

SOLUBLE REACTIVE PHOSPHORUS MEASUREMENTS IN LAKE MICHIGAN: FILTRATION ARTIFACTS

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ABSTRACT. Tests based on variations in vacuum pressure, volume of sample filtered, and filter pore size demonstrate that filtration artifacts seriously bias estimates of soluble reactive phosphorus (SRP) in water from Lake Michigan. Variations in vacuum pressure and filter pore size can bias estimates by approximately 100%, and variations in sample volume alone can produce nearly a twofold difference in SRP values. These biases are caused by differences in the relative amounts of molybdate-reactive PO_4-P released from particulate material and by the retention of P-containing "particles" by filters as pores clog with debris, and are partially responsible for method-specific differences in SRP estimation. Vacuum pressures ≤ 300 mm Hg, small sample volumes (≤ 100 mL), and filters of the same type and pore size should be used for routine SRP estimation.

ADDITIONAL INDEX WORDS: Phosphate, analytical methodology.

INTRODUCTION

Soluble reactive phosphorus (SRP) frequently is measured in surveillance programs on water quality and in phytoplankton studies in the Great Lakes and elsewhere. Two assumptions normally are made when comparing SRP values. (1) Seasonal variations in the same lake are thought to represent *real* changes in the size of the molybdate-reactive PO_4-P pool. (2) Method-specific differences in SRP values in the same or in different lakes (cf., Jones 1966, Robertson *et al.* 1974) are assumed to be caused by inherent differences in hydrolytic potentials once estimates are corrected for analytical interferences (cf., Olsen 1967, Jones 1966, Chamberlain and Shapiro 1969 and 1973).

Variations in filtration procedures may affect the accuracy of SRP estimates but are rarely evaluated. Rigler (1973), for example, states that SRP could be overestimated if algae are damaged and leak SRP into filtrates. King (1970) attempted to measure leakage by labeling phytoplankton with radiophosphorus ($^{32}P-PO_4$) and concluded that

the amount released may be equivalent to the original concentration in lake water. His results, however, are inconclusive because losses were measured indirectly by tracer rather than directly by chemical means and are further complicated by sorption of $^{32}P-PO_4$ onto filters (cf., Lean 1973, 1976). Detritus apparently has been ignored as a source of molybdate-reactive PO_4-P in filtrates even though large quantities generally are present in lakes (Wetzel 1975) and a relatively large fraction of P is associated with this material (Scavia 1979). If detritus fragments during filtration, small pieces may pass into filtrates and release molybdate-reactive PO_4-P when acid reagents are added. Leakage of SRP from microorganisms or fragmentation of detrital material could be induced by high vacuum pressures or by filtering large volumes of water, where compaction of materials on filter surfaces could damage algal cells or cause detritus to fragment (cf., Nalewajko and Lean 1972, Lean 1976).

A negative bias also will be introduced if SRP is removed from lake water during filtration. This could be a potentially serious error as the bulk of the molybdate-reactive PO_4-P pool in lake water appears to be "particulate" rather than truly dissolved PO_4-P . Chamberlain (1968), for ex-

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ample, demonstrated that 80% of the SRP and 90% of the $^{32}\text{P}-\text{PO}_4$ in 0.45- μm filtrates could be removed by refiltering 0.45- μm filtrates through 0.10- μm filters. More recently, Paerl and Downes (1978) and Stainton (1980) demonstrated that SRP in 0.45- μm filtered lake water is primarily colloidal P ($> 5,000$ mol weight). Large fractions of this material could be retained by sorption onto filters (Olsen 1967, Lean 1976) and progressively larger amounts of the material could be excluded from filtrates as filter pores gradually clog with debris as larger volumes of water are filtered (cf., Sheldon and Sutcliffe 1969). The importance of filter retention of SRP apparently has not been investigated.

A preliminary study was undertaken to evaluate the importance of filtration artifacts in SRP estimation using water from southern Lake Michigan. The purpose of this report is to demonstrate that SRP estimates can be seriously biased by variations in filtration procedures, that release of molybdate-reactive PO_4-P from abiotic particulate material can occur, and that filtration artifacts are partially responsible for method-specific differences in SRP estimation.

