The effects of post-persulfate-digestion procedures on total phosphorus analysis in water

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Abstract

There are differences between the EPA Method 365 and the APHA-AWWA-WEF's Standard Method 4500 with respect to the post-digestion treatment procedures of the persulfate-digested water. The effects on total phosphorus analysis of different post-digestion treatment procedures, such as neutralization and reacidification, and shaking/settling, were investigated in this study using the total phosphorus measurements of water samples from the Everglades Round Robin (ERR) study and comparing the results with the ERR study. The effects of the insoluble particles or phosphorus adsorption/precipitation on/with Al and Fe hydroxides in different post-digestion treatment procedures adequately accounted for the differences between the most probable value and the higher or lower total phosphorus measurements reported in the ERR study. Based on the results of this investigation we recommend that a clearly defined set of digestion and post-digestion treatment procedures be adopted as the standard for total phosphorus analysis using the ascorbic acid method.
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1. Introduction

Eutrophication is a worldwide water quality problem caused by nutrient enrichment in surface waters. Phosphorus, frequently identified as a nutrient limiting primary production, is responsible for algal blooms and invasions of exotic species in most surface water ecosystems. Therefore the reliable determination of total phosphorus (TP) is very important to ecosystem management and restoration.

There are many factors that contribute to uncertainty in the TP analysis of water containing inorganic and organic particles, which often are present in surface waters. Inorganic minerals that contain silica may be found in sediments; and such minerals are very difficult to dissolve during the digestion process. The efficiency of digestion, one of the major uncertainty factors, has been studied intensively in relation to TP analysis of water containing sediment (O'Connor and Syers, 1975; Hosomi and Sudo, 1986; Johnes and Heathwaite, 1992; Lambert and Maher, 1995; Woo and Maher, 1995; Maher et al., 2002). By means of the alkaline persulfate autoclave digestion method, Lambert and Maher (1995) obtained nearly complete recovery of TP in two certified reference sediment samples containing up to $100\,\mu g/L$ TP. However from turbid water samples, Woo and Maher (1995) recovered slightly lower amounts of TP by means of the alkaline persulfate autoclave digestion

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method than with the alkaline persulfate microwave and sulfuric acid/nitric acid digestion methods. We have found that both the acid persulfate autoclave digestion method and the sulfuric acid/nitric acid digestion method readily and successfully digested the certified reference sediment sample (NIST, 1646a), containing up to 200 μg/L TP. However, other uncertainty factors, such as, (a) the effects of insoluble particles on TP analysis and (b) precipitation with or adsorption of phosphorus on multivalent cations or hydroxides after neutralization of the digested water sample containing sediment, have not been studied in detail.

The post-treatment procedures after digestion, which influence the effects of insoluble particles and adsorption/precipitation of phosphorus in TP analysis, are quite different in the EPA methods 365.1, 365.2, 365.3 (USEPA, 1982) and the Standard Method (SM) 4500 (APHA-AWWA-WEF, 1998). The EPA method 365.2 requires neutralizing and filtering the digestates before TP analysis, while EPA method 365.1 requires cooling the digestates and EPA method 365.3 requires cooling and filtering the digestates before TP analysis. In contrast, SM 4500 requires neutralizing and mixing the digestates before TP analysis. An objective of this study was to establish reliably uniform standard digestion and post-digestion treatment procedures for TP analysis.

In this study, we statistically analyzed the data from the five most recent Everglades Round Robin (ERR) studies in order to identify samples analyzed for TP by various laboratories, which had unusually high relative standard deviations from the mean. We measured some physical and chemical properties of samples containing sediments, and related these measurements to the TP results. We studied the effects on TP analysis of the different post-digestion treatments (such as with/without neutralization and reacidification, shaking/settling, etc.). We compared the results from the different post-digestion treatments with the TP results from the various laboratories in the ERR study. Finally, we have recommended digestion and post-digestion treatment procedures for standard use in TP analysis.

