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ORIGINAL PAPER

The relationship between operational and bioavailable phosphorus fractions in effluents from advanced nutrient removal systems

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Abstract Because different phosphorus (P) forms vary greatly in their bioavailability, total phosphorus concentrations are a problematic predictor of the eutrophication potential of natural surface waters and wastewater treatment facility effluents. It is currently not known which operational P characterizations (i.e., dissolved/particulate and reactive/non-reactive) best predict effluent P bioavailability. We characterized the P speciation and directly measured the bioavailability of P (BAP) using algal bioassays for 14 full-scale advanced nutrient removal wastewater treatment plants representing a wide range of P removal technologies. A strong statistical relationship was observed between the effluent total BAP (tBAP) and total reactive P (TRP) ($r^2 \approx 0.81$), with a tBAP/TRP ratio of 0.61 ± 0.24, indicating that TRP can be used as a conservative surrogate predictor of tBAP. A comparison of different operational categories for phosphorus indicated that sBAP is consistently lower than both soluble P (SP) and soluble reactive P (SRP) with average ratios of 0.34 ± 0.19 and 0.62 ± 0.27, respectively. This shows a large fraction of the dissolved non-reactive P (i.e., SP−SRP), and $\geq$40 % of the P classified as SRP was not bioavailable. Total BAP concentrations were on average 30 % higher than soluble BAP (sBAP) concentrations, indicating that the particulate P fraction was an important component of the BAP for the tested effluents. Comparisons between different P removal technologies suggest the bioavailability, and P species composition varies with the nutrient removal process, and that in many cases, a large portion (>60 %) of the effluent P is recalcitrant to algal growth.

Keywords Phosphorus · Nutrient removal · Bioavailability of phosphorus · Tertiary treatment

Introduction

Phosphorus (P) is considered the proximal limiting macronutrient in many lakes, estuaries, and marine systems (Tyrrell 1999; Elser et al. 2007; Lewis and Wurtsbaugh 2008; Schindler et al. 2008). As a key driver of eutrophication, excessive P loading can lead to environmental problems such as harmful algal blooms, hypoxia and fish kills, resulting from biomass decay. To lessen P pollution and its environmental effects, various types of P removal processes have been developed in municipal wastewater treatment plants since these are often a major source of P to surface waters. Increasingly, ultra-low effluent P concentrations (i.e., <100 μg L^{-1}) in municipal wastewater treatment plant (WWTP) discharges are required (Ragsdale 2007). Advanced tertiary treatment with multiple stage chemical addition and filtration or membrane separation are commonly used to meet these low targets. It has been demonstrated that soluble reactive P (SRP) (often considered a measure of orthophosphate) in effluents from these systems only accounts for 33–53 % of TP (Guet al. 2007; Neethling et al. 2007). Lancaster and Madden (2008) suggested that refractory soluble organic P fractions have a greater tendency to persist and therefore have significantly lower removal efficiency than phosphates or inorganic P. In systems where P removal is the most effective, i.e., effluent

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TP ≈ 20 μg L^{-1}, soluble refractory P is commonly the dominant form in the discharge (Benisch et al. 2007; Gu et al. 2007; Neethling et al. 2007; Li and Brett 2012).

To quantify the effectiveness of P removal facilities, effluent monitoring for TMDL permitting is normally based on total P (TP) concentrations regardless, of the P composition, under the assumption that this is the most protective strategy for minimizing eutrophication in receiving surface waters. However, this assumption may greatly underestimate the environmental impact of P sources that are readily bioavailable (e.g., conventional secondary effluents) and over-estimate the importance of P sources with low bioavailability (e.g., P bound to inorganic particles) (Reynolds and Davies 2001). Furthermore, as noted above, in advanced P removal systems, much of the residual P may be recalcitrant. Therefore, the permitting process demands increased understanding of the composition and bioavailability of the nutrients in tertiary effluents. More precise and cost-effective P-response models would be possible if P bioavailability was fully accounted for.

The fraction of P that can be used to support algal growth is termed bioavailable P (BAP) and is typically quantified using bioassay experiments. Generally, P in the soluble orthophosphate form is the most easily utilized by phytoplankton and planktonic bacteria. It is well accepted that algae and bacteria are also capable of utilizing forms of P other than PO_4^{3-} to support their growth (Tarapchak and Moll 1990; Nausch and Nausch 2004). For instance, dissolved organic P sustained reasonable growth and yields of phytoplankton and bacteria under orthophosphate-depleted conditions (Berman, 1988; Thingstad et al. 1993; Monaghan and Ruttenberg 1999). Several studies on the bioavailability of phosphorus compounds in surface waters demonstrated efficient regeneration of phosphate from some organic and inorganic polyphosphate compounds (Bostrom et al. 1988; Bjorkman and Karl 1994, 2003). Consequently, utilization of organic P, and to a lesser extent recalcitrant inorganic P, could support algal productivity and control the BAP reservoir (Bjorkman and Karl 1994; Monaghan and Ruttenberg 1999). Bioassay experiments carried out with a wide variety of P-containing compounds showed that some compounds that are operationally classified as reactive phosphorus have very low %BAP (e.g., apatite), whereas other compounds that are classified as non-reactive are nearly entirely bioavailable (e.g., sodium tripolyphosphate and all true “organic” forms tested—DNA, RNA, and ATP.) (Li and Brett 2013). Furthermore, the dissolved phosphorus fraction associated with humic substances was almost entirely non-bioavailable (Li and Brett 2013). Therefore, from a management perspective, making decisions solely based on operational P speciation from chemical analyses is not sufficient without also considering phosphorus bioavailability.

