. For growing phosphorus-starved cultures, the usual total phosphorus content of algal growth media is approximately 30 ug P/L.

No attempt will be made to prevent growth of the natural bacterial flora that accompanies the sediments upon innoculation into the release vessel. Bioassay incubations will be done at ambient temperature in an air conditioned room (23 C +/- 10) with lighting supplied by approximately 4000 lux of cool-white fluorescent lamps.

After an initial three-day incubation period, which is lengthened to seven and ten days later in the test, the contents of the assay vessel are harvested and immedieatly replaced with another seven-day, phosphorus-starved culture of bioassay algae. The cycle of harvent and replacement of assay vessel contents is continued for a period of 21 to 30 days, depending on the extent and rate of available phosphorus release from the particulate matter.

The total phosphorus content of the initial and subsequently harvested or seeded algal cultures is known from measurements made directly on the cultures of interest. By assuming algal accumulation of phosphorus in the assay vessel is the result of available phosphorus release from the particulate matter and subsequent transport of that phosphorus through the membrane partition to the algae, followed by algal uptake, and by invoking the principle of mass conservation, the rate and extent of available phosphorus release may be calculated over each sampling interval. Past studies (De Pinto et al., 1981) have shown the release of sediment-bound phosphorus to be approximately first-order, dependent on the amount of phosphorus that may ultimately be released for algal uptake.

APPENDIX B

CONCENTRATION OF SRP DURING STORAGE EFFECTS STUDY

APPENDIX B

CONCENTRATION OF SOLUBLE REACTIVE PHOSPHORUS (ug P/L) IN WATER SAMPLES COLLECTED FROM THE HURON RIVER AS A FUNCTION OF TIME AND TEMPERATURE OF INCUBATION.

Elapsed Time of Incubation (days)						
Field Zero	Lab Zero	0.5	1.0	2.0	4.0	9.0
Incubation Temperature = 4C						
11.8	4.9	2.7	2.6	2.6	3.5	8.7
9.9	4.2	2.6	2.6	2.1	3.5	9.5
10.2	4.5	3.2	2.7	2.4	4.3	8.7
10.8	9.5	7.2	6.8	6.7	7.3	14.6
17.5	9.4	8.7	7.0	6.2	8.0	17.6
10.8	9.5	8.3	7.3	6.2	7.6	16.8
10.5	8.7	8.7	7.0	6.4	7.8	16.2
11.1	9.5	7.8	8.4	8.4	10.2	17.1
11.5	8.8	8.0	8.9	8.1	9.7	15.1
11.1	8.9	7.8	8.4	7.0	9.7	15.9
Incubation Temperature = 22C						
11.8	4.9	3.0	3.2	3.8	8.6	7.8
9.9	4.2	2.6	3.0	4.3	8.7	7.2
10.2	4.5	2.6	2.7	3.5	8.7	8.9
10.8	9.5	9.5	7.5	8.7	16.5	16.2
17.5	9.4	8.4	6.7	10.0	14.3	15.2
10.8	9.5	7.8	10.8	4.3	8.6	10.8
10.5	8.7	7.3	5.4	11.1	17.6	11.9
11.1	9.5	10.0	11.6	6.2	8.0	9.1
11.5	8.8	10.3	10.3	13.8	9.4	
11.1	8.9	9.9	12.4	13.5	9.9	21.4
Incubation Temperature = 45C						
11.8	4.9	14.8	3.5	2.9	3.8	2.9
9.9	4.2	18.9	2.7	2.1	3.2	1.5
10.2	4.5	15.9	2.1	2.4	2.6	1.1
10.8	9.5	4.0	2.9	0.8	2.6	1.1
17.5	9.4	4.3	1.3	2.1	2.9	1.1
10.8	9.5	13.6	1.8	1.3	3.2	0.8
10.5	8.7	6.7	2.7	2.3	2.1	1.0
11.1	9.5	24.3	2.7	1.0	0.7	0.4
11.5	8.8	25.0	4.8	0.8	0.8	0.7
11.1	8.9	24.3	2.4	0.8	0.7	0.5

APPENDIX B

CONCENTRATION OF SOLUBLE REACTIVE PHOSPHORUS (ug P/L) IN WATER SAMPLES COLLECTED FROM THE MAUMEE RIVER AS A FUNCTION OF TIME AND TEMPERATURE OF INCUBATION.

