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# SOLUBLE REACTIVE PHOSPHORUS MEASUREMENTS IN LAKE MICHIGAN: CAUSES OF METHOD-SPECIFIC DIFFERENCES

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***ABSTRACT.*** *Method-specific differences in soluble reactive phosphorus (SRP) determinations are thought to be caused in part by differences in acid strengths and exposure times. This premise was tested by comparing SRP concentrations measured in water from Lake Michigan and the Grand River by three methods differing in acid strength, exposure time, and molybdate concentration. Although longer exposure times often result in higher SRP values, more $PO_4$–P can be released from bound sources in lake water with 0.16 N HCl than with 0.4 N HCl. Method-specific differences in SRP values, therefore, rarely are proportional to differences in acid strength or exposure time and will vary with changes in the chemical composition of the SRP pool.*
*ADDITIONAL INDEX WORDS: Hydrolysis, orthophosphorus, ammonium molybdate.*

## INTRODUCTION

Eutrophication studies in the Great Lakes often include measurements of soluble reactive phosphorus (SRP) (Dobson *et al.* 1974, Torrey 1976). SRP can be estimated by several methods where orthophosphorus ($PO_4$–P) is complexed with molybdate under acid conditions to form 12-molybdophosphoric acid (Olsen 1967). This complex is then reduce to form a blue color that is measured along with standards in a spectrophotometer. Harvey's (1948) acid-molybdate stannous chloride method had been the time-honored method until the late 1960s (Olsen 1967, A.P.H.A. 1971). Currently, the ascorbic acid method (Murphy and Riley 1962) is the technique of choice (Strickland and Parsons 1972) because a single mixed reagent is added and color deveopment is stable for a much longer period of time than in Harvey's method.

Studies on molybdenum blue methodology have revealed the following. (1) SRP usually overestimates orthophosphorus ($PO_4$–P) concentrations in phosphorus-limited lakes by 10–100 times (Rigler 1966). This is thought to be caused by release of $PO_4$–P from bound dissolved or colloidal sources when acid reagents are added (cf., Rigler 1968, Paerl and Downes 1978, Stainton 1980). (2) Non-specific reduction or interference by arsenate (As) or silica (Si) may introduce positive biases because these and other ions also can complex with molybdate (Olsen 1967). (3) Different methods frequently yield dissimilar values even after corrections for analytical interferences are made (Jones and Spencer 1963, Jones 1966, Chamberlain and Shapiro 1969). The first and third observations have led to the commonly accepted premise that methods based on high acid levels (with the same exposure time) or long exposure times (with the same acid level) will hydrolyze more of the SRP pool and, therefore, yield higher SRP values than methods using low acid and short exposure times (cf., Chamberlain and Shapiro 1973). This belief has become so firmly entrenched that researchers have devised methods using low acid ($< 0.15$ N) or short exposure times ($< 30$ s) to minimize hydrolysis (cf., Golterman and Wurtz 1961; Chamberlain and Shapiro 1969, 1973).

The premise that high acid levels (e.g., 0.4 N) release more $PO_4$–P than low acid levels (e.g. $< 0.2$ N) has never been rigorously tested and may be incorrect. Jones (1966) and Jones and Spencer (1963), for example, observed that Harvey's

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method (1948) (0.4 N $H_2SO_4$, 9-min exposure) at times gave lower values in the Atlantic Ocean than several methods based on lower acid and shorter exposure times. Chamberlain and Shapiro (1969) reported that their extraction method (0.16 N HCl, 30-s exposure) sometimes gave higher values in Minnesota lakes than Harvey's method, but offered no explanation. Tarapchak and Rubitschun (1981) showed that, in offshore Lake Michigan water, the Chamberlain-Shapiro (1969) method usually gave lower SRP values than Harvey's method during spring and early summer, but yielded higher values during August through October. They postulated that the chemical composition of the SRP pool varies seasonally and at times may be composed of bound $PO_4$–P sources that are hydrolyzed more rapidly by weak rather than by strong acid. More recently, however, Tarapchak *et al.* (1982) demonstrated that SRP values in Lake Michigan also are partially dependent on molybdate concentration. The Chamberlain-Shapiro extraction method (0.048% ammonium molybdate), therefore, might yield higher values than Harvey's method (0.095% ammonium molybdate) if rates of $PO_4$–P release increase with decreasing molybdate concentrations.

SRP estimates have been used to assess eutrophication trends (Rockwell *et al.* 1980, EPA 1980) and to monitor spatial and temporal variations in biologically available P in localized regions of the Great Lakes (Smith *et al.* 1977). Conclusions drawn from these studies could be biased if different methods are used. Robertson *et al.* (1974), for example, showed that different laboratories measured dissimilar SRP values in the same samples of Lake Ontario water. Method-specific differences in SRP estimation, however, have not been adequately reported in the Great Lakes, nor has the underlying cause(s) of discrepancies been investigated.

The purpose of this study was: (1) to compare relative differences in SRP concentrations in water from Lake Michigan and the Grand River, Michigan, using three commonly used molybdenum blue methods, and to compare the rank order of values with expectations based on acid strength and exposure time, and (2) to investigate the cause of method-specific differences by evaluating the effects of acid, exposure time, and ammonium molybdate on SRP estimation. The Chamberlain-Shapiro extraction method and Harvey's method were selected because they had been used previously (Tarapchak and Rubitschun 1981). The ascorbic acid method was selected because it is used frequently in the Great Lakes and differs either in acid strength, exposure time, or molybdate concentration from at least one of the other methods.