A standard bioassay method (SM8011) using *Selenastrum* was developed based on Miller et al.’s algal toxicity bioassay protocol (Miller et al. 1978; American Public Health et al. 2005), which has been adapted to also include analyses of BAP. (Ellison and Brett 2006; Ekholm et al. 2007). In recent studies, algal bioassays were used to determine the presence of potential BAP from a wide variety of samples in various point and nonpoint sources, as well as sediments. These studies indicated the BAP % was highest in secondary WWTP effluents and lowest in lake and river sediments (Ekholm et al. 2007). Also, the bioavailability of particulates was studied in sixteen stream sites, which concluded the percent bioavailable particulate phosphorus averaged 15–30 % for streams draining catchments with forested, mixed use, and agricultural land cover (Ellison and Brett 2006). The BAP in the effluents and sediments could consist of inorganic P (such as phosphate), organic P (e.g., DNA, RNA), or particulate P (e.g., Ca–P) that commonly exist in wastewater effluents and storm water runoff.

The BAP concentration derived from bioassays is the most definitive way to estimate the eutrophication potential of a particular effluent. However, bioassays are problematic for routine measurements as they are costly, work intensive, and time-consuming (Ekholm et al. 2007), and bioassays are therefore not a practical method to monitor WWTP effluents. Several attempts have been made to correlate the results from algal bioassays with standard chemical extraction methods (Chamberlain and Shapiro 1969; Sharpley et al. 1991). Ideally, it is better to identify a widely measured chemical parameter to use as a predictor of the bioavailable fraction. Based on chemical reactivity with the acid molybdate reagents, operationally defined P fractions are usually used to characterize P speciation. For chemical analyses, the five most widely measured operational categories are TP, total reactive P (TRP) and SRP, soluble P (SP) and Particulate P (PP). There is no consensus on which chemical analysis provides the best predictor of the BAP pool. The most widely suggested estimators of BAP are SRP and TRP. It is commonly assumed that the fraction determined as SRP through the acid molybdate reaction is equal to the phosphate concentration and is also completely bioavailable to aquatic primary producers (Reynolds and Davies 2001). However, some studies have shown SRP underestimates BAP at low concentrations and overestimates BAP when SRP is greater than 20 μg L^{-1} (Twinch and Breen 1982). Further, using Rigler’s ^{32}P bioassay experiments, Hudson et al. (2000) concluded that operationally defined SRP concentrations may be two to three orders of magnitude higher than the actual phosphate concentration. Several authors have suggested that this disconnect between chemically determined SRP and actual phosphate concentrations could be because some organic P compounds are hydrolyzed to phosphate during the acid molybdate analysis (Rigler 1968; Bostrom et al. 1988; Kerouel and Aminot 1996). An alternative hypothesis for this mismatch is that part of the recalcitrant dissolved non-BAP fraction (e.g., humic–metal complexes or phytic acid) “disguise” as SRP because P is freed from humic–metal complexes at very low pH during the acid molybdate analysis (Li and Brett 2013). These and other studies suggest that SRP is not a reliable predictor of BAP or even phosphate.

Bradford and Peters (1987) developed a series of regression models predicting BAP from chemically determined fractions for 39 surface water samples. Their analysis indicated that TRP was the best predictor of BAP and explained 73 % of the overall variation in BAP in the lake and river samples with TP concentrations <30 μg L^{-1} that they studied (Bradford and Peters 1987). A study of the effluents from the City of Spokane’s advanced P removal pilot plant, which achieved TP concentrations ≤30 μg L^{-1}, also showed that TRP provided a better measure of the impact of effluent P on algae growth than did TP. However, none of these studies have investigated the relationship between different operational P categories for advanced WWTP treatment effluents with very low P concentrations. In this study, the statistical relationship between operational phosphorus categories and BAP for a wide range of effluents from phosphorus removal processes was investigated. This analysis could provide a basis for regulators to base effluent permits on phosphorus fractions other than TP. Moreover, currently, little is known about how effluent P composition varies for different nutrient removal processes and how this affects algae species composition and growth. Determining the relationship between the effluent phosphorus concentration and the percentage of BAP (%BAP) in effluents is particularly important when considering the balance between the environmental costs of additional energy consumption, chemical usage, and sludge disposal relative to the incremental BAP removal at low effluent TP concentrations. In our study, various types of P removal treatment processes were selected including enhanced biological phosphorus removal and chemical coagulant addition in secondary and tertiary treatment systems. Autoclaved and filtered samples were used in algal bioassays to estimate the total BAP (tBAP) and soluble BAP (sBAP), respectively. The effluent total and soluble phosphorus concentrations were also characterized as reactive or non-reactive and compared with BAP concentrations. Measured parameters were plotted against both sBAP and tBAP to determine which parameter provided the best correlation to biological response. The relationships between TP and BAP % in different effluents were also compared to identify the most efficient P removal process. Moreover, selected samples were filtered through different-sized filters to assess the impacts of filter size on P speciation.

Materials and methods

Sampling

A total of 75 samples from 14 plants were analyzed for this project. Based on the advanced filtration technologies employed in the WWTPs that participated in this study, 17 types of effluents from 14 plants were classified into four categories based on whether chemical addition and filtration were used in the treatment process. These categories included enhanced biological P removal (EBPR) process without chemical addition, membrane biological reactor (MBR) with chemical addition, single-stage tertiary treatment with chemical addition, and dual-stage tertiary treatment with chemical addition (Table 1). Subcategories were further classified based on the treatment technologies employed as described in SI Table 1 and Table 2.

All samples were 24-h composite samples collected in one liter acid-washed 0.1 M (HCl) polyethylene bottles from as near the final outfall as practical at each plant, from April 2011 to April 2012. Samples were stored at 4 °C immediately after collection and shipped to our laboratory on ice and in the dark within 24 h.