		Elapsed Time of Incubation (days)				
Field Zero	Lab Zero	0.5	1.0	2.0	4.0	9.0
Incubation Temperature = 4C						
94.7	92.2	92.1	90.4	92.0	87.2	93.1
96.9	95.5	91.2	92.5	91.4	89.6	92.5
96.3	93.5	91.4	91.8	90.7	91.7	91.8
75.7	74.5	73.0	75.7	73.3	74.4	75.8
75.1	73.3	75.4	75.8	78.2	73.3	81.5
77.0	73.1	74.4	76.3	74.9	74.3	76.0
79.2	73.5	74.6	76.5	74.3	74.3	75.7
73.8	71.6	67.6	70.6	74.6	73.2	73.8
75.1	70.5	70.9	71.7	73.3	74.3	72.5
72.2	76.6	71.7	70.0	84.9	71.7	71.6
Incubation Temperature = 22C						
94.7	92.2	89.8	90.9	87.6	89.5	91.2
96.9	95.5	90.6	91.5	86.1	88.8	89.8
96.3	93.5	90.6	91.4	86.6	90.9	91.4
75.7	74.5	74.7	74.7	71.7	71.3	74.9
75.1	73.3	76.0	75.5	72.0	70.8	70.6
77.0	73.1	74.3	74.4	71.6	72.2	69.7
79.2	73.5	74.4	73.0	71.2	70.1	72.0
73.8	71.6	73.0	70.5	66.2	72.8	65.7
75.1	70.5	72.5	73.8	70.8	73.2	74.4
72.2	76.6	74.4	73.5	72.2	72.5	73.2
Incubation Temperature = 45C						
94.7	92.2	77.6	36.4	21.2	22.0	29.5
96.9	95.5	77.1	41.0	21.4	13.9	33.1
96.3	93.5	70.9	41.5	22.2	21.1	31.8
75.7	74.5	42.3	32.3	14.9	4.9	1.8
75.1	73.3	45.9	23.6	13.6	3.8	1.9
77.0	73.1	46.9	22.8	10.0	2.3	1.6
79.2	73.5	44.0	21.6	8.6	2.3	2.3
73.8	71.6	81.1	39.0	1.8	1.8	24.9
75.1	70.5	84.2	47.8	31.4	16.3	23.6
72.2	76.6	83.9	48.3	28.7	25.5	24.6

APPENDIX C

CONCENTRATION OF BAPP DURING STORAGE EFFECTS STUDY

APPENDIX C

CONCENTRATION OF BAPP (ug P/L) AND SUSPENDED SOLIDS (mg/L) IN WATER SAMPLES COLLECTED FROM THE HURON RIVER AS A FUNCTION OF TIME AND TEMPERATURE OF INCUBATION.

Initial Suspended Solids	Elapsed Time of Incubation (days)				
	0.50	1.00	2.00	4.00	9.00
Incubation Temperature = 4C					
24	36.62	44.96	43.74	42.21	21.62
21	42.90	43.64	45.14	46.67	35.04
26	36.62	38.09	35.80	34.26	33.40
11	50.37	44.76	43.66	33.06	33.13
17	51.30	39.95	43.20	44.07	30.17
18	44.00	44.76	38.44	41.13	26.60
16	43.53	48.28	52.27	49.04	38.31
10	39.94	40.90	38.23	36.67	25.58
11	48.37	38.91	37.92	35.89	30.31
13	43.12	41.97	32.93	39.03	30.15
Incubation Temperature = 22C					
24	41.76	39.55	34.26	25.20	32.50
21	39.76	43.93	35.24	22.41	26.69
26	43.76	41.30	33.70	27.57	30.12
11	46.33	33.22	35.06	22.83	17.89
17	49.75	32.74	37.83	28.26	19.48
18	35.15	44.28	26.92	19.57	24.42
16	44.78	41.87	32.76	37.87	21.78
10	42.80	27.75	25.46	17.19	24.80
11	43.92	33.71	30.75	13.73	
13	42.49	38.61	24.05	24.73	16.45
Incubation Temperature = 45C					
24	25.63	41.45	38.86	31.19	55.76
21	20.49	34.59	36.63	26.73	43.83
26	28.20	40.28	35.24	30.91	42.64
11	56.74	36.70	34.29	28.57	37.65
17	44.78	36.59	38.44	37.09	31.12
18	33.90	55.66	43.20	33.99	44.18
16	35.46	50.21	49.65	40.04	53.20
10	18.14	38.30	32.15	24.89	39.83
11	20.53	37.84	39.79	40.44	24.40
13	21.01	42.13	30.44	38.24	49.90

