Spokane Regional Wastewater Phosphorus Bio-availability Study Final Report

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1. Introduction

Bio-available Phosphorus

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 (1). The speciation of P is quite complex, and for analytical purposes, four operational categories are commonly used to characterize phosphorus (2). These are: dissolved reactive P (DRP), dissolved non-reactive P, particulate reactive P, and particulate non-reactive P. These fractions are partitioned with dissolved P passing through a 0.45 µm filter, and reactive P determined via a colorimetric reaction with acid-molybdate. (2) But this classification scheme does not necessarily correspond to the role these forms play in the biotic cycling and utilization of P (2). 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 (3, 4). Bioavailable Phosphorus (BAP) is the component of total phosphorus (TP) which supports the growth of algae or other organisms (5). Previous studies have indicated that BAP rather than TP or DRP provides the most accurate measure of water quality conditions in lake systems (6, 7).

Numerous approaches have been applied to estimate BAP, including bioassays (5, 8), ion exchange resin-impregnated membranes (9) and NaOH and NH_4F based chemical extractions (10). Previous studies have shown that algal bioassays are the most reliable technique for quantifying BAP (11, 12). In batch assays, algae and the sample are directly mixed, thus allowing the activity of surface-bound algal enzymes to release particulate organic phosphorus (13).

Many studies suggest that P availability may vary between different sources of waters as a function of their physical, chemical and biological conditions (14, 17). Because of concern for the eutrophication problems caused by wastewater discharges (14), considerable effort is now being devoted at the national scale towards advanced P removal (15). 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 (15). Understanding of these questions is critical to ongoing efforts to control the negative consequences of widespread eutrophication on surface water bodies.

Background of Project

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 summer/fall period (16). Because Lake Spokane primary production is phosphorus limited, improving hypolimnetic dissolved oxygen concentrations will require significant reductions in total, and most importantly bioavailable (17), P loads.

The objective of this study was to use algal bioassays to determine the Bio-available Phosphorus (BAP) of effluent treated by the pilot projects at the main WWTP discharges to the Spokane River. This is critically important because it is currently unknown whether the effluents of facilities designed for advanced P removal tend to make the residual P more or less bioavailable compared to conventional treatment processes.

2. Experimental Section

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 persulfate digestion (18). 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.

Bioassay Analysis

P bio-availability was determined using the bioassay method described in Standard Method 8111 (18). The nutrient medium described by Miller (19) was used to maintain *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) algal cultures. Algae were centrifuged and re-suspended into P-free medium (which used KCl instead of K_2HPO_4) 7-10 days before the bioassays. Effluent samples were autoclaved for 45 mins to kill indigenous algae before the assay. Influent and intermediate process effluent samples were diluted with P-free media depending on their concentrations to alter P concentrations within an appropriate range. 50 mL of each test sample was placed into 125-mL Erlenmeyer flasks. All of the flasks 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 μg $P{\cdot}L^{-1}$) were incubated in triplicate to obtain a standard curve for 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 growth of algae was linear in the standard concentration 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 ºC 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 (18). Following incubation, algal cell density in each of the test samples and the standard curve solutions was determined using a Coulter Multisizer III particle size analyzer by passing the samples through a 100 µm aperture. As outlined by Standard Methods and Miller (18, 19), every sample was read three times. Prior to each reading, background particle concentrations were estimated by testing parallel samples which had not been inoculated with algae. The comparison of the algal cell yield in a set of standard assays and the test assay gave the level of BAP in samples.

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, from the algae's environment was stimulated (13). This creates a ready supply of enzymes which facilitate the release of all available phosphorus from the incubation media within the incubation period, which allows an accurate determination of total BAP without longer term incubations (13).

3. Report Organization

This report will begin with a Quality Assessment/ Quality Control (QA/QC) analysis. It will then proceed with chapter for each of the effluent types we processed (i.e., Spokane, City of Coeur d'Alene, Post Fall, Liberty Lake, Hayden Area Regional Sewer Board, Inland Empire Paper, Spokane River). We conclude with an executive summary of our study's key conclusion.

4. QA/QC

Quality Control Procedures

Field

Samples were collected at various points within the facilities assessed in this study using acid washed plastic bottles which were triple rinsed with deionized water immediately prior to sample collection. The one-liter polyethylene bottles for sample collection and storage were acid washed and triple rinsed with Deionized (DI) Water. All samples were cooled to 4 °C and shipped to the University of Washington on ice in insulated containers.