## METHODS AND EXPERIMENTAL TESTS

Reagent concentrations in Harvey's method (1948) were those used by Rigler (1964), the extraction technique was slightly modified (Tarapchak *et al.* 1982), and exposure time in the ascorbic acid method (Strickland and Parsons 1972) was held constant at 9 min (Table 1). Detection limits and precision were assessed by analyzing solutions of potassium dihydrogen phosphate ($KH_2PO_4$) ranging from 0.1 to 100 $\mu$g P/L in distilled, deionized water (Table 1). Solutions were analyzed in a Fisher Model II electrophotometer (Fisher Scientific Co.) and in a Bausch and Lomb, Model 88, spectrophotometer. Detection limits were identified by testing the mean optical density of reagent blanks against the mean optical density values of P standards beginning at the lowest concentration until a statistically significant difference ($P < 0.05$) was found (t-test based on weighted variance, Snedecor and Cochran 1967). Precision over a wide range of $PO_4$–P concentrations in distilled water was high (Table 1), and comparable estimates were obtained in $PO_4$–P standards prepared in 0.45-$\mu$m filtered lake water.

### Interferences

Tests were performed to determine if non-specific reduction (NSR) or salt effects bias SRP estimates and if silica (Si) or arsenate (As) interfere. NSR, i.e., formation of color in the absence of specific ions that complex with molybdate, could introduce serious errors into SRP estimation (Chamberlain and Shapiro 1973). Standard curves prepared in filtered lake water should yield slopes intercepting the positive y-axis if NSR is occurring (cf., Jones and Spencer 1963). Linear regression analysis performed on numerous standard curves prepared in 0.45-$\mu$m filtered lake and river water by all three methods failed to show positive y-axis intercepts at $P < 0.05$ (Snedecor and Cochran 1967), indicating that NSR did not introduce a serious bias. Salt effects, i.e., damping of color development in lake water *vs.* distilled water, were observed only in Harvey's method (Tarapchak and Rubitschun 1981) and were eliminated by using internal $PO_4$–P standards. Tests showed that Si did not interfere in any method at added concentrations up to 20 mg

***TABLE 1. Selected characteristics of Harvey's method, the ascorbic acid method, and the Chamberlain-Shapiro extraction technique: acid normality (N); the time that samples are exposed to reagents; concentration of ammonium molybdate; precision (standard error of the mean based on seven replicate distilled-water samples at concentrations between 0.2 and 100 μg P/L) in 5.0-cm in 2.3-cm cuvettes; interference by 50 μg As/L ($NaHAsO_4$) and 2.0 mg Si/L ($NaSiO_3 \cdot 9H_2O$) expressed in μg P/L. Salt effects are computed as the percent difference between slopes of standard curves prepared in filtered lake water and distilled water. ND = not netectable. Analyses performed at 21 ± 1°C.***

| Variable | Harvey | Ascorbic | Extraction |
|---|---|---|---|
| Acidity (N) | 0.40 | 0.22 | 0.16 |
| Exposure time | 9±0.25 min | 9±0.25 min | 30±2.0 s |
| Molybdate (%) | 0.095 | 0.055 | 0.048 |
| Salt effect | 20–25% | None | None |
| Detection limit | ~1.0 | ~0.8 | ~0.30 |
| Precision | | | |
| 1.1–1.5 μg P/L (5 cm) | ≤0.07 | ≤0.07 | — |
| >1.5 μg P/L (5 cm) | ≤0.05 | ≤0.05 | — |
| ≥(0.4) (5.0 cm) | — | — | ≤0.04 |
| ≤0.78 (2.3 cm) | ND | ND | ≤0.06 |
| >0.78 (2.3 cm) | — | — | ≤0.04 |
| Interference | | | |
| Arsenate | 25 | 4.0 | 0.3 |
| Silica | ≤1.0 | ≤0.8 | ≤0.3 |

Si/L. Arsenate seriously interferes in Harvey's method but not in the extraction method (Tarapchak and Rubitschun 1981), and tests as < 10% of the $PO_4$–P concentration in samples containing 6.3 and 400 μg As/L in the ascorbic acid method.

### Comparative Lake-Water Measurements

Comparative analyses were performed to verify results reported by Tarapchak and Rubitschun (1981) and to contrast values obtained by the ascorbic acid method with those measured by Harvey's method and the extraction method. Samples were collected between the surface and 30 m at an offshore station (43°05′12″N, 86°25′45″W) 15 km west of Grand Haven, Michigan, on 15 September 1976. On 23 November 1976, samples from the surface and 1 m above the bottom were taken at three stations in the mouth of the Grand River, Michigan, a station 0.4 km from shore, and a station 16 km from shore. On 20 August 1977, water was taken from the surface at 10 stations located within an 8- × 13-km grid adjacent to the shore and the mouth of the Grand River. Five hundred-mL samples were filtered through 0.45-μm Millipore filters (47-mm diameter) at 300 ± 10 mm Hg, refrigerated at 4 to 5°C, and analyzed within 24 h. SRP was measured in triplicate by Harvey's method (5-cm cuvettes) and the extraction technique (2.3-cm cuvettes) and in duplicate by the ascorbic acid method (5-cm cuvettes).