METHODS AND EXPERIMENTAL PROCEDURES

Analytical Methods

Samples were analyzed by Harvey's (1948) method and the Chamberlain-Shapiro (1969) extraction method. Both methods were slightly modified following Tarapchak and Rubitschun (1981) and Tarapchak *et al.* (1982a). All analyses were performed using common P standards prepared in 0.45- μm filtered lake water. Detection limits are ca. 1.0 $\mu\text{g P/L}$ (Harvey's method) and ca. 0.3 $\mu\text{g P/L}$ (extraction method) and precision (S.E.) of both methods is ≤ 0.07 $\mu\text{g P/L}$ at concentrations ≥ 1.1 $\mu\text{g P/L}$ (Tarapchak *et al.* 1982b). "Within run" precision based on triplicate analyses of filtered late-water samples > 1.1 $\mu\text{g P/L}$ was ≤ 0.8 $\mu\text{g P/L}$ for both methods. All glassware and sample bottles were washed thoroughly in hot water and Liquinox (Alconox, Inc., N.Y.), a phosphate-free detergent, soaked at least 12 h in concentrated nitric acid, rinsed six times in distilled, deionized water, and dried in a dust-free environment.

Filtration Tests

Water was obtained from an inshore (43°04'24"N, 86°16'15"W) and an offshore (43°05'12"N, 86°25'45"W) station in Lake Michigan with a 5-L Nisken bottle. Samples were filtered through pre-rinsed Millipore filters (47-mm diameter) to remove contaminant PO_4-P , refrigerated at 4–5°C, and analyzed within 24 h (Tarapchak and Rubitchun 1981, Tarapchak *et al.* 1982a). HA Millipore filters were chosen because they have been the most commonly used filter type (Golterman *et al.* 1978), and a pore size of 0.45 μm corresponds closely to the arbitrary separation of 0.5 μm between "dissolved" and "particulate" materials in natural waters (Strickland and Parsons 1965, Golterman *et al.* 1978).

Two preliminary tests were conducted in 1976 to determine if variations in vacuum pressure, filter pore size, and sample volume affect SRP measurements. On 8 July 1976, duplicate 60-mL subsamples of offshore water were filtered individually through 0.45-, 0.22-, and 0.1- μm filters at different vacuum pressures and each subsample was analyzed in duplicate. Offshore water was collected on 12 August and two different sample volumes were passed through filters of different pore size at different vacuum pressures. Duplicate subsamples were filtered for each test condition and each subsample was analyzed in triplicate.

Subsequent tests were designed to assess potential biases introduced by filtering different volumes of water and by using filters of different pore size. Volumes of water ranging from 100 to 500 mL or 1,000 mL from well-mixed samples were filtered in duplicate at a constant vacuum (300 ± 10 mm Hg) through 0.45- μm Millipore filters. SRP in each subsample was measured in triplicate by Harvey's method and the extraction method. In filter pore size tests, SRP was measured in subsamples of water filtered through 0.45-, 0.22-, and 0.10- μm Millipore filters. Duplicate 650-mL subsamples were passed individually through filters under a vacuum pressure of 300 ± 10 mm Hg and analyzed in triplicate by Harvey's method and the extraction method. In many tests, measurements on unfiltered lake water also were made to determine if molybdate-reactive PO_4-P is removed from water by 0.45- μm filters or if it is released from particulate material into filtrates. SRP in raw lake water was measured in six subsamples by Harvey's method and the extraction technique.

Sorption of orthophosphorus (PO_4-P), i.e. truly dissolved PO_4-P , on Millipore filters could bias results if sorption is dependent on sample volume

or filter porosity. We reject this as a potential error for two reasons. (1) Solutions of KH_2PO_4 in distilled water, ranging from 5.0 to 10 $\mu\text{g P/L}$, were filtered through pre-rinsed 0.45-, 0.22-, and 0.10- μm Millipore filters. $\text{PO}_4\text{-P}$ measurements on solutions before and after filtration by the extraction method demonstrated that $<6\%$ was sorbed. (2) True $\text{PO}_4\text{-P}$ concentrations in water from these stations are at least an order of magnitude lower than SRP from July through October (Tarapchak and Rubitschun 1981; Tarapchak, unpublished data).

