The Study Of Phosphorus Bioavailability In Effluents From Advanced Nutrient Removal Treatments

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Abstract

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Because phosphorus (P) is the main limiting nutrient in the majority of surface waters, developing protective and cost efficient P source control plans is crucial for reducing eutrophication risk. Currently, it is assumed all of the P discharged from Wastewater Treatment Plants (WWTP) is bioavailable. This study used standard algal bioassays to determine the extent to which the various forms of P in WWTP effluent are available for algal growth and how the mineralization kinetics of dissolved P should best be represented in water quality models. The effluents from a pilot plant with various alum-based process were tested. The bioassay indicated that percent bioavailable P (%BAP) declined as P removal level increased (r^2 = 0.98) and only 7 ± 4% of the P was bioavailable in the final effluent. The chemical speciation and biological uptake experiments for 21 selected P containing

compounds showed that in a majority of cases (81%) these species did not follow the classic assumption that Soluble Reactive P (SRP) is a representation of bioavailability. A new classification scheme is proposed to link the connection between bioavailability and operational chemical measures. After characterizing the P, both chemically and biologically, in effluents from 14 advanced nutrient removal facilities with a wide range of phosphorus removal technologies, a regression model was derived between the operational categories and bioavailability. This showed a strong statistical association between BAP and Total Reactive P (TRP) ($r^2 \approx 0.81$), with a BAP/TRP ratio of 0.61 ± 0.24, suggesting TRP could be used as a conservative predictor of BAP. Furthermore, this study indicated that the bioavailability and P speciation varies greatly from one treatment process to another, while in most cases the majority (> 60%) of the effluent P is recalcitrant for algal growth. Finally, the mineralization kinetics of dissolved P in effluents from tertiary process was assessed by bioassays. Model fitting results showed two-pool model and three-pool models fit the experimental data very well with $r^2 > 0.9$ and the mineralization rate determined in these first-order decay models could be seamlessly incorporated into existing Total Maximum Daily Loading (TMDL) models without structural modifications. This study also provided a scientific basis to consider the importance of recalcitrant P in tertiary effluents in future modeling practices.

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Acknowledgements

In literal translation, my name “Bo” given by my grandmother in Chinese means omniscience or infinite wisdom. Since birth, my family have always wished that I would obtain a Ph. D so that I can live up to the hype that was in my name. During my rebellious teenage years, I thought a doctorate was the furthest thing from what I wanted in life. However at this moment, as I am finally writing this last chapter of my Ph.D dissertation, I realized that my grandmother had much greater foresight decades ago than I do myself today. There are many people around me just like my grandmother that believed in me and made it possible for me to come this far today.

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encouragement and guidance when mistakes were made. I truly couldn't have finished my doctoral studies without his kind support.

Also, I consider myself fortunate enough to have Prof. Stensel and Prof. Benjamin as a part of my committee. I sincerely thank them for their consistent guidance and direction during the course of this dissertation despite their busy schedules. All the constructive feedback they provided on my research greatly improved my critical thinking as a scientist.

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Life is a journey. Without all of you, I most certainly would not have been where I am today.

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Introduction

It has long been known that phosphorus (P) is a key element for eutrophication control in freshwater systems. Although there is still a continuing debate regarding the nature of nitrogen (N) and P limitation (Tyrrell 1999, Schindler et al. 2008, Lewis and Wurtsbaugh 2008, Elser et al. 2007), P is considered the proximal limiting macronutrient in most cases. For instance, research has shown that because of nitrogen (N) inputs from atmospheric deposition, Lake Tahoe has shifted to persistent P limitation (Jassby et al. 1994). Also, by using factorial enrichment designs to estimate the strength of nutrient limitation, the growth of algae and bacteria in western Lake Superior was found to be P limited (Sterner et al. 2004). Moreover, a Long-term lake experiment has shown that reducing P inputs should be the focus of eutrophication control strategies because N input control would actually favor the growth of nitrogen-fixing cyanobacteria (Schindler et al. 2008).

Since excessive P availability to microbial populations can degrade water quality, common practice is to develop P sources control strategies to reduce eutrophication risk in the watershed planning process (EPA 2013a). Currently, in most P loading control plans, total P (TP) is assumed to be the end measure for monitoring and permitting (Cusimano 2004). This is plausible for effluents from secondary Wastewater Treatment Plants (WWTPs) where TP concentrations are high, and much of the reactive P compounds remain in the effluents. However, in order to control nutrient loading to maximize environmental protection, many WWTPs are or will be required to upgrade by implementing tertiary removal treatments (EPA 2013a, Cusimano 2004). With the help of intensive chemical addition and/or membrane filtration, effluent P concentrations can be dramatically decreased, while also leaving refractory dissolved P as the dominant fraction which barely contributes to algae growth (Neethling et al. 2007, Christen 2007). Using TP as the ultimate measure of eutrophication potential could greatly overestimate the effective P loading and lead to ineffective management strategies (Reynolds and Davies 2001). Thus, there is a need to re-configure water quality models with regards to P bioavailability and algae uptake kinetics for these advanced nutrient removal facility effluents. This is an essential step to provide scientific evidence in support of sound management decisions considering the high financial investments and secondary environmental effects associated with the operation of tertiary plants.

To achieve this goal, the main goal of this study is to characterize the P pool that supports algal growth, termed Bioavailable P (BAP), and its uptake kinetics in effluents from advanced nutrient removal facilities. Conventionally, wet chemical analyses using molybdate acid are used to characterize P (Murphy and Riley 1962). However, several studies have shown these operationally defined fractions do not necessarily correspond to the role these forms play in the biotic cycling and P utilization (Reynolds and Davies 2001, Baldwin 1998, Hudson et al. 2000, Rigler 1968). This study adopted a standard bioassay method (SM8011) using *Selenastrum*, which was developed based on Miller et al.'s (1978) algal toxicity bioassay protocol. This approach allows direct determination of the P pool assimilated by algae (American Public Health et al. 2005, Miller et al. 1978). This method has been applied widely to quantify the total BAP in surface water and sediment samples from natural systems (Ekholm et al. 2007). However, studies determining the BAP of wastewater effluents are very rare. As the regulatory process demands increased understanding of the bioavailability of the nutrients in tertiary effluents, particularly when discharging to extremely sensitive water system, direct estimation of BAP in these effluents using bioassays could be critical for implementing nutrient management schemes (Effler et al. 2012).

Another issue that is important for our understanding of P bioavailability is the disconnect between chemical measures and the biological characteristics of P species. In the P cycle in surface water systems, DRP is generally assumed to be entirely bioavailable and rapidly assimilated by phytoplankton. Organic bound P compounds can be hydrolyzed by enzymes which are synthesized by bacteria and phytoplankton, and subsequently assimilated (Berman 1988, Thingstad et al. 1993, Monaghan and Ruttenberg 1999). Dissolved inorganic and organic P can adsorb onto the particulate matter and settle from water column (Wetzel 2001). Factors known to regulate the internal P cycle within water systems include abiotic factors (pH, redox value, temperature and etc.) and biogenic mineralization (Wetzel 2001). There is considerable debate over which of these forms and conditions of phosphorus is bioavailable (Nausch and Nausch 2006, Cotner and Wetzel 1992, Bjorkman and Karl 2003, Bentzen et al. 1992). This issue should be resolved by distinguishing the contribution of each P species to the BAP pool.

In addition to the direct determination of the total BAP pool, finding a more practical and cost efficient approach to predict the bioavailable P fraction could improve monitoring of WWTP effluents because algal bioassays are too labor intense for routine measurements (Ellison and Brett 2006). The studies assessing the fit between chemical measures and BAP have shown TP could greatly overestimate BAP by including refractory P while Soluble Reactive P (SRP) could underestimate as it does not account for the potential bioavailable organic P (Reynolds and Davies 2001, Hudson et al. 2000). Yet, there is no solid conclusion on which operational chemical measure is the best predictor for BAP in WWTP effluents. Research on the relationship between operational categories and bioavailable fraction is needed to provide a basis for regulators to base effluent permits on phosphorus fractions other than TP.

Furthermore, in current watershed pollution plans, the analysis of point source and non-point source control strategies is mainly achieved by scientific modeling, in which an assumed mineralization rate is embedded to describe the degradation mechanism of P (Chapra 1997, EPA 2013b). There has been speculation that relying on this single rate to represent all uptake pathways could over-simplify the complex interactions of various P compounds in surface waterbodies (Christen 2007). Thus, after the determination of BAP in effluents and different P species, with the understanding of importance of BAP, the final step is to assess the mineralization kinetics of dissolved P. This could allow us to resolve the phosphorus bioavailability issue by incorporating these scientific findings into modeling application and thus presenting a science-based approach for permitting actions.

This dissertation is based on four publications with each corresponding to one scientific question I am aim to address in my Ph.D research:

1. ***What is the BAP fraction in the effluents from advanced nutrient removal facilities and how it is impacted by tertiary treatments?***

Paper I examined BAP in the effluents from a pilot tertiary plant that tested various alum based processes for achieving > 99% P removal. Effluents from different stages were assessed to test the influence of alum treatments on %BAP and tBAP.

2. ***What is the bioavailability of different P compounds that may commonly occur in WWTP effluents?***

Paper II tested 21 different P species with both algal bioassays and classic chemical operational methods to develop a new classification scheme for P compounds based bioavailability and chemical category.

3. ***Which operational chemical category best predicts the potential BAP pool in effluents and could be used as monitoring measure for WWTP.***

Paper III derived a regression model between BAP and chemical measures for effluents from 14 advanced nutrient removal facilities to assess the best correlates of biological response.

4. ***What are the mineralization kinetics of dissolved P and its associated biodegradation rate and how can this be embedded in current water quality models?***

Paper IV assessed the mineralization kinetics of dissolved P in algal uptake experiments. The experiment data were fit with three different first-order kinetic models and a Gamma model to identify which representation give the most parsimonious fit to the data and which mineralization rate derived from these models represents each of the effluents studied.

List of Publications

Paper I: Li, B., Brett, M. T. (2012). The impact of alum based advanced nutrient removal processes on phosphorus bioavailability. *Water Research, 46*(3), 837-844.

Paper II: Li, B., Brett, M. T. (2013). The influence of dissolved phosphorus molecular form on recalcitrance and bioavailability. *Environmental Pollution, 182*, 37-44.

Paper III: Li, B., Brett, M. T. (2013). The relationship between operational and bioavailable phosphorus fractions in effluents from advanced nutrient removal systems. *Journal of Environmental Management, under review.*

Paper IV: Li, B., Brett, M. T. (2014). The quantification of dissolved phosphorus mineralization kinetics. *In progress.*

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The impact of alum based advanced nutrient removal processes on phosphorus bioavailability

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ABSTRACT

Because eutrophication is a widespread consequence of wastewater discharges, there is a strong impetus to develop new approaches to remove phosphorus (P) from wastewater treatment plant (WWTP) effluents. We examined the effluents from a pilot plant that is testing various alum based processes for achieving > 99% P removal, however, it is not known how these advanced P removal technologies affect the bioavailability of P (BAP). We tested how the percent BAP (%BAP) varied with different P removal levels using an algal growth bioassay methodology. This facility reduced total P concentrations from ≈ 500 $\mu g\,L^{-1}$ in the pilot plant influent to 19 ± 4 (±SD) $\mu g\,L^{-1}$ in the final effluent, and our results showed that as the level of P removal increased, the %BAP of the product declined sharply, $r^2 = 0.98$. Prior to alum treatment, the influent had an average %BAP of $79 \pm 13\%$, and after three steps of alum-based removal the %BAP averaged $7 \pm 4\%$. Thus, this alum based P removal process was very effective at sequestering the P forms that most readily stimulate algal growth. Further, our results show the final BAP of the effluent was only ≈ 50% of the "reactive" P concentration. These results have important implications for nutrient management and trading schemes.

1. Introduction

Eutrophication is a global problem that causes a litany of problems including nuisance phytoplankton (and especially cyanobacteria) blooms, excessive periphyton accumulation, hypoxia and fish kills, taste and odor problems, and toxins and trihalomethanes in drinking water supplies (Correll, 1998; Smith 2003; Anderson et al., 2002). Excess nitrogen (N) and phosphorus (P) loading are the most common causes of eutrophication. Phosphorus is commonly thought to be the main cause of eutrophication in freshwaters as well as longer-term primary production in the world's oceans (Schindler, 1977; Tyrrell, 1999). Because high salinity positively impacts the speciation of P vis-à-vis phytoplankton bioavailability and negatively affects diazotrophic cyanobacteria, coastal areas are often thought to be N limited (Conley et al., 2009b), but can also be P limited (Nausch et al., 2004). However the nature of N and P limitation in fresh and marine waters is still the subject of considerable debate (Schindler et al., 2008; Lewis and Wurtsbaugh, 2008). For instance, there is considerably debate about whether the Gulf of Mexico and the Baltic Sea are N or P limited (Sylvan et al., 2006; Conley et al., 2009a).

Much emphasis has been placed on the quantification of phosphorus in water due to its fundamental importance as a plant nutrient and major cellular constituent (Sylvan et al., 2006). The speciation of P is quite complex, and for analytical purposes, four operational categories are commonly used to characterize phosphorus (McKelvie et al., 1995). These are: dissolved reactive P (DRP), dissolved non-reactive P, particulate reactive P, and particulate non-reactive P. These fractions

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are partitioned with dissolved P passing through a 0.45 μm filter, and reactive P determined via a colorimetric reaction with acid-molybdate (Murphy and Riley, 1962). But this classification scheme does not necessarily correspond to the role these forms play in the biotic cycling and utilization of P (Anderson et al., 2002). The use of chemical approaches to estimate eutrophication risk is problematic for management purposes as this approach does not actually estimate the amount of P that is biologically available to support phytoplankton and bacteria growth (Ekholm and Krogerus, 1998; Gerdes and Kunst, 1998). Bioavailable Phosphorus (BAP) is the component of total phosphorus (TP) which supports the growth of algae or other organisms (Boström et al., 1988), and previous studies have indicated that BAP rather than TP or DRP provides the most accurate measure of water quality conditions in lakes (Butkus et al., 1988; Gerdes and Kunst, 1998).

Numerous approaches have been applied to estimate BAP, including bioassays (Boström et al., 1988; Ekholm et al., 2007), ion exchange resin-impregnated membranes (Abrams and Jarrell, 1992) and NaOH and NH_4F based chemical extractions (Sharpley, 1993). However, most studies have concluded algal bioassays are the most reliable technique for quantifying BAP (Twinch and Breen, 1982; Ekholm and Krogerus, 2003). In batch assays, algae and the sample are directly mixed, thus allowing the activity of surface-bound algal enzymes to release particulate organic phosphorus into solution (Reynolds and Davies, 2001).

Many studies suggest that P availability may vary between different sources of waters as a function of their physical, chemical and biological conditions (Boström et al., 1988; Ekholm et al., 2007). Because of concern regarding eutrophication problems caused by wastewater discharges (Morse et al., 1998), considerable effort is now being devoted at the national scale toward advanced P removal (Neethling et al., 2010). One of the most important questions associated with these efforts is how these advanced nutrient removal processes affect the speciation and in particular the bioavailability of P for phytoplankton and planktonic bacteria (Cusimano, 2004) and this information is critical to ongoing efforts to control the negative consequences of widespread eutrophication on surface water bodies (Correll, 1998; Smith 2003; Anderson et al., 2002).

Advanced tertiary wastewater treatment can reduce TP down to $<500\ \mu g\,L^{-1}$ (Tchobanoglous et al., 2003), and in the most modern cases with multiple stages including filtration, coagulation and adsorption can reach effluent averages of $<50\ \mu g\,L^{-1}$ TP. However, direct infrastructure and operating costs, and secondary environmental impacts (*e.g.*, increased energy utilization and sludge disposal), can increase dramatically when attempting to achieve very low concentration targets. Therefore, there is a very strong incentive to better understand the merits of the various technologies available to remove nutrients, *e.g.*, ferric and aluminum salts, membranes and biological processes. Studies of chemical P characterization for tertiary effluents indicate that refractory dissolved organic P (rDOP) frequently becomes the dominant fraction in advanced treatment process effluents (Neethling et al., 2007). This chemically refractory P fraction may account for 5–25% of the residual TP for processes that are able to get phosphorus down to $<50\ \mu g\,L^{-1}$ level. (Neethling et al., 2007). This rDOP fraction is usually assumed to be non-bioavailable. However, this may not be the case because chemical phosphorus characterizations often correlated poorly with bioavailability derived from bioassays (Anderson et al., 2002). The regulatory process demands increased understanding of the biological characteristics of the nutrients in tertiary effluents particularly when discharging to extremely sensitive waterbodies. It is especially important that the best result for the cost be obtained, and to achieve this objective much more needs to be known about the properties of advanced WWTP effluents.

In the Spokane region (Washington State, USA), the hypolimnion of Lake Spokane (AKA Long Lake) commonly experiences hypoxia, and it is known that Spokane WWTP discharges contribute to this problem by approximately doubling the TP concentration in the Spokane river during the low flow summer/fall period (Cusimano, 2004). Because Lake Spokane primary production is phosphorus limited, improving hypolimnetic dissolved oxygen concentrations will require significant reductions in total, and most importantly bioavailable (Christen, 2007) P loads. The Spokane WWTP removes ca. 90% of the P from its influent during conventional treatment. Thus a pilot plant was constructed to aid in the design processes to meet these more rigorous permit limits (i.e., $TP < 50\ \mu g\,L^{-1}$).

The objective of this study was to use algal bioassays to determine the BAP of effluent treated by this pilot facility. This facility used multiple alum additions as aluminum sulfate ($AlSO_4$), and based on data collected at different steps along the treatment train we derived a general relationship between the level of P removal, and the percent BAP of the product generated by these processes. These results will serve as a baseline against which the results of other alum based approaches, and especially alternative processes (e.g., ferric, biological, and membrane based) can be compared. This study also tested whether more conventional, and easily carried out, measures of P composition could be used in place of BAP to quantify the eutrophication potential of this effluent.

2. Methods

2.1. Pilot plant processes

After the current secondary clarifier in the Spokane pilot plant, two parallel traditional sedimentation tube settlers and one magnetic based sedimentation unit were operated as intermediate processes followed by a granular media filter, an upflow sand filter or a membrane filter (Fig. 1). These combinations allowed us to test the P removal efficiency for various unit series. These advanced P removal technologies were based on alum, which reacts with P to form an aluminum phosphate precipitate which is insoluble within the pH range of typical wastewaters.

2.2. Sampling

The overall alum ($AlSO_4$) treatment process could be classified into three stages which include influent samples to the pilot plant (INF), intermediate effluents (INT) and final effluents of the pilot plant (EFFL) (Fig. 1). The influent to the pilot plant was

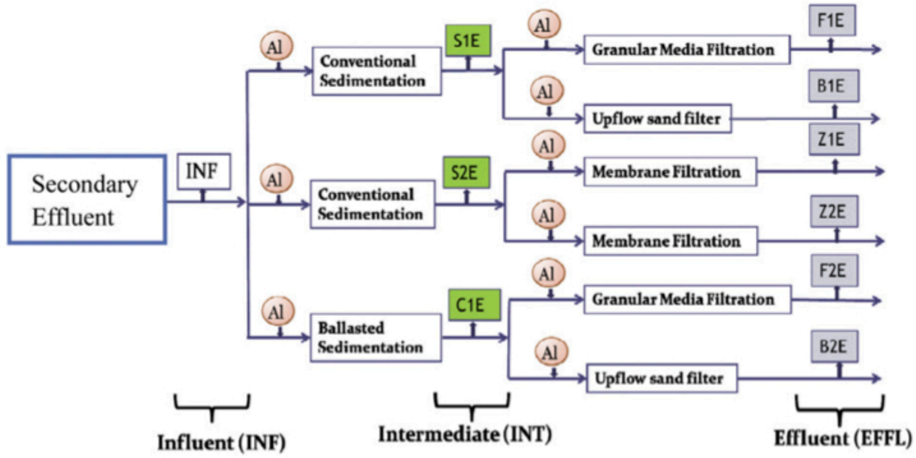

Fig. 1 – WWTP treatment process. Influent (INF) was the effluent from secondary WWTP. In the summer, there is one more alum addition in secondary WWTP. In the winter, there is no alum addition after secondary wastewater treatment. Intermediate (INT) was the mean of sample S1E, S2E and C1E. The final effluent (EFFL) was the mean of F1E, B1E, Z1E, Z2E, F2E, B2E.

the effluent from the conventional primary/secondary WWTP. The intermediate effluents category contains three samples. Two were sampled after two traditional sedimentation and one was obtained after magnetic enhanced based sedimentation. Six samples were collected from the six filtration units as final effluent samples.

24 h composite samples were collected in 1 L acid washed (HCl) polyethylene bottles from as near to the final outfall as practical at each of the units from August 2009 to April 2010. Three samples were collected between November 2009 and March 2010 when the secondary WWTP was under winter operation without alum addition. Another five samples were collected during summer operation when alum was added after the secondary treatment before the pilot treatment plant. Samples were stored at 4 °C immediately after collection and shipped to our laboratory within 24 h (Fig. 2).

2.3. *Chemical analysis*

All samples were analyzed for total reactive P (TRP) and TP. TRP was determined using the standard ascorbic acid colorimetric method outlined in Standard Methods 4500-P without filtering samples, and TP was determined with the same method following acidic persulfate digestion (APHA, 1998).

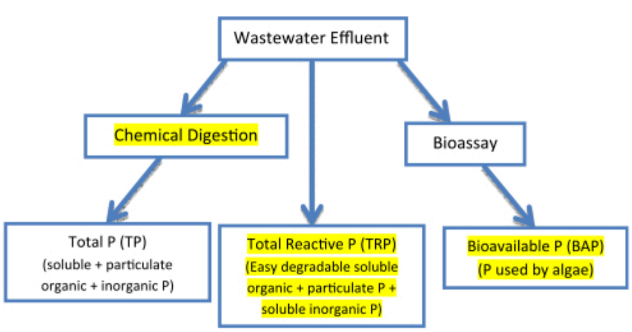

Fig. 2 – Description of P analysis. Note: TP and TRP are directly measured while BAP is estimated indirectly from algal growth.

Analysis of TRP allowed for speciation between the "reactive" and "non-reactive" fractions and provided a basis for comparison with the much more time intensive BAP assays.

2.4. *Bioassay analysis*

P bioavailability was determined using the bioassay method described in Standard Method 8111 (APHA, 1998). The nutrient medium described by Miller (Miller et al., 1978) was used to maintain *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) algal cultures. Algae were centrifuged and resuspended into P-free medium (which used KCl in place of K_2HPO_4) 7–10 days before the bioassays. Unfiltered effluent samples were autoclaved for 45 min to kill indigenous algae before the assay. Filtration through both 0.2 and 0.45 μm filters showed ca. 60% of the P in the final effluent samples was particulate (unpubl. data). Previous research has shown autoclaving BAP samples increases the estimated BAP somewhat, but that the linear relationship between autoclaved and unautoclaved samples was strong, i.e., $r^2 = 0.9$ (Brandon and Frederick, 2005), this is consistent with our test results which show a strong linear relationship between the autoclaved and 0.45 μm filtered samples ($r^2 = 0.996$, $n = 15$) with the autoclaved samples giving an average 7% higher estimate of BAP. Thus the BAP results reported in this paper are likely to be conservative.

Influent and intermediate samples were diluted with P-free media to bring their P concentrations within an appropriate range (i.e., $<100\ \mu g\ L^{-1}$). 50 mL of each test sample was placed into 125-mL Erlenmeyer flasks, which were acid-washed (0.1 M HCl) and autoclaved between each experiment. Standard media with a known concentration series of KH_2PO_4 (0, 5, 10, 15, 20, 25, 30, 40 and 50 $\mu g\ P \cdot L^{-1}$) 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. Algal growth was linear in the 0–50 $\mu g\ L^{-1}$ range ($r^2 \approx 0.99$).

P-starved algae were added to the 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 lm ± 10% in a horizontal shaker at 110 rpm for 14 days. The 14-day incubation period is based upon the maximum growth potential for the study algae in laboratory conditions (APHA, 1998). 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 (APHA, 1998; Miller et al., 1978). Prior to each reading, background particle concentrations were estimated by testing parallel samples which were not inoculated with algae. The correlation function between the BAP and algal cell density can be derived from the standard solution concentrations and algal counts in these standard samples as followed.

$$BAP = (\text{Cell Density}) * A + B$$

where A is the slope of the standard curve, B is the interception of the standard curve.