2. Materials and methods

2.1. Samples and TP results in ERR

The standard operating procedure for ERR sample collection is described on the web site: www.dep.state.fl.us/labs/everglades and the TP results for the ERR samples were obtained from this same web site. All ERR samples were collected from structures in South Florida Water Management District (SFWMD). The TP results from the five most recent ERR studies (VII, VIII, IX, X

and XI) have been used in this paper. Additional water samples were collected from site G342A and G342C in SFWMD for this study.

2.2. Statistical analysis

The relative standard deviation (RSD) of TP results among the different laboratories was calculated after removal of outliers. The Z-value was used to identify the outliers.

$$Z = (\text{Reported Value} - \text{Mean})/S$$

where the mean and the S value are the mean and standard deviation of all the data points for the sample collected from a sampling site, respectively. If the Z value(s) reported by a certain laboratory for one or more samples from a given sampling site were each more than 2, the results of this laboratory for this site were treated as outliers (in 95% confidence). After removal of the outliers, the mean of the TP concentration (most probable value, MPV) was calculated by averaging the averaged replicated results from all of the laboratories for a given sampling site. In addition the standard deviation (SD) was also calculated from the averaged replicated results of each laboratory. The RSD was calculated as follows:

$$\text{RSD}(\%) = \text{SD} \times 100/\text{MPV}.$$

2.3. Digestion, neutralization and reacidification

The water samples in ERR XI study from the S5A and the G253 sites and additional samples collected from the G342A and G342C sites in the Everglades were digested using the acid persulfate autoclave method (SFWMD, 1997) and the clean hood hot block acid persulfate digestion methods (Zhou and Struve, 2002). Water samples digested using the autoclave method were analyzed for their TP concentrations without neutralization and reacidification. An aliquot of digestate for the hot block method was neutralized with NaOH and reacidified with H_2SO_4 to 0.15 N acid concentration, and another aliquot was neutralized with NaOH, but not reacidified, before TP analysis.

2.4. TP analysis

The TP concentration was determined using the modified Murphy and Riley method (1962). Reagent A was prepared using 9, 16 and 24.5 mL concentrated H_2SO_4 for non-neutralized, neutralized/acidified and neutralized/unacidified samples, respectively, plus 2.136 g ammonium molybdate, and 0.088 g antimony potassium tartarate, and made up to 100 mL with DI

water. Reagent B consisted of 0.39 g ascorbic acid dissolved in 10 mL of DI water.

Disposable polystyrene 1-cm cuvettes with covers were used as reaction vessels and for spectrophotometric measurement. To each 3 mL of digested sample, 0.15 mL reagent A and 0.15 mL reagent B were added, and mixed. The color development time was 30 min. The absorbance was measured at 880 nm using a PE Lambda 40 spectrophotometer.

2.5. Physical and chemical properties

The turbidity was determined using a turbidimeter (Hach 2100AN). Ca, Mg, Fe and Al ion concentrations were measured in the persulfate-digested samples before and after neutralization using an ICP-OES (PE Optima 3000 DV).
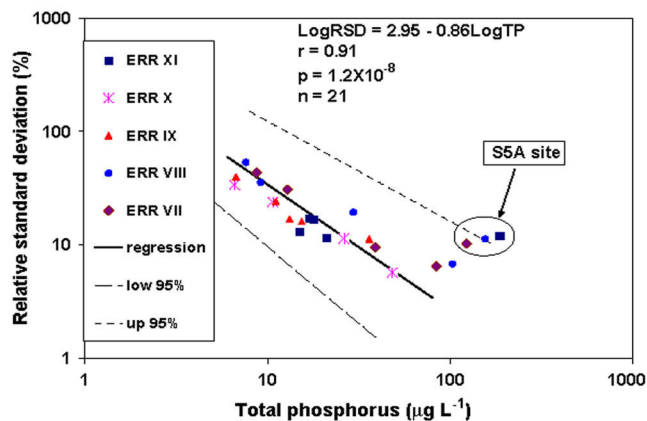

Fig. 1. The relationship between the log total phosphorus (TP) concentrations (Most Probable Values) in water samples and relative standard deviations of TP concentrations. Measurements were made by a number of laboratories participating several Everglades Round Robin Studies.