APPENDIX C

CONCENTRATION OF BAPP (ug P/L) AND SUSPENDED SOLIDS (mg/L) IN WATER SAMPLES COLLECTED FROM THE MAUMEE RIVER AS A FUNCTION OF TIME AND TEMPERATURE OF INCUBATION.

Initial Suspended Solids	Elapsed Time of Incubation (days) 0.50	1.00	2.00	4.00	9.00
Incubation Temperature = 4C					
189	125.54	122.78	120.59	119.05	120.35
199	130.39	127.31	116.68	118.56	129.75
170	128.96	113.73	118.36	121.84	119.16
127	95.88	100.52	92.51	90.28	95.31
133	98.36	97.64	88.36	95.87	98.89
130	96.03	89.31	85.60	96.80	92.67
139	97.90	102.77	89.84	94.94	101.37
198	136.67	121.35	136.21	125.31	137.05
204	139.69	129.46	133.57	133.01	129.33
215	142.39	130.23	135.28	117.29	145.86
Incubation Temperature = 22C					
189	137.38	105.55	109.15	108.32	108.87
199	134.39	117.96	127.42	114.68	110.36
170	128.96	118.11	119.47	118.71	119.61
127	89.04	90.91	92.36	84.08	86.47
133	85.16	98.12	94.81	84.86	91.27
130	91.06	81.45	87.29	79.43	87.07
139	97.58	98.12	83.60	83.31	80.39
198	141.12	120.44	124.69	116.35	123.82
204	138.42	124.57	130.92	120.28	123.03
215	128.71	115.85	122.66	122.95	117.52
Incubation Temperature = 45C					
189	129.82	164.39	160.06	153.08	147.64
199	135.24	180.89	167.17	185.23	137.20
170	142.81	130.96	134.67	149.43	
127	107.68	125.68	130.60	151.85	134.66
133	106.90	152.12	147.96	155.57	161.71
130	100.69	140.58	155.33	150.14	140.57
139	117.78	134.49	148.73	157.43	159.53
198	130.78	137.11	204.60	194.61	138.39
204	121.08	137.11	166.59	169.15	191.60
215	126.96	136.96	186.06	147.31	139.02

APPENDIX D

CONCENTRATIONS OF EXTRACTABLE AND BIOASSAY DETERMINED BAPP

APPENDIX D

RESULTS OF BAPP ANALYSES, ug P/g

SOURCE	SAMPLE	TOTAL P	CCTCENT	CCTFILT	BAKER	ARMSTRONG	CCIW	BIOASSAY
MAUMEE R.	1	1128.6	225.9	222.1	422.6	365.1	563.8	310.0
		1118.0	231.5	227.8	425.7	372.4	597.4	299.0
CUYAHOGA R.	2	1135.7	399.3	385.1	489.6	500.2	670.4	367.0
		1131.2	397.4	385.1	492.3	509.2	691.1	350.0
		1134.3						449.0
		1107.2						
SANDUSKY R.	3	977.6	294.5	288.7	324.3	341.9	581.2	236.0
		998.2	297.5	289.7	322.3	346.8	604.3	219.0
RAISIN R.	4	1271.1	137.3	133.5	428.6	459.1	628.5	
		1271.1	138.2	136.3	429.7	450.1	643.1	
		1247.8						
		1255.9						
MAUMEE R.	5	825.5	189.1	183.1	219.6	221.5	405.9	231.0
		848.1	189.1	183.1	223.2	224.2	416.9	233.0
								235.0
SANDUSKY R.	6	888.8	263.6	256.9	265.6	271.8	520.0	164.0
		911.1	263.6	254.9	256.2	280.9	530.4	203.0
L. ERIE	7	2169.0	409.4	388.3	1201.5	1618.9	1928.3	1462.0
		2279.6	420.8	404.6	1192.5	1527.7	1931.1	1665.0
		2195.6				1592.2		1453.0
		2195.6				1498.9		
L. ERIE	8	2470.4	271.7	257.0	1300.3	1794.6	2245.0	1955.0
		2468.4	258.0	243.3	1291.3	1785.9	2215.8	2140.0
		2470.2		213.7				2026.0
		2482.7		234.8				