Through this regression equation, the algal cell yield in the test assay gave the BAP concentration in the samples.

3. Results

The TP, TRP and BAP results were presented according to two different operation phases (winter and summer scenario) and three stages within the treatment process (INF, INT, EFFL) (Fig. 3). The results for INT and EFFL were the means for the samples in those categories.

3.1. *Winter operation*

The TP in the INF sample (*i.e.*, primary and secondary treatment effluent) averaged 2750 ± 116 μg L^{-1} during winter operation (Fig. 3a). The TP removal efficiency in the pilot processes for the first alum addition was approximately 96% while the second alum addition with filtration removed around 74% from the intermediate effluent (TP = 116 ± 56 μg L^{-1}). Hence, in the final effluent, there was only 30 ± 21 μg L^{-1} TP. The influent TRP (2480 μg L^{-1}), which was the predominant fraction of P, was reduced by 99% to only 15 ± 17 μg L^{-1} in the final effluent. The bioassay reveals that average BAP for the influent sample (2180 μg L^{-1}), was markedly decreased in the final effluent to only 6 μg L^{-1}. Overall, without alum addition in secondary WWTP, the pilot plant reduced the TP concentration to 30 μg L^{-1} with only 6 μg L^{-1} as BAP.

3.2. *Summer operation*

Because alum was added after secondary treatment during the summer, TP was reduced compared to winter samples by a factor of 5 (*i.e.*, to 470 μg L^{-1} for influent TP sample) and by a factor of 2 for the EFFL TP (*i.e.*, 18 μg L^{-1}) (Fig. 3b). The TP in the influent samples (post primary and secondary treatment) ranged widely as did the concentration of the different fractions as shown in Fig. 3b. After the alum addition in secondary

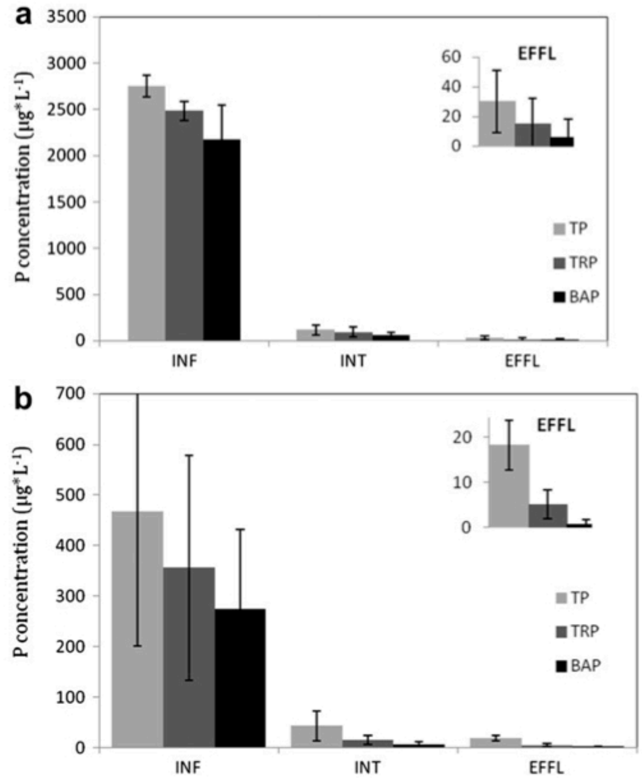

Fig. 3 – TP, TRP, BAP concentration profiles for the samples when the pilot plant was under winter when there is no alum addition in secondary WWTP (a) and summer when there is alum addition in secondary WWTP (b) operation. INF-Influent, INT-Intermediate Effluent, EFFL-Final Effluent, Error bars represent standard deviations.

wastewater treatment, the second alum addition in the pilot plant reduced TP by 91%, while the third step removed another 58%. Thus, this pilot facility was able to get TP concentrations down to 18 ± 6 μg L^{-1} in the final stage. Also, the TRP concentrations were reduced 99% from 350 ± 223 μg L^{-1} in the influent to only 5 ± 3 μg L^{-1} in the final effluent. BAP concentrations declined from 280 ± 156 μg L^{-1} to 7 ± 4 μg L^{-1} after the first alum addition with only ≈ 1 μg L^{-1} BAP in the final effluent. Overall, after three alum additions (one in secondary wastewater treatment, two in the pilot plant), the P concentration in the final product was reduced to 18, 5, 1 μg L^{-1} for TP, TRP, BAP, respectively.

3.3. *Relationship between %BAP and P removal level*

We divided the BAP concentration by TP to determine how the fraction of TP that was bioavailable varied with different P removal levels (Fig. 4). Prior to any alum treatment, the influent to the pilot plant in winter had an average %BAP of 79 ± 1%. When alum was added to secondary wastewater treatment plant in the summer, the %BAP in the influent to the pilot plant decreased to 61 ± 21%. For the final product, the %BAP was reduced to 14 ± 14% for the winter and 4 ± 5% for the summer. Because the BAP bioassays are based on a biological approach, as opposed to the more typical chemical assays used to quantify nutrient concentrations, the expected

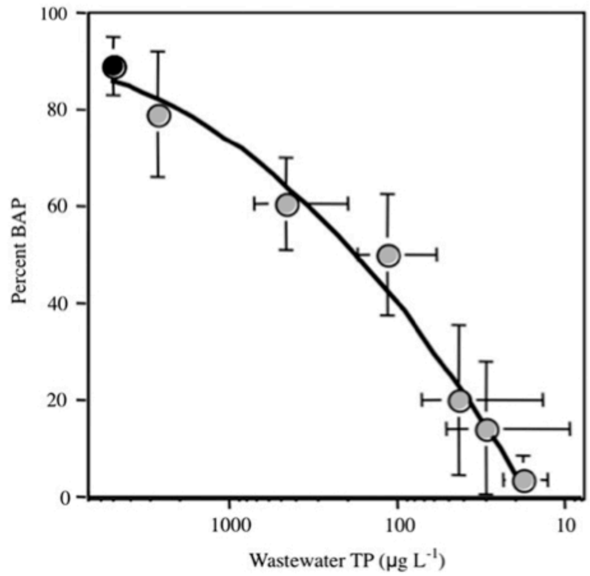

Fig. 4 – Relationship between the %BAP of TP and TP concentrations in the effluents. Error bars represent standard deviations.

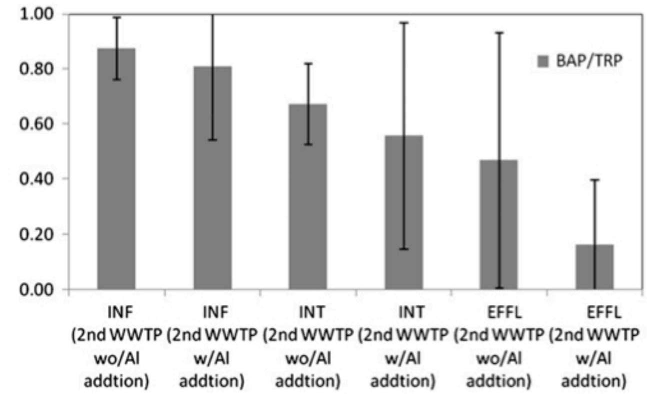

Fig. 5 – BAP/TRP for six different sample types. INF-Influent, INT-Intermediate Effluent, EFFL-Final Effluent. Error bars represent standard deviations. W represents "with" while WO represents "without".

variation in BAP bioassay results is larger especially at very low BAP values. The %BAP vs. TP regression model we derived for the overall alum treatment process is shown as below:

$$\%BAP = -12.2 * \log(TP)^2 + 92.0 * \log(TP) + 94.2\%;$$
$$r^2 = 0.98, \ n = 7, \ MSE = 10.3\%$$

3.4. BAP to TRP ratio

We also tested whether TRP can be used as a conservative measure of BAP. The BAP/TRP results for different treatment steps and scenarios are shown in Fig. 5. The average of BAP/TRP for all the samples was 0.44 ± 0.40 and BAP is consistently less than TRP for all situations ($P < 0.01$). As P removal increased the ratio of BAP to TRP declined from 0.87 ± 0.11 to 0.16 ± 0.23. Variability in the BAP/TRP ratio was higher at high levels of wastewater treatment because both methods were approaching their analytical limits.

4. Discussion

Because the test algae were deprived of phosphorus prior to incubation, the production of alkaline phosphatase enzymes, which are used by algae to convert organic forms of P to inorganic P, was stimulated to facilitate the release of available phosphorus to allow a more accurate determination of total BAP without longer term incubations (Reynolds and Davies, 2001).

The phosphorus fractionation and bioassay results indicate that in the pilot plant the removal efficiency for the first alum addition was approximately 90% TP while the second alum addition with filtration removed around 60% TP from the intermediate effluent. In the final product, the pilot plant decreased TP concentrations to amongst the lowest levels ($20\ \mu g\,L^{-1}$) we are aware for WWTP facilities in the United States. The overall removal from the initial influent to the conventional plant to the final effluent for the tertiary plant was 99.6%. The P concentration profile for the winter operation followed the same pattern as the summer condition albeit with a higher initial concentration. Without alum addition in secondary treatment, the pilot plant was able to reduce TP to $30\ \mu g\,L^{-1}$ with only 20% BAP.

The TP versus alum dose plots for the effluent samples provide additional information about the treatment system performance (Fig. 6). The first alum addition in the secondary treatment processed removed $2200\ \mu g\,L^{-1}$ and left $470\ \mu g\,L^{-1}$ in the influent to the pilot plant. In the pilot plant, the first alum addition in tertiary floc/settling process removed an additional $\approx 400\ \mu g\,L^{-1}$ TP, while final alum addition and filtration step only removed a further $\approx 20\ \mu g\,L^{-1}$ TP. The alum dose for the first two steps was similar (≈ 100 mg alum L^{-1}), whereas the final step used $50\ mg\,L^{-1}$, so the total alum dose for the whole process is $250\ mg\,L^{-1}$. Fig. 6 also shows the trends for the different P forms (i.e., TP, TRP and BAP) deviated more at higher levels of treatment. This suggests it would be warranted to investigate the optimal balance between nutrient removal, economic costs and secondary environmental impacts (e.g., energy consumption, sludge disposal, etc) (Dodds et al., 2009). It is also interesting that the results show the percentage of TRP (%TRP) relative to TP declined from $\approx 80\%$ in the influent to $\approx 30\%$ in the final effluent. These decreases in %TRP and %BAP indicate that, not only was TP reduced to very low levels, but the composition of the P was changed markedly as well.

Both the quantity of P as well as the availability of P in the environment is crucial for eutrophication. In both the winter and summer scenarios BAP was reduced 99% with only $\approx 6\ \mu g\,L^{-1}$ and $1\ \mu g\,L^{-1}$ left in the final effluent for winter and summer scenarios, respectively. This suggests that the P forms which most readily stimulate algal growth were sequestered by this P removal process. From the regression model characterizing the relationship between %BAP and TP for the whole alum treatment process, it is clear that as the aggressiveness of P removal increased, the %BAP of the

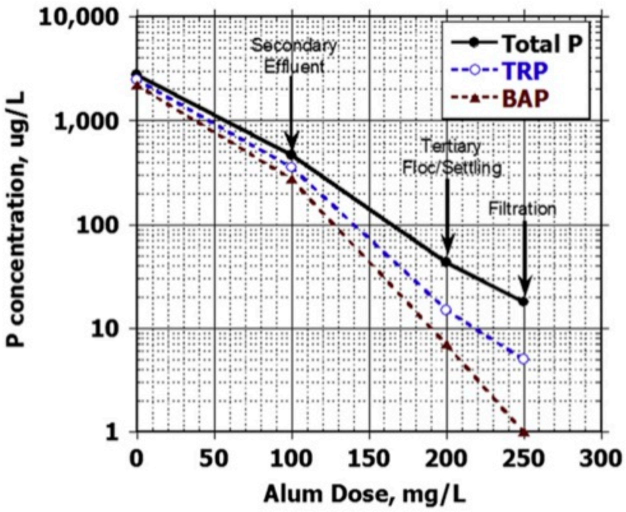

Fig. 6 – P concentration and average Alum dose in each treatment process.

effluent declined sharply. Furthermore, this alum based model will provide an important baseline against which the results of other alternative approaches (e.g. ferric, biological, and membrane based) can be compared.

Most importantly, as the results above show at high treatment levels the concentration of TP greatly over-estimates effluent BAP. However, it is possible to approximate BAP with other chemical P analyses, such as TRP – which is a more conventional and less time intensive analysis than BAP bioassays. The BAP/TRP ratios indicate that BAP is consistently less than TRP with BAP/TRP averaging 0.44. This suggests TRP could be used in place of BAP as a conservative measure of the eutrophication potential of alum treated wastewater effluents. These results also suggest that 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 and cause eutrophication problems.

Further, although TRP is generally assumed to be mostly bioavailable for algal growth, our results indicated that BAP was only about half of TRP. This suggests that there is a large portion of TRP which cannot be utilized for algal growth. Our current understanding of the bioavailability of various P species is rudimentary but evolving. Dissociated orthophosphate ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) is commonly believed to be entirely bioavailable for planktonic algae and bacteria, and it is generally assumed these fractions correspond to the P quantified by the DRP colorimetric assay (Rydin, 2000). However, previous bioassay data suggest both very high and very low DRP concentrations may overestimate BAP because a fraction of the DRP may actually be unavailable forms such as colloidal or polymerized P rather than dissolved orthophosphate (Ekholm et al., 2007; Twinch and Breen, 1982). Furthermore, sorption–desorption reactions between orthophosphates and redox-sensitive metals, such as iron and manganese, can result in substantial immobilization of orthophosphate making it unavailable for biological uptake (Reynolds and Davies, 2001). However, if water column or sediment dissolved oxygen concentrations decline below $2\ mg\ L^{-1}$, some of this immobilized P will be re-released to the water column (Reynolds and Davies, 2001).

Recent studies have shown dissolved organic phosphorus is the dominate component of the dissolved non-reactive phosphorus which is estimated by subtracting DRP from the total dissolved P concentration (Mark et al., 1995; Mark and Roger, 1997; Cade-Menun et al., 2006). Several studies have shown phytoplankton can utilize some forms of dissolved organic P in the absence of inorganic P (Tarapchak and Moll, 1990; Cotner et al., 1991). For example, it is shown that nucleotides were the most readily utilizable of the combined phosphorus compounds investigated (Björkman and Karl, 1994). Meanwhile, only a minor proportion of NaOH extractable P (including alum-bound P and iron-bound P) has been found to be algal extractable and its bioavailability is influenced to pH and dissolved oxygen (Boström et al., 1988). This suggests the change in %BAP is caused by alum precipitation converting orthophosphate into particulate Al-bound phosphorus. Also, particulate organic P is often relatively stable and only bioavailable after protracted sedimentary diagenesis (Reynolds and Davies, 2001).

These results also suggest the biochemical and eutrophication promoting characteristics of P discharged from advanced nutrient removal processes may be very different than for conventional WWTPs. The results of this study, showing much lower %BAP values for the final alum treated effluents than for the secondary treatment discharges, begs the question of how %BAP results like these should be used when trying to control eutrophication risk in receiving water-bodies. One option is to envision the low %BAP in aggressively treated effluents as a safety factor and continue to manage nutrient loading to sensitive surface waters as if TP is the principle measure of eutrophication risk. This would generally lead to favorable outcomes vis-à-vis recipient water-bodies, but could also lead to "over-treatment" in regards to secondary environmental impacts such as chemical and energy consumption and solid waste generation. In cases where multiple advanced technologies for removing P are economical, there should be a strong incentive to select the approach that results in the lowest total BAP. Ultimately, %BAP results like the present will achieve greater credence in the management decision making process when it is shown in the field that effluents with very low %BAP values lead to more favorable outcomes in receiving waters than effluents with similar TP values but higher %BAPs. All other things being equal, it is clearly better to have effluents with recalcitrant phosphorus.

Most phosphorus management plans focus on TP loading based on the assumption that accounting for all P forms is the most conservative approach. However, several studies suggest agricultural and urban runoff, and natural stream flows commonly have %BAP values in the 20–40% range (Ekholm and Krogerus, 2003; Reynolds and Davies, 2001; Ellison and Brett, 2006) whereas secondary WWTP effluents have %BAP averaging 80–90% (Ekholm and Krogerus, 1998; this study). Therefore, comparing the eutrophication potential of these different sources without accounting for %BAP greatly underestimates the true risk associated with wastewater discharges (Reynolds and Davies, 2001). Further, our results show the effluents of alum-based tertiary P removal processes have %BAP of ≈10% which suggest that this P source is likely to promote eutrophication a factor two less than urban, agricultural and natural P sources and much less than conventional WWTP effluents. It is especially important to consider %BAP in nutrient trading schemes (Paul, 2000) where P sources with vastly different bioavailability may be treated equivalently based on the false assumption quantifying all nutrient sources as TP is the most protective approach for minimizing eutrophication.

Further studies need to be carried out in order to identify the species of phosphorus which are not bioavailable in this alum treated WWTP effluent. It would be useful to analyze the bioavailability of defined P species directly. Also effluent samples from other advanced P removal processes other than alum addition will allow us to obtain a comparison between different approaches for P removal, and enable a better understanding of the bioavailability of P and wastewater treatment processes. Our results suggest aggressive alum treatment is very effective at obtaining very low %BAP values, at least for the waste-stream treated at the Spokane WWTP, but it remains to see how the %BAP of effluents varies with other advanced P removal processes.

5. Conclusion

Our results showed intensive P removal using an alum-based process greatly reduced the proportion of the P in the final effluent that was bioavailable to algae. Knowing how total BAP in effluents varies with the level of treatment will be very important when selecting particular treatment technologies for advanced P removal and especially for deciding on final treatment strategies to balance eutrophication risk from effluents with secondary factors such as cost, energy consumption and greenhouse gas and sludge production. Knowing the %BAP of a particular effluent type will also be critical for implementing nutrient trading schemes. Future research should compare the bioavailability of P from alternative advanced tertiary processes and determine which forms of P are present in the non-BAP fraction of these final effluents.

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The influence of dissolved phosphorus molecular form on recalcitrance and bioavailability

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ABSTRACT

Several studies have shown Soluble Reactive Phosphorus (SRP) analyses provide a poor index of dissolved phosphorus (P) bioavailability in natural systems. We tested 21 inorganic and organic P containing compounds with series of nutrient uptake and bioavailability bioassay experiments and chemical characterizations. Our results show that in 81% of cases, these compounds did not fit the classic assumption that SRP approximately equals Bioavailable P (BAP). Many organic compounds were classified as non-reactive, but had very rapid uptake kinetics and were nearly entirely bioavailable (*e.g.,* several nucleic acids, ATP, RNA, DNA and phosphatidylcholine). Several inorganic compounds also classified as non-reactive but had high bioavailability (*i.e.,* sodium tripolyphosphate and phosphorus pentoxide). Conversely, apatite was operationally classified as reactive, but had low bioavailability. Due to their tendency to alias as SRP, but recalcitrance and very low bioavailability, humic-(Al/Fe)-phosphorus complexes may play an especially important role in the dissolved phosphorus dynamics of natural systems.

1. Introduction

Phosphorus plays a critical role in the growth of algae and aquatic macrophytes, and is recognized as the most common limiting macronutrient in many freshwater systems (Redfield, 1934; Schindler et al., 2008). Elevated phosphorus (P) concentrations can lead to eutrophication resulting in excessive algal growth which can lead to a suite of negative responses including harmful algal blooms, hypoxia, and in severe cases fish kills (Smith et al., 1999; Lewis and Wurtsbaugh, 2008).

What compounds comprise dissolved P, and the relative bioavailability of this fraction for primary producers, is one of the most long-standing questions in freshwater and marine science (Hudson et al., 2000). Also, recent research has shown a high portion of the dissolved P in advanced P removal treatment plants is non-bioavailable, but it is unclear which P forms comprise the recalcitrant dissolved P (Reynolds and Davies, 2001; Li and Brett, 2012). It is often assumed that the soluble reactive P (SRP) fraction is nearly entirely bioavailable, and some authors have claimed that this is also the case for the total dissolved P fraction (Hatch and Reuter, 1999; Reynolds and Davies, 2001). Conversely, the classic results of Rigler (1968) and more recently Hudson et al. (2000) suggest measured phosphate may comprise only a minuscule portion of dissolved phosphorus. Because of its importance for aquatic ecosystems, a variety of methods have been developed to characterize the P forms that are most prevalent in natural systems (Murphy and Riley, 1962; Hedley et al., 1982; Cade-Menun et al., 2002). Although these fractionation techniques can identify certain broadly defined P pools, they cannot identify specific P containing compounds. This is important because different P species may vary greatly in their bioavailability for algae and bacterioplankton (Nausch and Nausch, 2006; Björkman and Karl, 2003). The large majority of studies use filtration and the acid-molybdate method to operationally define P pools according to whether they are "dissolved" (=soluble) and/or "reactive" (Murphy and Riley, 1962; Hedley et al., 1982; Cade-Menun et al., 2002). Many studies assume soluble reactive P (SRP) represents orthophosphate, and that this fraction can be readily utilized by aquatic primary producers (Reynolds and Davies, 2001). However, whether the SRP estimate given by the acid-molybdate method provides a reasonable estimate of orthophosphate concentrations has long been in dispute (Rigler, 1968; Hudson et al., 2000). Hudson et al. (2000) showed using Rigler ^{32}P bioassay experiments that SRP concentrations in 14 lakes exceeded actual phosphate concentrations by 2–3 orders of magnitude. These authors suggested the disparity between lake water SRP and phosphate concentrations could be artifacts due to

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sample filtration releasing particulate P to the dissolved phase and the acidification step of the SRP analyses releasing bound phosphate. For example, the hydrolysis of non-orthophosphate in compounds such as phytic acid, riboflavin monophosphate and adenosine 5′ diphosphate, can contribute to the formation of heteropoly blue in SRP analyses (Boström et al., 1988; Kerouel and Aminot, 1996; Hudson et al., 2000).

Research has shown that various forms of P can be used as P sources and support primary production (Boström et al., 1988; Berman et al., 1991; Björkman and Karl, 1994, 2003) Dissolved non-reactive P, which is commonly assumed to represent dissolved organic P (DOP), represents a major phosphorus reservoir in soils and surface waters (Monbett et al., 2007). However, the DOP pool is generally poorly resolved because suitable measurement techniques are not available to deduce its composition. Studies examining the bioavailability of inorganic and organic phosphorus compounds to marine microorganisms identified several forms, such as adenosine-5′-triphosphate, adenosine monophosphate, and glucose-6-phosphate which can be readily converted to orthophosphate and thus made bioavailable (Hedley et al., 1982; Berman, 1988; Boström et al., 1988; Björkman and Karl, 1994, 2003). Research with freshwater phytoplankton showed some organic P forms can also support high algal growth rates (Boström et al., 1988; Berman et al., 1991; Cotner and Wetzel, 1992). When studying P-limited limnetic systems, Tarapchak and Moll (1990) showed several naturally occurring DOP compounds were hydrolyzed to orthophosphate and utilized by phytoplankton and bacteria. Although not well resolved, the fraction of DOP that is susceptible to enzymatic hydrolysis is considered to be an important component of the bioavailable P pool (Cooper et al., 2009; Monbett et al., 2009). However, to date, only a small number of defined P species have been analyzed using bioassays to characterize their uptake kinetics and bioavailability.

We examined the chemical and biological properties of a wide variety of pure phosphorus containing compounds, including inorganic, organic and humic substance associated P. The objective of these analyses was to determine how closely the bioavailability of pure phosphorus containing compounds corresponds to the conventional operational categorization used to classify the P present in surface water samples. We tested the conventional assumption that primary classification as SRP indicates that a phosphorus form is highly bioavailable. Bioassays were used to determine algal uptake rates and bioavailability, and these compounds were also assessed using the classic operational characterization scheme. Based on these results, a novel classification scheme for dissolved P was also suggested.

2. Methods

2.1. P containing compounds

A wide range of phosphorus containing compounds were used for this study based on their prevalence in nature and chemical classification (Table 1). Humic substances were obtained from International Humic Substances Society. These compounds were prepared as stock solutions and combined with a synthetic P-free nutrient medium as described by Miller et al. (1978) (SI Table 1). KCl instead of K_2HPO_4 was used as a potassium source to assure the compounds tested were the only P source for phytoplankton. Information for the P compounds used for these experiments and their final concentration in the test solutions are listed in Table 1. Fresh solutions were prepared prior to each chemical analyses and bioassay experiment.