To ensure valid comparisons, all tests were based on determinations from the same sample; therefore, potential artifacts arising from bottle effects are constant across each test. Common $PO_4$–P standards ($KH_2PO_4$) were prepared in 0.45-μm filtered water; color blanks (all reagents except molybdate) were run with each test; Millipore filters were prerinsed to remove contaminant P (Tarapchak *et al.* 1982); and samples were filtered in a Fisher Filtrator (Fisher Scientific Co.) fitted with a 300-mL Millipore funnel to minimize handling of filtrates. Samples were permitted to equilibrate to room temperature (21 ± 1°C) to avoid temperature-dependent variations in color development (To and Randall 1977). Glassware and sample bottles were washed in dilute Liquinox (Alconox, Inc., N.Y.), a phosphate-free detergent, soaked at least 12 h in concentrated nitric acid, and rinsed six times in distilled, deionized water.

### Hydrolysis Tests

Causes of method-specific differences in SRP estimation were evaluated by (1) analyzing synthetic solutions, (2) determining if differences in exposure time affect amounts of $PO_4$–P release, (3) evaluating the relative effects of differences in acid strength and molybdate concentration on SRP estimates, and (4) manipulating acid and molybdate levels in one method to confirm results generated in (2) and (3). Tests on synthetic compounds were based on high-purity sodium salts (Sigma Chemical Co., St. Louis, Mo.) ranging from acid-stable compounds (e.g., glycerophosphates) to acid-labile carbohydrate esters (e.g., 2-Deoxy-α-D-ribose-1-phosphate) (Leloir and Cardini 1957, Colowick and Kaplan 1957). Except for two highly labile compounds, stock (25 to 100 mg P/L) and working solutions (50 to 500 μg P/L) were prepared in distilled water (buffered with 10 mg/L sodium bicarbonate to slow hydrolysis) in volumetric flasks 1 to 8 h before analysis. Standards and reagent blanks also were made in buffered, distilled, deionized water and run in triplicate. Measurements by all methods were made in 5-cm cuvettes within 15 min of one another. Solutions of acetyl phosphate and 2-Deoxy-α-D-

Ribose-1-phosphate were analyzed by all methods within 1 to 3 min using stock and working solutions < 10 min old. Analyses were performed in quadruplicate by Harvey's method and the ascorbic acid method and in triplicate by the extraction method. Each test was repeated at least once using freshly prepared solutions to verify method-specific differences.

The effects of exposure time on SRP estimation were evaluated by measuring SRP by Harvey's method and at two exposure times in the Chamberlain-Shapiro extraction method. Samples were taken from a depth of 4 m at the offshore and from an inshore station (43°04′24″ N, 86°16′15″ W) 4 km west of Grand Haven, Michigan, and filtered as described above. Estimates using the prescribed 30-s exposure in the extraction method were made by adding 25 mL isobutanol, 5 mL 1% aqueous ammonium molybdate (0.048% in sample) (pH 5.5–6.0), and 5 mL 3.3 N NCl in that order within 8 ± 1 s to 100 mL of lake water in a 250-mL separatory funnel. Samples were shaken immediately for 30 s, and allowed to stand 60 s for phase separation. The organic phase was recovered, washed with 100 mL 0.5 N NCl, recovered again, and then reduced (Tarapchak and Rubitschun 1981). Estimates for 9-min exposures were made by adding reagents and allowing samples to stand for 8.5 min before shaking and completing the analysis (cf., Shapiro *et al.* 1970, Tarapchak *et al.* 1982). Estimates at all exposure times are based on replicate analysis (N = 4) of samples, standards, and reagent blanks in 2.3-cm cuvettes.

Concentration-dependent effects of molybdate on SRP determinations were assessed by varying ammonium molybdate concentrations in each method (cf., Tarapchak *et al.* 1982). Ammonium molybdate concentrations used in Harvey's method (0.055–0.143%), the extraction technique (0.024–0.143%), and the ascorbic acid method (0.024–0.095%) were within the acid-molybdate "plateau" and did not affect the accuracy of SRP estimation (Going and Eisenreich 1974). Measurements at each molybdate concentration are based on replicate analysis of samples (N = 4) and standards and reagent blanks (N = 3) in 5-cm (Harvey's method and the ascorbic acid method) and 2.3-cm cuvettes (extraction method).

Progressive hydrolysis tests were performed by the extraction technique to directly evaluate the effects of "high" *vs.* "low" acid and molybdate levels on SRP estimates. Acid levels of 0.16 N and 0.4 HCl were selected to match those used in the extraction method and Harvey's method, respectively. Ammonium molybdate concentrations of 0.075 and 0.125% were used as "low" and "high" levels, respectively, as concentrations < 0.075% do not produce sufficient color development in Harvey's method. Tests were performed by adding reagents in the prescribed sequence and allowing samples to stand for 0 to 12 min before shaking and completing analysis. Measurements at each exposure time are based on replicate analysis of samples (N = 3) in 2.3-cm cuvetts.