Estimates of Biological and Abiotic SRP Release From Particulate Material

The relative amounts of SRP released by damaged microorganisms or from abiotic particulate material $> 0.45 \mu\text{m}$ during filtration cannot be measured directly. Qualitative tests, however, were designed to determine if SRP is also released from abiotic particulate materials. These analyses are based on the assumption that Surplus P, i.e. polyphosphates and stored P-containing organic compounds (Kuhl 1974), represents the maximal amount of molybdate-reactive $\text{PO}_4\text{-P}$ that can be released from microorganisms during filtration. If the increase in SRP in 0.45- μm lake-water filtrates over the concentration in unfiltered lake water is \leq Surplus P, release of SRP could be solely from biological sources. If the difference between SRP in 0.45- μm filtrates and in unfiltered lake water is $>$ Surplus P, release also must occur from abiotic particulate material.

Surplus P in microorganisms was measured by the method of Fitzgerald and Nelson (1966). Subsamples of raw lake water and 0.45- μm filtered lake water were boiled for 1.0 h, and SRP was measured in duplicate by the extraction method. Surplus P was calculated by subtracting the SRP concentration in boiled filtrates from that in boiled subsamples of raw lake water.

RESULTS AND DISCUSSION

Preliminary Tests

In July, SRP increased with increasing filter pore size and increasing vacuum pressure (Table 1). A two-way ANOVA (Zar 1974) showed statistically significant ($P < 0.001$) main effects and a significant ($P < 0.001$) two-way interaction between filter pore size and vacuum pressure. The second test, conducted on 12 August, showed that SRP in-

TABLE 1. Effects of filtering offshore Lake Michigan water collected from 4 m on 8 July 1976, at different vacuum pressures through Millipore filters of different pore size on SRP concentrations measured by Harvey's method. Mean values are based on duplication determinations in each of two filtered subsamples for each condition. Results are tested with a two-way ANOVA (Zar 1974).

Filter Pore size	$\mu\text{g P/L}$				
	100	200	300	400	600
0.10	—	≤ 1.0	≤ 1.0	2.9	3.1
0.22	≤ 1.0	≤ 1.0	≤ 1.0	4.0	4.5
0.45	2.4	3.5	5.9	6.4	6.4

Source of variation	Sum of squares	d.f.	Mean square	F ¹
Main effects	202	6	33.7	1584****
Filter	114	2	57.1	2688****
Vacuum	105	4	26.2	1124****
Two-way interactions	16.8	7	2.41	113****
Filter vacuum	16.8	7	2.41	113****
Explained	219	13	16.8	792****
Residual	0.89	42	0.02	
Total	220	55	4.01	

¹Significance at $P < 0.001$ ****

creased with increasing vacuum pressure and variations in sample volume and filter pore size also yielded different SRP values (Table 2). A three-way ANOVA (Zar 1974) showed statistically significant main effects, significant two-way interactions (particularly contrasts involving vacuum pressure and sample volume), and a significant three-way interaction ($P < 0.001$).

Both tests clearly demonstrate that variations in filtration procedures, especially vacuum pressure, can result in large differences in SRP. The first test shows that a large fraction of molybdate-reactive $\text{PO}_4\text{-P}$ in 0.45- μm filtrates must be "particulate" because SRP concentrations decreased as filters of smaller pore size filters were used (Table 1). Note that the SRP concentration in the 0.10- μm filtrate was over 80% lower than in the 0.45- μm filtrate at 300 mm Hg. Both tests also demonstrate that large amounts of SRP were released from particulate material into filtrates. This is evident by comparing SRP values in 0.45- μm filtrates measured at different vacuum pressures. (Also see below.)

Effects of Sample Volume on SRP Determinations

SRP determinations usually are highly dependent on the volume of water filtered. Results of 11 tests,

TABLE 2. Effects of filtering different volumes of offshore Lake Michigan water collected from 4 m on 12 August 1976, at different vacuum pressures through Millipore filters of different pore size on SRP concentrations measured by Harvey's method. Mean values are based on triplicate determinations in each of the two filtered subsamples for each condition. Results are tested with a three-way ANOVA (Zar 1974).