2.2. Chemical analyses

The chemical analyses for each compound determined whether they were classified as reactive and/or dissolved according to the acid-molybdate spectrophotometric method described in Standard Methods 4500-P. This yielded the four classic operational categories, *i.e.*, total P (TP), soluble P (SP), total reactive P (TRP) and soluble reactive P (SRP). 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 (APHA, 1967). TRP was determined using the same reaction on unfiltered samples without persulfate 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 digestion while SRP was determined without persulfate digestion. Soluble non-Reactive P (SnRP) was calculated as the difference between SP and SRP.

2.3. Algal bioassays

The freshwater alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) was used for these experiments. As indicated by Standard Method 8111 (APHA, 1967), *P. subcapitata* was maintained in synthetic nutrient growth media prior to and during the bioassay experiments. Seven to ten days prior to the bioassays, algae cultures were centrifuged and resuspended into P-free medium to induce P-stress. 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 (0, 5, 10, 15, 20, 25, 30, 40 and 50 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. Algal cell yield was linear in the 0–50 μg L^{-1} range ($r^2 \approx 0.99$).

Table 1
P species tested.

Category	Chemical name	Molecular formula	P content (μg P/L)	Producer, product number
Inorganic P	Aluminum phosphate (Al-P)	$AlPO_4$	71	ALDRICH, 341452
	Calcium phosphate (Ca-P)	$CaHPO_4$	58	MP Biomedicals, ICN 19380480
	Ferric pyrophosphate (Pyro-P)	$Fe_4(P_2O_7)_3$	59	MP Biomedicals, ICN211191
	Sodium tripolyphosphate (Tripoly-P)	$Na_5P_3O_{10}$	122	ALROS, AC39396
	Phosphorus pentoxide (P_4O_{10})	P_4O_{10}	103	ALROS, AC31582
	Apatite	$Ca_5(PO4)_3(OH,F,Cl)$	158	Alfa Aesar, 42535
	Ca-hydroxypatite	$Ca_5(PO4)_3(OH)$	219	Spectrum, C1264
Organophosphate	Adenosine 5′monophosphate (AMP)	$C_{10}H_{14}N_5O_7P$	105	Spectrum, AD113
	Guanosine diphosphate (GDP)	$C_{10}H_{15}N_5O_{11}P_2$	120	MP Biomedicals, ICN15121325
	Uridine diphosphate (UDP)	$C_9H_{14}N_2O_{12}P_2$	100	MP Biomedicals, ICN10120525
	Adenosine-5′-triphosphate (ATP)	$C_{10}H_{14}N_5O_{13}P_3Na_2\bullet_3H_2O$	126	Affymetrix, NC9948088
	Deoxyribonucleic acid (DNA)		118	Spectrum, DE115
	Ribonucleic acid (RNA)		121	Spectrum, RI104
	Lecithin		67	Alfa Aesar, AA3648630
	Liposome	$C_{44}H_{88}NO_8P$	78	Sigma sterile pyrogen-free preliposome formulation 5
	Phytic Acid	$C_6H_{18}O_{24}P_6$	161	TCI, P0409
Humic substance	Elliott soil humic acid standard		261	IHSS, 1S102H
	Wackish peat humic acid reference		201	IHSS,1R107H
	Leonardite humic acid standard		663	IHSS, 1S104H
	Pahokee peat humic acid standard		503	IHSS, 1S103H
	Pahokee peat humic acid reference		520	IHSS, 1R103H

P-starved algae were added to the 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 (APHA, 1967). 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 (APHA, 1967; Miller et al., 1978). 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:

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

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

2.4. SP uptake rate experiments

According to initial bioassay and P speciation results, twelve P compounds which tended to be in the soluble phase were selected to assess their uptake kinetics during fourteen day algal bioassays. A 300 mL stock solution with a synthetic P-free nutrient medium was prepared in triplicate for each P species. Then a 20 mL algal inoculum was added to these solutions to initiate these experiments. The incubation conditions for the bioassays were the same as previously described for our BAP experiments. In another set of experiments, the P species with persistently high SP concentration were selected to test for potential interactions (*e.g.*, inorganic particle formation or re-precipitation) between the P species and growth media during the fourteen day experiment. The same stock solution preparation and incubation conditions were used except these solutions were incubated in the dark without algae. On days 0, 1, 2, 4, 8 and 14, 50 mL of sample was collected and filtered for soluble P analyses, and the percentage of soluble P remaining in the solution was calculated relative to the initial P concentration.

3. Results

Four types of P were measured during chemical analyses, total P (TP), soluble P (SP), total reactive P (TRP) and soluble reactive P (SRP). Based on these analyses, TP was then divided into four operational categories: SRP, soluble non-reactive P (SnRP), particulate reactive P (PRP), and particulate non-reactive P (PnRP) (Fig. 1). The percentage BAP (BAP%) was calculated relative to TP. Some compounds showed BAP% greater than 100% which could be caused by analytical error or incomplete recovery during TP analyses. These results were rounded to 100%. P speciation and BAP% results were presented according to three different groups of P species (inorganic, organic and humic substances) as listed in Table 1.

3.1. Inorganic P compounds

The results for the seven inorganic P species analyzed are shown in Figs. 2 and 3. The majority of the P in the $CaHPO_4$ solution was classified as reactive according to the acid molybdate analyses with TRP accounting for 86% of TP. In the TRP pool, nearly half of the P was in the particulate phase with the remainder soluble (*i.e.*, passed through a 0.45 μm filter). The BAP experiment indicated all of the P in the $CaHPO_4$ solution was bioavailable for algal growth. $AlPO_4$, which is commonly found as a particulate, was as expected mostly classified as PnRP (88% of TP). Very little Al bounded P was used by the algae in the bioassay, with only 8% BAP. PP comprised a large percentage of the TP in the ferric pyrophosphate ($Fe_4(P_2O_7)_3$) solution with 42% of PRP and 48% of PnRP, however, the percentage of P in ferric pyrophosphate that was bioavailable was very low (≈0%). Phosphorus pentoxide (P_4O_{10}) and sodium tripolyphosphate (Tripoly-P) were classified as inorganic P based on their molecular structure, however, they were chemically quantified as SnRP which would operationally classify both as organic P. Approximately half of P in phosphorus pentoxide and all of P in sodium tripolyphosphate supported algal growth even though they were categorized as non-reactive P. Apatite is the most common phosphorus form in many soils and sediments. Two forms of apatite, naturally occurring apatite ($Ca_5(PO4)_3(OH,F,Cl)$) and Ca-hydroxypatite ($Ca_5(PO4)_3(OH)$), were tested in this study. The majority of P in naturally occurring apatite classified as SRP, which comprised 85% of TP. Conversely, PRP accounted for 62% of the Ca-hydroxyapatite. Of the remainder, 18% and 14% of the Ca-hydroxypatite was classified as PnRP and SRP, respectively. Both types of apatite had low bioavailability with natural apatite ≈0% bioavailable and Ca-hydroxyapatite only 24% BAP.

3.2. Organic P compounds

Nine commonly occurring organic P compounds were tested for this study (Figs. 2 and 3). Six, including AMP, UDP, ATP, GDP, RNA and DNA (see Table 1 for acronyms), were classified as 93%–98% SnRP (which is generally interpreted to represent dissolved organic P). Furthermore, these species were nearly entirely bioavailable with a range of BAP% of 89–100%. Lecithin is a heterogeneous group of substances found in animal and plant tissues that is comprised of a variety compounds, such as fatty acids, phosphoric acid, glycolipids and phospholipids. In the chemical analyses, 52% of lecithin was classified as PnRP, whereas SnRP accounted for another 30%. Thus, over 80% of P in lecithin was non-reactive with the acid-molybdate reagents. In the bioassay, approximately half of TP in lecithin was bioavailable. For phosphatidylcholine liposomes, 90% of the P was particulate with most of this fraction non-reactive. Nevertheless, all of the P in the

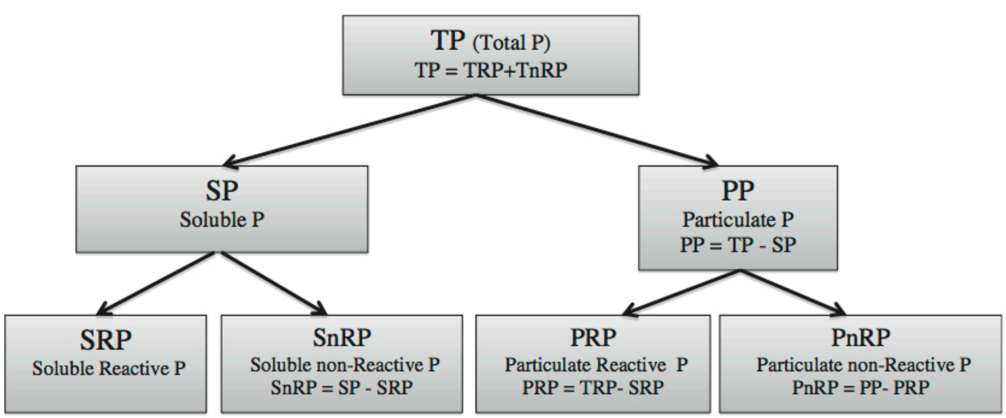

Fig. 1. Schematic of operational P speciation based on the chemical analyses. The total P and soluble P were determined through acid-molybdate spectrophotometric method after persulfate digestion. The reactive fractions (TRP, SRP) were determined without persulfate digestion.

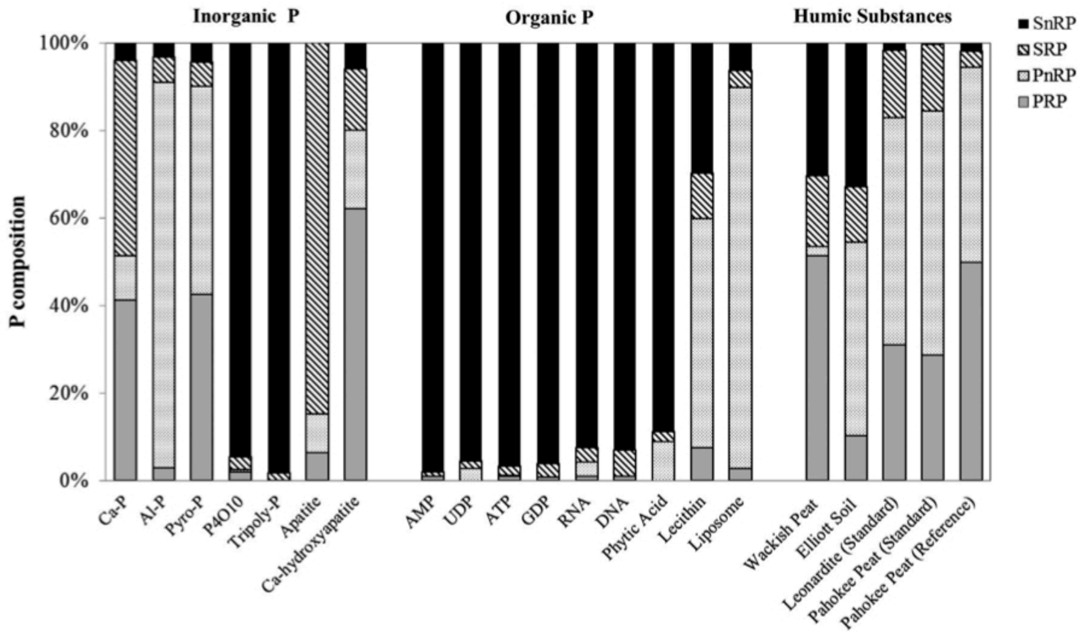

Fig. 2. P speciation results. The percentages of each category were calculated based on total phosphorus concentration.

liposomes tested was bioavailable. Phytic acid, which is also known as inositol hexakisphosphate, is commonly found in plant tissues. A very high proportion of the P in phytic acid was classified as SnRP (*i.e.*, 89%) and this form also had low bioavailability (*i.e.*, 17% BAP).

3.3. Humic substances

Five humic substance standards with relatively high and known P contents were tested (Figs. 2 and 3). The P composition of these humic substances varied between different types tested. The Wackish humic acid reference has ≈50% PRP with neglectable PnRP, and also included 30% and 15% SnRP and SRP, respectively. For the Elliott Soil humic acid standard, PnRP was 44% of TP and SnRP contributed 33%. For the Leonardite and Pahokee Peat humic acid standards, and the Pahokee Peat humic acid reference, PP was the main component (≈80% of TP). Further, for these three humic substances, approximately half of the particulate P was classified as PRP in the chemical analyses. For all the humic substances, a small (4%–16%), but non-neglectable, fraction was indicated as SRP. For

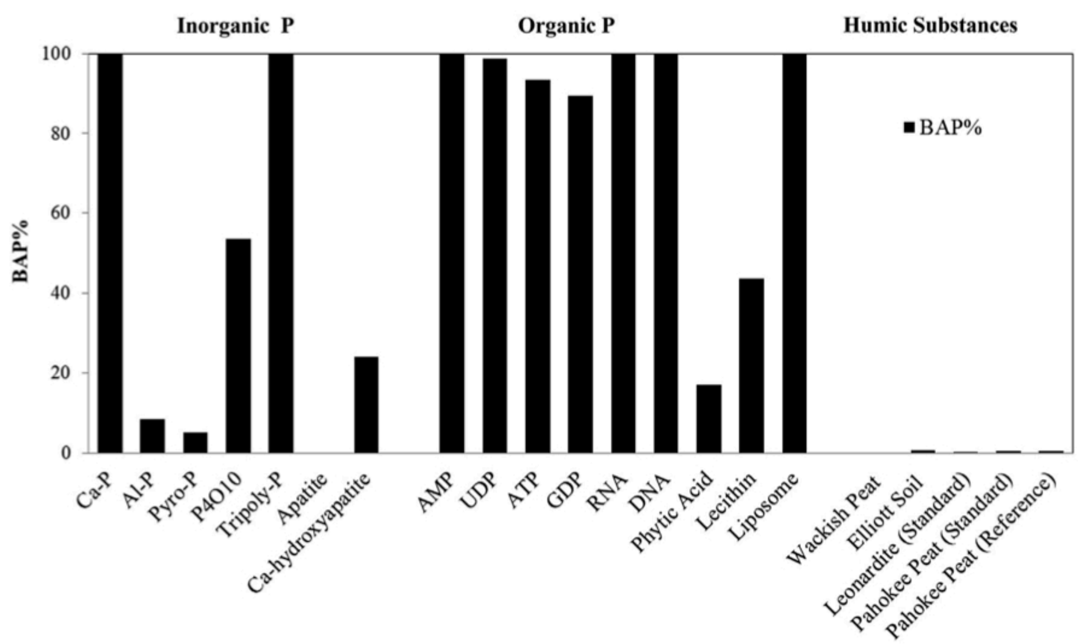

Fig. 3. BAP% for P species. BAP% is calculated based on TP.

all of the BAP analyses using the humic substances standards, no algal growth was observed during the 14 day incubation period which indicates none of the P in the humic substances was bioavailable even when they were chemically classified as reactive.

3.4. SP uptake rate experiments

Three inorganic P species were tested, including Ca-hydroxyapatite, phosphorus pentoxide and sodium tripolyphosphate. The uptake of SP was in the order of Ca-hydroxyapatite < P_4O_{10} ≈ sodium tripolyphosphate. After two days of incubation (Fig. 4), the uptake of Ca-hydroxyapatite reached a plateau with around 63% of the soluble fraction converted to the particulate phase. For P_4O_{10} and sodium tripolyphosphate, after 8 days of incubation, nearly all of SP had been converted to the particulate phase (96% and 98%, respectively).

Experiments with organic P compounds showed very rapid uptake of P (Fig. 5). Most of the organic P species tested (ATP, AMP, UDP, GDP) were almost entirely depleted within the first 2 days of the incubation. Less than 3% of these organic P forms remained as SP after 14 days which is consistent with the previous bioassay results that indicated these organic P species were nearly 100% bioavailable. The uptake of DNA was slower; however, it was also almost entirely used up day 14. The uptake of phytic acid reached a plateau after one week of incubation. The utilization of SP in phytic acid was incomplete with 27% of the SP in phytic acid treatment remaining during the second week of these experiments. Three humic acid standards were tested as well (Leonardite, Pahokee Peat and Elliott Soil humic acid standard), and the uptake trajectory of these complexes were similar to each other (Fig. 6). The SP concentrations decreased rapidly in the first 2 days of the experiments, and remained relatively constant thereafter. The final results at 14 days indicated that 67 ± 12% of the SP in these humic acid standards remained.

To account for non-biological conversion of SP to particulate P, which would be impossible for us to distinguish from algal uptake in our original experiment design, four P compounds which had persistently high SP concentration in the algal uptake experiments were incubated without algae and analyzed for changes in SP concentrations (Fig. 7). This was necessary to account for inorganic conversion of SP to particulate P due to physical–chemical reactions between the ions in our algal growth media and the humic standards we tested. This experiment indicated that the SP concentration in the Ca-hydroxyapatite solution continued to increase throughout the bioassay experiment. This implied that some particulate P dissolved in the medium, thus the actual SP uptake was likely higher than reported in the original SP uptake experiment. In the phytic acid solution, 7% of SP was converted to the particulate phase. This suggests that although SP decreased 63% in the algal uptake analysis, a small portion of that loss might have been caused by an absorption or particle formation mechanism. For the humic substances, SP% decreased 37% and 26% for the Pahokee Peat and Elliott Soil humic acid standards, respectively. This was very similar to the initial loss of SP in the original algal uptake experiments, and indicates that almost all of the initial loss of SP from the previous experiment could be explained by inorganic transformation of SP to particulate P rather than algae utilization. Thus, after accounting for absorption and particle formation, the SP in humic substance solutions did not appear to be used by algae during the prior uptake bioassay.

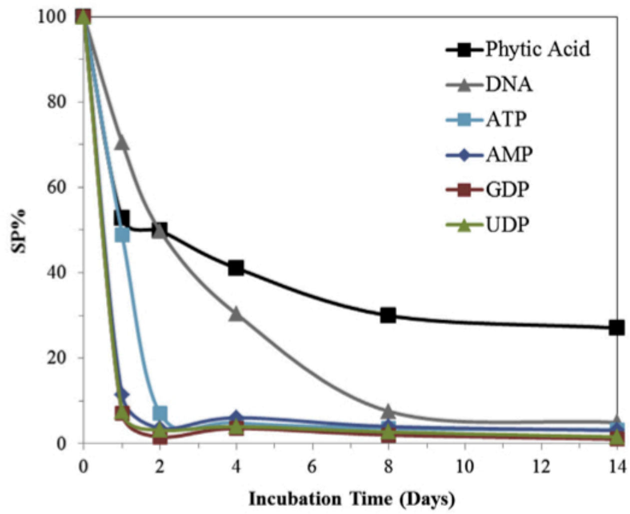

Fig. 5. SP% of selected organic P species during SP uptake bioassay. SP% is calculated based on initial SP value.

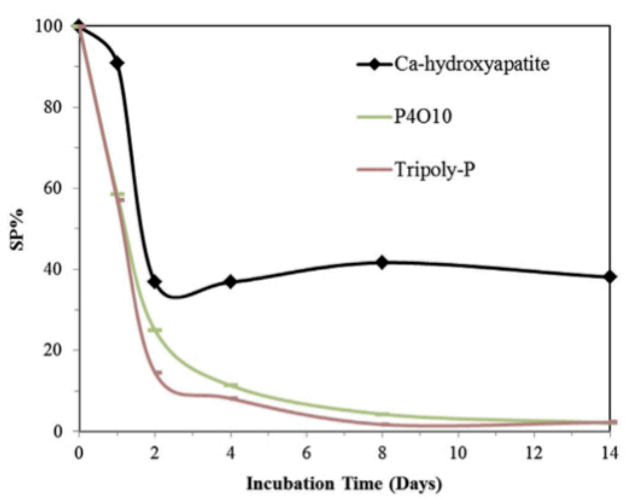

Fig. 4. SP% of selected inorganic P species during SP uptake bioassay. SP% is calculated based on initial SP value.

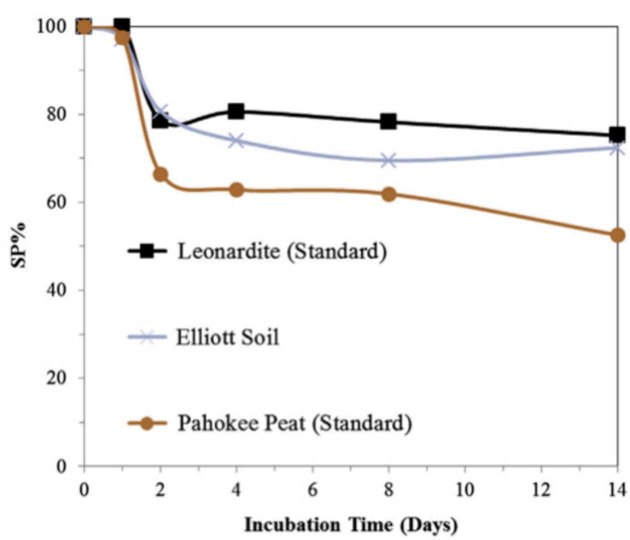

Fig. 6. SP% of selected humic substances during SP uptake bioassay. SP% is calculated based on initial SP value.

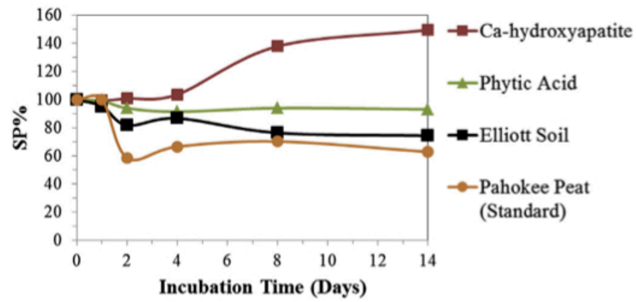

Fig. 7. SP% of selected P during SP uptake bioassay without algal incubation. SP% is calculated based on initial SP value.

4. Discussion

The principal method of characterizing P speciation in water samples is via filtration to separate particulate from "dissolved" P and the classic molybdenum blue method to differentiate "reactive" from non-reactive P (Murphy and Riley, 1962; John, 1970; Dick and Tabatabai, 1977). Using these operational categories, the most common assumption is that the soluble reactive fraction represents bioavailable orthophosphate (Reynolds and Davies, 2001). However, whether this chemical classification approach truly represents the bioavailability of P has been questioned by several authors (Rigler, 1968; Hatch and Reuter, 1999; Reynolds and Davies, 2001; Li and Brett, 2012). Our results showed several compounds that were operationally classified as reactive P had very low %BAP, whereas other compounds that were classified as non-reactive were nearly entirely bioavailable.

Because orthophosphate is commonly accepted as the most readily bioavailable phosphorus species, it is generally believed that the orthophosphate concentration represents a minimum value for BAP (Reynolds and Davies, 2001). The orthophosphate concentration has also traditionally, but incorrectly, been assumed to be equal to the fraction which develops the heteropoly blue species with ascorbic acid. Several authors have noted that the fraction termed SRP in this manner may overestimate the true orthophosphate concentration by including labile condensed and organic phosphates that are hydrolyzed during the analytical process (Rigler, 1968; Hatch and Reuter, 1999; Reynolds and Davies, 2001; Cade-Menun et al., 2002; Li and Brett, 2012). Apatites commonly occur in soils and sediments and are believed to have very low bioavailability, which is consistent with our BAP results that indicated the bioavailability of natural apapite and Ca-hydroxyapatite is 0% and 24%, respectively (Fig. 3). However, a large fraction of the P in the apatites we tested reacted with acid molybdate and was classified as SRP or PRP, with reactive P in natural apapite and Ca-hydroxyapatite comprising 91% and 76% of TP, respectively. In the naturally occurring apatite, the majority of the P was classified as SRP. This result also suggested using SRP as an indicator of the bioavailable fraction may greatly overestimate the true BAP for certain compounds, further suggesting using SRP to approximate BAP is problematic. This inference is similar to that of several classic studies that employed completely different methodologies to examine the bioavailability of naturally occurring SRP, *e.g.*, the Rigler ^{32}P bioassay (Rigler, 1968; Hatch and Reuter, 1999; Reynolds and Davies, 2001; Cade-Menun et al., 2002; Björkman and Karl, 2003; Li and Brett, 2012).