## RESULTS AND DISCUSSION

### Comparisons of SRP Concentrations

Based on the premise that SRP values increase with increasing acid concentrations and longer exposure times, the rank order of SRP concentrations in lake water should be: Harvey's method > ascorbic acid method > Chamberlain-Shapiro extraction method (provided that materials are not completely hydrolyzed by the method with the lowest acid level and shortest exposure time) (Table 1). SRP concentrations in water from Lake Michigan and the Grand River were method-dependent and confirm our previous observations (Tarapchak and Rubitschun 1981). In the September 15 profile, values measured by the extraction method were statistically higher than those measured by the ascorbic acid method and most values determined by Harvey's method (Table 2). In the November 1976 samples, the extraction technique usually gave statistically higher values than the ascorbic acid method and Harvey's method, but in several samples, the ascorbic acid method yielded values equal to or higher than those measured by Harvey's method (Table 3). Analyses of the samples taken from the grid near the mouth of the Grand River showed that SRP measured by Harvey's method and the extraction method generally were higher by a factor of two or more than values measured by the ascorbic acid method (Table 4). These discrepancies cannot be attributed to analytical errors because total As at these stations is < 0.5 μg As/L (Chambers and Eadie 1980) and the estimates apparently are not subject to other known biases (see Interferences).

### Potential Causes of Method Specific Differences

Method-specific differences in SRP could be caused by differences in acid strength, exposure

*TABLE. 2. Comparison of SRP concentrations measured by Harvey's method, the Chamberlain-Shapiro extraction method (prescribed exposure time), and the ascorbic acid method in filtered water collected on 15 September 1976 through the euphotic zone at an offshore station in southern Lake Michigan. Results are treated with a one-way ANOVA and the Newman-Kuels multiple range test ($P < 0.05$) (Zar 1974). Groups with difference numbers in parentheses represent statistically dissimilar mean values. Analyses performed at 21 ± 1° C.*

| Sample depth (m) | Harvey | Extraction | Ascorbic | [1]F |
|---|---|---|---|---|
| | μg P/L | | | |
| 0 | 5.3 (1) | 5.4 (1) | 1.8 (2) | 1437*** |
| 3 | 4.0 (1) | 5.2 (2) | 1.7 (3) | 731*** |
| 6 | 3.3 (1) | 5.4 (2) | 1.3 (3) | 2926*** |
| 9 | 1.8 (1) | 5.7 (2) | ≤0.8 (3) | 1968*** |
| 12 | 4.0 (1) | 5.7 (2) | ≤0.8 (3) | 1809*** |
| 15 | 4.8 (1) | 5.9 (2) | ≤0.8 (3) | 2568*** |
| 20 | 1.8 (1) | 5.9 (2) | ≤0.8 (3) | 7129*** |
| 30 | 6.0 (1) | 6.3 (2) | ≤0.8 (3) | 22.5*** |

[1]F Statistic from one-way ANOVA with 2,5 d.f.: $P < 0.01$***

time, or molybdate concentration (Chamberlain and Shapiro 1973, Tarapchak and Rubitschun 1981, Tarapchak *et al.* 1982). Four types of tests were performed to evaluate the importance of each factor. (1) The premise that high rather than low acid and long rather than short exposure times cause more hydrolysis was tested by analyzing synthetic solutions of organic and condensed inorganic compounds. If a lower rather than a higher acid regime causes more hydrolysis, the ascorbic acid method and extraction method should give higher values than Harvey's method. (2) The hypothesis that the low acid-molybdate regime of the extraction can hydrolyze more of the SRP pool than that used in Harvey's method could be true, but masked, if comparable exposure times are not used. Samples, therefore, were analyzed by comparing SRP values measured at 30 s and 9 min

*TABLE 4. Comparison of SRP concentrations measured by Harvey's method, the Chamberlain-Shapiro extraction method (prescribed exposure time), and the ascorbic acid method in filtered water collected in 16 August 1977 from stations located in an 8- × 13-km sampling grid adjacent to shore and the Grand River in southern Lake Michigan. Results are tested with a one-way ANOVA and the Newman-Kuels multiple range test ($P < 0.05$) (Zar 1974). Groups with different numbers in parentheses represent statistically dissimilar mean values. Analyses performed at 21 ± 1° C.*

| Station | Harvey | Extraction | Ascorbic | [1]F |
|---|---|---|---|---|
| | μg P/L | | | |
| 1 | 3.1 (1) | 5.4 (2) | 4.0 (3) | 1593*** |
| 2 | 2.8 (1) | 3.0 (1) | 1.0 (2) | 461*** |
| 3 | 2.7 (1) | 3.5 (2) | 1.5 (3) | 667*** |
| 4 | 2.9 (1) | 4.0 (2) | 1.5 (3) | 601*** |
| 5 | 3.3 (1) | 2.9 (2) | 1.5 (3) | 345*** |
| 6 | 4.4 (1) | 3.2 (2) | 1.0 (3) | 1924*** |
| 7 | 4.3 (1) | 3.3 (2) | 1.0 (3) | 2539*** |
| 8 | 3.7 (1) | 3.0 (2) | 1.0 (3) | 1735*** |
| 9 | 2.0 (1) | 2.9 (2) | ≤0.8 (3) | 711*** |
| 10 | 47 (1) | 45 (2) | 43 (2) | 8.52** |