Laboratory

Laboratory QC for wet chemistry tests included standard solution samples for standard curve, reagent blanks, analytical duplicates (18). The TP/TRP samples were all run in duplicate. A reagent blank using de-ionized water (DI water) was analyzed with every set of samples processed for TP/TRP analyses. This reagent blank included all reagents that were used in the analytical process and was carried through the entire process, including extraction and digestion (when applicable).

To account for the greater variability of the bioassay procedure, four replicates were run for each bioassay sample. The average algal density of the four samples was used to represent the bio-available phosphorus concentration and the variability amongst these replicates was used to qualify sample precision. The bioassay standard curves were run in triplicate. P-free medium was used as a blank for the bioassays.

Quality Assessment Procedures

The data quality assessment in which the data used for decision-making was evaluated in terms of its relationship to expected norms of variability. The TRP and TP concentrations of samples were either within the 0-100 µg/L range for standards or were diluted to the applicable range. The bioassay method is applicable in the range from 0-50 µg/L. Samples were diluted with P-free medium to obtain the appropriate concentration.

Table 1 The standard deviation and coefficient of variation in different concentration ranges

TP						
Concentration Range (µg/L)	0—30		30—100		> 100	
	Mean	Median	Mean	Median	Mean	Median
±SD (µg/L)	1.4	1	2.9	2.3	102	27
CV (%)	8	5	6	7	4	3

TRP						
Concentration Range (µg/L)	0—30		30—100		> 100	
	Mean	Median	Mean	Median	Mean	Median
±SD (µg/L)	0.6	0.3	1.1	0.8	80	31
CV (%)	6	3	2	1	3	2

BAP						
Concentration Range (µg/L)	0—30		30—100		> 100	
	Mean	Median	Mean	Median	Mean	Median
±SD (µg/L)	0.8	0.4	10.4	5.6	189	119
CV (%)	19	13	16	9	11	9