3. Results and discussion

There was a linear relationship between the log relative standard deviation of TP results obtained by the different laboratories with the log TP concentration (MPV), with the exception of two out of three water samples from site S5A, which were the outliers since they were outside the up 95% confidence interval (Fig. 1). One difference between the samples from S5A and those from other sites was that S5A samples contained visible sediments. The presence of sediments is also indicated from the high turbidity reading of a S5A (ERR XI) sample (Table 1). The amount of sediment in the samples may contribute to the unusually high relative standard deviation of TP results obtained by the different laboratories, which will be discussed in this paper.

There are at least two uncertainty factors that may explain the unusually large relative standard deviations of the water samples from the S5A site. The first factor is the difference in the TP digestion efficiency of the methods employed in the various laboratories. Thus, if the persulfate and/or acid concentrations used for digestion were insufficient to oxidize and extract the phosphorus from the water samples (Zhou and Struve, 2002), then the TP measurements in the water samples would be lower than the actual concentrations. Indeed, insufficient digestion can be expected to compromise the TP recovery in almost all of the water samples. However, the sediment in the S5A water sample may further increase the deviation of the obtained measurement from the actual value, since the phosphorus in the sediment is more difficult to solubilize during the digestion (O'Connor and Syers, 1975; Hosomi and Sudo, 1986; Johnes and Heathwaite, 1992; Lambert and Maher, 1995; Woo and Maher, 1995). However, when sufficient amounts of persulfate and acid were added during digestion, the $99 \pm 5\%$ recovery of TP from the samples with sediment TP concentrations of

Table 1
Some physical and chemical properties of the water samples obtained from various structures in the South Florida Water Management District

Sample name	Turbidity (NTU)	Ca[a] (mg L^{-1})	Ca[b] (mg L^{-1})	Mg[a] (mg L^{-1})	Mg[b] (mg L^{-1})	Fe[a] (µg L^{-1})	Fe[b] (µg L^{-1})	Al[a] (µg L^{-1})	Al[b] (µg L^{-1})
S5A (ERR XI)	25	82	79	24	23	826	40	552	41
G253 (ERR XI)	1	42	41	14	14	8	ND	94	13
G324A	29	28	27	1	1	2019	201	2282	708
G324C	16	17	16	1	1	1229	151	1429	590

ND: not detected.
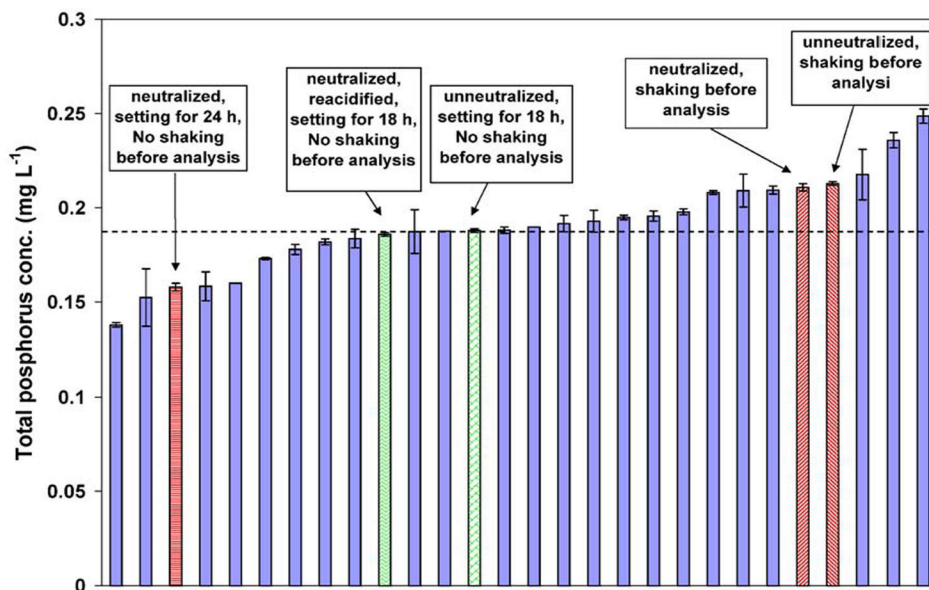[a]Non-neutralized digested sample.
[b]Neutralized digested sample.