APPENDIX D

RESULTS OF BAPP ANALYSES, ug P/g

SOURCE	SAMPLE	TOTAL P	CCTCENT	CCTFILT	BAKER	ARMSTRONG	CCIW	BIOASSAY
OSWEGO R.	9	994.5	223.4	225.3	227.3	231.5	322.9	106.0
		1019.9	224.3	213.1	224.9	232.1	345.7	112.0
GENESEE R.	10	605.7	92.2	85.6	124.2	130.9	218.2	52.0
		609.3	90.3	85.6	125.3	126.8	229.6	61.0
CATTARAUGUS CREEK	11	429.3	29.7	29.7	33.7	32.9	79.7	5.0
		418.5	30.7	29.7	33.7	34.7	80.0	1.0
NBS STANDARD SEDIMENT	12	930.0	37.0	43.7	111.8	118.6	289.7	
		922.7	40.8	42.7	106.3	114.4	293.1	
		1001.1			109.2	115.6		
		966.5			108.2	115.0		
		1071.5						
		1022.1						
NUMBER OF DETERMINATIONS		36	24	26	26	28	24	29
AVERAGE		1277.0	214.8	209.4	403.5	550.5	709.7	518.6
STANDARD DEVIATION		605.8	119.9	110.4	383.7	583.8	639.7	667.0
MINIMUM		418.5	29.7	29.7	33.7	32.9	79.7	1.0
MAXIMUM		2482.7	420.8	404.6	1300.3	1794.6	2245.0	2140.0

APPENDIX E.0

STATISTICAL ANALYSES: ANALYSIS OF VARIANCE TABLES

APPENDIX E.1

DOUBLE SPLIT-PLOT ANOVA OF STORAGE EFFECTS ON SRP

APPENDIX E.1

DOUBLE SPLIT-PLOT ANOVA OF STORAGE EFFECTS ON SRP

DEPENDENT VARIABLE: SRP

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	83	340981.47384995	4108.21052831
ERROR	215	2912.77553400	13.54779318
CORRECTED TOTAL	298	343894.24938395	

MODEL F =	303.24		PR > F = 0.0001
R-SQUARE	C.V.	ROOT MSE	SRP MEAN
0.991530	10.5964	3.68073270	34.73555184

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	225232.51210329	16625.03	0.0001
SAMPLE(RIVER)	18	8876.76468743	36.40	0.0001
TEMP	2	46754.02848090	1725.52	0.0001
TEMP*RIVER	2	33983.69568510	1254.22	0.0001
SAMPLE*TEMP(RIVER)	36	3629.14174980	7.44	0.0001
TIME	4	4510.69087345	83.24	0.0001
RIVER*TIME	4	2758.68393520	50.91	0.0001
TEMP*TIME	8	11217.64289398	103.50	0.0001
TEMP*RIVER*TIME	8	3172.55765340	29.27	0.0001

TESTS OF HYPOTHESES USING THE TYPE III MS FOR SAMPLE(RIVER) AS AN ERROR TERM:

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	225232.51210329	456.72	0.0001

TESTS OF HYPOTHESES USING THE TYPE III MS FOR SAMPLE*TEMP(RIVER) AS AN ERROR TERM:

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SAMPLE(RIVER)	18	8876.76468743	4.89	0.0001
TEMP	2	46754.02848090	231.89	0.0001
TEMP*RIVER	2	33983.69568510	168.55	0.0001