Chemical analyses

Chemical analyses for each sample determined whether the phosphorus pool was reactive and/or dissolved according to the acid molybdate spectrophotometric method described in Standard Methods 4500-P. Four classic operational categories (TP, TRP, SP, and SRP) could be directly measured and determined. TP was determined after 45 min of autoclave-mediated digestion (120 °C, 100 kPa, with $K_2S_2O_8$ and H_2SO_4) of an unfiltered sample. TRP was

Table 1 Treatment process classification

Treatment process category	Plants	Chemical addition
EBPR without chemical addition	3	
MBR processes	2	√
Single-stage tertiary	6	√
Dual-stage tertiary	6	√

determined using the same reaction on unfiltered samples without digestion. Samples for SP and SRP analyses (120 mL) were first filtered through a 0.45 µm polycarbonate membrane filter (Millipore®). SP was measured after persulfate and acid digestion, while SRP was measured without digestion. Soluble non-reactive P (SnRP) was calculated as the difference between SP and SRP. Values for PP were calculated by subtracting SP from TP to represent the particulate phase. To assess the effect of different filter sizes, selected samples were also filtered through a 0.2 µm polycarbonate membrane filter (Millipore®), analyzed for SP and SRP, and compared with samples passed through the 0.45-µm filter.

Algal bioassays

The freshwater algal species *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was used for these experiments. As indicated by Standard Method 8111, *P. subcapitata* was maintained in synthetic nutrient growth media prior to and during the bioassay experiments (American Public Health et al. 2005). Seven to ten days prior to the bioassays, algae cultures were centrifuged and resuspended in P-free media to induce P-stress according to Ellis and Stanford (1988). Fifty mL of each test sample was placed into 125-mL Erlenmeyer flasks, which were acid-washed (0.1 M HCl) and autoclaved prior to each experiment. Standard media with known concentrations of KH_2PO_4 in the range of 0–100 µg P L^{-1} were incubated in triplicate to obtain a standard curve for the algal growth yield. Because the precision of this method is lower than for standard wet chemistry approaches, four replicates of each sample were incubated and the results averaged for the final calculations. The algal cell yield was observed to be linear in the 0–100 µg L^{-1} range ($r^2 \approx 0.99$).

Two-hundred milliliters effluent samples were autoclaved for 45 min, and paired 200-mL samples were filtered through a 0.45-µm membrane filter. P-starved algae were added to both the autoclaved and filtered samples at a starting concentration of 10^4 cell·mL^{-1} to initialize the experiments. Samples were incubated at 24 ± 2 °C under continuous fluorescent lighting of 4300 ± 430 lm in a horizontal shaker at 110 rpm for 14 days. The 14-day incubation period is based upon the maximum growth potential for the test algae in laboratory conditions (American Public Health et al. 2005). Following incubation, algal cell density in the test and standard curve samples was determined using a Coulter Multisizer III particle size analyzer by passing the samples through a 100-µm aperture, with every sample read three times (Miller et al. 1978; American Public Health et al. 2005). Prior to each reading, background particle concentrations were estimated by testing parallel samples which were not inoculated with algae. The regression equation between algal cell density and BAP can be derived from the standard solution concentrations and algal counts accordingly:

$$\mathrm{BAP}\ \left(\mu\mathrm{g\ L}^{-1}\right) = (\mathrm{Cell\ Density}) * \mathrm{A} + \mathrm{B},$$

where A represents the slope and B the intercept of the standard curve.

The BAP derived from the autoclaved samples represented the value for tBAP, while BAP in filtered sample indicates the soluble BAP (sBAP) fraction. The percentage BAP is calculated relative to TP for tBAP and relative to soluble P for sBAP.

Results and discussion

Comparison of chemical measures with bioassay results

Our results showed that tBAP averaged only 34 ± 17 % of TP with a moderate correlation between these fractions ($r^2 = 0.77$) (Fig. 1a). The tBAP/TRP ratio showed tBAP was also consistently less than TRP, with this ratio averaging 0.61 ± 0.24. There was a moderately strong statistical relationship between tBAP and TRP ($r^2 \approx 0.81$) (Fig. 1b). Furthermore, in our study, sBAP was consistently much lower than SP, with an average sBAP/SP ratio of 0.34 ± 0.19 (Fig. 1c). sBAP also had a moderately strong statistical association with SP ($r^2 \approx 0.83$). Similarly, most of the sBAP values were lower than SRP with only a few exceptions (Fig. 1d). The r^2 between sBAP and SRP was 0.88, and the average sBAP/SRP ratio was 0.62 ± 0.24.

It has long been known that P is the main cause of eutrophication in many freshwater ecosystems (Schindler et al. 2008). Since the availability of P to the phytoplankton community often determines primary production rates, understanding the factors that affect bioavailability and determining the best predictor of BAP have important implications for developing eutrophication reduction strategies. Our study examined the statistical association between operational phosphorus categories and BAP in order to determine an alternative predictor for the bioavailable fraction. We found that TRP, among all the operational categories tested, was the best predictor for the total bioavailable fraction in the WWTP effluents we tested ($r^2 \approx 0.81$). Furthermore, the tBAP/TRP ratio of 0.61 ± 0.24 indicates that while TRP is a good predictor of tBAP, “reactive” P is not synonymous with BAP as commonly assumed. The comparison of the TP and tBAP % in the different processes indicated that higher