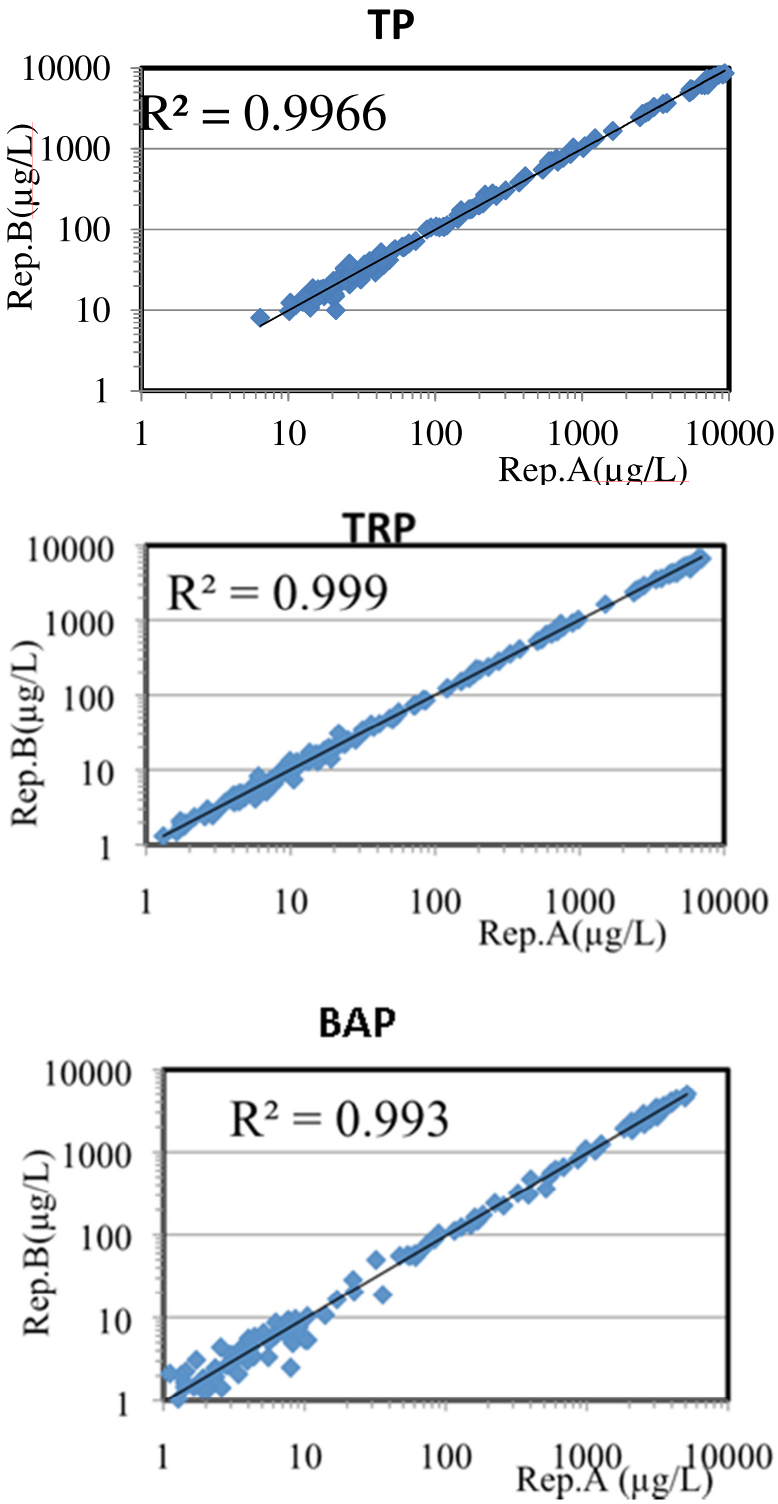


Figure 1 Correlation between the two replicates

(Note: four replicates were used for BAP analysis, the correlation is shown between the average of first two samples and second two samples.)

In the QA/QC data sheets, the TRP and TP standard curves had average r^2 values of 0.9995 and 0.9993 respectively. The average r^2 value for the bioassays was 0.98. All of the samples had TP and TRP concentrations above the method detection limits of 2 and 1 µg/L, respectively. The TP and TRP concentrations had average standard deviations of 6 and 2 µg/L respectively. The average standard deviation for BAP was 20 µg/L. However, in table 1, when we classified the concentration into different concentration ranges, the high end of BAP concentrations gave large SD values which skewed the overall average SD. Conversely, in Table 1, it is shown that in the low BAP concentration range (0-30 µg/L) the CV was relatively high (19%) when the corresponding SD values were actually quite low (i.e., ± ≈ 1 µg/L). At the other extreme, high mean BAP concentrations gave large SD values (i.e., ± ≈ 190 µg/L), but relatively low CVs (i.e., ± 11%). The same tendency was seen in SD and CV results of TP and TRP, although this tendency was more muted. The two replicates within experiments showed good correlation with r^2 = 0.999, 0.997, 0.993 for TP, TRP, and BAP, respectively (see Figure 1).

Sample variability most likely originated from two sources, first the variability introduced by fluctuations in the operation of the pilot plants (*i.e.,* process uncertainty) and second variability introduced by analytical uncertainty for the P characterizations conducted at the Brett UW water chemistry laboratory. Because a very large number of replicated samples have been processed within the UW lab for this project, it is straightforward to quantify the analytical uncertainty that has on average been introduced to the concentrations we report for these samples. It should also be noted that this source of "analytical error" is an unavoidable consequence of analytical procedures and should be similar for most sample types.

Process uncertainty is a result of fluctuations in the operation of the pilot plant processes from which we obtained our samples. Process uncertainly could also potentially be very large, for example, if a particular P removal process was not operating properly on a day for which we obtained samples the effluent values could be similar to influent concentrations. Furthermore, whereas we have a very large number of samples from which we can infer the expected analytical uncertainty, we usually have a very small number of samples (*e.g.,* n = 5) from which we can infer process uncertainty for a particular process. However, because we know the expected analytical uncertainty, in most cases we can with a high degree of confidence say whether the variability we observed for a particular group of samples might have originated from analytical error introduced in our lab. In cases were the variability within a subset of samples is much larger than could be reasonably expected based on analytical error, we identify "outliers" attributable to process uncertainly. For example, our analytical uncertainty for all of the TP samples averaged ± 3% (*i.e.,* ± 1 CV = sample SD/sample mean), whereas the concentration variability for a particular set of five TP samples from a Spokane region pilot plant might be ±17%. Furthermore, because our TP samples were all duplicated, the true analytical uncertainly introduced to the values we report is on average the within sample variability divided by the square root of the number of replicates we processed for each sample, *i.e.,* two for TP and TRP, and four for BAP. Because we have a large QA/QC database for analytical uncertainly, we can use simple statistical tests to determine whether the uncertainty we observed for any group of five samples could conceivably have been solely due to analytical error. For example, given our QA/QC data, we can say there is only a 1 chance in 50 that a CV of 17% would be solely due to analytical error.