Volume mL	µg P/L			
	Vacuum pressure (mm Hg)			
	100	250	400	600
0.45-µm pore size				
125	5.3	5.6	5.9	7.8
500	6.3	6.4	6.5	6.7
0.22-µm pore size				
125	4.2	6.7	6.7	7.4
500	—	6.2	6.4	8.1
.....				
Source of variation	Sum of squares	d.f.	Mean square	F ¹
Main effects	56.3	5	11.3	340 ****
Filter	0.11	1	0.18	5.7
Vacuum	49.9	3	16.6	503 ****
Volume	2.38	1	2.38	72 ****
Two-way interactions	14.5	7	2.05	62 ****
Filter vacuum	5.58	3	1.66	56.3****
Filter volume	0.09	1	0.08	2.6
Vacuum volume	2.25	3	0.75	22.7****
Three-way interaction				
Filter vacuum volume	8.11	2	4.06	123 ****
Explained	78.8	14	5.63	170 ****
Residual	2.48	75	0.03	
Total	81.3	99	0.91	

¹Significance at P < 0.10*; P < 0.001****

illustrated for three paired analyses in Table 3, generally showed one of three statistically verifiable patterns. (1) SRP concentrations remained unchanged or varied only slightly (e.g., 17 August, offshore, Harvey's method). (2) SRP increased as large volumes of water were filtered (e.g., 21 September, offshore, both methods). (3) SRP decreased as larger volumes were filtered (e.g., 2 November, offshore, extraction method). This pattern also was evident in inshore water collected on 14 November, 1977 (extraction method). Filtered subsamples of 100, 200, 300, and 500 mL gave values of 7.7, 7.7, 5.9, and 3.9 µg P/L, respectively (F_{3,20} = 481, P < 0.001). The first pattern can occur if the SRP pool is composed primarily of small P-containing "particles" that pass unimpeded through filters. The second pattern can be caused by a release of molybdate-reactive PO₄-P from particulate material, and the third by retention of P-containing "particles" by filters as pores clog.

TABLE 3. Effects of filtering different volumes of offshore Lake Michigan water from 4 m on SRP concentrations as measured by Harvey's method and the extraction method and Surplus P determinations. Samples were filtered through 0.45-µm Millipore filters at 300 ± 10 mm Hg. Mean values for each condition are the result of six determinations. 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 are statistically dissimilar.

Volume mL	µg P/L		
	Harvey	Surplus P	Extraction
17 August 1977 (Offshore)			
100	2.9 (1)		2.6 (1)
250	2.9 (1)		2.6 (1)
500	3.2 (2)	0.5	3.9 (1)
1000	3.2 (2)		2.4 (2)
F ¹	7.67***		108***
21 September 1977 (Offshore)			
100	1.8 (1)		3.8 (1)
200	1.8 (1)		4.0 (1)
300	2.0 (1)	1.6	4.8 (2)
500	2.2 (2)		4.9 (2)
F ¹	8.06***		77.7***
2 November 1977 (Offshore)			
100	3.1 (1)		6.3 (2)
200	3.2 (1)		6.3 (2)
400	3.7 (2)	3.2	3.4 (1)
500	3.6 (2)		3.4 (1)
F ¹	28.4***		755***

¹F statistic from one-way ANOVA with 3,20 d.f.: P < 0.01****

Two tests clearly showed that molybdate-reactive PO₄-P could be released from particulate material during one phase of filtration (elevating filtrate SRP), but could be retained by filters in another phase (lowering filtrate SRP). This pattern was apparent in the 17 August offshore sample (extraction method) (Table 3), where filtration of 500 mL produced a 50% increase over the 250-mL subsample but filtration of 1,000 mL resulted in a 38% decrease relative to the 500-mL subsample. A similar result was obtained in offshore water collected on 14 November (extraction method). Filtered subsamples of 100, 200, 300, and 500 mL gave values of 3.1, 5.7, 5.0, and 3.7 µg P/L, respectively (F_{3,20} = 184, P < 0.001). The increase in the 200-mL subsample represents a 46% increase over the 100-mL subsample, and the decrease in SRP in the 500-mL subsample, relative to the 200-mL subsample, represents a 54% reduction (see below). Both samples were refiltered and reana-

lyzed with 1.5 h. The Newman-Kuels multiple range test (Zar 1974) identified the same number and statistical grouping of treatment means ($P < 0.05$).