[1]F statistic from one-way ANOVA with 2,5 d.f.: $P < 0.05$**; $P < 0.01$***

*TABLE 3. Comparison of SRP concentrations measured by Harvey's method, the Chamberlain-Shapiro extraction method (prescribed exposure time), and the ascorbic acid method in filtered water collected on 23 November 1976, at the surface and near the bottom from three stations in the Grand River and at two stations in southern Lake Michigan. Results are treated with a one-way ANOVA and the Newman-Kuels multiple range test ($P < 0.05$) (Zar 1974). Groups with different numbers in parentheses represent statistically dissimilar mean values. Analyses performed at 21 ± 1° C.*

| Station | Harvey | Extraction | Ascorbic | [1]F |
|---|---|---|---|---|
| | μg P/L | | | |
| Surface (1 m) | | | | |
| Offshore (16 km) | ≤1.0(1) | 1.3(2) | 1.0(1) | 13.9*** |
| Inshore (0.4 km/mouth) | ≤1.0(1) | 3.2(2) | 1.0(1) | 1201*** |
| River mouth | ≤1.0(1) | 4.3(3) | 2.0(2) | 2309*** |
| River 1.6 km upstream | 62 (2) | 63 (3) | 56 (1) | 1416*** |
| River 3.2 km upstream | 43 (2) | 45 (3) | 38 (1) | 1258*** |
| Bottom (1 m above bottom) | | | | |
| Offshore (16 km) | 3.7(2) | 3.7(2) | 1.0(1) | 821*** |
| Inshore (16 km) | ≤1.0(1) | 3.6(3) | 2.8(2) | 1354*** |
| River mouth | 44 (1) | 54 (3) | 45 (2) | 4975*** |
| River 1.6 km upstream | 58 (2) | 61 (3) | 52 (1) | 2138*** |
| River 3.2 km upstream | 36 (1) | 44 (2) | 36 (1) | 2633*** |

[1]F statistic from one-way ANOVA with 2,5 d.f.: $P < 0.01$***.

*TABLE 5. Comparison of hydrolysis potentials of Harvey's method, the Chamberlain-Shapiro extraction method (prescribed exposure time), and the ascrobic acid method based on synthetic solutions of P-containing organic and condensed inorganic compounds. Results are treated with a one-way ANOVA and Newman-Kuels multiple range test ($P < 0.05$) (Zar 1974). Mean values are statistically similar or dissimilar as indicated by the rankings given as subheadings above each groups of compounds. Analyses performed at 21 ± 1°C.*

| | Conc. | Harvey | Extraction | Ascorbic | [1]F |
|---|---|---|---|---|---|
| | | μg P/L | | | |
| | | Harvey > Extraction > Ascorbic | | | |
| Adenosine-5-triphosphate | 100 | 3.9(1) | 1.2(2) | 0.9(3) | 1729*** |
| 2,3-Diphosphoglyceric acid | 100 | 1.9(1) | 1.3(2) | 0.9(3) | 250*** |
| D-Fructose-6-phosphate | 200 | 4.5(1) | 2.7(2) | 2.0(3) | 1576*** |
| D-Glucose-6-phosphate | 500 | 2.9(1) | 1.8(2) | 2.2(3) | 363*** |
| β-Glycerophosphate | 400 | 2.6(1) | 1.4(2) | ≤0.8(3) | 758*** |
| α-D-Ribose-1-phosphate | 50 | 37 (1) | 7.8(2) | 6.5(3) | 2741*** |
| Sodium tripolyphosphate | 200 | 3.1(1) | 2.7(2) | 1.3(3) | 596*** |
| | | Harvey > Ascorbic > Extraction | | | |
| α-D-Glucose-1-phosphate | 200 | 4.8(1) | 1.8(2) | 3.6(3) | 1820*** |
| β-D-Glucose-1-phosphate | 100 | 3.5(1) | 1.3(2) | 2.1(3) | 738*** |
| D-L-α-Glycerophosphate | 100 | 2.5(1) | 1.2(2) | 1.9(3) | 193*** |
| L-α-Glycerophosphate | 200 | 2.2(1) | 0.8(2) | 1.4(3) | 284*** |
| Phosphocreatine | 100 | 36 (1) | 4.1(2) | 27 (3) | 1793*** |
| | | Harvey > Extraction = Ascorbic | | | |
| D-Fructose-1,6-diphosphate | 200 | 5.1(1) | 1.8(2) | 1.7(2) | 1718*** |
| Phospho(enol)pyruvate | 500 | 2.4(1) | 1.3(2) | 1.4(2) | 310*** |
| D-Ribose-5-phosphate | 100 | 2.4(1) | 1.4(2) | 1.3(2) | 302*** |
| Tetrasodium pyrophosphate | 200 | 7.2(1) | 2.6(2) | 2.6(2) | 4027*** |
| | | Harvey = Ascorbic > Extraction | | | |
| Acetyl phosphate | 100 | 100 (1) | 88 (2) | 100 (1) | 525*** |
| | | Harvey = Extraction > Ascorbic | | | |
| 2-Deoxy-α-D-Ribose-1-phosphate | 50 | 50 (1) | 50 (1) | 46.4(2) | 345*** |
| | | Ascorbic > Extraction > Harvey | | | |
| D-Arabinose-5-triphosphate | 100 | 1.9(1) | 2.5(2) | 3.0(3) | 195*** |

[1]F statistic from one-way ANOVA with 2,8 d.f.: $P < 0.01$***

by the extracction method with values detected by Harvey's method at 9 min. (3) The relative importance of acid and molybdate was evaluated by holding acid levels constant in each method and varying the molybdate concentrations in each method. Molybdate effects can be assessed by determining if SRP estimates increase or decrease with increasing molybdate concentrations, and acid effects can be deduced by comparing SRP values measured at the same molybdate concentration. (4) Direct tests on the effects of "low" *vs.* "high" acid and molybdate levels on the time course of $PO_4$–P release were performed to verify results generated in (2) and (3).