Finally, it should be noted that whereas we have an excellent frame of reference to quantify the expected analytical error for our reported data, a sample size of five is entirely inadequate to quantify process uncertainty. [But we are working under the assumption that the pilot plants are collecting far more TP and TRP data than we have access to]. For example, if a process were operating as intended 80% of the time, and it was only sampled at five random times, it is quite likely that one set of data might contain zero "outliers", two datasets might contain two outliers and a fourth dataset might contain two outliers. Whereas a dataset with no outliers might at first inspection seem much different than a dataset with 40% outliers, if the overall sample is only 5 these outcomes are statistically indistinguishable.

Overall, from Figure 2, the coefficients of variation (CV) were shown for all the samples (including the Spokane pilot plant, other plants, Spokane River samples and samples from Inland Empire Paper). The top of the box represents the 75th percentile, the bottom of the box represents the 25th percentile, and the line in the middle represents the 50th percentile. The whiskers (the lines that extend out the top and bottom of the box) represent the highest and lowest values that are not outliers or extreme values. Outliers (values that are between 1.5 and 3 times the interquartile range) and extreme values (values that are more than 3 times the interquartile range) are represented by circles beyond the whiskers.

Our analytical uncertainty for the samples, after taking into account the replicates for each analyte, was ± 2.9% for TP, ± 2.7% for TRP, and 7.0% for BAP. As expected, the BAP results are more variable than TP and TRP, because this method is based on a biological as opposed to a chemical assay. Furthermore, the highest CV values for BAP where generally for cases where the mean BAP estimate was very low and uncertainly was also low in absolute terms. Because the CV is the ratio between the sample SD and mean, even a moderately low SD value can result in a high CV if the mean is also very low.