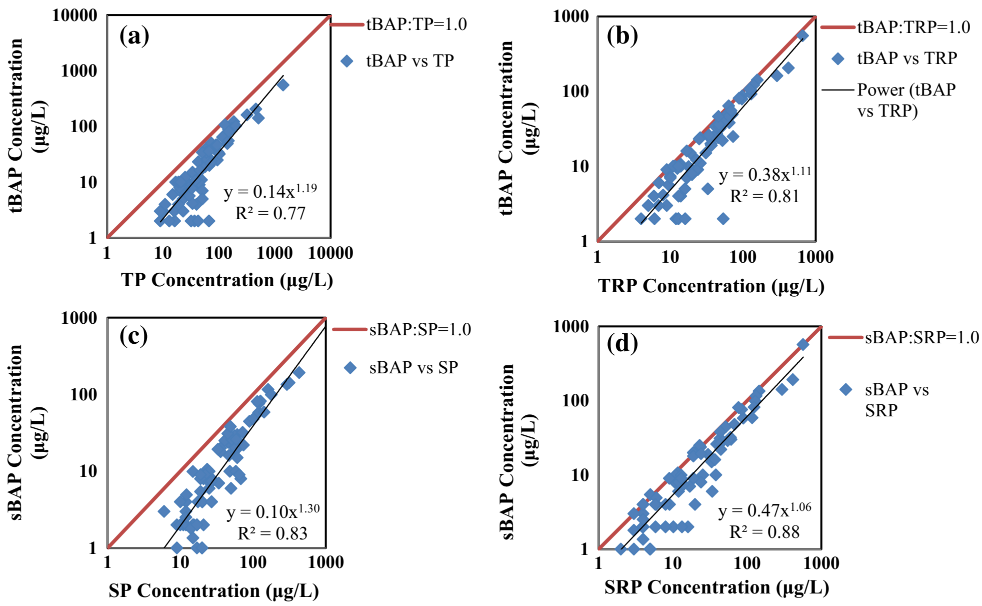

Fig. 1 Comparison of tBAP and TP (**a**), tBAP and TRP (**b**), sBAP and SP (**c**), sBAP and SRP (**d**)

chemical doses might be able to achieve lower TP with a smaller fraction of the P being bioavailable. Our results also show that certain tertiary processes are more efficient in reducing tBAP than others.

The algal bioassay method has the potential to resolve some of the missing links between the chemical P analyses and the P species that can be utilized by algae, thereby promoting eutrophication. However, algal bioassays are quite time-consuming and are therefore not practical for routine analyses. Therefore, this study tested whether more conventional and easily carried out measures of P composition from classic chemical analyses could be used in place of BAP to quantify the eutrophication potential of effluents. This was done by comparing the BAP values with operational phosphorus characterizations, such as SRP, SP, or TRP, which are much faster and less laboratory and cost intensive than algal bioassays. It has been previously noted that P concentrations derived from classic acid molybdate analyses are a poor estimator of the actual bioavailable fraction (Bradford and Peters 1987; Smith et al. 1999; Hudson et al. 2000; Ekholm and Krogerus 2003). It is conceivable that a significant fraction of TP is not readily bioavailable for algae. Thus, merely using TP as an indicator of BAP is problematic from a management perspective as this would greatly underestimate the eutrophication potential of some phosphorus sources, such as effluents from conventional primary/secondary wastewater treatment processes with relatively high bioavailability, and overestimate the potential of others, such as nonpoint sources and effluents from advanced tertiary treatment facilities with very low TP and BAP % (Ellison and Brett 2006; Li and Brett 2012).

The average ratio of 0.61 ± 0.24 between tBAP and TRP is similar to previous results, where the tBAP/TRP ratio averaged 0.44 ± 0.40 for effluents from a Spokane City Pilot Plant (Li and Brett 2012). This ratio suggests that TRP could be used in place of BAP as a conservative measure of the eutrophication potential of wastewater effluents. Moreover, this result indicated that the tBAP of the effluent was only ≈60 % of the "reactive" P concentration averaged across all the effluent samples we assessed. Thus, we conclude the P conventionally categorized as chemically reactive was not entirely bioavailable for algal utilization. The fraction that was molybdate acid reactive could be comprised of recalcitrant P forms such as large humic–metal–P complexes that are too large to be utilized by algae or bacteria, or by apatite which also alias as reactive but has low bioavailability (Li and Brett 2013).

Because it is impossible to physically separate the "dissolved organic P" (which is generally assumed to be equivalent to DOP = SP−SRP) from SRP, the individual bioavailability could not be calculated for these two components of the dissolved P pool. One interpretation of these data is that most of the P that is classified as SRP was bioavailable (i.e., ≈60 %), whereas much of what would classify as DOP was not bioavailable. Regardless, our results clearly show that much of the dissolved P in the advanced P removal effluents we tested, whether SRP or DOP, has low algal bioavailability. This observation is consistent with the results from experiments conducted on individual P-containing compounds that showed several dissolved P compounds had very low bioavailability (i.e., ferric pyrophosphate and apatite.) (Li and Brett 2013). Bradford and Peters (1987) also noted that a substantial proportion of the SP from lake water was not utilized by the phytoplankton community. sBAP is generally thought to be lower than SP because not all of the soluble P is bioavailable, and there may be a substantial pool of both inorganic and organic P, which is biologically recalcitrant. SRP is generally assumed to be mostly orthophosphate, which is commonly believed to be entirely bioavailable for planktonic algae and bacteria. However, Hudson et al. (2000) suggested that some phosphorus that operationally classifies as SRP when environmental P concentrations are low may actually consist of recalcitrant colloidal or polymerized P rather than true phosphate (Hudson et al. 2000). Furthermore, in lake sediments, phosphate could be released to the soluble phase due to changes in oxygen concentration or released from metal complexes because of the sorption–desorption reactions between orthophosphate and redox-sensitive metals (Bostrom et al. 1988; Reynolds and Davies 2001). Our results suggest using SRP to estimate sBAP will overestimate the real sBAP but much less so than using SP to estimate sBAP. Our results also suggest that there are components of the dissolved P pool, which alias as SRP in the analytical protocol, but which are not actually bioavailable to algae.