These tests reveal two important phenomena. (1) Variations in sample volume can bias SRP estimates by up to nearly 100%, and reductions in SRP concentration due to filter clogging can introduce biases equivalent to those caused by release of SRP from particulate material. (2) SRP concentrations in filtrates should be viewed as the *net* result of two processes that could occur simultaneously during filtration: leakage of molybdate-reactive $\text{PO}_4\text{-P}$ from particulate material into filtrates and exclusion of P-containing "particles" by filters as clogging occurs. In the 17 August test (Table 3, extraction method), for example, compaction of particulate materials, releasing molybdate-reactive $\text{PO}_4\text{-P}$ into the filtrate, must have occurred between filtration of 250 and 500 mL. This release should continue as larger volumes are filtered. The 38% reduction in SRP observed in the 1,000-mL subsample could only occur if a much larger fraction of P-containing "particles" in lake water is retained by filters due to severe clogging of pores.

Leakage of SRP from particulate material, coupled with increased filter clogging as larger volumes are filtered, also must account for the pattern detected in the November 14 test on offshore water discussed above.

Effects of Filter Pore Size on SRP Determinations

Filtering water through different sized filters gave one of three statistically verifiable patterns: (1) SRP concentrations were unaffected (e.g., 21 September and 2 November, offshore, extraction method), (2) SRP decreased (e.g., 19 August and 30 September, offshore, extraction method), and (3) concentrations were higher in 0.22- μm filtrates (relative to 0.45- μm filtrates) or in 0.10- μm filtrates (relative to 0.22- μm filtrates) (Table 4). The first pattern will occur if P-containing "particles" pass unrestricted through all filter sizes. The second pattern will occur if a significant fraction of SRP in 0.45- μm filtrates is "particulate" and is retained by smaller filters. The third pattern was unexpected and cannot be attributed to analytical errors or P contamination. Samples showing this pattern were refiltered and reanalyzed within 1.5 h. The

TABLE 4. Effects of filtering offshore and inshore Lake Michigan water collected at a depth of 4 m through Millipore filters of different pore size at 300 ± 10 mm Hg on SRP concentrations as measured by the extraction method and Harvey's method (*) and Surplus P determinations in raw lake water. Mean values 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 are statistically dissimilar.

Date	Station	Surplus P	Unfiltered	$\mu\text{g P/L}$			F ¹
				0.45 (μm)	0.22 (μm)	0.10 (μm)	
18 October 1976	Offshore	0.9	—	3.0(1)	4.2(2)	4.2(2)	97.0 ***
18 October 1976	Offshore*	—	—	2.4(2)	3.2(3)	$\leq 1.0(1)$	378 ***
20 October 1976	Offshore*	1.0	4.1	2.1	—	—	—
4 December 1976	Offshore	—	—	6.1(3)	3.5(2)	2.5(1)	867 ***
4 December 1976	Offshore*	—	—	4.0(2)	4.0(2)	3.1(1)	37.9 ***
12 August 1977	Offshore*	—	4.4	2.9	—	—	—
19 August 1977	Offshore	0.3	2.3(2)	2.6(3)	$\leq 0.3(1)$	$\leq 0.3(1)$	1089 ***
21 September 1977	Offshore	1.3	5.6(1)	7.8(2)	7.8(2)	7.8(2)	458 ***
21 September 1977	Offshore*	—	1.5(1)	1.7(2)	1.7(2)	1.7(2)	4.15**
22 September 1977	Inshore	1.9	6.5(2)	7.4(3)	7.5(3)	6.1(1)	159 ***
22 September 1977	Inshore*	—	2.4(2)	2.4(2)	2.3(2)	1.9(1)	21.0 ***
30 September 1977	Offshore	2.1	4.7(3)	4.9(4)	4.0(2)	3.1(1)	166 ***
30 September 1977	Inshore	2.0	5.2(2)	5.7(3)	5.2(2)	4.3(1)	128 ***
20 October 1977	Inshore	2.2	5.4(1)	7.9(3)	6.5(2)	6.5(2)	262 ***
20 October 1977	Inshore*	—	3.8(3)	3.0(1)	3.8(3)	3.5(2)	56.5 ***
2 November 1977	Offshore	3.2	2.3(1)	4.8(2)	4.7(2)	4.7(2)	485. ***
2 November 1977	Offshore*	—	5.4(1)	5.4(1)	5.9(2)	5.4(1)	18.4 ***
2 November 1977	Inshore	3.4	5.2(1)	5.7(2)	5.1(1)	6.1(3)	92.9 ***
2 November 1977	Inshore*	—	4.7(3)	4.7(3)	4.2(2)	2.9(1)	241 ***