Fig. 2. Measurements of total phosphorus (TP) concentrations in water samples taken at site S5A. The solid bars are measurements from different lab in Everglades Round Robin (ERR) Study XI, and the cross-hatched bars are the measurements with different treatment made in this study ($n = 4$). The horizontal line represents the MPV ($188\,\mu g\,L^{-1}$) of S5A (ERR XI) sample.

$25–200\,\mu g\,P\,L^{-1}$ were achieved, and this implies that nearly full recovery of TP can also be achieved from the ERR samples through the use of an adequate digestion method. A second uncertainty factor pertains to the post-digestion treatment procedures, and these will be discussed here in detail.

The acid persulfate digestion method is the standard procedure for sample digestion in both the EPA 365 methods (USEPA, 1982) and SM 4500 (APHA-AWWA-WEF, 1998). The main difference among these methods lies in the post-digestion treatment procedures. The SM 4500 requires both neutralization and shaking before TP analysis. EPA method 351.1 and 365.3 does not include neutralization prior to TP analysis. It is quite possible that the different laboratories participating in the ERR studies used different combinations of the post-digestion treatment procedures (neutralization/ non-neutralization and shaking/settling).

TP results of site S5A sample from different labs in Everglades Round Robin (ERR) Study XI ranged from 138 to $249\,\mu g\,L^{-1}$ with the MPV of $188\,\mu g\,L^{-1}$ (Fig. 2). TP concentration results of the same S5A (ERR XI) sample with different pre-digestion treatment were within the concentration range of the ERR Study XI (Fig. 2). For S5A samples that had been shaken after digestion prior to analysis (shaking before analysis), the total P concentration measurements were nearly 13% higher than the MPVs calculated from ERR XI study (Fig. 2). The 13% increase in the apparent TP concentrations, which is due to increase of the absorbance readings from the insoluble particulates in the sample, adequately accounts for the difference between

the MPV and the high total P measurements reported in the ERR XI study (Fig. 2). However, when the TP was analyzed after the digested solution had been neutralized and allowed to stand overnight (neutralized, settled, not shaken before analysis), the TP measurement was 16% lower than the MPV (Fig. 2), which is also well accounted for in the difference between the MPV and the reported low TP results (Fig. 2). Other researchers have reported that neutralization step tended to decrease recovery of total phosphorus in water samples (Morton et al., 2003). The precipitation of phosphorus with Ca^{2+} had been recognized as an important mechanism of phosphorus loss from solution when the pH of the digested samples were increased to near 7–8 (House, 1999). However, in this study, the Ca and Mg concentrations in neutralized digestates were similar to those in non-neutralized digestates (Table 1), and this was true even for the water samples from the sites, S5A, G342A and G342C, that have high pH (>7) and alkalinity ($>100\,mg/l$). Therefore, the concentration of Ca^{2+} per se may not cause phosphorus to precipitate in these samples. On other hand, the Fe and Al ions may play an important role in the loss of phosphorus from solution after neutralization. Fe and Al concentrations in neutralized digested samples were much lower than those in non-neutralized digestates (Table 1). These differences are caused by the tendency of Fe and Al ions to form insoluble Fe and Al hydroxides as the pH is increased. This is significant because some phosphorus can be lost by adsorption/precipitation on/with the Fe and Al hydroxides (Cabrera et al., 1982). However the precipitation of phosphate directly with Fe^{3+} and Al^{3+}