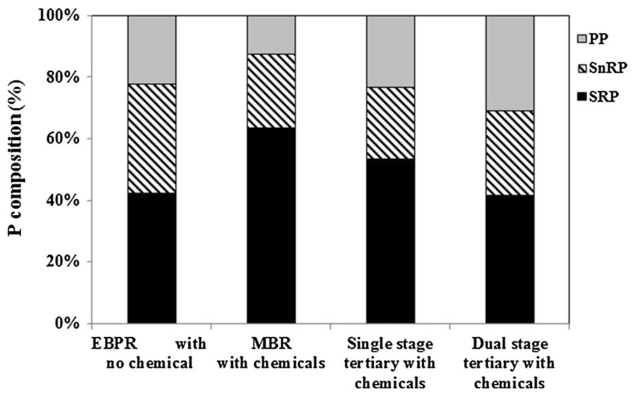

Fig. 2 Comparison of P concentrations for four P removal process categories

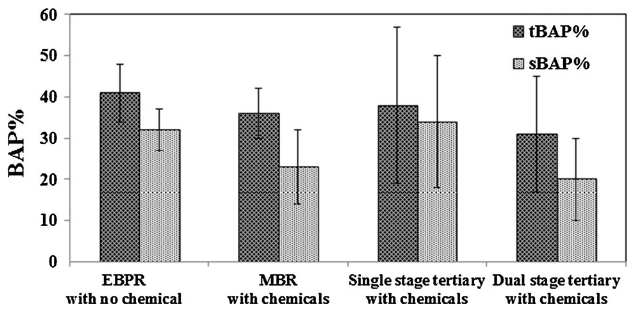

Fig. 3 Comparison of BAP % for four P removal process categories

Impact of different P removal processes

Figures 2 and 3 below show the average difference in P speciation and BAP for the effluents from the four main treatment processes assessed in this study. Higher chemical doses in dual-stage filtration systems, like continuous backwash gravity sand filters and other filtration processes, were quite effective at reducing inorganic P and especially SRP, via flocculation and coagulation with alum or ferric iron. In the final effluents from these systems, SRP averaged only 34 ± 16 % of TP. The P speciation results also indicated that the MBR systems were particularly efficient in removing PP (which was only 13 ± 9 % of TP). However, this was expected since these systems employed membranes for filtration. There was a substantial portion of SnRP (35 ± 14 %) in the EBPR effluents without chemical addition. The SnRP fraction is commonly assumed to include recalcitrant P components, which are both difficult to remove from wastewater and less bioavailable for algae. The average BAP fraction varied from 31 % (for dual-stage tertiary with chemicals) to 41 % (for EBPR without chemical addition).

In general, tBAP % declined with the final effluent P. The ten effluents with the lowest tBAP are shown in the Fig. 4. In the first stage of the continuous backwash gravity sand filters process in the Hayden Wastewater Research Facility (Hayden WRF), TP was reduced to 69 ± 18 μg L^{-1}. After the second stage, TP was reduced to 36 ± 9 μg L^{-1}, with only 33 ± 15 % bioavailable (tBAP = 12 ± 7 μg L^{-1}). In the effluent from the Ruidoso Village Regional WWTP (Ruidoso Village RWTP), TP was 51 ± 17 μg L^{-1} with 32 ± 13 % bioavailable. In the final effluent from the Broad Run Water Reclamation Facility (Broad Run WRF), TP was only 24 ± 7 μg L^{-1} with 9 ± 3 μg L^{-1} tBAP. Of the three technologies tested in the Coeur d'Alene pilot Advanced Wastewater Treatment Facility (Coeur d'Alene AWTF) [Membrane Bioreactor (Coeur d'Alene-MBR), Tertiary Membrane Filtration (Coeur d'Alene-TMF), and continuous backwash gravity sand filters process (Coeur d'Alene-CUMF)], effluents from the Coeur d'Alene-TMF process had the lowest tBAP (i.e., 4 ± 2 μg L^{-1}) out of 13 ± 5 μg L^{-1} of TP compared with Coeur d'Alene-MBR and Coeur d'Alene-CUMF (tBAP = 15 ± 8 μg L^{-1} and 6 ± 3 μg L^{-1}, respectively). Three chemical filtration systems, i.e., the P removal process at the Farmers Korner WWTP (Farmers Korner WTP), the Metropolitan Syracuse WWTP (Metropolitan Syracuse WTP), and the Iowa Hill WWTP (Iowa Hill WTP) obtained extremely low tBAP concentration (i.e., 5 ± 3 μg L^{-1}, 3 ± 2 μg L^{-1}, and 3 ± 1 μg L^{-1}, respectively). These systems also had very low tBAP % values (i.e., 15 ± 8, 8 ± 4 , and 17 ± 2 %, respectively).