Dissolved organic P (DOP) is usually calculated as the difference between soluble P and SRP, which we refer to as SnRP. It has been reported that certain pyrophosphate, inorganic polyphosphates, and other inorganic derivatives may be included in this classification (John, 1970; Dick and Tabatabai, 1977; Karl and Yanagi, 1997). This is in agreement with our results where the inorganic P, pentoxides and sodium tripolyphosphate, were classified mostly as SnRP (95% and 98% of TP, respectively). Although these were non-reactive compounds, high bioavailability was observed in our bioassays. This suggests some condensed phosphate compounds in the inorganic P pool besides orthophosphate are also readily available inorganic P sources for algae and bacteria.

Interestingly, almost all the dissolved organic P compounds tested in our study were nearly entirely bioavailable for phytoplankton. This agrees with the hypothesis that DOP can be utilized as a supplemental P source in the absence of inorganic P. This hypothesis has been substantiated by various studies using different analytical approaches (Hedley et al., 1982; Boström et al., 1988; Tarapchak and Moll, 1990; Berman et al., 1991; Cotner and Wetzel, 1992; Björkman and Karl, 2003; Monbett et al., 2007). For instance, in Björkman and Karl's study (1994), the bioavailability factor (BF), a bioavailability index to quantify the equivalence of selected compounds relative to orthophosphate, was evaluated using marine bacteria and phytoplankton for 7 organic and 2 inorganic phosphorus species. Of these compounds, ATP, GDP, and UDP were found to have high BF values, as we also observed in study. It has been hypothesized that the hydrolysis process mediated by alkaline phosphatase enzymes is the main driver cleaving P free from organic molecules in aqueous systems (Solorzano and Strickland, 1968; Kobori and Taga, 1979). Our bioassay results indicate most organic forms of dissolved P are highly bioavailable, which further suggests using only operational P categories from chemical analyses is not sufficient to characterize P bioavailability.

Our nutrient uptake experimental results suggest that dissolved P in most nutrient limited lakes in streams will be comprised mostly of forms that have low bioavailability because readily used forms of P will be very rapidly taken up by primary producers and hence unlikely to persist in the dissolved phase within the euphotic zone of P limited systems. Conversely, most P in storm runoff is in the particulate phase which generally has a lower tendency to be utilized by phytoplankton (Karl and Craven, 1980; Ellison and Brett, 2006). Also, Sonzogni et al. (1982) hypothesized that the phosphorus forms that are typically in the particulate phase (*i.e.*, P associated with inorganics such as sand particles and clays) or phosphorus compounds inclined to form precipitates (*i.e.*, $AlPO_4$, $FePO_4$, etc.) are likely to rapidly settle to lake sediments and be disassociated from euphotic zone biological P cycling in thermally stratified lakes. Thus, low phosphorus bioavailability is a common characteristic of P limited systems (Ekholm and Krogerus, 2003).

Humic substances are often the dominant form of organic matter in rivers and lakes (Jones, 1992; Wetzel, 2001; Steinberg et al., 2006). Thus interactions between humic substances and phosphorus have the potential to greatly affect the P cycle (Aiken, 1985; Stevenson, 1994). In natural systems, phosphate associated with humic substances may account for >50% of the dissolved P pool (Eisenreich and Armstrong, 1980; Gerke, 2010). There is strong evidence that P in these complexes is bound via Al (III) and Fe (III) bridges rather than directly to the humic matter (Gerke, 1992; Gerke and Hermann, 1992). Thus equilibrium reactions between the humic, Al, Fe and orthophosphate content of the water can be of central relevance to P bioavailability, and the binding between orthophosphate and the humic-metal-complexes may be modified depending on the humic and metal content as well as the chemical conditions in the water (Gerke, 1992; Gerke and Hermann, 1992).

A series of experiments using a double isotope labeling method indicated pH and ionic strength can influence the interaction of humic substances with iron and phosphate (Jones et al., 1993), and low pH values will decrease the tendency of P and Al/Fe to bind to humic complexes (Gerke, 2010). For example, when samples are acidified to $pH < 1$ during the acid-molybdate assay, metal-P complexes on the outer surface of these molecules can be hydrolyzed, thereby releasing the Al/Fe and associated P into solution. This results in humic bound P being falsely classified as SRP. Conversely metal-P complexes in the inner areas of the humic molecules would not be readily hydrolyzed and would thus be classified as non-reactive dissolved organic P. In actuality, the P in these humic metal complexes is neither reactive nor truly organic because the P is primarily bound to Al or Fe. Previous studies have shown that up to 50% of the humic bound P may be hydrolyzed and therefore falsely classified as SRP during the acid molybdate analyses (Gerke, 2010). This can explain why our results indicated that there was a large portion of reactive P in the humic substance solutions we tested (≈50% of P as TRP). This also suggests that some of the SRP in natural water systems could be humic bound-P aliasing as orthophosphate. Our results also suggest most of the dissolved P associated with humic matter which operationally classifies as SnRP, which is classically considered to be a measure of organic P, may not actually be "organic" because the P in these complexes is actually bound to Al and/or Fe within the inner matrix of the humic complexes.

Our bioassays indicated the P fractions in the humic substances we tested had very low algal uptake and did not support algal growth. All of the humic standards tested in our study showed very low bioavailability suggesting the P in these humic-Al-Fe complexes is not utilized by algae. This is because humic-metal complexed phosphorus is associated with macromolecules that are far too large (*i.e.*, 10–30 K Daltons) to cross algal cell membranes (Battin et al., 2008; Gerke, 2010). Moreover, the P bound in these complexes cannot be cleaved free by the enzymes typically used by phytoplankton or bacteria because this P is actually tied to the Al/Fe rather than the organic matter. If much of the dissolved non-BAP in advance tertiary wastewater treatment plant (WWTP) effluents (Li and Brett, 2012) and natural waters are actually associated with humic-metal-complexes, it would be easier to hypothesize how these forms might behave and affect algal production in receiving water bodies. This might also suggest ways of targeting this non-BAP fraction in P removal processes, if that was deemed warranted.

Even though most of the compounds tested indicated SRP is a problematic index of bioavailable P, the SRP results for some P species were consistent with our BAP results. For instance, most of P in the calcium phosphate solution reacted with acid molybdate and also stimulated high yields in the algae bioassay. Also, aluminum phosphate, ferric pyrophosphate and phytic acid, which were classified as non-reactive or particulate P, had low bioavailability. Phytic acid, *i.e.*, inositol hexakisphosphate or phytate, is a primary phosphorus form in plant tissues, especially seeds and bran which are also a very important component of livestock and human diets. From a management perspective, there could be considerable quantities of phytic acid in WWTP effluents and other wastewaters such as industrialized animal feed operation discharges (Mallin and Cahoon, 2003). Phytic acid is a common phosphorus compound in lake sediments with concentrations ranging from 30 to 150 μg P g^{-1} of dry sediment (Degroot and Golterman, 1993; Golterman, 2006). Decomposition of aquatic and terrestrial plants is a major contributor of phytic acid to sediments (Golterman, 2006). Non-ruminant animals are incapable of digesting phytic acid because they lack the enzyme phytase which is required to release phosphate from inositol, which can result in excess phosphorus excretion to the environment (Mallin and Cahoon, 2003). The low bioavailability of phytic acid suggests that it might be an important P compound in sediments, as well as an important component of recalcitrant DOP (SnRP) in rivers and lakes.

The SP uptake experiments suggest that the uptake rate of some P compounds (such like P_4O_{10}, tripoly-P and most of organic P compounds tested) are much higher than the rate at which water is advected in most lakes, *i.e.*, <3% d^{-1} for lakes with water retention times > 1 month, this strongly suggests these compounds will not persist in natural systems even if they have high fluxes. These experiments re-confirmed that most forms of dissolved organic P forms were readily utilized, as noted by previous studies. However, the humic solutions tested had much slower uptake suggesting they are likely to persist in natural systems. The soluble P fraction of the humic substances decreased in the first two days, but most of the SP persisted in the solution as a non-bioavailable fraction. The dark experiments without algae suggested the decrease shown in the light bioassays was independent of algal uptake, *i.e.*, caused by chemical–physical reactions between the humic substances and the ions in the algal growth media leading to particle formation.

Table 2
Classification scheme.

Classification	Chemical category	Operational category	Bioavailability	Example compounds
1	Inorganic	Reactive	Bioavailable	Ca-P
2	Inorganic	Non-reactive	Non-bioavailable	Al-P, Pyro-P
3	Inorganic	Reactive	Non-bioavailable	Apatite and Ca-hydroxyapatite
4	Inorganic	Non-reactive	Mostly Bioavailable	Tripoly-P
5	Organic	Non-reactive	Bioavailable	ATP, DNA, RNA
6	Organic	Non-reactive	Non-bioavailable	Phytic acid
7	Humic substances	Halfly reactive	Non-bioavailable	Humic complexes

A new classification scheme was derived from our chemical analyses and bioassay results (Table 2), including 1) Dissolved phosphorus species which are inorganic, operationally classified as mostly reactive, and entirely bioavailable, *e.g.*, calcium phosphate; 2) Phosphorus species which are inorganic, operationally classified as both non-reactive and particulate, and non-bioavailable, *e.g.*, aluminum phosphate; 3) Dissolved phosphorus which is inorganic, operationally classified as reactive, and not bioavailable, *e.g.*, apatite; 4) Phosphorus which is inorganic, operationally classified as non-reactive, and mostly or entirely bioavailable, *e.g.*, sodium tripolyphosphate; 5) Dissolved phosphorus species which are organic, operationally classified as non-reactive, and almost entirely bioavailable, *e.g.*, nucleic acids; 6) Dissolved phosphorus which is organic, operationally classified as non-reactive and low bioavailability, *e.g.*, phytic acid; and 7) Dissolved phosphorus associated with humic substances, which was classified as reactive or non-reactive, but was almost entirely non-bioavailable. However, it would also be worth noting that the classification of P species might change under different conditions or if tested with different phytoplankton species. This classification scheme could serve as a baseline for comparing different P species with emphasis on the limitations of chemical analyses to characterize the bioavailability of P.

5. Conclusions

In conclusion, the results from our study clearly show the operationally defined P classification scheme from classic chemical methods is problematic from a management perspective. We hypothesize much of the dissolved phosphorus in surface waters operationally classified as dissolved and reactive according to the acid molybdate method is likely non-bioavailable because the phosphorus bound to humic metal complexes is not released by phosphatase enzymes and these complexes are too large to cross algal cell membranes. By comparing alternative chemical indicators, a faster and less labor intensive approach to estimate BAP independent of bioassays would be a welcome development. Using ultrafiltration to distinguish P associated with large sized humic complexes (*i.e.*, >10 K Daltons), may be an effective means to separate humic bound non-BAP which aliases as SRP or dissolved organic P from other dissolved P forms which are more likely to be bioavailable.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.06.024.

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III

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. Further, it is currently not known which operational P characterizations (*i.e.,* dissolved/particulate and reactive/non-reactive) best predict effluent phosphorus 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 phosphorus 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 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 ≥ 40% of the P classified as SRP was not bioavailable. Total BAP concentrations were on average 30% higher than soluble BAP (sBAP) concentrations, indicating the particulate P fraction was an important component of the bioavailable P pool for the effluents we tested. 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. Our results showed four tertiary processes which all used filtration combined with high chemical doses (> 30 mg L^{-1} as Fe or Alum) achieved extremely low effluent total BAP concentrations (> 5 µg L^{-1}) and also reduced the percentage total BAP (tBAP%) down to less than 30% of TP.

Key Words: Phosphorus, Nutrient Removal, Bioavailability of Phosphorus, Tertiary Treatment

1. **Introduction**

Phosphorus (P) is considered the proximal limiting macronutrient in many lakes, estuaries and marine systems (Tyrrell 1999, Schindler et al. 2008, Lewis and Wurtsbaugh 2008, Elser et al. 2007). As a key driver of eutrophication, excessive P loading can lead to environmental problems such as harmful algal blooms, and 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 discharges are required (Ragsdale 2007). Advanced tertiary treatment with multiple stages using chemical addition and filtration or membrane separation are commonly used to meet these low targets. Studies of the effluents from these systems have shown Soluble Reactive P (SRP), which is often assumed to be a measure of orthophosphate, only accounts for 33-53% of TP (Neethling et al. 2007, Gu et al. 2007). Lancaster et al., (2008) suggested 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 TP ≈ 20 μg L^{-1}, soluble refractory P is commonly the dominant form in the discharge (Neethling et al. 2007, Gu et al. 2007, Benisch 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. This assumption may greatly underestimate the environmental impact of P sources that are readily bioavailable (e.g., conventional secondary WWTP effluents), and over-estimate the importance of P source with low bioavailability (e.g., P bound to inorganic particles) (Reynold and Davis, 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 could be possible if these models fully accounted for P bioavailability.

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 indicated an efficient regeneration of phosphate from some organic and inorganic polyphosphate compounds (Bjorkman and Karl 2003, Bjorkman and Karl 1994, Bostrom et al. 1988). Consequently, utilization of organic P, and to a lesser extent recalcitrant inorganic P, could support algal productivity and control the bioavailable P reservoir (Monaghan and Ruttenberg 1999, Bjorkman and Karl 1994). 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, ATP, etc.) (Li and Brett 2013a). Furthermore, the dissolved phosphorus fraction associated with humic substances was almost entirely non-bioavailable (Li and Brett 2013a). 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 (American Public Health et al. 2005, Miller et al. 1978), which has been adapted to also include analyses of BAP. (Ekholm et al. 2007, Ellison and Brett 2006). In recent studies, algal bioassays were used to determine the presence of potentially bioavailable P from a wide variety of samples in various point and nonpoint sources as well as sediments. These studies indicated the BAP% is highest in secondary wastewater treatment plant effluents and lowest in lake and river sediments (Ekhom, 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, respectively (Ellison and Brett, 2006).

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 reagent, 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 soluble reactive P (SRP), soluble P (SP) and Particulate P (PP). There is no consensus on which particular 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 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 $\mu g\ L^{-1}$ (Twinch and Breen 1982). Further, using Rigler's ^{32}P bioassay experiments, Hudson et al. (2000) concluded operationally defined SRP concentrations may be 2-3 orders of magnitude higher than the actual phosphate concentration (Hudson et al. 2000). Several authors have suggested this disconnect between chemically determined SRP and actual phosphate concentrations could be due to the hydrolysis organic P compounds generating the blue color in chemical molybdate acid analysis (Rigler 1968, Bostrom et al. 1988, Kerouel and Aminot 1996). An alternative hypothesize for the mis-match is that some of the recalcitrant dissolved non-BAP fraction (e.g., humic-metal complexes or phytic acid) "aliases" as SRP because P is freed at very low pH during the acid molybdate analysis (Li and Brett 2013a). These and other studies suggest that SRP is not a reliable predictor of BAP or phosphate.

Bradford and Peters (1987) developed regression models predicting bioavailable P 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 TRP provided a better measure of the impact of effluent P on algae growth than did total P. The statistical relationship between operational phosphorus categories and BAP on a wide range of effluents from phosphorus removal processes was investigated in this study. 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 nonreactive and compared to BAP concentrations. These analytically measured parameters were regressed against the both sBAP and tBAP to assess the best correlates of biological response. The relationships between TP and BAP% in different effluents were compared to identify the most efficient P removal treatment processes. Also, selected samples were filtered through different filters to assess the impacts of filter size on P speciation.

2. Methods

2.1 Sampling

A total 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 was 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-hr compose samples collected in one liter acid washed (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 hours.

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	√

2.2 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 minutes of autoclave-mediated digestion (120 °C, 100 kPa, with $K_2S_2O_8$ and H_2SO_4) of an unfiltered sample. TRP was 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®) and analyzed for SP and SRP and compared with samples passed through the 0.45 µm filter.

2.3 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 ($r^2 \approx 0.99$).

200 mL effluent samples were autoclaved for 45 mins and paired 200 mL samples were filtered through a 0.45µm 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 ºC 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 (American Public Health et al. 2005, Miller et al. 1978). 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:

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

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

The BAP derived from the autoclaved samples represented the value for total BAP (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.

3. Results

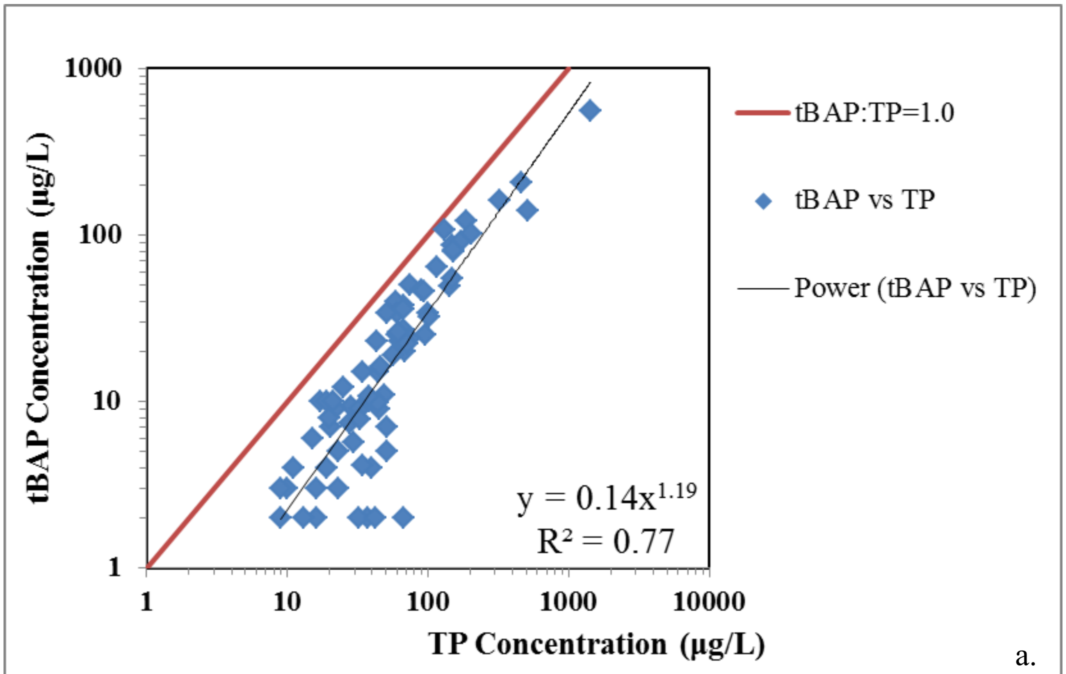
3.1 Comparison of chemical measures with bioassay results

3.1.1 tBAP vs. TP and TRP

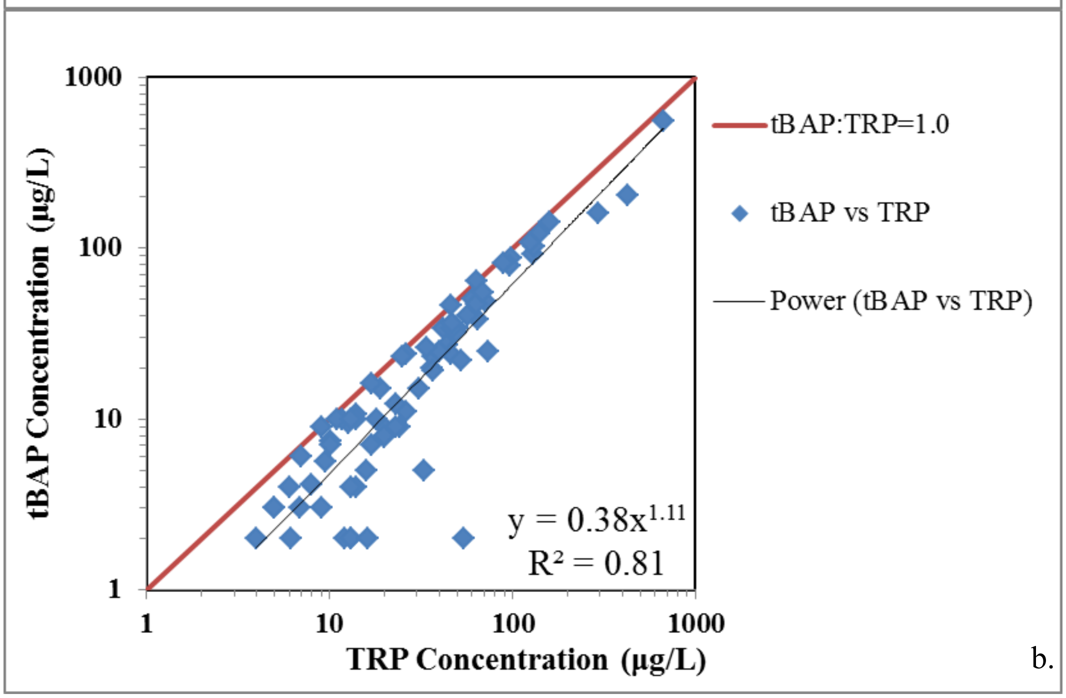
Our results showed that tBAP averaged only 34 ± 17% of TP with a moderate correlation between these fractions ($r^2 = 0.77$) (Fig. 1a). The tBAP/TRP ratio showed tBAP was also consistently less than TRP, with this ratio averaging 0.61 ± 0.24. There was a moderately strong statistical relationship between tBAP and TRP ($r^2 \approx 0.81$) (Fig. 1b).

3.1.2 sBAP vs. SP and SRP

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.



1000
100
10
1
tBAP Concentration (µg/L)
1
10
100
1000
10000
TP Concentration (µg/L)
tBAP:TP=1.0
tBAP vs TP
Power (tBAP vs TP)
y = 0.14x^1.19
R² = 0.77
a.



1000
100
10
1
tBAP Concentration (µg/L)
1
10
100
1000
TRP Concentration (µg/L)
tBAP:TRP=1.0
tBAP vs TRP
Power (tBAP vs TRP)
y = 0.38x^1.11
R² = 0.81
b.

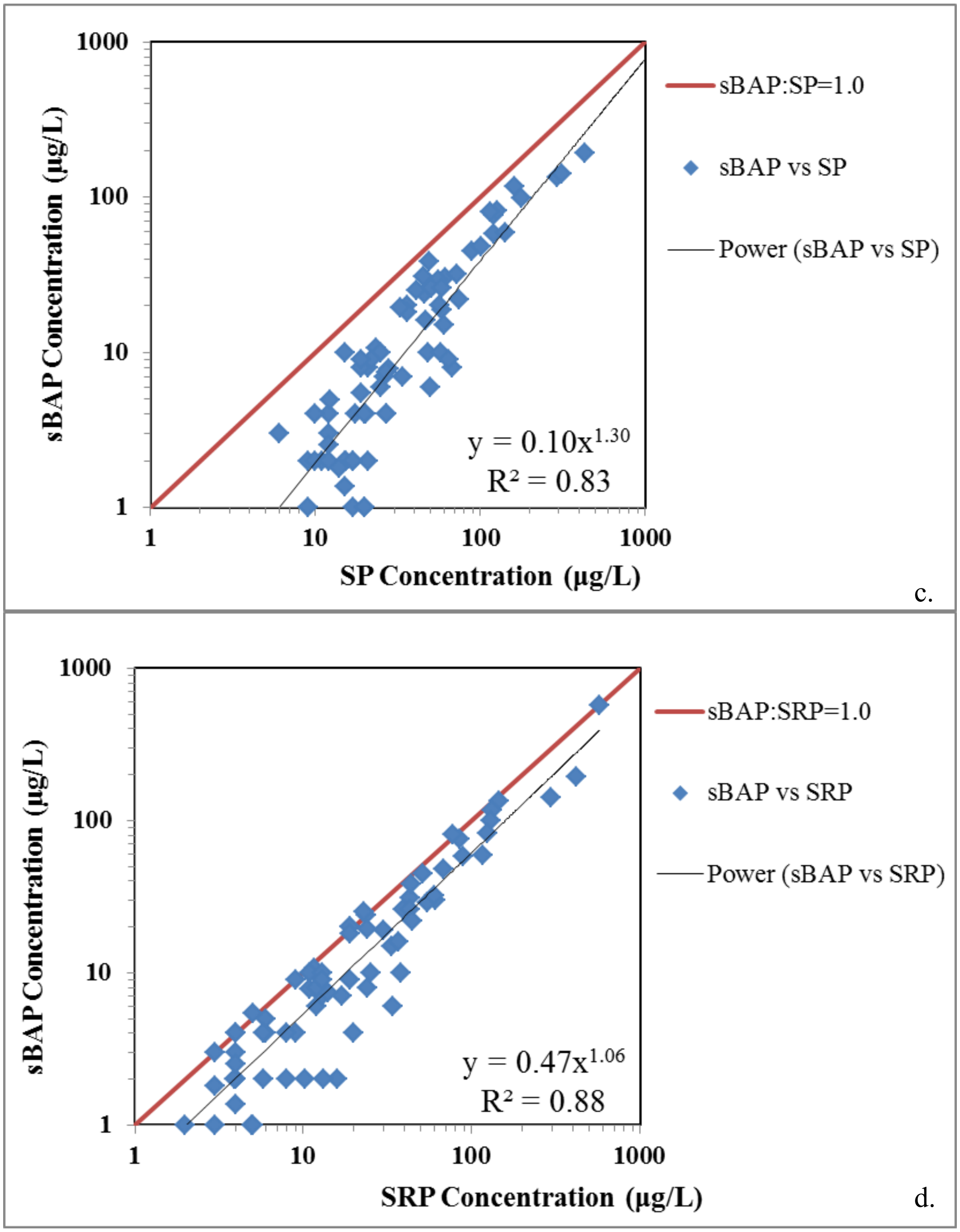


Fig. 1. Comparison of tBAP and TP (a), tBAP and TRP (b), sBAP and SP (c), sBAP and SRP (d).