¹F statistic from one-way ANOVA with 2,15 d.f. (three-way comparison) or 3,20 d.f. (four-way comparison); $P < 0.05^{**}$; $P < 0.01^{***}$

Newman-Kuels multiple range test (Zar 1974) identified the same number and statistical grouping of treatment means ($P < 0.05$).

Higher concentrations in 0.22- or 0.10- μm than in 0.45- μm filtrates could occur in one of two ways. (1) Small, delicate algae may be damaged to a greater degree by impacting on 0.45- μm filters (where they may be partially drawn into filter pores). (2) 0.45- μm filters have a greater porosity than smaller filters; therefore, sorption sites saturate with "particulate" $\text{PO}_4\text{-P}$ more slowly, allowing less SRP to pass into filtrates (c.f., Nalewajko and Lean 1972). According to the Millipore Corporation (Catalog MF-74), porosity of HA (0.45 μm), GS (0.22 μm), and VC (0.10 μm) filters is 79, 75, and 74%, respectively. Differences in filter porosity, tested by filtering solutions of $^{33}\text{P}\text{-PO}_4$ through each filter size, were found to vary from specifications by up to $\pm 10\%$ (Tarapchak, unpublished data). These porosity differences, however, are too small to account for observed differences in SRP concentrations, suggesting that leakage from microorganisms is less when filters of smaller pore size are used.

The interpretation of filtration tests (Tables 1-4) hinges primarily on the assumption that the major fraction of the SRP pool in unfiltered water and in 0.45- μm filtrates is in a "particulate" rather than in a dissolved phase. SRP in 0.10- μm filtrates averaged just over 30% of the SRP in 0.45- μm filtrates where concentrations in 0.45- μm filtrates were greater than in 0.10- μm filtrates (Table 4), and only two analyses showed that SRP in 0.10- μm filtrates was 80% less than in 0.45- μm filtrates [19 August 1977, (Table 4), and 8 July 1976 (Table 1)]. Two explanations can account for the phenomenon. (1) The bulk of the SRP pool in Lake Michigan consists primarily of truly dissolved $\text{PO}_4\text{-P}$ or dissolved organic compounds that are partially sorbed by filters. (2) Leakage of SRP from particulate materials masks retention of P-containing "particles" when water is filtered directly through 0.10- μm filters. Evidence for the second explanation was obtained by refiltering several 0.45- μm filtrates through 0.10 μm filters (Table 4). SRP in 0.10- μm filtrates was 2.3 (21 September, offshore, extraction method), 1.4 (20 October, inshore, extraction method), 1.0 (2 November, offshore, extraction method), and 0.7 $\mu\text{g P/L}$ (2 November, inshore, extraction method), showing that 71 to 88% of the SRP in 0.45- μm filtrates can be removed by refiltration through 0.10- μm filters. These analyses clearly demonstrate the "particulate"

nature of SRP in 0.45- μm filtrates, and also show that leakage from particulate material masks the removal of large quantities of P-containing "particles" when water is filtered directly through 0.10- μm filters.