### Hydrolysis Tests: Synthetic Solutions

Results of hydrolysis tests based on synthetic solutions are at variance with lake-water analyses (Tables 2–4) and with expected rankings (Table 1). Harvey's method yielded the highest values in 16 solutions (Table 5), and the extraction method yielded values higher than or statistically equivalent to values measured by the ascorbic acid method in 12 of 19 solutions (Table 5, note that subheading rankings indicate statistical differences among mean values at $P < 0.05$). Materials releasing $PO_4$–P in lake water, therefore, must differ from these artifical compounds in their suseptability to acid molybdate.

***TABLE 6. Hydrolysis tests, based on the Chamberlain-Shapiro extraction method, and SRP estimates by Harvey's method in filtered water from an inshore and an offshore station in southern Lake Michigan in 1977. Measurements were at the prescribed exposure of 30 s and at 9 min in the extraction method and at the prescribed exposure of 9 min in Harvey's method. Results are treated with a one-way ANOVA and the Newman-Kuels multiple range test ($P < 0.05$) (Zar 1974). Groups with different numbers in parentheses represent statistically dissimilar mean values. All analyses performed at 21 ± 1°C.***

| | Extraction | | Harvey | |
|---|---|---|---|---|
| | 30 s | 9 min | 9 min | F[1] |
| Inshore | | | | |
| 3 June | 1.2(1) | 2.8(2) | 2.3(2) | 452*** |
| 12 June | 1.8(1) | 2.9(2) | 2.7(2) | 62.6*** |
| 11 July | 1.3(1) | 1.9(2) | 2.4(3) | 172*** |
| 17 August | 0.3(1) | 1.9(2) | 1.9(2) | 726*** |
| 22 September | 0.8(1) | 4.6(2) | 4.7(2) | 2391*** |
| 20 October | 3.0(1) | 5.2(2) | 5.1(2) | 719*** |
| 2 November | 5.2(1) | 5.6(2) | 4.7(3) | 92.6*** |
| Offshore | | | | |
| 22 October | 0.3(1) | 4.5(2) | 2.4(3) | 2936*** |
| 15 December | 1.2(1) | 1.9(2) | 4.0(3) | 446*** |
| 12 June | 0.3(1) | 3.8(2) | 1.0(3) | 1599*** |
| 13 July | 0.6(1) | 2.0(2) | 2.3(3) | 511*** |
| 19 August | 1.3(1) | 2.7(2) | 2.8(2) | 590*** |
| 21 September | 2.0(1) | 4.6(2) | 4.5(3) | 1363*** |
| 21 October | 4.2(1) | 5.6(2) | 2.3(3) | 578*** |
| 14 November | 2.8(1) | 4.0(2) | 3.3(3) | 139*** |

[1]F statistic from one-way ANOVA with 2,8 d.f.: P 0.01***.

## Hydrolysis Tests: Low *vs.* High Acid Molybdate

Comparisons of SRP values measured by the two methods show that differences in exposure time can be responsible for the higher SRP values measured by Harvey's method (Table 6). Harvey's method yielded higher values than routine 30-s estimates by the extraction method in all but two analyses; however, the extraction method based on 9-min exposures gave statistically higher values than Harvey's method in 6 of 15 samples and equivalent values in three other comparisons. These tests show that a low rather than a high acid-molybdate regime must hydrolyze more of the SRP pool, but do not differentiate between the relative importance of acid strength and molybdate concentration.

## Hydrolysis Tests: Molybdate *vs.* Acid

Tests on the concentration-dependent effects of molybdate on SRP analysis provide two important insights into the causes of method-specific differences in SRP estimation (Table 7). (1) The possibility that the extraction method yields higher values than Harvey's method because more of the bound $PO_4$–P is released by low rather than high molybdate levels are not substantiated by our analysis. SRP values increase rather than decrease with increasing molybdate concentrations, and, therefore, should be higher in Harvey's method. (2) Differences in SRP values measured at the same molybdate level in all three methods, therefore, must be caused by differences in acid strength. The high acid level of Harvey's method obviously caused more hydrolysis in the 12 June, 13 July, and 19 August samples. However, the SRP pool in the 2 November sample apparently was more suceptible to hydrolysis by the lower acid level of the extraction method.

## Experimental Manipulation of Acid and Molybdate Levels

Our argument that more $PO_4$–P is released from bound sources by 0.16 N rather than by 0.4 N acid levels might be challenged because tests were performed by comparing values measured by two different methods rather than by manipulating acid levels in the same method. To verify this conclusion, we performed tests using the extraction method to evaluate the effects of 0.4 N *vs.* 0.16 N acid and 0.125% *vs.* 0.075% ammonium molybdate in three samples analyzed in Table 7. These tests clearly demonstrate that "low" rather than "high" acid levels can yield higher SRP values (Fig. 1). Analysis of the 2 November sample clearly showed that SRP values were higher when subjected to 0.16 N instead of 0.4 N NCl. Note that 0.16 N acid in the 2 November sample actually caused a more rapid initial hydrolysis rate (Fig. 1 A, B), and that the 0.16 N acid in the 14 November sample caused a slower release of $PO_4$–P although an equal amount of the SRP pool was hydrolyzed after 9 min (Fig. 1 C, D). In contrast, analysis of the 19 August sample gave results showing that more hydrolysis occurred at higher acid levels (Table 1). These tests verify our observations that more $PO_4$–P can be released by the lower acid level of the extraction method than by the strong acid regime of Harvey's method.