3.2 Impact of different P removal processes

Figs. 2 and 3 below showed the average difference in P speciation and BAP for the effluents from the four different treatment processes. 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 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 additions).

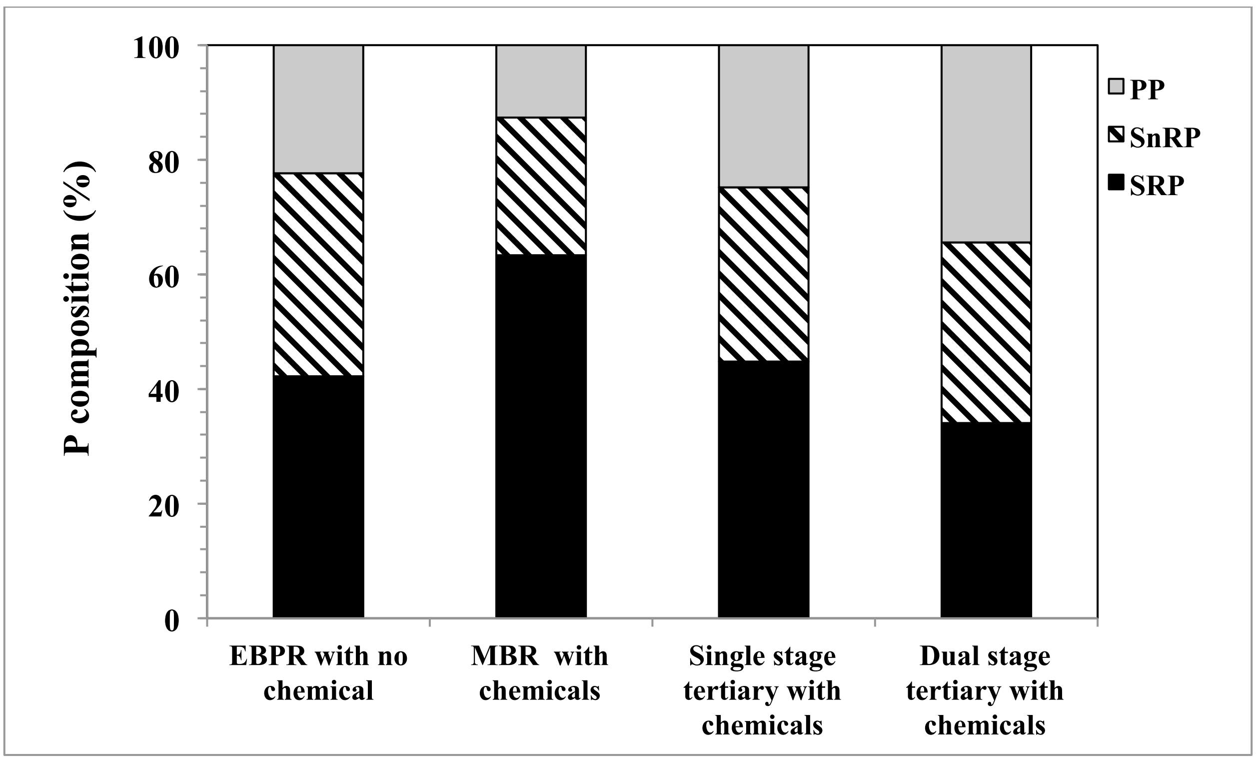


Fig. 2. P concentrations comparison for four P removal process categories.

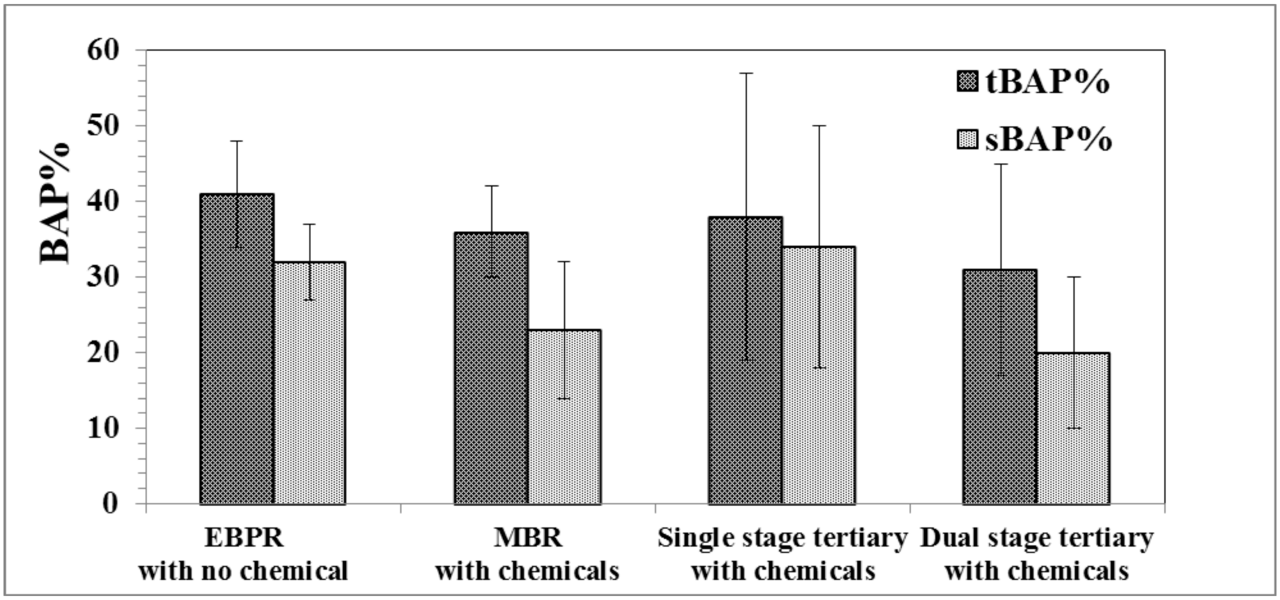


Fig. 3. BAP% comparison for four P removal process categories.

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 Hayden Wastewater Research Facility (Hayden WRF), TP was reduced to 69 ± 18 $\mu g \cdot L^{-1}$. After the second stage, TP was reduced to 36 ± 9 $\mu g \cdot L^{-1}$, with only 33 ± 15% bioavailable (tBAP = 12 ± 7 $\mu g \cdot L^{-1}$). In the effluent from the Ruidoso Village Regional Wastewater Treatment Plant (Ruidoso Village RWTP), TP was 51 ± 17 $\mu g \cdot L^{-1}$ with 32 ± 13% bioavailable. In the final effluent from Broad Run Water Reclamation Facility (Broad Run WRF), TP was only 24 ± 7 $\mu g \cdot L^{-1}$ with 9 ± 3 $\mu g \cdot L^{-1}$ tBAP. Of the three technologies tested in the Coeur d'Alene pilot Advanced Wastewater Treatment Facility (Coeur d'Alene AWTF) [Membrane Bio-reactor (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 (which had 4 ± 2 $\mu g \cdot L^{-1}$) out of 13 ± 5 $\mu g \cdot L^{-1}$ of TP compared to Coeur d'Alene-MBR and Coeur d'Alene-CUMF (tBAP = 15 ± 8 $\mu g \cdot L^{-1}$ and 6 ± 3 $\mu g \cdot L^{-1}$, respectively). Three chemical filtration systems, i.e., the P removal process at the Farmers Korner Wastewater Treatment Plant (Farmers Korner WTP), the Metropolitan Syracuse Wastewater Treatment Plant (Metropolitan Syracuse WTP) and the Iowa Hill Wastewater Treatment Plant (Iowa Hill WTP) obtained extremely low tBAP concentration (i.e., 5 ± 3 $\mu g \cdot L^{-1}$, 3 ± 2 $\mu g \cdot L^{-1}$ and 3 ± 1 $\mu g \cdot L^{-1}$, respectively). These systems also had very low tBAP% values (i.e., 15 ± 8%, 8 ± 4% and 17 ± 2%, respectively).

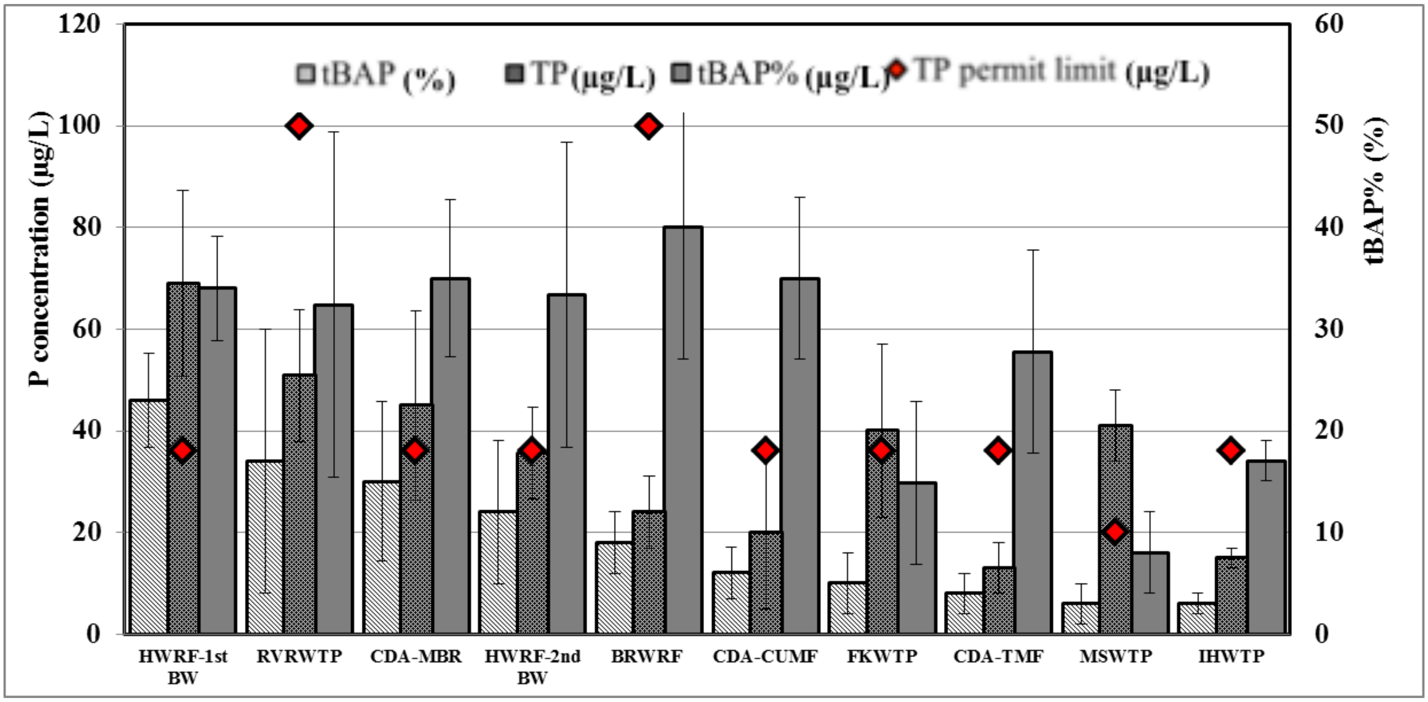


Fig. 4 tBAP concentration (µg/L), TP concentration (µg/L), tBAP% (%) and TP permit limit (µg/L) for 10 effluents with lowest tBAP. (Note: **HWRF-1st BW:** Hayden Wastewater Research Facility continuous backwash gravity sand filters process 1st stage. **RVRWTP**: Ruidoso Village Regional Wastewater Treatment Plant. **CDA-MBR:** Coeur d'Alene Advanced Wastewater Treatment Plant Membrane Bio-Reactor. **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.)

3.3 Impact of chemical addition

The effect of primarily biological and primarily chemical based P removal processes (alum or ferric based) was assessed by comparing the phosphorus characteristics of two biological based processes (North Durham Water Reclamation Facility and Snoqualmie Wastewater Reclamation Facility) against 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 concentrations were statistically significantly higher in the biologically based systems for all forms considered, and the proportion of the phosphorus that was bioavailable was also higher. However, the percent composition as operationally defined was not different. The net effect of higher concentrations and higher relative bioavailability was a 5.5 times higher tBAP concentration in the biologically based systems.

The biological and chemical-based membrane systems at Coeur d'Alene Advanced Wastewater Treatment Plant 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 particulate P - which was extremely low in both effluents as expected for membrane systems.

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[a]
	SP	**293** ± 386	**47** ± 53	0.01[a]
	TRP	**201** ± 225	**42** ± 48	0.00[a]
	SRP	**177** ± 202	**32** ± 48	0.00[a]
	SnRP	**115** ± 208	**15** ± 12	0.02[a]
	PP	**87** ± 89	**17** ± 13	0.00[a]
	tBAP	**151** ± 172	**27** ± 33	0.00[a]
	sBAP	**140** ± 183	**20** ± 30	0.00[a]
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[a]
	sBAP%	**49** ± 11	**34** ± 17	0.00[a]
Ratio	tBAP/TRP	**0.79** ± 0.18	**0.59** ± 0.24	0.01[a]
	sBAP/SRP	**0.78** ± 0.20	**0.62** ± 0.28	0.05[a]
	sBAP/SP	**0.49** ± 0.11	**0.34** ± 0.20	0.00[a]

[a] the difference is significant with P less than 0.05

Table 3 Comparison of MBR and 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[a]
	SP	**43** ± 18	**13** ± 5	0.00[a]
	TRP	**25** ± 14	**7** ± 3	0.02[a]
	SRP	**23** ± 13	**6** ± 3	0.01[a]
	SnRP	**20** ± 5	**7** ± 3	0.00[a]
	PP	**1** ± 1	**0** ± 0	0.15
	tBAP	**15** ± 8	**4** ± 2	0.00[a]
	sBAP	**10** ± 3	**2** ± 1	0.00[a]
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

[a] the difference is significant with P less than 0.05

3.4 Comparison of tBAP and sBAP

Phosphorus bioavailability studies that focus on particulate P 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. 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; $r^2 = 0.90$, $n = 75$. 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) (Fig. 5).

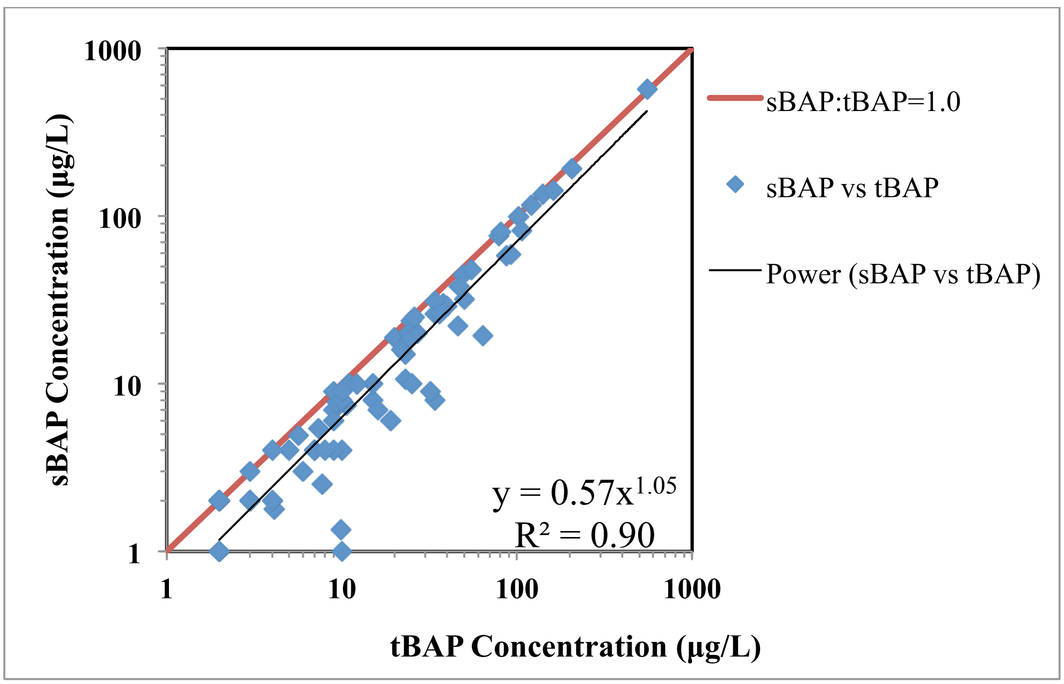


Fig. 5. Comparison of autoclaved and filtered BAP.

3.5 Comparison of filter size

In this study, two filter pore sizes (0.45 µm and 0.2 µm) were used to determine if 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 2011 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).

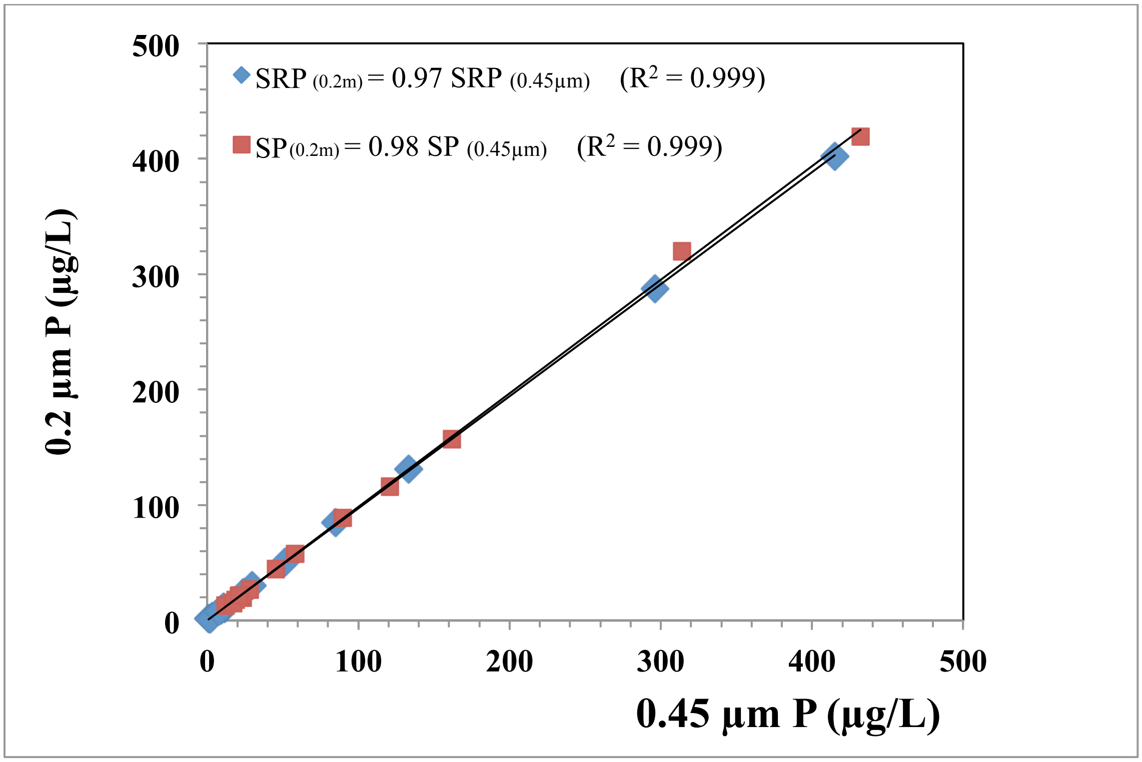


Fig. 6. Comparison of SP and concentration from samples passed through 0.45 µm and 0.2 µm filter size. (n=14)

4. Discussion

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 microbial plankton community can impact 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, amongst all the operational categories tested, was the best predictor for the total bioavailable fraction in the WWTP effluents ($r^2 \approx 0.81$). 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 bioavailable P as commonly assumed. The comparison of the TP and tBAP% in the different processes indicated higher chemical doses might be able to achieve lower TP with a smaller fraction of the P being bioavailable. Our results also show 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 lab 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 (Hudson et al. 2000, Bradford and Peters 1987, Smith et al. 1999, 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 non-point sources and effluents from advanced tertiary treatment facilities with extremely 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 total BAP 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 is 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 aliases as reactive but has low bioavailability (Li and Brett 2013a).

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 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 that made from experiments conducted on individual P containing compounds that showed several dissolved P compounds had very low bioavailability (i.e., ferric pyrophosphate and etc.) (Li and Brett 2013a). 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. (2008) suggested that some phosphorus that operationally classifies as SRP when environmental P concentrations are low may actually consist of recalcitrant colloidal or polymerised 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 orthophosphates and redox-sensitive metals (Reynolds and Davies 2001, Bostrom et al. 1988). 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 there are components of the dissolved P pool which alias as SRP in the analytical protocol, but which are not actually bioavailable for algae.

This study clearly showed that phosphorus bioavailability and composition varies with the nutrient removal process as well as between individual treatment plants. Overall an average >50% of the effluent P was recalcitrant relative to 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 tBAP% below 50%. In some cases, the tertiary treatment processes decreased tBAP to ≈ 10% of TP. This suggests that tertiary treatment processes can be very efficient in removing the most bioavailable P components thereby significantly reducing the potential impact on receiving waters. Chemical addition during filtration markedly decreased the portion that can be easily utilized by phytoplankton as the SRP in effluents from the plants with chemical addition was 33% lower than for the effluents without chemical treatment. Although there was more particulate P from the chemical treatment plants, this fraction can be readily removed through filtration. The bioavailable phosphorus fraction was also substantially decreased by chemical addition. The average tBAP% in the EBPR treatment systems without chemical addition was 41 ± 7% while the average tBAP% for the processes with chemical addition was reduced to 35 ± 15%. These results indicate chemical addition is warranted if low %BAP in the effluent is necessary to protect receiving waters.

In the ten processes, which had lowest effluent tBAP, relatively high chemical doses (ranging from 6 $mg \cdot L^{-1}$ to 100 $mg \cdot L^{-1}$ 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 the Fig. 4. In six cases, a future limit of 36 $\mu g \cdot L^{-1}$ has been proposed. In particular, a TMDL permit limit of 20 $\mu g \cdot L^{-1}$ starting December, 2015, has been proposed for the Metropolitan Syracuse WTP.

A variety of tertiary systems here 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 Bio-Reactor (MBR) and media or membrane filtration with high chemical doses, appeared to have the highest potential to remove most bioavailable P. A two-stage continuous backwash gravity sand filters 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 $mg \cdot L^{-1}$ as Fe) and second pass (10 $mg \cdot L^{-1}$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 $mg \cdot L^{-1}$ at the first stage and 45 $mg \cdot L^{-1}$ 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 $\mu g \cdot L^{-1}$, 12 ± 7 $\mu g \cdot L^{-1}$ and 6 ± 3 $\mu g \cdot L^{-1}$, 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 $mg \cdot L^{-1}$) 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 Enhanced Biological P removal (EBPR) with 9.4 $mg \cdot L^{-1}$ of Alum. Even though there was no or low chemical addition in these MBR systems, the tBAP concentrations were still below 20 $\mu g \cdot L^{-1}$ in the effluents. These results suggest biological P removal with modest chemical addition, which removes bioavailable P, plus a membrane system to target particulate P can also achieve efficient tBAP removal.