APPENDIX E.2

DOUBLE SPLIT-PLOT ANOVA OF STORAGE EFFECTS ON BAPP

APPENDIX E.2

DOUBLE SPLIT-PLOT ANOVA OF STORAGE EFFECTS ON BAPP

DEPENDENT VARIABLE: RNAOHP

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	83	659716.79449932	7948.39511445
ERROR	214	15093.66124195	70.53112730
CORRECTED TOTAL	297	674810.45574128	
MODEL F =	112.69		PR > F = 0.0001
R-SQUARE	C.V.	ROOT MSE	RNAOHP MEAN
0.977633	10.5249	8.39828121	79.79439597

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	565189.37586957	8013.33	0.0001
SAMPLE(RIVER)	18	29045.08712331	22.88	0.0001
TEMP	2	25113.66489230	178.03	0.0001
TEMP*RIVER	2	19824.03399125	140.53	0.0001
SAMPLE*TEMP(RIVER)	36	5052.63030753	1.99	0.0015
TIME	4	658.26984750	2.33	0.0568
RIVER*TIME	4	2177.31410798	7.72	0.0001
TEMP*TIME	8	10415.28314372	18.46	0.0001
TEMP*RIVER*TIME	8	3599.76112477	6.38	0.0001

TESTS OF HYPOTHESES USING THE TYPE III MS FOR SAMPLE(RIVER) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	565189.37586957	350.26	0.0001

TESTS OF HYPOTHESES USING THE TYPE III MS FOR SAMPLE*TEMP(RIVER) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SAMPLE(RIVER)	18	29045.08712331	11.50	0.0001
TEMP	2	25113.66489230	89.47	0.0001
TEMP*RIVER	2	19824.03399125	70.62	0.0001

APPENDIX E.3

DOUBLE SPLIT-PLOT ANOVA OF STORAGE EFFECTS ON BAPP/TSS

APPENDIX E.3

DOUBLE SPLIT-PLOT ANOVA OF STORAGE EFFECTS ON BAPP/TSS

DEPENDENT VARIABLE: BAPP/TSS

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	83	303.65548909	3.65849987
ERROR	214	23.31876430	0.10896619
CORRECTED TOTAL	297	326.97425339	
MODEL F =	33.57		PR > F = 0.0001
R-SQUARE	C.V.	ROOT MSE	NORM MEAN
0.928683	21.2050	0.33010027	1.55671292

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	199.25856678	1828.63	0.0001
SAMPLE(RIVER)	18	66.30799813	33.81	0.0001
TEMP	2	5.77614755	26.50	0.0001
TEMP*RIVER	2	3.57659199	16.41	0.0001
SAMPLE*TEMP(RIVER)	36	3.84485061	0.98	0.5071
TIME	4	3.94091205	9.04	0.0001
RIVER*TIME	4	4.72058190	10.83	0.0001
TEMP*TIME	8	8.54298049	9.80	0.0001
TEMP*RIVER*TIME	8	5.57195851	6.39	0.0001

TESTS OF HYPOTHESES USING THE TYPE III MS FOR SAMPLE(RIVER) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	199.25856678	54.09	0.0001

TESTS OF HYPOTHESES USING THE TYPE III MS FOR SAMPLE*TEMP(RIVER) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SAMPLE(RIVER)	18	66.30799813	34.49	0.0001
TEMP	2	5.77614755	27.04	0.0001
TEMP*RIVER	2	3.57659199	16.74	0.0001

APPENDIX E.4

ANCOVA OF SOURCE VARIABLES AND SRP DURING STORAGE

APPENDIX E.4

ANCOVA OF SOURCE VARIABLES AND SRP DURING STORAGE

STORAGE PERIOD: TIME = 0 TO TIME = 0.5 DAYS

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	90.54002949	1.66	0.2035
TEMP	2	84.52749000	0.78	0.4661
TEMP*RIVER	2	1551.86614333	14.23	0.0001
TP	1	61.30429782	1.12	0.2941
TSS	1	4.79320779	0.09	0.7681
PH	1	43.79826328	0.80	0.3744
COND	1	112.32486374	2.06	0.1575
FIELD	1	343.16361263	6.30	0.0155