## Explanation for Method-Specific Differences in SRP Estimation

Our tests show that method-specific differences in SRP are caused primarily by variations in acid

*TABLE 7. Hydrolysis tests based on variations in ammonium molybdate concentrations in Harvey's method, the Chamberlain-Shapiro extration method (30-s and 9-min estimates), and the ascorbic acid method in filtered water from an inshore and an offshore station in southern Lake Michigan. Prescribed ammonium molybdate concentrations are 0.095% Harvey's method (Har), 0.048% extraction method (Ext), and 0.055% ascorbic acid method (Asc). Ext (9) is the SRP concentration measured after a 9-min exposure in the extraction method using the prescribed sequence of reagent additions. Results are tested with a one-way ANOVA and the Newman-Kuels multiple range test ($P < 0.05$) (Zar 1974). Groups with different numbers in parentheses represent statistically dissimilar mean values. All analyses performed at 21 ± 1°C.*

| Date | Station | Method | % Molybdate concentration | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0.024 | 0.034 | 0.041 | 0.048 | 0.055 | 0.095 | 0.119 | 0.143 | F[1] |
| | | | μg P/L | | | | | | | | |
| 12 June 1977 | Inshore[2] | Har | | | | | 2.2(1) | 2.7(2) | 2.7(2) | 2.9(3) | 38.1*** |
| | | Ext | 1.8(1) | | | 1.7(1) | | 2.3(2) | 3.5(3) | 4.0(4) | 436*** |
| | | Ext(9) | | | | 2.8 | | 3.5 | | | — |
| 13 July 1977 | Offshore[2] | Har | | | | | 2.1(1) | 2.3(2) | 2.3(2) | 3.1(3) | 7.88*** |
| | | Ext | 0.9(1) | | | 0.6(2) | | 2.3(3) | 2.6(4) | 3.0(5) | 388*** |
| | | Asc | 0.8(1) | ≤0.8(1) | 1.2(2) | 1.2(2) | 1.4(3) | | | | 58.6*** |
| | | Ext(9) | | | | 0.9 | | 2.7 | | | — |
| 19 August 1977 | Offshore[2] | Har | | | | | 2.7(1) | 2.8(1) | 3.3(2) | 3.6(3) | 105*** |
| | | Ext | 1.2(1) | | | 1.3(1) | | 1.3(1) | 1.6(2) | 1.6(2) | 11.6*** |
| | | Ext(9) | | | | 1.7 | | 2.0 | | | |
| 2 November 1977 | Inshore | Har | | | | | 4.3(1) | 4.7(2) | 4.8(2) | 5.6(3) | 170*** |
| | | Ext | 4.8 | | | 5.2(2) | | 5.3(2) | 5.4(2) | 6.5(3) | 77.5*** |
| | | Asc | ≤0.8(1) | ≤0.8(1) | 0.9(1) | 0.9(1) | 1.2(2) | | | | 17.9*** |
| | | Ext(9) | | | | 5.6 | | 6.0 | | | — |
| 14 November 1977 | Offshore | Har | | | | | 3.1(1) | 3.3(2) | 3.6(3) | 4.1(4) | 61.3*** |
| | | Ext | 2.3(1) | | | 2.8(2) | | 3.9(3) | 4.0(3) | 4.5(4) | 251*** |
| | | Ext(9) | | | | 4.0 | | 4.4 | | | — |

[1] F statistic from one-way ANOVA with 3,12 d.f. (Harvey's method); 4,10 d.f. (extraction method); and 4,15 d.f. (ascrobic acid method): $P < 0.01$***

[2] Har and Ext values From Tarapchak *et al.* (1982)

strength that can be modified by differences in exposure time. Contrary to expectations, however, 0.16 N acid sometimes yielded higher SRP values than 0.40 N acid. Moreover, our tests show that the chemical composition of the SRP pool in Lake Michigan must vary temporally and spatially because proportional differences among methods are variable (Tables 2–4). Further analyses are required to identify the chemical forms of bound $PO_4$–P in lake water and how they vary seasonally.

The only inconsistency in our explanation for method-specific differences is that the ascorbic acid method frequently yields lower values than the extraction method even though it has a higher molybdate level and a longer exposure time (Table 1). This could occur in one or both of at least two ways. First, the extraction method could yield higher values if $PO_4$–P release rates from bound sources are accelerated by rapid removal of phosphomolybdate into isobutanol. This phenomenon could be pronounced if bound $PO_4$–P is in equilibrium with free $PO_4$–P. Radiotracer experiments show that "particulate" materials can release $PO_4$–P when suspended in distilled water (Tarapchak *et al.* 1981, Tarapchak unpublished data), suggesting that an equilibrium between $PO_4$–P and the bound pool exists. Second, analysis by the extraction method involves addition of ammonium molybdate before acidification, whereas all reagents are added simultaneously in the ascorbic acid method. Weil-Malherbe and Green (1951) and Lutwak and Sacks (1952) have demonstrated that molybdate can hydrolyze some organic compounds in the absence of acid. Consequently, significant amounts of $PO_4$–P might be released into solution from bound sources at ambient pH levels when samples are analyzed by the extraction method. Both phenomena could contribute to comparatively high SRP values as measured by the extraction method; however, our conclusions are not discredited because "low" acid can promote more "hydrolysis" than "high" acid (Fig. 1).