The last four treatment plants (Farmer Korner WTP, Coeur d'Alene-TMF, Metropolitan Syracuse WTP and 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 alum) 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 is 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 Iowa Hill WTP is accomplished by activated sludge biological treatment, biological aerated nitrification, chemical coagulation using alum and polymer (100 mg·L^{-1} as dry alum), 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.

Previous research showed autoclaving soil samples increased the estimated BAP by approximately 60% compared to 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 organic P might be hydrolyzed to soluble reactive phosphate during autoclaving (Jansson 1977). Our results were similar to previous studies showing sBAP averages 70% of tBAP (Bradford and Peters 1987). We interpret this as evidence that a substantial fraction of the particulate P is bioavailable. Because tBAP determinations were generally higher than sBAP, we feel it is best to base our main conclusions on the bulk bioavailable P fraction in order to provide the most conservative estimates of WWTP effluent eutrophication potential.

Standard Methods suggests using a 0.45 µm filter to separate the dissolved and particulate P fractions (American Public Health et al. 2005). However, some authors have suggested colloidal P species in the 0.2-0.45 µm size range could in some cases be a substantial fraction of the dissolved P (Turner et al. 2004). Conversely, our results indicate that P species in the 0.2 to 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 only had 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.

5. Conclusions

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. A high percentage (≥ 40%) of TP classified as SRP but much of this SRP was not bioavailable. This study indicates that P removal processes employing high chemical doses for flocculation followed by filtration most effectively removed the bioavailable P fraction. Furthermore, our results show that BAP concentrations in autoclaved samples were consistently higher than for filtered samples suggesting autoclaved BAP samples are a more conservative because they include the particulate P fraction which may also be bioavailable. Future research on the conservative estimates of soluble organic P mineralization rates from bioassay experiment could provide better insights to integrate bioavailability of P into eutrophication management models.

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Supplemental Information:

Table 1. General Plant Information

Plant	Abbreviation	Capacity	Chemical Addition	Permit (mg/L)	Effluent TP conc. (mg/L)	Location	Sampling #
EBPR with no chemical addition							
Coeur d'Alene Advanced Wastewater Treatment Plant - Membrane Bio-Reactor	CDA-MBR	0.05 MGD	None	0.036	0.045	Coeur D'Alene, ID	5
Snoqualmie Wastewater Reclamation Facility	SWRF	3 MGD	None	NA	0.139	Snoqualmie ,WA	3
North Durham Water Reclamation Facility	NDWRF	20 MGD	None	0.5	0.524	Durham, NC	5
MBR Processes with chemical addition							
Broad Run Water Reclamation Facility	BRWRF	11 MGD	9.4 mg/L as Alum	0.1	0.029	Loudoun, VA	5
Ruidoso Village Regional Wastewater Treatment Plant	RVRWTP	1.9 MGD	Alum	0.1	0.051	Ruidoso, NM	5
Single stage tertiary - with chemical addition							
Coeur d'Alene Advanced Wastewater Treatment Plant – Tertiary Membrane Filter	CDA-TMF	0.05 MGD	50 mg/L as Alum	0.036	0.013	Coeur D'Alene, ID	5
Metropolitan Syracuse Wastewater Treatment Plant	MSWTP	84.2 MGD	30 mg/L as Ferric Chloride	0.020	0.041	Syracuse, NY	5
Blue Plains Advanced Wastewtaer Treatment Plant	BPAWTP	370 MGD	2-3 mg/L as Ferric Chloride	0.18	0.068	Washington, DC	5
Hayden Wastewater Research Facility continuous backwash gravity sand filters process 1st stage	HWRF-1st BW	1.2 MGD	15 mg/L as Fe	0.036	0.069	Hayden, ID	5
City of Las Vegas Water Pollution Control Facility	LVWPCF	91 MGD	Alum	0.1	0.094	Las Vegas, NV	1
South Durham Water Reclamation Facility	SDWRF	20 MGD	Alum	0.23	0.195	Durham, NC	5

Dual stage tertiary - with chemicals									
Iowa Hill Wastewater Treatment Plant	IHWTP		No, –	Yes, –		Yes, alum		Yes, –	
Coeur d'Alene Advanced Wastewater Treatment Plant - continuous backwash gravity sand filters process	CDA-CUMF	Yes, –	No, alum	Yes, –				Yes, ferric	Yes, ferric
Hayden Wastewater Research Facility continuous backwash gravity sand filters process 2nd stage	HWRF-2nd BW		No, –	Yes, –				Yes, ferric	Yes, ferric
Farmers Korner Wastewater Treatment Plant	FKWTP		No, –	Yes, –		Yes, alum		Yes, –	
Rock Creek Wastewater Treatment Plant	RCWTP	Yes, alum	Yes, –	Yes, –		Yes, alum		Yes, –	
Durham Advanced Wastewater Treatment Facility	DAWTP	Yes, alum	Yes, –	Yes, –		Yes, alum		Yes, –	

Table 2 Treatment Process Information.(Note: "Yes" - the process is applied; "No" - the process is not applied; "-" - no chemical addition; "alum" - with alum addition; "ferric" -with ferric addition.)

Plant	Abbreviation	Primary	Secondary			Tertiary			
			Secondary EBPR	Clarifier	Membrane	Floc/ Sedimentation	Membrane	Filter	2nd Filter
EBPR with no chemical addition									
Coeur d'Alene Advanced Wastewater Treatment Plant - Membrane Bio-Reactor	CDA-MBR	Yes, –	Yes, –		Yes, –				
Snoqualmie Wastewater Reclamation Facility	SWRF		Yes, –	Yes, –				Yes, –	
North Durham Water Reclamation Facility	NDWRF	Yes, –	Yes, –	Yes, –				Yes, –	
MBR Processes with chemicals									
Broad Run Water Reclamation Facility	BRWRF	Yes, alum	Yes, –		Yes,alum				
Ruidoso Village Regional Wastewater Treatment Plant	RVRWTP		Yes , –		Yes,alum				
Single stage tertiary - with chemicals									
Coeur d'Alene Advanced Wastewater Treatment Plant – Tertiary Membrane Filter	CDA-TMF	Yes, –	No, alum	Yes, –			Yes, alum		
Metropolitan Syracuse Wastewater Treatment Plant	MSWTP	Yes, –	No, –	Yes, ferric		Yes, ferric			
Blue Plains Advanced Wastewtaer Treatment Plant	BPAWTP	Yes, ferric	No, –	Yes, –				Yes, –	
Hayden Wastewater Research Facility continuous backwash gravity sand filters process 1st stage	HWRF-1st BW		No, –	Yes, –				Yes, ferric	
City of Las Vegas Water Pollution Control Facility	LVWPCF	Yes, ferric	Yes, partial	Yes, –				Yes, alum	
South Durham Water Reclamation Facility	SDWRF	Yes, –	Yes, –	Yes, alum				Yes, –	
Dual stage tertiary - with chemicals									
Iowa Hill Wastewater Treatment Plant	IHWTP		No, –	Yes, –		Yes, alum		Yes, –	
Coeur d'Alene Advanced Wastewater Treatment Plant - continuous backwash gravity sand filters process	CDA-CUMF	Yes, –	No, alum	Yes, –				Yes, ferric	Yes, ferric
Hayden Wastewater Research Facility continuous backwash gravity sand filters process 2nd stage	HWRF-2nd BW		No, –	Yes, –				Yes, ferric	Yes, ferric

IV

The quantification of dissolved phosphorus mineralization kinetics for effluents from advanced nutrient removal processes

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ABSTRACT:

Given the importance of the watershed protection plans, direct determination of phosphorus (P) mineralization rates in advanced wastewater treatment facility effluents is crucial for developing the most protective strategies for minimizing eutrophication in receiving surface waters. In this study, bioassays were used to determine the mineralization rate of dissolved P in effluents from a broad range of advanced nutrient removal technologies (Membrane Biological Reactor, traditional biological, tertiary membrane, Blue PRO™, and etc.). Mineralization kinetics were described by a gamma model and three first-order decay models. A traditional one-pool model correlated poorly with the experimental data (i.e., $r^2 = 0.73 \pm 0.09$), whereas two-pool model and three-pool model performed much better (i.e., $r^2 > 0.9$). These models provided strong evidence for the existence of recalcitrant P in the effluents from these tertiary facilities. The Gamma model showed the mineralization of organic P followed a reactive continuum and further suggested the partitioning of P loads with different bioavailability levels should be accounted for the future modeling practices. From the modeling perspective, although the gamma model should be considered as theoretically correct model, the results also suggested simpler two-pool model and three-pool model could provide similar fit depending on the effluents.

Key Words: Phosphorus Uptake Model, Bioavailability of Phosphorus, TMDL, Nutrient Removal

1. Introduction

Among the environmental factors acting as accelerators of eutrophication, phosphorus (P) is the most widely investigated because of the recognition that it is the most controllable of the limiting nutrients (Schindler et al. 2008, Gurkan et al. 2006). Extensive efforts have been devoted to developing management schemes for P loading control in impaired surface water systems. Phosphorus in Wastewater Treatment Plant (WWTP) effluents and natural systems is found in a variety of forms including dissolved inorganic phosphate, dissolved organic compounds, and both organic and inorganic molecules attached to particulate matter (Bradford and Peters 1987, Dodds 2003, Li and Brett 2013b). Several studies have demonstrated that certain organic P forms are highly bioavailable for phytoplankton (Cotner and Wetzel 1992, Bostrom et al. 1988, Li and Brett 2013a). Other studies have demonstrated that a majority of the dissolved P in some advanced P removal effluents is recalcitrant and probably undergoes much slower uptake and utilization (Christen 2007, Effler et al. 2012, Li and Brett 2012, 2013b). Bioassays with pure P compounds further suggested this recalcitrant fraction consist of molecules that are too large to be hydrolyzed or are bounded in humic complexes that cannot be cleaved free by the enzymes typically used by phytoplankton or bacteria (Li and Brett 2013a, Gerke 2010). Assigning the same uptake rate for this recalcitrant pool as for other organic P fractions is problematic and could introduce a substantial prediction error in water quality models. Understanding and characterizing the uptake kinetics of P with slower turn overtimes is therefore crucial from management perspective.

Although several studies have investigated potential phosphorus mineralization rates in natural waters or sediments, little is known about the biological uptake mechanism for the dissolved organic P in advanced WWTP effluents (Wilson et al. 2010, Reitzel et al. 2007). Nevertheless, the function embedded in most water quality models to represent the mineralization process from organic to inorganic P is generally described by first-order kinetics with a single constant decay coefficient, k. This statement that all organic P compounds decompose at same rate has long been challenged since numerous studies on natural organic matter have shown considerable heterogeneity for the biodegradation kinetics of different organic species (Guillemette and del Giorgio 2011, Giorgio and Davis 2003, Vahatalo et al. 2010). Ahlgren et al. (2005) distinguished individual components of organic P by ^{31}P NMR technology and found certain forms of organic P in lake systems, such as pyrophosphate, are mineralized to PO_4 with a half-life time of 10 years; whereas mono- and diseter-P compounds may persist for several decades in sediments (Ahlgren et al. 2005). To partition the bioavailability gradient that might exist in the organic compounds, first-order decay functions with multiple terms (commonly two or three) have been proposed to represent the various pools of organic matter (Ogura 1975, Harmon et al. 2009, Westrich and Berner 1984). This approach can differentiate the organic compounds into several classifications with specific decay rates designated individually.

$$C_{tot,t} = \sum_{1}^{i} C_{i,0} \exp(-k_i t)$$

Where, $C_{tot,t}$ = total bioavailable fraction;

$C_{i,0}$ = bioavailable fraction in i pool;

k_i = mineralization rate in i pool;

t = mineralization time.

Another alternative is the Gamma model which was first used to characterize the decay of recalcitrant organic matter by Boudreau and Ruddick (Boudreau and Ruddick 1991). These authors hypothesized that organic matter bioavailability should vary along a continuum of highly available to very low availability according to the following function:

$$C(k, 0) = C_0 \, \Gamma(\nu)^{-1} \, k^{\nu-1} \exp(-a*k)$$

Where, C = total bioavailable fraction;

C_0 = initial concentration;

k = mineralization rate;

a = the average life-time of the more reactive components of the organic pool;

ν = the shape of the distribution near k = 0.

This reactivity continuum approach is featured with a time-variable function for the decomposition rate, k, and a spectrum of reactive forms that is a continuous definition of an infinite distribution (Boudreau and Ruddick 1991). In studies on natural organic matter (NOM), the Gamma model has been shown to characterize the biodegradation continuum in a realistic manner (Vahatalo et al. 2010). However, those models primarily characterized with the biodegradation of NOM, while the kinetics associated with the transformation of dissolved P remains unexplored. Although these Gamma models for NOM could potentially characterize dissolved P mineralization, they could not be used as surrogate due to the C to P ratio of NOM could vary greatly.

In Total Maximum Daily Loading (TMDL) analyses, an analysis of the non-point and point source pollution control actions that are needed to attain water quality standards are assessed with water quality models (Chapra 1997, EPA 2013b). To meet the increasing needs of addressing a broad range of watershed pollution issues, various modeling approaches have been developed, such as Stream Water Quality Model (QUAL2K), Water Quality Analysis Simulation Program (WASP), CE-QUAL-W2 etc.(EPA 2013b) The majority of TMDL models adapted for regulating nutrient discharge permits estimate the P fraction in Wastewater Treatment Plant (WWTP) discharges that can be utilized by phytoplankton in natural systems using an effective mineralization rate for total P (TP) concentrations to soluble reactive P, regardless of the P composition (Water Quality Research Group 2010, Berger 2009). This assumption that a single rate can represent all potential mineralization paths greatly simplifies the complex phosphorus cycle. To improve evidence-based decision-making to minimize eutrophication potential from WWTP effluent discharges, there is a need for more sophisticated TMDL modeling approaches backed by sound science. Thus, direct determination of dissolved P mineralization rate is critical in the TMDL assessment process and could influence the management decisions.

In our study, algal nutrient uptake bioassay experiments were used to determine the loss rate of dissolved P from advanced WWTP effluents. It is assumed that the loss of P in this experimental system approximately corresponds to the mineralization of organic P to phosphate. We used conventional statistical tools to identify which P uptake kinetic models give the most parsimonious fit to the data in order to obtain a conservative measure of dissolved P mineralization rates. Ultrafiltration was used to size-fractionate P in the effluents and could enhance our understanding of recalcitrant P. The potential consequences of replacing the current two-pool P kinetic model with a more comprehensive Gamma model are discussed from a management perspective. The findings presented in this study could be used to guide future TMDL analyses and eutrophication modeling in lakes and reservoirs.

2. Methods

2.1 Sampling

A total of 22 samples from five main Spokane Region effluents were analyzed: Spokane County Regional Water Reclamation Facility (SCRWRF), City of Post Falls Water Reclamation Facility (PFWRF), Hayden Wastewater Research Facility (HWRF), Coeur d'Alene Advanced Wastewater Treatment Plant (CDA) and Inland Empire Paper Company (IEP). The facility information are listed in Table 1. All the samples were 24-hour composite samples collected in one-liter acid washed (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 hours.

Table 1. Facility Information.

Plant	Advanced Nutrient Removal Technology	Capacity	Chemical Addition	Location	Bioassay #	Ultra-filtration #
City of Post Falls Water Reclamation Facility (PFWRF)	Biological Nutrient Removal	4 MGD	None	Post Fall, ID	5	2
Spokane County Regional Water Reclamation Facility (SCRWRF)	Chemically Enhanced Primary Treatment (CEPT) + Membrane Bioreactor (MBR)	8 MGD	CEPT: 20 mg/l as $FeCl_3$ MBR: 45 mg/l as $FeCl_3$	Spokane, WA	5	3
Hayden Wastewater Research Facility (HWRF)	Blue Pro® process	1.2 MGD	15 mg/L as Fe	Hayden, ID	2	1
Coeur d'Alene Advanced Wastewater Treatment Plant (CDA)	Tertiary Membrane Filtration	0.05 MGD	50 mg/L as Alum	Coeur D'Alene, ID	5	2
Inland Empire Paper Company (IEP)	Trident HS	0.8 MGD	1.6 – 2.1 mg/L as Alum	Spokane, WA	5	2

2.2 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, total P (TP), total reactive P (TRP), dissolved P (DP), and dissolved reactive P (DRP) in the effluents could be directly measured and determined. TP was determined after 45 minutes of autoclave-mediated digestion (120 °C, 100 kPa, with $K_2S_2O_8$ and H_2SO_4) of an unfiltered sample. TRP was determined using the same reaction on unfiltered samples without persulfate digestion. Samples for DP and DRP analyses (120 mL) were first filtered through a 0.45 µm polycarbonate membrane filter (Millipore®). DP was measured after persulfate digestion while DRP was measured without persulfate digestion. Dissolved organic P (DOP) was calculated as the difference between DP and DRP. Values for PP were estimated by subtracting DP from TP to represent the particulate phase.

2.3 Algal bioassays

The freshwater alga *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, Miller et al. 1978). Seven to ten days prior to the bioassays, algae cultures were centrifuged and resuspended into P-free medium to induce P-stress according to Ellis and Stanford (1988) (Ellis and Stanford 1988).

The effluents were filtered through a 0.45 μm filter. Eight hundred mL samples were transferred into 1-L Erlenmeyer flasks, which were acid-washed (0.1 M HCl) and autoclaved prior to each experiment. 0.8 mL of each component of the synthetic nutrient stock solution (1000X), except P, was added into the samples. Each treatment was run in triplicate. P-starved algae were added to the samples at a starting concentration of $2*10^5$ cell·mL^{-1} to initialize the experiments. Samples were incubated at 24 ± 2 ºC under continuous fluorescent lighting of 4300 ± 430 lm in a horizontal shaker at 110 rpm for 21 days (American Public Health et al. 2005, Miller et al. 1978). During the experiment, 50mL samples were transferred for DP analysis on days 0, 0.33, 1, 2, 4, 7, 10, 14 and 21. 80 mL samples were transferred for DRP analysis on day 0 and day 2. In the time course experiments, all nutrients (except P) were amended weekly to avoid limitation by other elements. Through maintaining optimum incubation environment and providing abundant nutrients, it is ensured that the potential growth rate of algae reached maximum and the utilization rates derived from these experiments were conservative compared to *in situ* condition.

2.4 Model fitting

The loss of dissolved P in these experiments was fit to three first-order decay functions to obtain estimates of phosphorus mineralization kinetic (in units of day^{-1}) (Table 2).

One-pool model is the simplest with only single term with single mineralization rate. The initial DP concentration (C_0) changes through the time with the same velocity k_1. According to the theory of reactive continuum, the two terms in two-pool model could be interpreted as readily bioavailable and slowly bioavailable with their corresponding degradation rates (k_1 and k_2). A three-pool model increases complexity by introducing an additional term representing a recalcitrant P pool that might exist.

Table 2. G models formula.

First-Order Decay Model	Formula
One-pool model	$C_t = C_1 exp\ (-k_1\ t)$
Two-pool model	$C_t = C_1 exp\ (-k_1 t) + C_2 exp\ (-k_2 t)$
Three-pool model	$Ct = C_1 exp\ (-k_1 t) + C_2 exp\ (-k_2 t) + C_3$

A Gamma model was also tested to characterize a biodegradation continuum. This infinite k-range Gamma distribution is characterized by two free parameters, "a" measures the average life-time of the more reactive components of the organic P pool and "ν" describes the shape of the distribution near k = 0.

$$C_t = C_0\ (a\ (a+t)^{-1})^{\ \nu}$$

The average first-order decay coefficient k is time dependent. Its value was estimated as below:

$$k = \nu\ (a+t)^{-1}$$

The Solver in Excel was used to minimize the error sum of squares in order to determine which model gives the most parsimonious fit to the data.

The Akaike Information Criterion (AIC) statistic was used to determine which P kinetics model gave the most parsimonious fit to the experimental data (Akaike 1974). The AIC determine which model gives the best fit with the fewest parameters (Akaike 1974).

$$AIC = NLog(ESS/N) + 2(n_v+1),$$

Where, N = the sample size,

ESS = the error sum of squares, and

$n_v + 1$ represents the number of variables in the model plus the variance.

These models were then assessed according to the Δ AIC criterion (Burnham 2002), which is calculated as:

$$\Delta AIC = AICi - \min AIC,$$

Where, AICi represents the AIC value for model i, and

min AIC represents the lowest AIC value observed in the population of models assessed.

When there are multiple models with Δ AIC values < 3, it is recommended to develop weighted multi-model estimates (Burnham 2002). In order to do this it is necessary to calculate Akaike Weights which indicate the probability that a particular model is the best model amongst the population of models considered. Akaike Weights were calculated accordingly:

$$\text{Akaike Weight} = \exp(-\Delta AICi/2)/\,\Sigma \exp(-\Delta AIC/2)$$

According to the Akaike weight criteria, models with higher Akaike Weights can be interpreted to have a higher probability of being the best model.

2.5 Ultrafiltraion

The effluent samples were fractionated into five fractions using a Millipore 8400 ultrafiltration system. The ultrafiltration membranes (Millipore) used had nominal molecular weight cutoffs of 3, 10, 30 and 100 kDa. The membranes were pre-soaked at least overnight and filtered using Milli-Q water for half an hour prior to each filtration to remove organic matter attached to the membranes. 150 mL effluent samples were filtered through the membrane at a maximum pressure of 20 psi for phosphorus characterization.

3. Results

The chemical P speciation suggested SCRWRF and CDA removed most of PP through membrane treatment while PP still comprised a significant portion (>30%) of the TP for samples from PFWRF, HWRF and IEP (Figure 1). After filtration, the starting dissolved P concentrations in the effluents from PFWRF and SCRWRF (103 ± 54 μg/L and 55 ± 8 μg/L, respectively) were higher than IEP, HWRF, and CDA (27 ± 4 μg/L, 23 ± 2 μg/L, and 12 ± 4 μg/L respectively). In the dissolved phase, the fractions operationally identified as reactive P in effluents from HWRF, CDA and IEP were remarkably low (<7 μg/L) compared to others (Figure 1).

During the course of experiment, the DRP was depleted in first 2 days as shown in Figure 2. On day 2, the DRP concentration in the effluents from SCRWRF, HWRF and CDA was only 1 μg/L. In the effluents from IEP and PF, the averaged DRP were 3 μg/L and 4 μg/L, respectively.

Figure 1. P speciation in the effluents. Note: the error bars represented the standard deviations of total P.