STORAGE PERIOD: TIME = 0 TO TIME = 1.0 DAYS

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	4.40797891	0.19	0.6657
TEMP	2	7574.06262333	162.37	0.0001
TEMP*RIVER	2	5208.31260333	111.66	0.0001
TP	1	12.05587451	0.52	0.4756
TSS	1	0.60905374	0.03	0.8723
PH	1	0.56131775	0.02	0.8774
COND	1	1.95032945	0.08	0.7737
FIELD	1	120.93768348	5.19	0.0272

STORAGE PERIOD: TIME = 0 TO TIME = 2.0 DAYS

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	0.81960001	0.04	0.8394
TEMP	2	14501.62623000	367.39	0.0001
TEMP*RIVER	2	10469.99858333	265.25	0.0001
TP	1	19.86049355	1.01	0.3207
TSS	1	4.22025522	0.21	0.6458
PH	1	0.91340833	0.05	0.8306
COND	1	6.82838717	0.35	0.5591
FIELD	1	167.72751549	8.50	0.0053

APPENDIX E.4

ANCOVA OF SOURCE VARIABLES AND SRP DURING STORAGE

STORAGE PERIOD: TIME = 0 TO TIME = 4.0 DAYS

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	0.46361422	0.03	0.8534
TEMP	2	17898.25454333	666.13	0.0001
TEMP*RIVER	2	11890.68314333	442.54	0.0001
TP	1	64.90952628	4.83	0.0327
TSS	1	41.65454603	3.10	0.0845
PH	1	84.46284448	6.29	0.0155
COND	1	9.25050180	0.69	0.4107
FIELD	1	13.34825249	0.99	0.3238

STORAGE PERIOD: TIME = 0 TO TIME = 9.0 DAYS

SOURCE	DF	TYPE III SS	F VALUE	PR > F
RIVER	1	82.59397964	3.59	0.0640
TEMP	2	17832.48047204	388.06	0.0001
TEMP*RIVER	2	8065.69424048	175.52	0.0001
TP	1	49.65860240	2.16	0.1481
TSS	1	6.87744634	0.30	0.5868
PH	1	9.77979789	0.43	0.5172
COND	1	0.58474204	0.03	0.8739
FIELD	1	11.21721784	0.49	0.4881

APPENDIX E.5

FACTORIAL ANOVA OF CHEMICAL EXTRACTION DATA

APPENDIX E.5

FACTORIAL ANOVA OF CHEMICAL EXTRACTION DATA

DEPENDENT VARIABLE: BAP

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE
MODEL	69	41498802.79288702	601431.92453459
ERROR	85	65193.38577504	766.98100912
CORRECTED TOTAL	154	41563996.17866206	
MODEL F =	784.15		PR > F = 0.0001

R-SQUARE	C.V.	ROOT MSE	BAP MEAN
0.998431	6.3011	27.69442199	439.51529032

SOURCE	DF	TYPE III SS	F VALUE	PR > F
PROC	5	4355958.29279255	1135.87	0.0001
SAMPLE	11	23266129.09088487	2757.70	0.0001
PROC*SAMPLE	53	9385673.82885074	230.89	0.0001

APPENDIX E.6

CORRELATIONS AMONG CHEMICAL EXTRACTION AND BIOASSAY RESULTS

APPENDIX E.6

CORRELATIONS AMONG CHEMICAL EXTRACTION AND BIOASSAY RESULTS

All Pairwise Comparisons, all data.