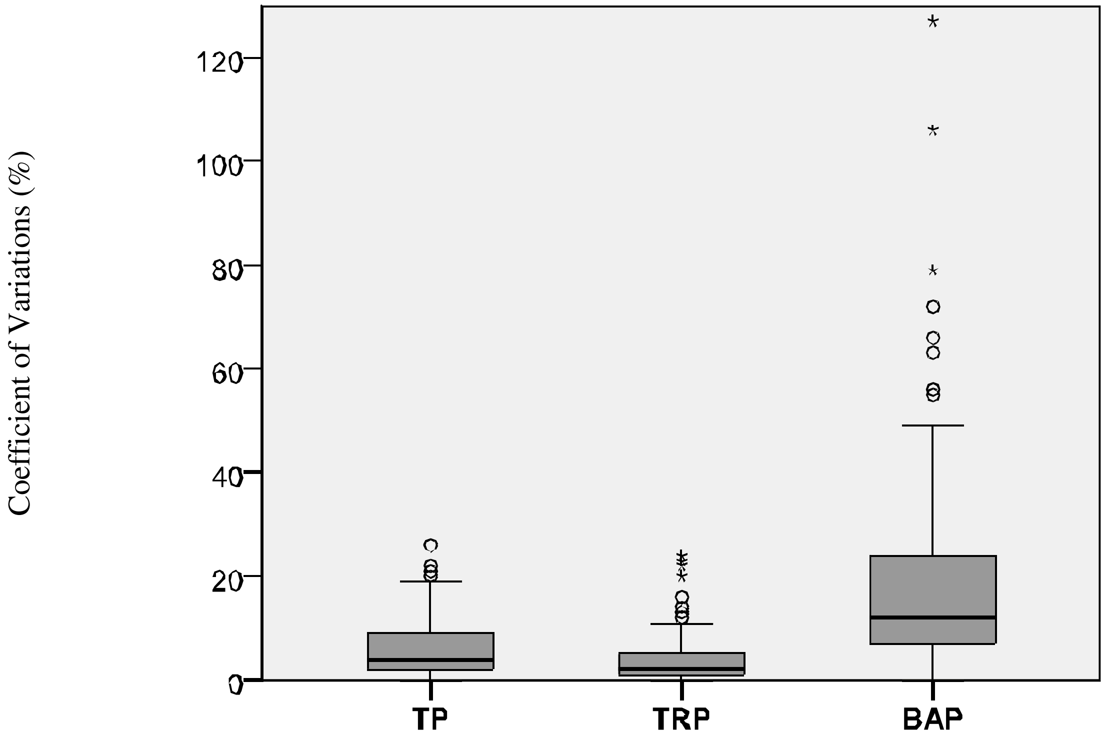


Figure 2 Coefficient of Variations within the treatment For all samples

Because the data we obtained for the Coeur d'Alene, Post Falls, Hayden Area Regional Sewer Board and Liberty Lake WWTP all contained values suggesting outliers relative to analytical uncertainty, we first identified the samples we thought represented process uncertainty for each facility. We then differentiated between the data that suggested optimal operation and those data which suggested process upsets. However, bifurcating our discussion of the data this way is clearly problematic because our sample size for these pilot facilities is so small, even a series of five very consistent values does not by itself prove the advanced P removal process was operating as intended. But by focusing on the "optimal case" data we feel we can provide some insights into what types of outcomes these facilities might be able to achieve if they were operating as intended. But clearly in cases where one or more "outlier" observations were apparent, caution is warranted. Ideally these cases would be followed up with additional samples collected from time periods when the pilot plant operators believe the plants are operating as designed. We suggest all pilot plants should have a sample size of at least 10 constant samples before strong generalization can be drawn.

Thus, the analytical uncertainties of all the analysis were in appropriate range. As discussed in individual section, the analytical uncertainties were much smaller than the variations of the results for certain samples. So the uncertainty we observed could not conceivably have been solely due to analytical error and is likely in part due to process uncertainty.

5. Spokane Pilot plant

Sampling

The Spokane WWTP removes approximately 85% of the P from its influent. A major upgrade to tertiary treatment, scheduled to start in 2011, will remove > 99% of P. Thus a pilot plant has been constructed to aid in the design processes to meet these more rigorous permit limits.

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. The overall alum treatment process could be differentiated into three stages which include influent samples to the pilot plant (INF), intermediate effluents (INT) and final effluents of the pilot plant (EFFL). But it is important to note that in the case of the Spokane Pilot Plant, the samples we call the "Influent" are actually the effluents from the conventional WWTP. The intermediate effluents category contains three samples. Two were sampled after two traditional sedimentation tube settlers and one was obtained after magnetic-based sedimentation. Six samples were collected from six filtration units as final effluent samples. These combinations allow us to test the P removal efficiency for the various unit series. These advanced P removal technologies were based on alum additions which reacted with P to form an aluminum phosphate precipitate which is insoluble within the pH range of typical wastewater.

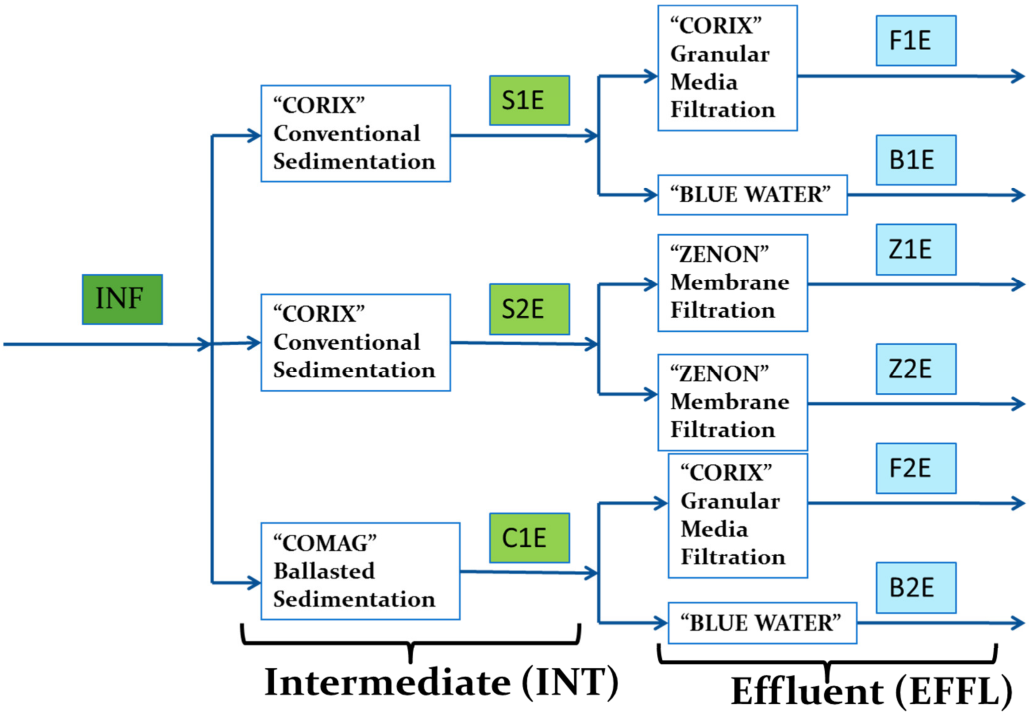


Figure 3 Spokane Pilot Treatment Plant Treatment Process

Composite samples were collected in one liter acid washed (HCl) polyethylene bottles from as near 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 hours.