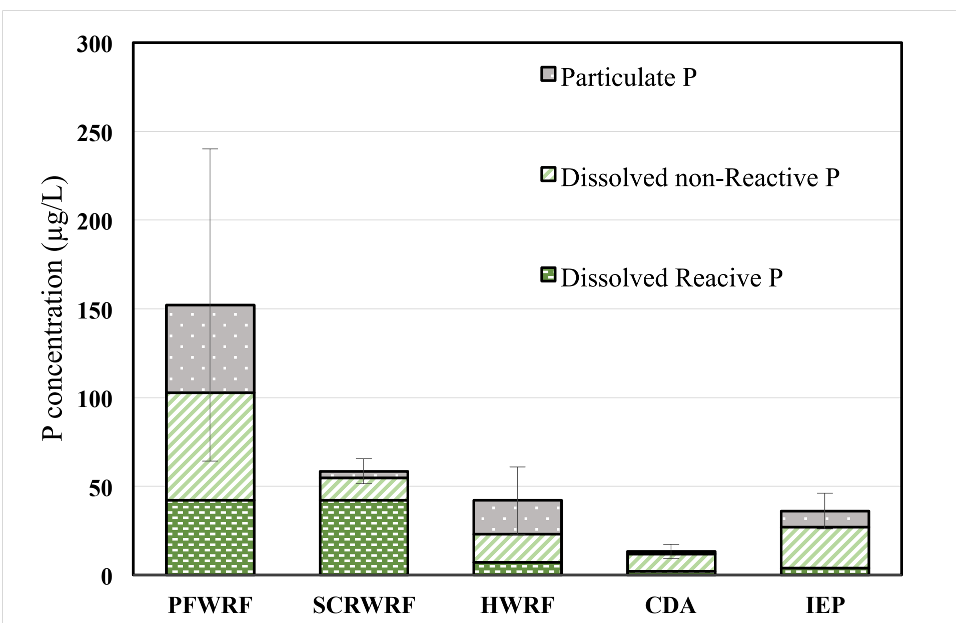
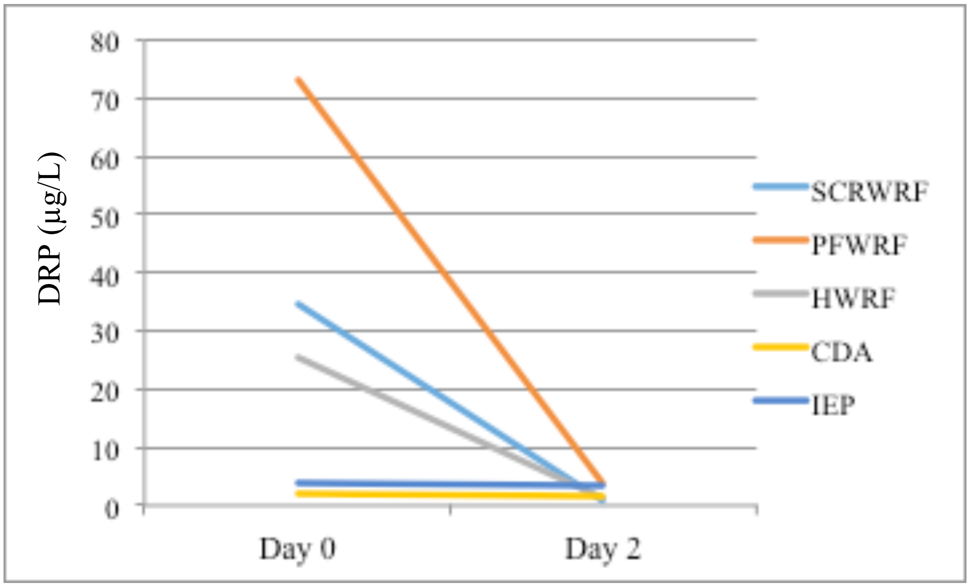


Figure 2. DRP concentration in Day 0 and Day 2.



The model fitting results for first-order kinetic models and Gamma model were listed in Table 3 and Table 4.

Table 3. G model results for 5 different effluents

Facility		C1	C2	C3	K1	K2	r^2
PF	1 pool	**103** ± 54			**0.39** ± 0.35		**0.741** ± 0.138
	2 pool	**61** ± 44	**42** ± 10		**5.00** ± 2.07	**0.04** ± 0.02	**0.988** ± 0.005
	3 pool	**48** ± 23	**38** ± 32	**17** ± 10	**8.31** ± 3.69	**0.21** ± 0.22	**0.995** ± 0.004
SCRWRF	1 pool	**55** ± 8			**0.97** ± 0.60		**0.835** ± 0.062
	2 pool	**37** ± 9	**18** ± 2		**4.16** ± 1.50	**0.03** ± 0.00	**0.990** ± 0.007
	3 pool	**34** ± 7	**12** ± 4	**9** ± 5	**6.49** ± 5.70	**0.20** ± 0.16	**0.992** ± 0.008
HWRF	1 pool	**23** ± 2			**0.06** ± 0.01		**0.757** ± 0.077
	2 pool	**8** ± 0	**14** ± 2		**0.85** ± 0.52	**0.01** ± 0.00	**0.971** ± 0.002
	3 pool	**8** ± 0	**14** ± 2	**0** ± 0	**0.86** ± 0.53	**0.01** ± 0.00	**0.971** ± 0.002
CDA	1 pool	**12** ± 4			**0.07** ± 0.05		**0.727** ± 0.059
	2 pool	**7** ± 4	**6** ± 0		**0.37** ± 0.19	**0.00** ± 0.00	**0.898** ± 0.087
	3 pool	**7** ± 4	**6** ± 0	**0** ± 0	**0.37** ± 0.19	**0.00** ± 0.00	**0.898** ± 0.087
IEP	1 pool	**27** ± 4			**0.03** ± 0.02		**0.586** ± 0.092
	2 pool	**8** ± 5	**19** ± 3		**0.62** ± 0.39	**0.00** ± 0.00	**0.915** ± 0.075
	3 pool	**8** ± 5	**19** ± 3	**0** ± 0	**0.62** ± 0.39	**0.00** ± 0.00	**0.915** ± 0.075

Table 4. Gamma model results.

	Gamma model											
	C_0			a			v			r^2		
PF	103	±	54	0.04	±	0.04	0.23	±	0.13	0.986	±	0.009
SCRWRF	55	±	8	0.02	±	0.01	0.22	±	0.05	0.991	±	0.007
HWRF	23	±	2	0.55	±	0.55	0.19	±	0.04	0.972	±	0.034
CDA	12	±	4	0.82	±	0.49	0.25	±	0.10	0.871	±	0.085
IEP	27	±	4	0.44	±	0.60	0.09	±	0.08	0.890	±	0.095

3.1 One-pool model.

This simplest first-order kinetic model assumes all the dissolved P in the effluent degrades at the same rate (k_1). Due to the higher initial concentrations in effluents from PFWRF and SCRWRF, the model predicted degradation coefficients were relatively higher (0.39 ± 0.35 day^{-1} and 0.97 ± 0.60 day^{-1}, respectively) than other three effluents, which had degradation rates < 0.1 day^{-1}. The average coefficients of determination (r^2) for five one-pool models was only 0.730 ± 0.090.

3.2 Two-pool model

The two-pool model divided the P into two fully bioavailable fractions (C_1 and C_2) with their individual degradation coefficients (k_1 and k_2). In the effluents from PFWRF and SCRWRF, the rapidly decomposing fractions were almost fully utilized by the phytoplankton in first two days with rates of 5.00 ± 2.07 day^{-1} and 4.16 ± 1.50 day^{-1}, respectively. In contrast, the slow degrading pools, which consisted 40% and 32% of dissolved P, were estimated to degrade one hundredth as fast with degradation rates at 0.04 ± 0.02 day^{-1} and 0.03 ± 0.00 day^{-1}. The performance of the two-pool model describing P uptake in HWRF followed a similar pattern. 64% of the dissolved P underwent a much slower decomposition process with k_2 averaging 0.01 day^{-1} in contrast with the more readily bioavailable fraction which was utilized at 0.85 day^{-1}. Notably, the k_2 rate was ≈ zero for both effluents from CDA and IEP which strongly suggested that the second pool (44% and 66% of dissolved P) was almost entirely recalcitrant. The two-pool model correlated with the experimental data much better than one-pool model with an average coefficient of determination (r^2) of 0.954 ± 0.045.

3.3 Three-pool model.

The three-pool model introduced an additional pool to two-pool model by assuming a certain fraction of the P will never be utilized. The estimated values for the non-degradable pools (C_3) were 9 ± 5 μg/L and 17 ± 10 μg/L for SCRWRF and PFWRF, respectively, indicating that 17% and 16% of P in these effluents was not utilized by algae and thus could persist in the water column. The correlation coefficient between the predicted values and experimental data in the three-pool model was the highest among the three for these two effluents (0.992 ± 0.008 and 0.995 ± 0.004 for SCRWRF and PFWRF correspondingly). However, the three-pool model fit the data similar to the two-pool G model for the effluents from HWRF, CDA and IEP.

3.4 Gamma model.

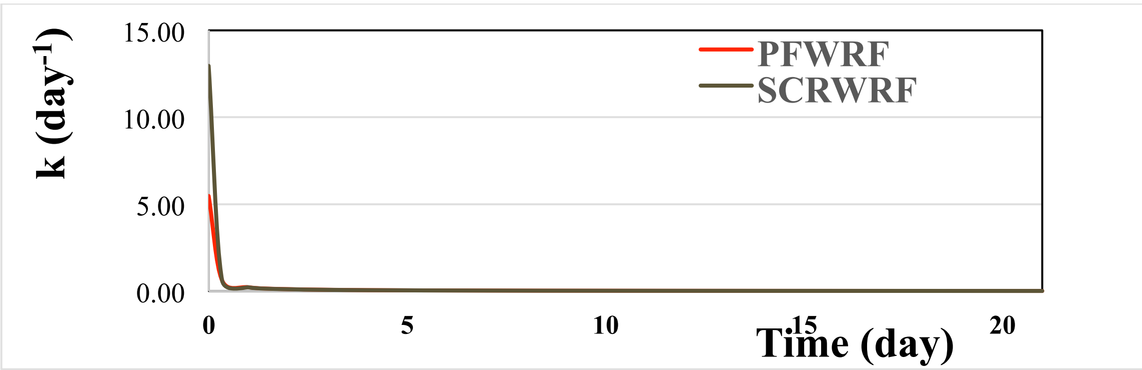
The Gamma model fit the dissolved P time series well and had comparable correlation coefficients to the three-pool models as shown in Table 4. The averaged r^2 was 0.942 ± 0.057 and the degradation pattern was almost identical to three-pool model.

The average first-order decay coefficients (k) were calculated for the five effluents. These rate constants describe how the dissolved P degradation slowed during the experiments (Figure 3). The higher portion of readily bioavailable P in the PFWRF and SCRWRF effluent triggered a fast uptake mechanism with a utilization rate of 5.46 day^{-1} and 12.94 day^{-1}, respectively (Figure 3a). Yet, the mineralization rate quickly dropped to 0.62 day^{-1} and 0.63 day^{-1} after the first 8 hrs and continued to decrease to only 0.01 day^{-1} by the end of experiment. Similarly, initially, the dissolved P in HWRF, CDA and IEP effluent mineralized very rapidly with k values equals to 0.35 day^{-1}, 0.30 day^{-1} and 0.21

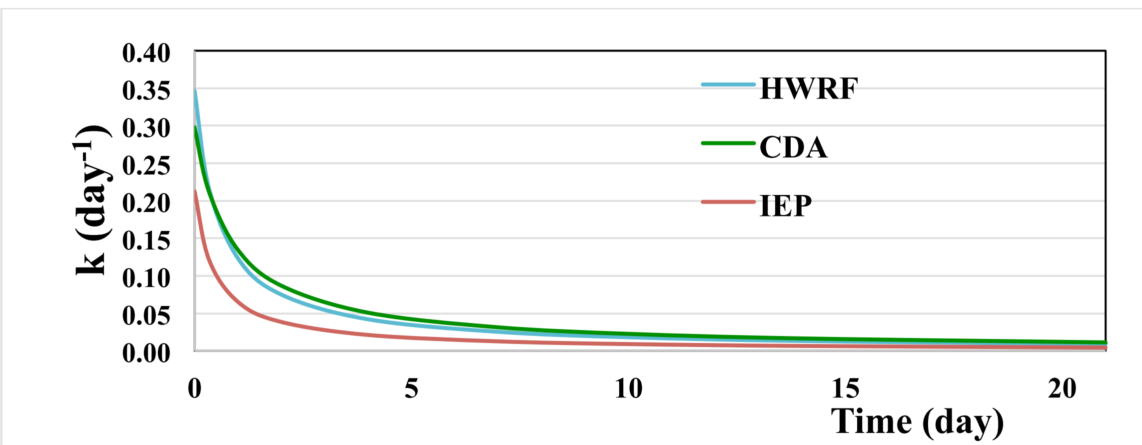
day^{-1}, respectively (Figure 3b). The degradation process gradually slowed down and the decomposition rate reached only 0.01 day^{-1} after 21 days, which was similar to the other effluents.

Figure 3. The average first-order kinetic coefficient k for Gamma model.

a.



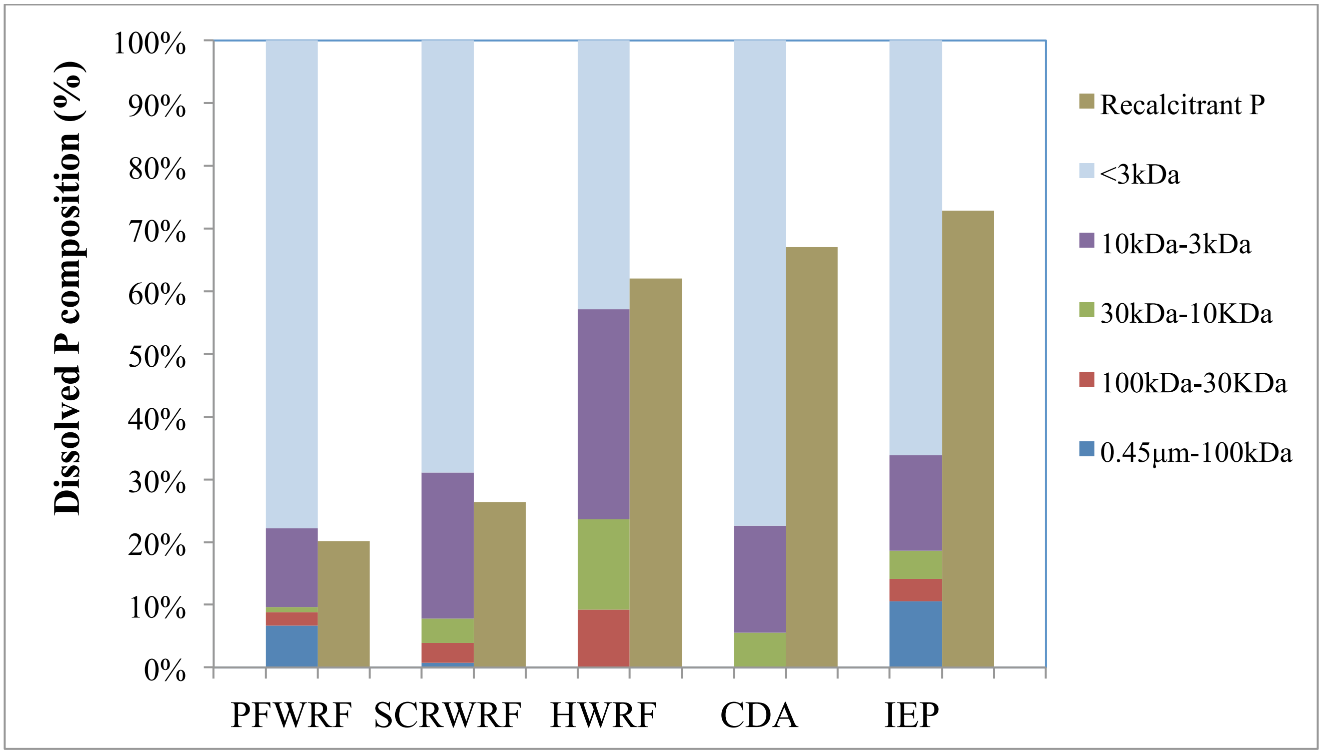
b.



3.5 P size fractionation.

From the results of ultrafiltration for all the effluents (Figure 4), a large fraction (33 ± 14%) of the dissolved P is larger than 3 kDa in the dissolved phase. For effluents from PFWRF, SCRWRF and HWRF, the percentage of P fraction larger than 3 kDa is similar with the recalcitrant P predicted by three-pool model. For effluents from CDA and IEP, within the P fraction that was smaller than 3 kDa, 71 ± 0% and 69 ± 22% of the P was non-reactive.

Figure 4. Size fractionation of P in the effluents.

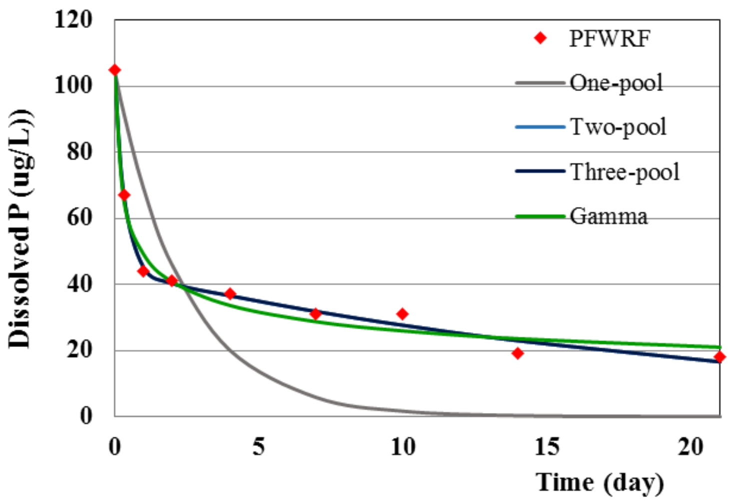


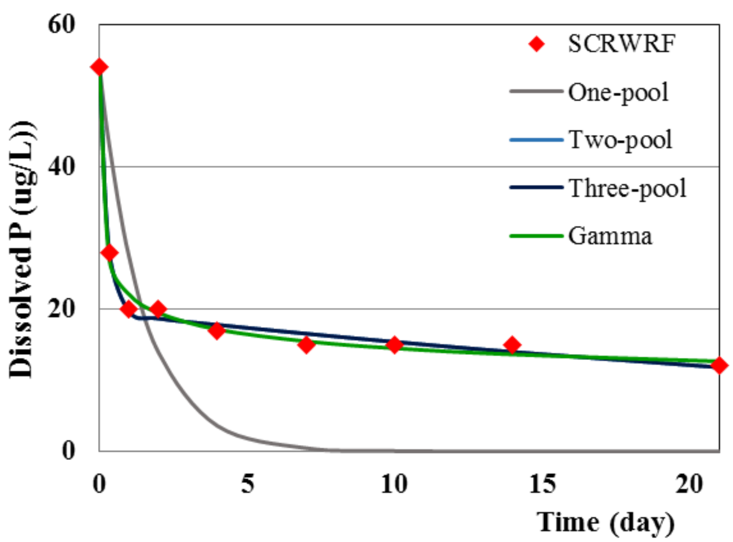
4. Discussion

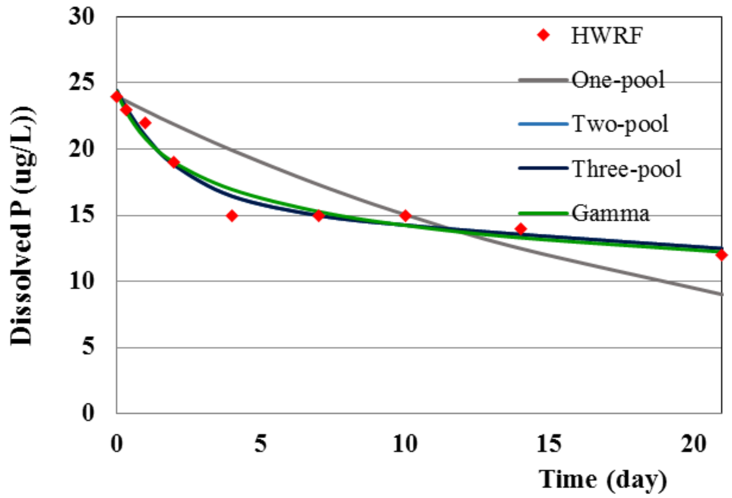
4.1 Implication of model results

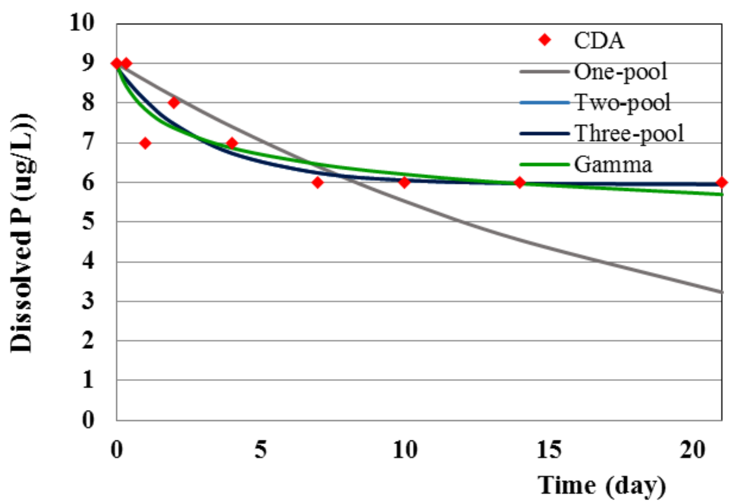
In the three first-order decay functions, the one-pool model had weakest statistical association with the experiment results as shown in Table 3 ($r^2 = 0.729 \pm 0.090$). This suggests the use of a one-pool model to characterize phosphorus availability is a considerable oversimplification of the phosphorus kinetics in lakes. Figure 5 is shown as an example to demonstrate how the model predicted dissolved P concentrations, this pattern is consistent for all of the effluents studies. The first-order model underestimated the dissolved P bioavailability during the first 4 days of the experiment, but greatly overestimated bioavailability thereafter. Using a two-pool model to differentiate the slower bioavailable from the readily bioavailable pool dramatically increased the fit between the experimental and predicted values.

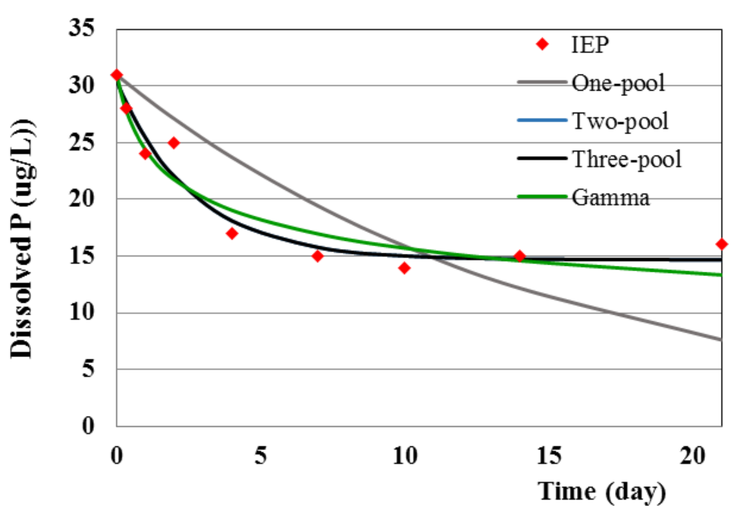
Figure 5. Model fitting results for five effluents. These figures were selected from one of the sampling dates which has dissolved P results most closed to the average.






HWRF
One-pool
Two-pool
Three-pool
Gamma
Dissolved P (ug/L))
Time (day)


CDA
One-pool
Two-pool
Three-pool
Gamma
Dissolved P (ug/L))
Time (day)


IEP
One-pool
Two-pool
Three-pool
Gamma
Dissolved P (ug/L))
Time (day)

In this modeling exercise, the three-pool model had highest correlation with the experimental results for effluents from PFWRF and SCRWRF. Although the modeling results for the three-pool model and two-pool model were the same for the effluents from HWRF, CDA and IEP, this could be due to the low effluent concentrations in these tertiary treatments which were very close to the detection limit for the analysis (2 μg/L) and thus posed difficulties in model fitting when distinguishing the slight difference between two- and three-pool models. However, considering the substantially lower uptake rate in second pool ($k_2 < 0.01$ day^{-1}) and the third term (C_3) to be approximately zero, the second fraction (C_2) could be assumed to be recalcitrant and less likely to contribute to eutrophication. These results indicated the three-pool model, which includes two kinetic rates for two-P pools (one readily bioavailable and one slowly bioavailable) and a third pool that is assumed to be inert for algal growth, are more appropriate description of the dissolved P uptake in receiving waters. Contrary to the traditional approach which models dissolved P as a single pool with a constant decay rate, the three-pool model shown that recalcitrant P should be accounted for in water quality modeling.

The Gamma model provides a more comprehensive description of the degradation pattern and demonstrates a sharp shift towards lower bioavailability during bioassay. Previous exercises compared the predictive power of the various first-order models and Gamma model have shown multiple-pool models had a weaker predictive power than the Gamma model, in which the estimation should be insensitive to the experiment length and requires less experimental data to predict uptake kinetics (Vahatalo et al. 2010).

Based on the ΔAIC and Akaike weight criteria (see Table 5), the Gamma model clearly outperformed the others. Furthermore, the ΔAIC values suggest the following overall ranking in model performance: Gamma model > 2 Pool model > 3 Pool model > 1 Pool model. The fact that the Akaike weights strongly favored the Gamma model, despite the fact that Gamma model and 3 pool model had very similar correlation of coefficients, was because the Gamma model achieved the same fit with three fewer variables. This result strongly suggests the Gamma model is the optimal model with better overall performance and fewer variables.