VARIABLE	N	MEAN	STD DEV	SUM	MINIMUM	MAXIMUM
TP	12	1161.7333	598.59102	13940.800	423.9000	2472.9000
CCTCENT	12	214.7333	125.07213	2576.800	30.2000	415.1000
CCTFILT	12	208.1250	119.79774	2497.500	29.7000	396.5000
BAKER	12	428.1333	406.77749	5137.600	33.7000	1295.8000
ARMSTRNG	12	502.9917	566.63827	6035.900	33.8000	1790.2000
CCIW	12	710.5333	668.01307	8526.400	79.9000	2230.4000
BIOASSY	10	508.5100	692.59261	5085.100	2.7000	2040.3000

Correlation Coefficients / Type I Error Rate / Number of Observations

	TP	CCTCENT	CCTFILT	BAKER	ARMSTRNG	CCIW	BIOASSY
TP	1.00000	0.57817	0.56583	0.97713	0.97472	0.97808	0.97402
	0.0000	0.0489	0.0552	0.0001	0.0001	0.0001	0.0001
	12	12	12	12	12	12	10
CCTCENT	0.57817	1.00000	0.99964	0.64719	0.59088	0.60550	0.49848
	0.0489	0.0000	0.0001	0.0229	0.0431	0.0369	0.1425
	12	12	12	12	12	12	10
CCTFILT	0.56583	0.99964	1.00000	0.63261	0.57468	0.59012	0.47754
	0.0552	0.0001	0.0000	0.0273	0.0506	0.0434	0.1628
	12	12	12	12	12	12	10
BAKER	0.97713	0.64719	0.63261	1.00000	0.99365	0.99250	0.98115
	0.0001	0.0229	0.0273	0.0000	0.0001	0.0001	0.0001
	12	12	12	12	12	12	10
ARMSTRNG	0.97472	0.59088	0.57468	0.99365	1.00000	0.99718	0.99353
	0.0001	0.0431	0.0506	0.0001	0.0000	0.0001	0.0001
	12	12	12	12	12	12	10
CCIW	0.97808	0.60550	0.59012	0.99250	0.99718	1.00000	0.99058
	0.0001	0.0369	0.0434	0.0001	0.0001	0.0000	0.0001
	12	12	12	12	12	12	10
BIOASSY	0.97402	0.49848	0.47754	0.98115	0.99353	0.99058	1.00000
	0.0001	0.1425	0.1628	0.0001	0.0001	0.0001	0.0000
	10	10	10	10	10	10	10

APPENDIX F

COMPARISON OF FIELD AND LABORATORY FILTRATION UNITS FOR SRP ANALYSIS

APPENDIX F

COMPARISON OF FIELD AND LABORATORY FILTRATION UNITS FOR SRP ANALYSIS

Sample	Filtration Unit	Sample Absorbance	Concentration (ug P/L)
Maumee #9	Field	0.471	74.2
Maumee #9	Laboratory	0.471	74.2
	Difference =	0.000	0.0
Huron #9	Field	0.128	19.0
Huron #9	Laboratory	0.122	20.0
	Difference =	0.006	1.0

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16 ABSTRACT

Sediments were assayed for biologically available particulate phosphorus (BAPP) by bioassay and chemical extractions to permit comparisons among the procedures. Although the procedures extracted widely differing amounts of phosphorus (P), accurate estimate conversions, using regression relationships, could be made among procedures for freshly collected samples. Samples stored for several years gave anomalous extractable P results by some procedures. In fresh samples amounts of P extracted by the De Pinto procedure were consistently closest in magnitude to the amounts taken up by algae during bioassays. In both fresh and stored samples the Armstrong procedure gave results that correlated most closely with bioassays.

Effects of storage time (0 to 9 days) and temperature (4, 22, and 45 C) on soluble reactive P (SRP) and BAPP were examined using unfiltered samples from the Maumee and Huron Rivers. Significant changes occurred for most combinations of time and temperature. Major changes occurred at 45 C; any period of sample storage could affect the reliability of SRP estimates in samples held at 45 C. The results support standard procedures for handling of water samples collected for P analysis. Observed changes in SRP during storage, however, were offset partially by inverse changes in BAPP, indicating that for storage times of no more than 9 days, the total bioavailable P (BAP) of water samples (SRP+BAPP) may be partially conserved.

17 KEY WORDS AND DOCUMENT ANALYSIS

a DESCRIPTORS	b IDENTIFIERS/OPEN ENDED TERMS	c COSATI Field/Group
Phosphorus, sediments, river, nutrients, fluvial, suspended solids, bioavailability, soluble, particulate		

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