## SUMMARY

The chemical composition of the SRP pool in Lake Michigan varies seasonally, and at times, is com-

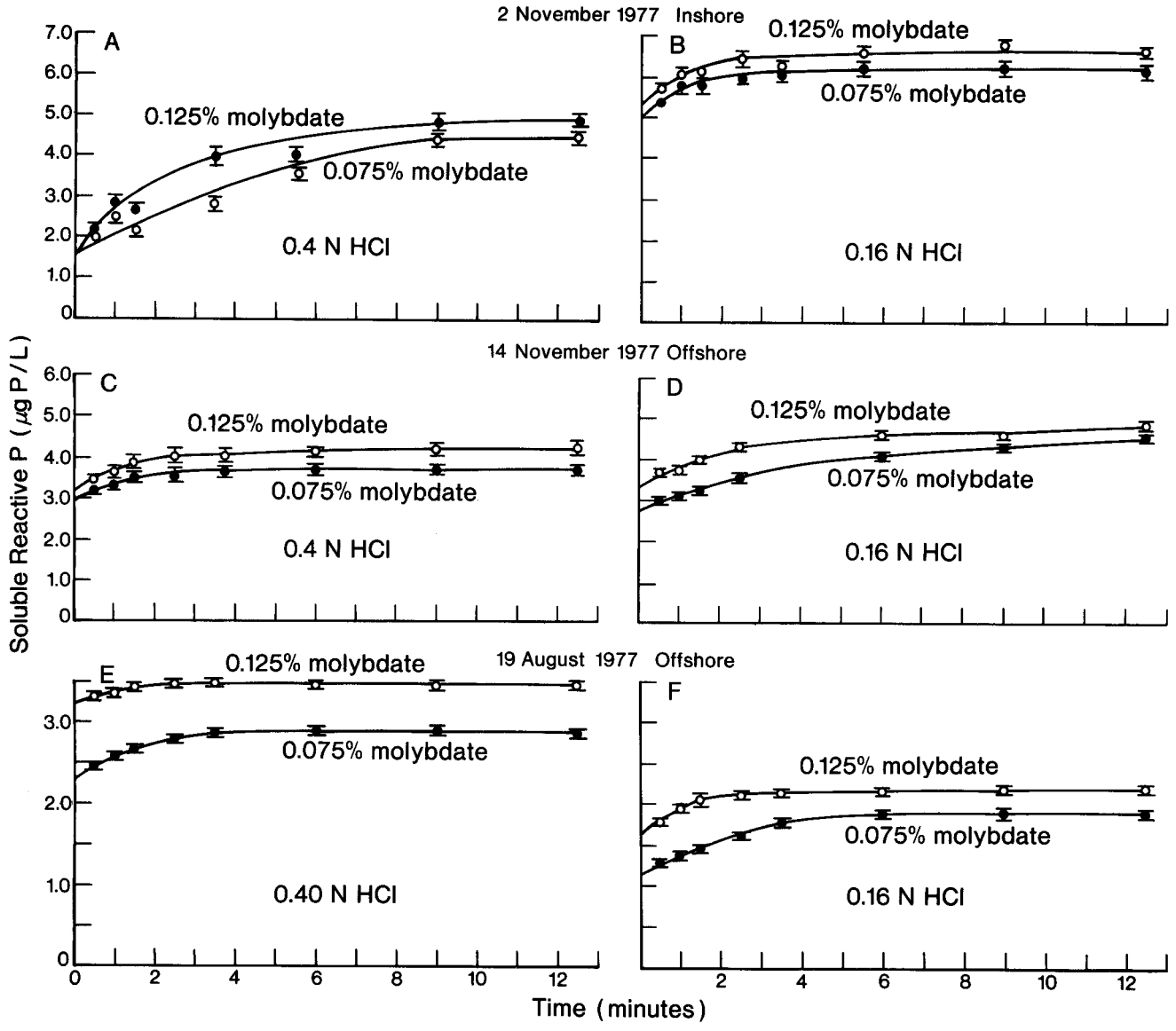


*FIG. 1. Progressive hydrolysis tests based on combinations of exposure to "high" (0.40 N) and "low" (0.16 N) acid and "high" (0.125%) and "low" (0.075%) ammonium molybdate concentrations. A, B 2 November 1977 inshore water; C, D 14 November 1977 offshore water; E, F 19 August 1977 offshore water. Precision of estimates is shown as the standard error of the mean (vertical bars). Analyses performed at 21 ± 1°C.*

posed of materials that release more $PO_4$–P in 0.16 N than in 0.40 N acid. Three conclusions can be drawn from our tests. (1) Apparent "anomolies" in SRP estimation detected in other environments could be caused by relatively rapid $PO_4$–P release under low acid concentrations (c.f., Jones 1966, Chamberlain and Shapiro 1969). (2) Harvey's method on the average will give higher values than the ascorbic acid method. Because method-specific discrepancies in SRP are not proportional to variations in acid strength, exposure time, and molybdate concentrations, however, data generated by different methods cannot be validly compared. Assessment of eutrophication trends in the Great Lakes, therefore, will be biased if different methods are used to measure SRP. (3) Attempts to minimize hydrolysis by using methods based on weak acid concentrations or short exposure times

will fail in environments where "low" acid causes more hydrolysis than "high" acid or when $PO_4$–P is released rapidly into solution.

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