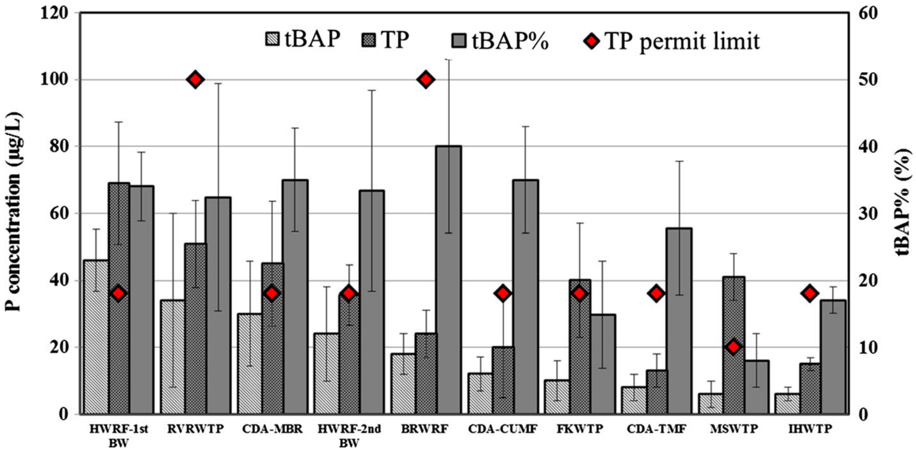

Fig. 4 tBAP %, TP concentration and TP permit limit for ten effluents with lowest tBAP. (*Note*: *HWRF-1st BW* Hayden wastewater research facility continuous backwash gravity sand filters process 1st stage. *RVRWTP* Ruidoso Village regional wastewater treatment plant. *CDA-MBR* Coeur d'Alene advanced wastewater treatment plant membrane bioreactor. *HWRF-2nd BW* Hayden wastewater research facility continuous backwash gravity sand filters process 2nd stage. *BRWRF* broad run water reclamation facility. *CDA-CUMF* Coeur d'Alene advanced wastewater treatment plant continuous backwash gravity sand filters process. *FKWTP* Farmers Korner wastewater treatment plant. *CDA-TMF* Coeur d'Alene advanced wastewater treatment plant membrane filter. *MSWTP* Metropolitan Syracuse wastewater treatment plant. *IHWTP* Iowa Hill wastewater treatment plant.)

This study clearly showed that phosphorus bioavailability and composition vary with the nutrient removal process as well as between individual treatment plants. Overall, an average of >50 % of the effluent P was recalcitrant for algal growth. Conversely, the effluents from conventional secondary wastewater treatment plants usually have BAP % >80 % (Li and Brett 2012). Most of the advanced wastewater treatment technologies were able to get the fraction of BAP to <50 %. In some cases, the tertiary treatment processes decreased tBAP to ≈10 % of TP. This suggests that tertiary treatment processes can be very efficient at removing the most bioavailable phosphorus components, thereby significantly reducing the potential impact on receiving waters.

In the ten processes with the lowest effluent tBAP, relatively high chemical doses (ranging from 6 mg L^{-1} to 100 mg L^{-1} Fe or Al) and intensive chemical P removal processes were used to achieve low TP concentrations. Most of these facilities have been recently built or upgraded to comply with more rigorous TMDL permit limits as indicated in Fig. 4. In six cases, a future limit of 36 µg L^{-1} has been proposed. In particular, a TMDL permit limit of 20 µgL^{-1} starting December, 2015, has been proposed for the Metropolitan Syracuse WTP.

A variety of tertiary systems here have been tested in these plants to determine the most efficient P removal processes. In these processes, three types of technologies, i.e., continuous backwash gravity sand filters, membrane bioreactor (MBR), and media or membrane filtration with high chemical doses, appeared to have the highest potential to remove BAP. A two-stage continuous backwash gravity sand filter reactive filtration process was installed as tertiary treatment for a slipstream of the secondary effluent at the Hayden WRF. Ferric iron (Fe) was dosed before the first stage (15 mg L^{-1} as Fe) and the second pass (10 mg L^{-1} as Fe). Samples were collected immediately before and directly after the second Fe dose. The same process was tested at the Coeur d'Alene pilot AWTF with a Ferric addition of 76 mg L^{-1} at the first stage and 45 mg L^{-1} at the second stage. The effluents from the two stages of Hayden WRF and the final effluent from Coeur d'Alene pilot AWTF reached a similar low tBAP % of around 35 % with tBAP of 23 ± 5 µg L^{-1}, 12 ± 7 µg L^{-1}, and 6 ± 3 µg L^{-1}, respectively.

In the Ruidoso Village RWTP, there is an anaerobic tank before biological nitrogen removal (BNR) followed by a MBR, which used A2O (Anaerobic–Anoxic–Oxic) treatment plus membrane filtration. Alum (6.3 mg L^{-1}) is added in this BNR process. The effluent samples were collected from the MBR system tested in the Coeur d'Alene AWTF when it did not apply any chemical addition. Broad Run WRF uses a MBR with EBPR with 9.4 mg L^{-1} of Alum. Even though there was low chemical addition in these MBR systems, the effluent tBAP concentrations were still below 20 µg L^{-1}. These results suggest that biological P removal with modest chemical addition, which removes BAP, plus a membrane system to target PP can also achieve efficient tBAP removal.