is not an important mechanism of phosphorous loss, since the Fe^{3+} and Al^{3+} concentrations are controlled by the Al and Fe hydroxides. Also very high phosphate concentrations ($mg\,L^{-1}$ level) in solutions are required for $Fe(PO_4)$ and $Al(PO_4)$ to precipitate (Galarneau and Gehr, 1997; House and Denison, 2000). It should be pointed out that the effects of mixing digestates with insoluble particles and the precipitation/adsorption phenomenon adequately account for the deviation of reported TP measurements from the MPV. However, this does not necessarily mean that all of the laboratories participating in the ERR studies have exactly the same problem, since other factors, such as the efficiency of digestion and final acid concentration of the digested solution/reagent A/B mixture in phosphorus analysis, also play important roles. Unfortunately, we do not know precisely the methods of digestion, the post-digestion treatment and TP analysis procedures that were used in the various participating laboratories.

TP measurements from the non-neutralized digested samples and neutralized/reacidified samples with 2 h or more of setting time without shaking before analysis were almost the same as the MPV (Table 2 and Fig. 2). The increase of setting time from 2 to 18 h did not significantly change the TP results of non-neutralized digested samples (Table 2, Figs. 3 and 4). The TP concentrations of neutralized digested samples tended to decrease as the setting time increased (Figs. 3 and 4) due to the kinetics of P adsorption/precipitation on/with the Fe and Al hydroxides. Even though the TP measurements from the neutralized samples with shaking before analysis and blank corrections were also were comparable to the MPV (Table 2), the neutralization and blank correction steps take time and blank corrections are not feasible when using a flow analytical instrument, and especially not for samples with sediments.

A settling time of 2 h was sufficient for the insoluble particles in the unneutralized samples to settle to the bottom of the digestion tube (Table 2, Figs. 3 and 4). On other hand, more time was needed for the insoluble particles in the neutralized samples to settle to the bottom of the tube. In the neutralized samples, the

phosphorus may already have been adsorbed/precipitated with the multivalent cation hydroxides before the insoluble particles had settled to the bottom of the tube.
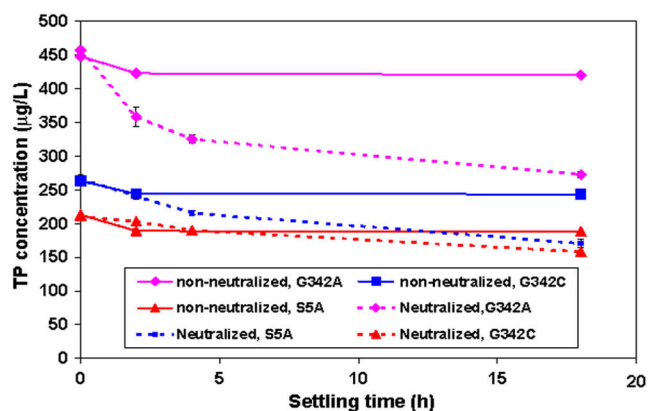

Fig. 3. The effect of neutralization and setting times after digestion or neutralization on total phosphorus (TP) analysis of water samples containing sediments ($n=4$ for S5A, and $n=3$ for G342A and G342C).
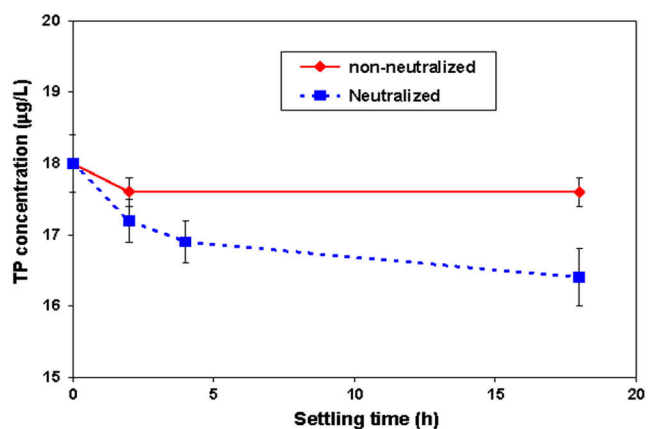