Table 5 Model performance based on AIC criteria. ESS represents the error sum of squares, N represents sample size, n_v represents the number of variables in the models, AIC is the Akaike Information Criterion value, and Ak. Wt. represents the Akaike Weight.

		ESS	N	n_v	AIC	ΔAIC	Ak. Wt.%
PFWRF	***1 Pool***	24797	45	1	127	36	0
	2 Pool	3585	45	4	96	5	9
	3 Pool	3585	45	5	98	7	3
	Gamma	3464	45	2	91	0	88
SCRWRF	***1 Pool***	19735	45	1	123	46	0
	2 Pool	1729	45	4	81	5	8
	3 Pool	1729	45	5	83	7	3
	Gamma	1657	45	2	76	0	89
HWRF	***1 Pool***	3311	18	1	45	10	1
	2 Pool	632	18	4	38	3	17
	3 Pool	632	18	5	40	5	6
	Gamma	711	18	2	35	0	77
CDA	***1 Pool***	14807	45	1	117	10	1
	2 Pool	7925	45	4	111	3	14
	3 Pool	7925	45	5	113	5	5
	Gamma	8144	45	2	108	0	80
IEP	***1 Pool***	9758	45	1	109	9	1
	2 Pool	5348	45	4	103	4	14
	3 Pool	5348	45	5	105	6	5
	Gamma	5483	45	2	100	0	80

4.2 Recalcitrant P

As shown in this study and increasingly suggested in related studies, the fundamental role of recalcitrant P in water systems should not be ignored. As predicted by three-pool model, For the SCRWRF and PFWRF effluents, the remaining portions at the end of 21 days were 17% and 16%, respectively, which could be assumed to be more or less inert P. For the effluents discharged from CDA and IEP, three-pool model predicted a nearly complete cessation of biodegradation after 10 days (Figure 4) with residual recalcitrant dissolved P pools of 48% and 71%, respectively.

This strongly re-affirmed the conclusion from previous bioassays, that a substantial fraction of P in the effluents from advanced water reclamation facilities has very low bioavailability (Li and Brett 2012, 2013b). This hypothesis is also supported by a wide variety of previous studies in natural systems. It is commonly accepted that dissolved organic P (DOP) mainly consists of polymers (Cembella et al. 1982). Incubation experiments with polymeric DOP detected this pool was assimilated at a much longer turnover time (over 20 days) (Thingstad et al. 1993). Within this pool, it is speculated that there is another fraction which decomposed much slower than typical organic forms such as ATP or nucleotides (Nausch and Nausch 2006). By studying the P cycle in the ocean, a relatively constant portion of DOP was reported to be refractory in several studies, which is an indication that this slowly mineralized pool could persist in the environment for a fairly long time (Thingstad et al. 1993, Bjorkman and Karl 2003, Benitez-Nelson 2000, Paytan and McLaughlin 2007). More detailed and directed research with selected compounds using specific labeling technologies has also shown certain organic P compounds (mostly phosphoesters) were resistant to algal uptake (Berman 1988, Bjorkman and Karl 1994, Huang and Hong 1999, Hernández et al. 2000). Phosphorus bounded with humic-(Al/Fe) were also believed to contribute to this recalcitrant pool most likely due to its association with large macromolecules (Li and Brett 2013a).

This hypothesis is in agreement with the ultrafiltration result for effluents from PFWRF, SCWRF and HWRF, which suggested there is a potential connection between P compounds with larger molecule size (>3 kDa) and recalcitrant P. Phosphorus species which are larger than 3 kDa are too large to cross phytoplankton cell membranes (Gerke 2010). The ultrafiltration results for the CDA and IEP effluents also suggested that the P compounds that were smaller than 3 kDa were non-reactive.

These findings provided experimental evidence to support that the recalcitrant P should not be neglected in the modeling exercise; otherwise modeling outputs could lead to unrealistic management strategies.

4.2 Bioavailability Continuum

The modeling results also consistently suggested there is a reactive continuum for the dissolved P in wastewater effluents. By reviewing the uptake pattern in natural systems, one can shed light onto this phenomenon. Extensive studies investigated the mineralization rate in lake sediments and water column due to its important role in P dynamics in surface water systems. By developing diagenetic models based on non-reactive P (nrP) and reactive P (rP) concentrations as a function of sediment depth, Wilson et al. (2010) quantified that the constants for the transformation (k_f) of nrP to rP ranged from 3 x 10^{-5} to 8 x 10^{-5} day^{-1} in three oligotrophic lake sediments (Wilson et al. 2010). Several other studies of the mesotrophic Lake Erken (Reitzel et al. 2007, Ahlgren et al. 2005, Reitzel et al. 2006) using similar methods showed a slightly higher net loss of P from sediments over time with lower half-life times reported for P mineralization. Particularly, in the biogenic P fraction, the NaOH-soluble humic compounds were shown to be degraded at a much lower rate (3 x 10^{-4} to 2 x 10^{-5} day^{-1}) than the non-humic fraction (1.9 x 10^{-3} to 6 x 10^{-5} day^{-1}), which further suggested that humic associated P is more recalcitrant than other fractions.

In sediments from hypertrophic Lake Onandaga (New York) (Penn et al. 1995), two fractions of P were classified with the fast fraction mineralized at 0.014 day^{-1} while the second pool mineralized at only 0.003 day^{-1}. Compared to sediment P, phosphorus in lake water is usually regenerated into biomass at a higher rate. Based on the reported half-life values, a range of regeneration rate (0.003 - 0.014 day^{-1}) was found (Penn et al. 1995). Research on 20 temperate freshwater lakes using a radioisotope technique derived a statistical relationship between TP and dissolved P regeneration with an average of approximate 0.126 day^{-1} calculated from the regression model ($n = 20$, $r^2 = 0.96$) (Hudson et al. 1999). From these studies, one could speculate that bioavailability of P transitioned from low for particulate P and humic associated P in the sediments to high in the more reactive forms in dissolved phase.

Studies on pure P compounds provided another perspective into this discussion. Bioassays with two inorganic forms of dissolved inorganic P, four organic P and three humic associated dissolved P solutions determined that uptake rates of some inorganic P species (0.59 ± 0.06 day $^{-1}$) and organic P compounds (0.77 ± 0.06 day $^{-1}$) were much higher than for the humic solutions tested (0.01 day^{-1}) suggesting these humic complexities are likely to persist in natural systems. Representing the organic P decay rate with a single value could result in a value that is much too low for true organic forms of DOP (such as AMP, GDP, UDP and ATP), and way too high for recalcitrant forms of DOP (rDOP) such as humic-metal complexed P. These results imply that multiple degradation rates, or even time-dependend coefficients, should be considered when representing mineralization kinetics.

4.3 Implication for Watershed Management

Both the Spokane River and Long Lake suffer low dissolved oxygen (DO) levels in the summer during dry years (Department of Ecology 2010). Portions of Lake Spokane do not meet the requirement of the Washington State water quality standards for DO and are listed as impaired and subject to a dissolved oxygen TMDL (Department of Ecology 2010). A water quality model (CE-QUAL-W2) has been used to characterize the DO dynamic of the Spokane River and Lake Spokane (Berger 2009). This model has been used to assess DO changes under various discharge scenarios (Berger 2009). A key component of the P uptake kinetics in the existing TMDL model is a two-pool model with one pool representing DRP, which can be utilized immediately by phytoplankton, while the other pool is organic bound P which is mineralized to phosphate when organic matter is oxidized. Thus the organic decay rate for carbonaceous BOD (CBOD) in secondary effluents has been used to represent the organic P to phosphate mineralization rate in the TMDL model (Berger 2009). This model also assumes that the concentration of organic bound P (both dissolved and particulate) can be represented as the difference between the TP and dissolved reactive P (DRP) concentrations (Berger 2009).

Although it is commonly assumed that dissolved inorganic phosphate is entirely bioavailable, the dissolved reactive P (DRP) term commonly measured in contemporary monitoring programs corresponds poorly to true phosphate (Hudson et al. 2000, Li and Brett 2013a). Extensive research has shown DRP is not a reliable indicator of phosphate due to potential analytical problems (Li and Brett 2013a, Dodds 2003). The results from this study suggest one should characterize dissolved P according to its uptake kinetics instead of operationally classifying P fractions merely based on wet chemistry analyses (Hudson et al. 2000, Rigler 1968).

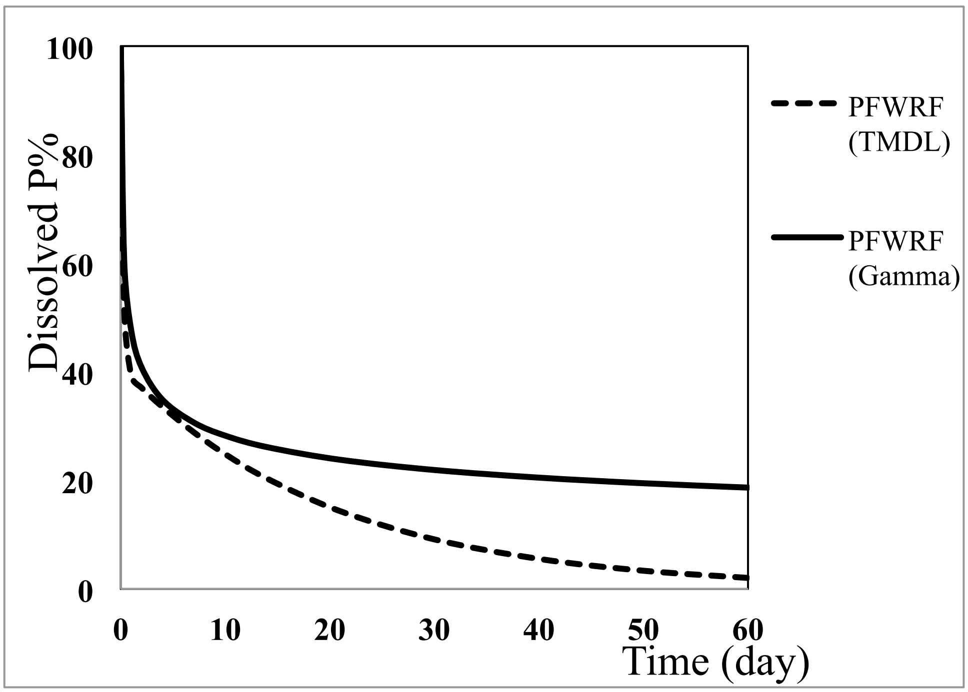
Also, recent research has shown that particulate P (PP) in municipal effluents may have a lesser contribution to effective P loading due to sediment deposition (Effler et al. 2012, Effler et al. 2009). The assumption that the mineralization of particulate P is similar to dissolved P is also problematic. Further, there is no *a priori* reason to assume that the mineralization of recalcitrant phosphorus in the effluents from advanced phosphorus removal processes will follow the same kinetics as the degradation of CBOD in conventional secondary effluents. Different uptake patterns with different rates constants indicate the variation of treatment process and P species in the effluents.

For the WWTP effluents, Berger et al. (2009) reported CBOD decays rates averaged 0.064 ± 0.026 day^{-1} and assumed these are equal to the effective mineralization rate for organic P in the current model setting. Due to the water retention time in Long Lake, which averages ≈ two months in late summer, assuming a 6% per day organic P mineralization rate effectively assumes a P input to this reservoir will be sued to support algal production. The bioassay results from this study suggest the degradation rates might be much lower than the pre-set values in the current.

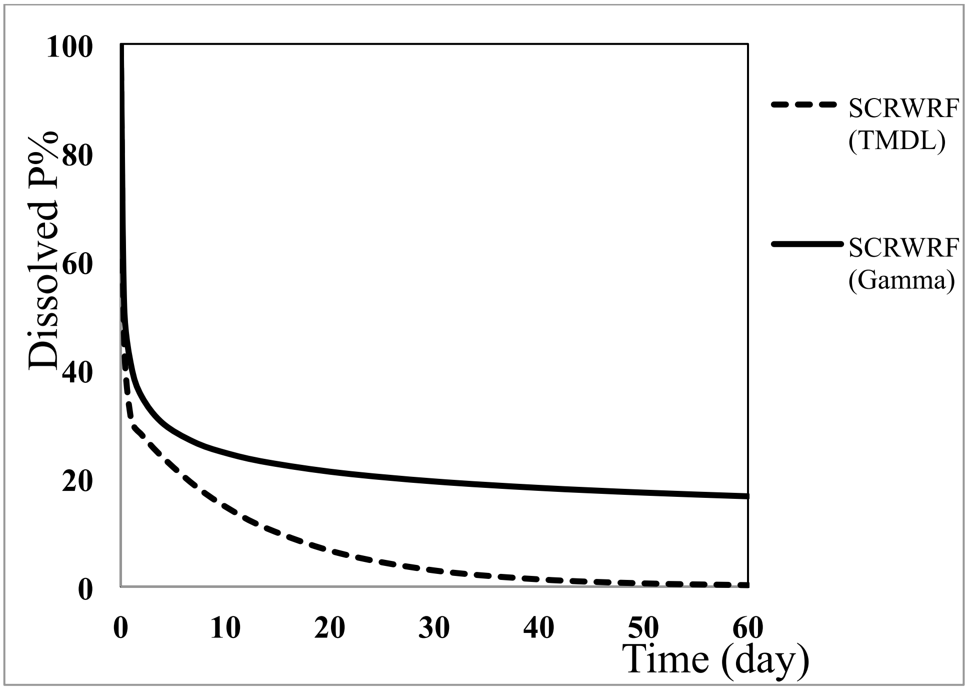
To demonstrate the difference between the two pool model in the current existing TMDL model and Gamma model indicated by in this study, the modeling results of these two models were normalized based on total P concentration and extrapolated to 60-days which is approximately the Hydrological Retention Time for Lake Spokane in the late summer (Figure 6). During this modeling time, approximately half of the dissolved P was depleted within the first two days while the rest underwent a much slower uptake rate. For effluents from PFWRF, SCRWRF, HWRF and CDA, after 60 days, the current two pool model, indicated almost all of the phosphorus in the effluents would be used up by algae, and less than 3% of the P would be left after 60 days. However, the Gamma model, which takes recalcitrant P into account, predicted there was a large fraction of P which degraded at much slow rates and left a significant amount of P in the lake system unused. For the industrial wastewater effluent from IEP, due to its higher humic and large molecule weight contents, it might undergo a slower mineralization as indicated in the current TMDL model. However, the Gamma model suggests the current TMDL over-estimates the bioavailable P by over 40%. Therefore, it is foreseeable that a difference of this magnitude could substantially change model outputs.

Figure 6 Comparison of the predicted P kinetics for the current TMDL model and the Gamma model. (Note: a. PFWRF, b. SCRWRF, c. HWRF, d. CDA, e. IEP.)

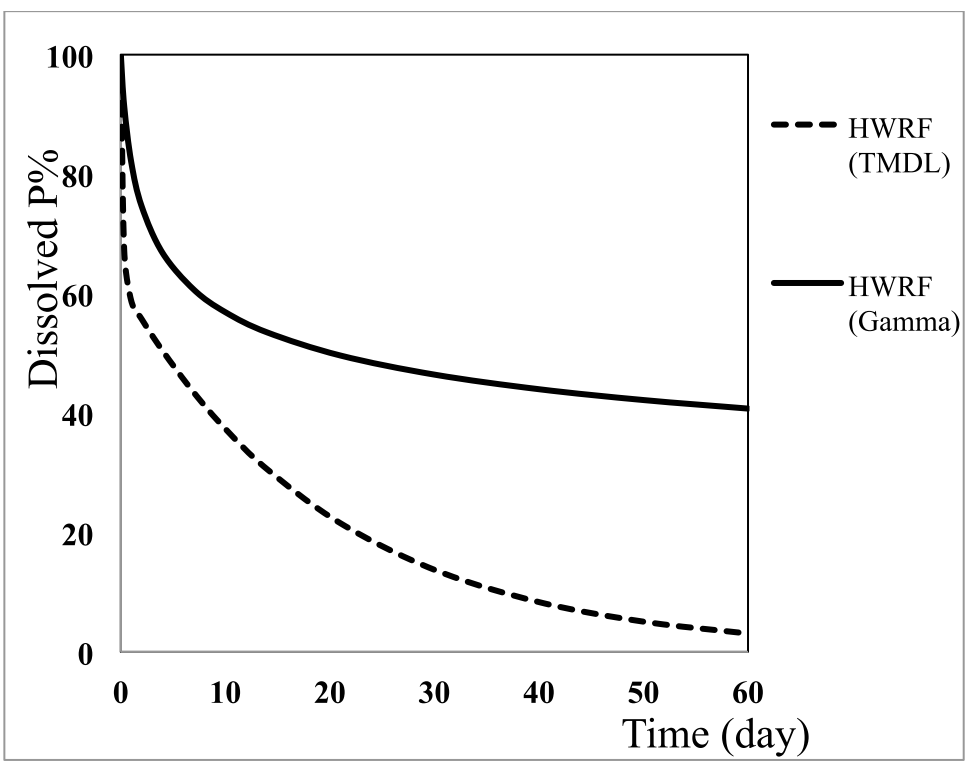
a.



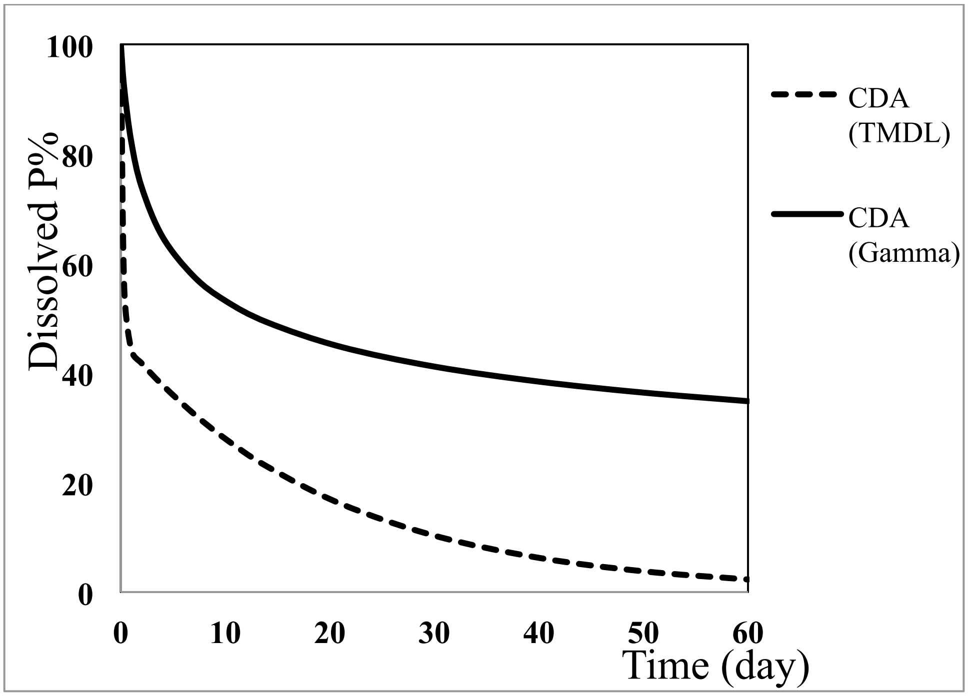
b.


100
80
60
40
20
0
Dissolved P%
0
10
20
30
40
50
60
Time (day)
SCRWRF
(TMDL)
SCRWRF
(Gamma)

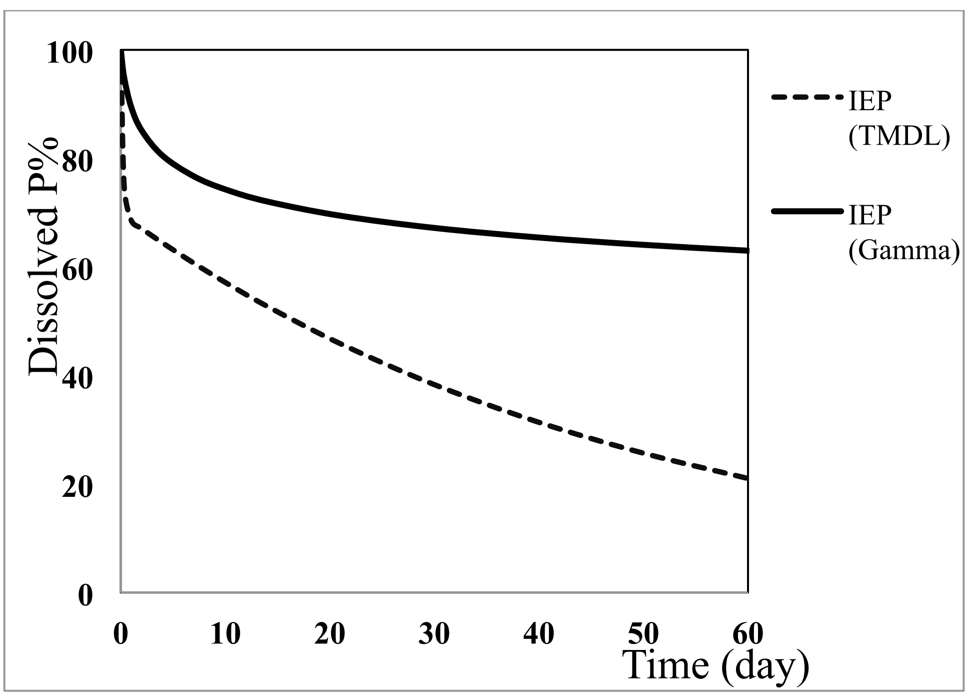
c.


100
80
60
40
20
0
Dissolved P%
0
10
20
30
40
50
60
Time (day)
HWRF
(TMDL)
HWRF
(Gamma)

d.



e.



5. Conclusions

Bioassay uptake experiments developed in this study could be a useful tool to describe the mineralization of P quantitatively and thereby better determine the mineralization kinetics of dissolved P in the environment. The modeling results suggested that the three-pool model and the dissolved P mineralization rate constants associated with it could be used, in place of the assumption that organic P is mineralized at the same rate that CBOD is degraded. The mineralization rate determined in these first-order decay models could be seamlessly incorporated into the existing TMDL model without structural modifications. However, it also strongly suggested that the current first-order decay models to be replaced with the more comprehensive Gamma model to fully describe the bioavailability continuum exists in the effluents tested. Moreover, the modeling results suggested that the outcome of current TMDL model could be significantly modified with the updated mineralization rates and exclusion of inner fraction. This study presents a sound approach for managing similar nutrient-impacted water bodies.

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Conclusions

Refining our understanding of fractionation and bioavailability of external P source will improve our fundamental knowledge for water quality modeling and developing the most protective eutrophication management plans. This dissertation contribute to our understanding of this critical issue by addressing these scientific question with following key findings:

Key Findings:

1. ***What is the BAP fraction in the effluents from advanced nutrient removal facilities and how it is impacted by tertiary treatments?***

Paper I indicated that less than 10% of P was bioavailable in the final effluent, and as the level of P removal increased, the %BAP of the product declined sharply, $r^2 \approx 0.98$.

2. ***What is the bioavailability of different P compounds that may commonly occur in WWTP effluents?***

Paper II proposed an objective classification scheme to more clearly describe P containing compounds based on their bioavailability.

3. ***Which operational chemical category best predicts the potential BAP pool in effluents and could be used as monitoring measure for WWTP.***

Paper III suggested TRP could be used as a surrogate predictor for BAP with a strong statistic relationship observed ($r^2 \approx 0.81$), and with a BAP/TRP ratio of 0.61 ± 0.24,

4. ***What are the mineralization kinetics of dissolved P and its associated biodegradation rate and how can this be embedded in current water quality models?***

Paper IV showed two-pool and three-pool models correlated better with the experimental data ($r^2 > 0.9$) and provided strong evidence for the existence of recalcitrant P in effluents from these tertiary facilities.

Key Benefits:

- Provided a more scientific method for setting wastewater treatment plant (WWTP) discharge permit limitations for effluent P based on actual algae bioavailability.
- Provided a simple, quick method to estimate bioavailable phosphorus in treated effluents.
- Provided a basis to avoid unnecessarily high chemical use and reduce operation costs, sludge production and greenhouse gas footprint for wastewater treatment.
- Showed the classic SRP chemical characterization is a poor predictor of the bioavailability of P containing compounds.
- Proposed a new approach to estimate the mineralization rate and describe the uptake kinetic of dissolved P.

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