The last four treatment plants (Farmers Korner WTP, Coeur d'Alene-TMF, Metropolitan Syracuse WTP and

Table 2 Comparison of biological process and chemical process with *t* test

		Biological process ($n = 8$)	Chemical process ($n = 57$)	*t* test log
P speciation (μg/L)	TP	380 ± 454	64 ± 56	*0.00*
	SP	293 ± 386	47 ± 53	*0.01*
	TRP	201 ± 225	42 ± 48	*0.00*
	SRP	177 ± 202	32 ± 48	*0.00*
	SnRP	115 ± 208	15 ± 12	*0.02*
	PP	87 ± 89	17 ± 13	*0.00*
	tBAP	151 ± 172	27 ± 33	*0.00*
	sBAP	140 ± 183	20 ± 30	*0.00*
P composition (%)	SP %	71 ± 21	69 ± 20	0.90
	TRP %	56 ± 18	60 ± 20	0.62
	SRP %	47 ± 21	41 ± 25	0.20
	SnRP %	24 ± 14	28 ± 15	0.71
	PP %	29 ± 21	31 ± 20	0.80
	tBAP %	43 ± 12	35 ± 19	*0.02*
	sBAP %	49 ± 12	34 ± 17	*0.00*
Ratio	tBAP/TRP	0.79 ± 0.18	0.59 ± 0.24	*0.01*
	sBAP/SRP	0.78 ± 0.20	0.62 ± 0.28	*0.05*
	sBAP/SP	0.49 ± 0.11	0.34 ± 0.20	*0.00*

Italics indicates the difference is significant with $P < 0.05$

Iowa Hill WTP), which used aggressive chemical filtration or membrane treatment with a chemical dose over 30 mg L^{-1} of Alum, had tBAP ≤ 5 μg L^{-1} in their final effluents. In the FKWTP, a system with high-rate settling and mixed media filtration was used as the tertiary process after biological nutrient removal. This process includes chemical coagulation and flocculation using a polymer and alum (100 mg L^{-1} as Al) followed by clarification via tube settlers and filtration through mixed media bed filters. In the Coeur d'Alene-TMF process, the secondary clarifier effluent is treated with a 50 mg L^{-1} alum addition followed by membrane filtration. In the full-scale (84.2 MGD capacity) Metropolitan Syracuse WTP in Syracuse, New York, active sludge BOD removal and biological aerated nitrification are used for secondary treatment followed by a high-rate flocculated settling (HRFS) process with a ferric chloride addition (≈ 15 mg L^{-1} as Fe). The P removal in the Iowa Hill WTP was accomplished by activated sludge biological treatment, biological aerated nitrification, chemical coagulation using alum and polymer (100 mg L^{-1} as Al), flocculation and clarification using a tube settler, followed by sand single-stage filtration. The bioassay results from these effluents indicated chemical filtration or membrane processes with intensive chemical addition substantially modified P speciation in the final effluent and resulted in the P in the effluents being much less bioavailable.

Impact of chemical addition and biological treatment

The effect of primarily biological and primarily chemical-based phosphorus removal processes (alum or ferric based) was assessed by comparing the P characteristics of two biological-based processes (North Durham Water Reclamation Facility and Snoqualmie Wastewater Reclamation Facility) against the 13 chemical-based processes. We also compared the effluents from the combined biological removal/membrane and chemical (alum) removal/membrane systems operated in Coeur d'Alene, which is particularly insightful since these systems treated the same waste stream. The differences between the biologically and chemically based processes were assessed using t tests (two-tailed, heteroscedastic) of log-transformed concentrations. Table 2 compares the P speciation and bioavailability for the two plants that did not have chemical addition to the thirteen systems that used alum or ferric addition. These data show that P concentrations and the proportion of the phosphorus that was bioavailable were statistically significantly higher in the biologically based systems for all forms considered. However, the percent composition as operationally defined was not different. The net effect of higher concentrations

Table 3 Comparison of membrane biological reactor (MBR) and tertiary membrane reactor (TMR) in Coeur d'Alene advanced wastewater treatment plant with *t* test

		CDA-MBR ($n = 5$)	CDA-TMF ($n = 5$)	*t* test log
P speciation (μg/L)	TP	45 ± 19	13 ± 5	*0.00*
	SP	43 ± 18	13 ± 5	*0.00*
	TRP	25 ± 14	7 ± 3	*0.02*
	SRP	23 ± 13	6 ± 3	*0.01*
	SnRP	20 ± 5	7 ± 3	*0.00*
	PP	1 ± 1	0 ± 0	0.15
	tBAP	15 ± 8	4 ± 2	*0.00*
	sBAP	10 ± 3	2 ± 1	*0.00*
P composition (%)	SP %	98 ± 2	99 ± 2	0.47
	TRP %	53 ± 10	56 ± 11	0.65
	SRP %	49 ± 10	44 ± 11	0.43
	SnRP %	48 ± 11	55 ± 14	0.53
	PP %	2 ± 2	1 ± 2	0.37
	tBAP %	35 ± 8	28 ± 10	0.28
	sBAP %	26 ± 9	17 ± 5	0.08
Ratio	tBAP/TRP	0.68 ± 0.21	0.53 ± 0.21	0.29
	sBAP/SRP	0.56 ± 0.29	0.39 ± 0.13	0.30
	sBAP/SP	0.26 ± 0.09	0.17 ± 0.05	0.08

Italics indicates the difference is significant with $P < 0.05$

and higher relative bioavailability was a 5.5 times higher tBAP concentration in the biologically based effluents.

The biological- and chemical-based membrane systems at the Coeur d'Alene Advanced WWTP both had very low phosphorus concentrations, probably on account of the effectiveness of the membrane component of these combined systems (Table 3). However, in this case, differences in the effectiveness of biological versus chemically based processes were also clearly evident. The biologically based process had 3–4 times higher concentrations for all of the P forms considered, except PP—which was extremely low in both effluents as was expected for membrane systems. These results indicate that chemical addition is warranted when low %BAP in the effluent is necessary to protect receiving waters.