Fig. 4. The effects of neutralization and setting time after digestion or neutralization on total phosphorus (TP) analysis of samples without visible sediments. Samples ($n=3$) were taken at the G253.

Table 2
Total phosphorus measurements in sample from site S5A in ERR XI ($n=4$)

Neutralization	Reacidification	Settling time (h)	Shaking before analysis	Blank-correction	TP^a ($\mu g\,L^{-1}$)
No	—	0	Yes	No	213 ± 2
No	—	2	No	No	189 ± 2
No	—	18	No	No	188 ± 1
Yes	Yes	18	No	No	186 ± 1
Yes	No	0	Yes	No	211 ± 12
Yes	No	0	Yes	Yes	185 ± 2
Yes	No	24	No	No	158 ± 16

[a]The MPV of the S5A samples is $188\,\mu g\,L^{-1}$ based on statistical analysis.

The pH and sodium ion concentration of the sample may affect the settling speed of insoluble particles. Since the zero point charge of the sediment is at a low pH, then as the pH of solution increases, the sediment becomes negatively charged, and this prevents the flocculation of sediment. The sodium ion added during neutralization also tends to disperse the sediment. For these reasons, the settling speed of sediment is the slowest for neutralized/unreacidified-digestates, and is the fastest for non-neutralized digestates.

The most popular colorimetric method (ascorbic acid method) for P analysis, may not require the neutralization of the digested sample, as long as the pH of the preserved water sample is above 1.2 (Zhou and Struve, unpublished data). The neutralization step may be required when using a more sensitive cationic dye based method, such as that with malachite green, which is sensitive to the sample pH. The reacidification of the neutralized digested sample not only prevents the phosphorus adsorption/precipitation on/with the Fe and Al hydroxides, but also increases the settling speed of the sediment. The neutralization and reacidification steps after digestion are recommended for the cationic dye-based methods (Zhou and Struve, 2002).

The presence of the insoluble particles and precipitation/adsorption of phosphorus may also affect the analysis of water samples without visible sediment (such as the sample from site G253 (Table 1, Fig. 4). Especially subject to these influences are those water samples with very low TP. A small change in the absorbance reading, caused by the presence of insoluble particles and/or precipitation/adsorption of phosphorus, will also greatly affect the low level TP analyses in water samples (Fig. 4).

4. Conclusion

Standardized digestion and post-digestion treatment procedures are needed for analysis of TP in water samples. The procedures must specify and describe not only the acid and persulfate concentrations, but also treatment procedures immediately after sample digestion and before colorimetric analysis. As standard digestion and post-digestion treatment procedures for TP analysis using the ascorbic acid method, we recommend the following: (a) transfer a 15 mL sample into a 30 mL digestion tube, (b) add 0.3 mL 10.8 N sulfuric acid and 0.3 mL 40% ammonium persulfate solution to the sample, (c) immediately after mixing the sample with the acid persulfate solution, digest the sample in closed vessels in an autoclave for 30 min at 125°C and 14 kg/cm^2 pressure, (d) allow the digested sample to cool and set at least 1 h, (e) carefully decant/transfer an aliquot of the clear solution in digestion tube into a cup or vial without shaking/mixing, (f) analyze the TP concentration of the sample from the cup or vial using a spectrophotometer or an automatic colorimetric flow analyzer.

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