Biotic Versus Abiotic Release of SRP into Filtrates

Evidence for SRP release from abiotic particulate material during filtration is apparent in several tests. SRP in unfiltered water on 8 July (Table 1) and 12 August (Table 2) was 4.3 and 4.4 $\mu\text{g P/L}$, respectively, and Surplus P on these dates was 0.6 and ≤ 0.3 $\mu\text{g P/L}$. Leakage of SRP from damaged microorganisms alone, therefore, cannot account for observed SRP increases in 0.45- μm filtrates at vacuum pressures > 300 mm Hg in July and ≥ 250 mm Hg in August. Filtration of 250 versus 500 mL in the 17 August offshore sample (by the extraction method) showed an increase greater than the Surplus P measurement (Table 3), and two filter pore-size tests (21 September, offshore, extraction method and 20 October, inshore, extraction method) showed that the increase in 0.45- μm filtrates could not have been caused solely by biological SRP release (Table 4).

Our tests probably underestimate SRP release from abiotic sources. Surplus P represents an upper limit for the amount of molybdate-reactive $\text{PO}_4\text{-P}$ that can be released from microorganisms. Many thick-walled algae, e.g., green and blue-green algae, are resistant to damage, and fractions of the molybdate $\text{PO}_4\text{-P}$ released into filtrates may not be completely hydrolyzed by these analytical methods (Tarapchak, unpublished data). If less than one-half of the Surplus P is actually released into filtrates and less than one-half of the material is hydrolyzed, observed increases in 0.45- μm filtrates in other tests will exceed the amount of SRP that could have been released by microorganisms (Table 4).

Small fragments of detritus are the most likely source of abiotically-released SRP during filtration. Although only a small fraction of the detrital mass in lake water is likely to be released into filtrates, relatively large amounts of molybdate-reactive $\text{PO}_4\text{-P}$ could be released because small fragments will have exceptionally high surface area-to-volume ratios in comparison with the parent material. SRP release from abiotic sources appears to be more pronounced when water is filtered at high vacuum pressures (Tables 1 and 2), but it is probable that significant amounts also were

released into 0.45- μm filtrates even at vacuums of 300 mm Hg.

SUMMARY, CONCLUSION, AND RECOMMENDATIONS

Several conclusions regarding the accuracy of SRP estimation can be drawn from our tests. (1) Variations in the true size of the molybdate-reactive $\text{PO}_4\text{-P}$ pool can be obscured by filtration artifacts. Estimates can differ by up to ca. 100% depending on variations in vacuum pressure, sample volume, or filter pore size. (2) Rigler's (1973) concern that microorganisms might release molybdate-reactive $\text{PO}_4\text{-P}$ during filtration is justified. Abiotic particulate material, however, apparently can release P-containing "particles" into filtrates. Relative amounts released from these two sources cannot be estimated accurately, but it is probable that fragmentation of loosely aggregated detrital material contributes to filtrate SRP. (3) Reductions in filtrate SRP can be caused by progressive clogging of filter pores, resulting in negative biases equivalent to positive biases introduced by release of SRP from particulate material. (4) Method-specific differences in SRP concentration (Jones 1966, Robertson *et al.* 1974, Tarapchak and Rubitschun 1981) can be partially attributed to filtration-induced alterations in the chemical composition of the SRP pool. Examination of the sample volume test of 2 November (Table 3), for example, shows that SRP increased as measured by Harvey's method, but decreased as measured by the extraction method when larger volumes of water were filtered. Similarly, Harvey's method gave higher SRP values in unfiltered lake water than in 0.45- μm filtrates in three of seven analyses, but the extraction method gave higher values in 0.45- μm filtrates than in raw lake water in five of eight analyses (Table 4). Moreover, inspection of Table 4 shows that the ratio of SRP measured by Harvey's method to values measured by the extraction method changes as samples are passed through filters of different pore size. Consequently, filtration artifacts will affect the relative differences between SRP concentrations measured by different methods.

The best set of filtration conditions for SRP estimation cannot be specified from our analyses. Leakage of molybdate-reactive $\text{PO}_4\text{-P}$ from fragile organisms will vary seasonally depending on variations in their abundance and Surplus P content. Similarly, reductions in SRP caused by filter

clogging will vary with the amount of debris in the water and on the density and size of P-containing particles. Vacuum pressures ≤ 300 mm Hg and sample volumes ≤ 100 mL should reduce the amount of SRP released from particulate materials, and the use of small sample volumes should decrease filter clogging. For routine SRP estimation, we recommend the use of constant vacuum pressure and sample volumes and the same filter type and size should be used.

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