Comparison of tBAP and sBAP

Phosphorus bioavailability studies that focus on PP autoclave the samples prior to conducting the bioassay experiments to kill endogenous algae (e.g., Ellison and Brett 2006), whereas some studies that examine the bioavailability of dissolved P only use filtration to remove endogenous algae. Previous research showed that autoclaving soil samples increased the estimated BAP by approximately 60 % compared with un-autoclaved samples, but that the linear relationship between autoclaved and un-autoclaved samples was quite strong, i.e., $r^2 = 0.9$ (Anderson and Magdoff 2005). There is evidence that phosphorus may be liberated by phosphatase enzymes when lake water is autoclaved, indicating that organic P might be hydrolyzed to soluble reactive phosphate during autoclaving (Jansson 1977). In this study, we conducted bioassay experiments on both bulk autoclaved and filtered dissolved samples for every single sample processed. The total bioavailable P (tBAP) as determined for the bulk autoclaved samples was very highly correlated with the soluble bioavailable P (sBAP) for the filtered samples ($r^2 = 0.90$, $n = 75$) (Fig. 5). The average percentage BAP for the bulk fraction was similar to that for the soluble fraction (i.e., tBAP/TP = 36 ± 17 % vs. sBAP/SP = 34 ± 19 %, respectively). Our results also suggested sBAP was always lower than tBAP and averaged approximately 70 % of tBAP. In the case of biological-based membrane systems at Coeur d'Alene Advanced Wastewater Treatment Plant, where the PP in the effluent is very low (e.g., 2 ± 1 %), the sBAP (26 ± 9 %) is 74 % of tBAP (35 ± 9 %). This is important because it provides evidence that a substantial fraction of the difference between tBAP and sBAP could be solely due to autoclaving. Moreover, because tBAP determinations

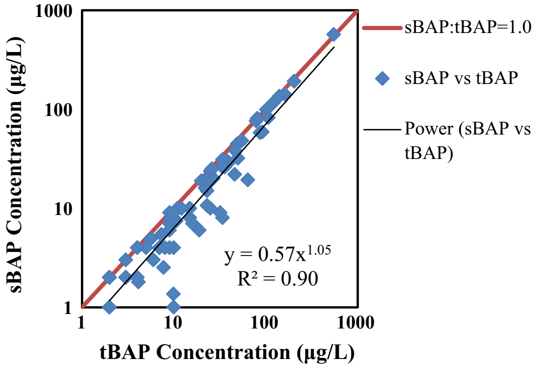

Fig. 5 Comparison of autoclaved and filtered BAP

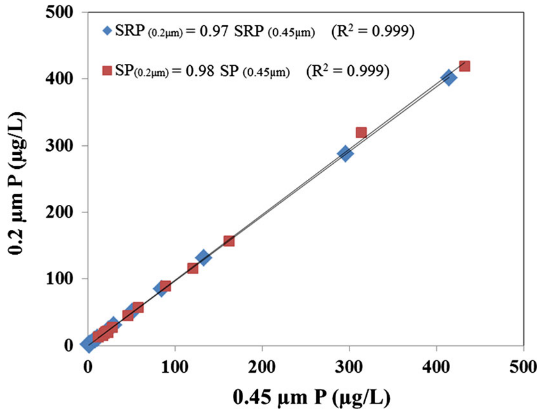

Fig. 6 Comparison of SP concentration from samples passed through 0.45- and 0.2-µm filter size. (n = 14)

were generally higher than sBAP, this result indicated that it is best to base our main conclusions on the bulk BAP fraction in order to provide the most conservative estimates of WWTP effluent eutrophication potential.

Comparison of filter size

Standard Methods suggest using a 0.45-µm filter to separate the dissolved and PP fractions (American Public Health et al. 2005). However, some authors have suggested that colloidal P species in the 0.2–0.45 µm size range could be a substantial fraction of the dissolved P (Turner et al. 2004). In this study, two filter pore sizes (0.45 and 0.2 µm) were used to determine whether using different filter sizes to partition the dissolved from the particulate fractions had a significant impact on P classification. Fourteen effluent samples received between April and June 2011 were filtered through both filter sizes and analyzed for SP and SRP. In the results shown in Fig. 6, the filtrate of effluent samples after 0.2-µm filtration had slightly lower SP and SRP concentrations than the samples passed through a 0.45-µm filter, with a very high correlation between the two sets of samples ($r^2 = 0.999$). Our results indicate that P species in the 0.2–0.45 µm size range only accounted for 2–3 % of the TP. Thus, using either a 0.2- or 0.45-µm filter pore size had only a minimal effect on our P classification. Further, using a 0.45-µm filter to differentiate the soluble and particulate fractions is more advisable since it will provide a more conservative value for the soluble fraction which normally has higher bioavailability.

Conclusion

The results of this study should encourage water quality modelers and TMDL permit writers to consider the importance of BAP when assessing the likely ecological impacts of municipal nutrient removal facility effluent discharges. The use of TRP in lieu of the BAP bioassay may provide a fast, simple, and conservative monitoring parameter for effluent P and thus could serve as the basis of effluent permits. A high percentage (≥40 %) of TP classified as SRP, but much of this SRP was not bioavailable. The results indicate that certain filtration processes with high chemical doses (such as tertiary membrane system, Trident® HS System, Actiflo® or multiple-stage filtration) achieved extremely low effluent tBAP concentrations (>5 µg L^{-1}) and also reduced the percentage total BAP (tBAP %) to less than 30 % of TP. These processes would be the preferred design for dischargers that must meet the most stringent P removal goals. Furthermore, our results show that BAP concentrations in autoclaved samples were consistently higher than that for filtered samples, suggesting that autoclaved BAP samples are a more conservative because they include the PP fraction, which may also be bioavailable. We found TRP was the best predictor of tBAP. It is worth noting that this result is based on comparisons with other parameters for the effluents tested, and therefore, caution should be exercised when interpreting and applying this to other systems. Future research on conservative estimates of soluble organic P mineralization rates from bioassay experiments could provide better insights to integrate the bioavailability of P into eutrophication management models.

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