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).(Table 2-3, Figure 4)

Table 2 Spokane Pilot Plant Overall P removal Performance in Summer (µg·L^{-1})

Table 2a Spokane Pilot Plant Overall TP removal Performance in Summer (µg·L^{-1})

TP								
		8/27/2009	9/10/2009	9/24/2009	10/8/2009	4/15/2010	AVE	STDEV
INF	INF	180	765	245	716	434	468	266
INT	S1E	144	36	39	25	45	43	30
	S2E	33	25	29	30	45		
	C1E	42	17	42	37	62		
EFFL	B1E	25	19	7	17	21	18	5.6
	B2E	29	18	29	15	17		
	F1E	25	23	22	24	17		
	F2E	25	17	13	17	16		
	Z1E	23	14		13	13		
	Z2E	17	14		10	12		

Table 2b Spokane Pilot Plant Overall TRP removal Performance in Summer (µg·L^{-1})

TRP								
		8/27/2009	9/10/2009	9/24/2009	10/8/2009	4/15/2010	AVE	STDEV
INF	INF	123	706	211	398	342	356	223
INT	S1E	37	7	13	8	22	15	8.3
	S2E	19	7	12	12	24		
	C1E	14	6	9	17	17		
EFFL	B1E	13	4	4	2	9	5	3.1
	B2E	8	4	5	2	5		
	F1E	16	5	5	6	5		
	F2E	8	3	5	3	4		
	Z1E	5	4		2	4		
	Z2E	4	4		2	4		

Table 2c Spokane Pilot Plant Overall BAP removal Performance in Summer (µg·L^{-1})

BAP								
		8/27/2009	9/10/2009	9/24/2009	10/8/2009	4/15/2010	AVE	STDEV
INF	INF	86	436	234	436	184	275	156
INT	S1E	5	2	4	11	4	7	4.1
	S2E	7	8	17	8	12		
	C1E	8	3	3	5	4		
EFFL	B1E	0	0	0	1	0	1	1.0
	B2E	3	2	0	0	0		
	F1E	2	0	0	2	1		
	F2E	3	1	0	0	0		
	Z1E	0	0		1	0		
	Z2E	3	1		0	0		

Table 3 Spokane Pilot Plant Overall P removal Performance in Winter (μg·L^{-1})

Table 3a Spokane Pilot Plant Overall TP removal Performance in Winter (μg·L^{-1})

TP						
		11/19/2009	12/3/2009	3/4/2010	AVE	STDEV
INF	INF	2868	2757	2636	2754	116
INT	S1E	200	112	107	116	56
	S2E	199	148	106		
	C1E	73	61	42		
EFFL	B1E	32	48	100	30	21
	B2E	23	25	20		
	F1E	55	37	25		
	F2E	34	28	28		
	Z1E	18	11	14		
	Z2E	18	19	13		

Table 3b Spokane Pilot Plant Overall TRP removal Performance in Winter (μg·L^{-1})

TRP						
		11/19/2009	12/3/2009	3/4/2010	AVE	STDEV
INF	INF	2478	2590	2384	2484	103
INT	S1E	170	85	86	92	54
	S2E	174	121	85		
	C1E	49	41	19		
EFFL	B1E	9	33	72	15	17
	B2E	6	10	8		
	F1E	33	23	16		
	F2E	13	13	18		
	Z1E	2	1	3		
	Z2E	3	7	2		

Table 3c Spokane Pilot Plant Overall TRP removal Performance in Winter (μg·L^{-1})

BAP						
		11/19/2009	12/3/2009	3/4/2010	AVE	STDEV
INF	INF	2060	2593	1882	2178	370
INT	S1E	89	59	56	60	31
	S2E	113	76	59		
	C1E	36	41	9		
EFFL	B1E	6	1	55	6	13
	B2E	3	3	2		
	F1E	6	1	2		
	F2E	4	10	2		
	Z1E	2	2	0		
	Z2E	1	5	1		

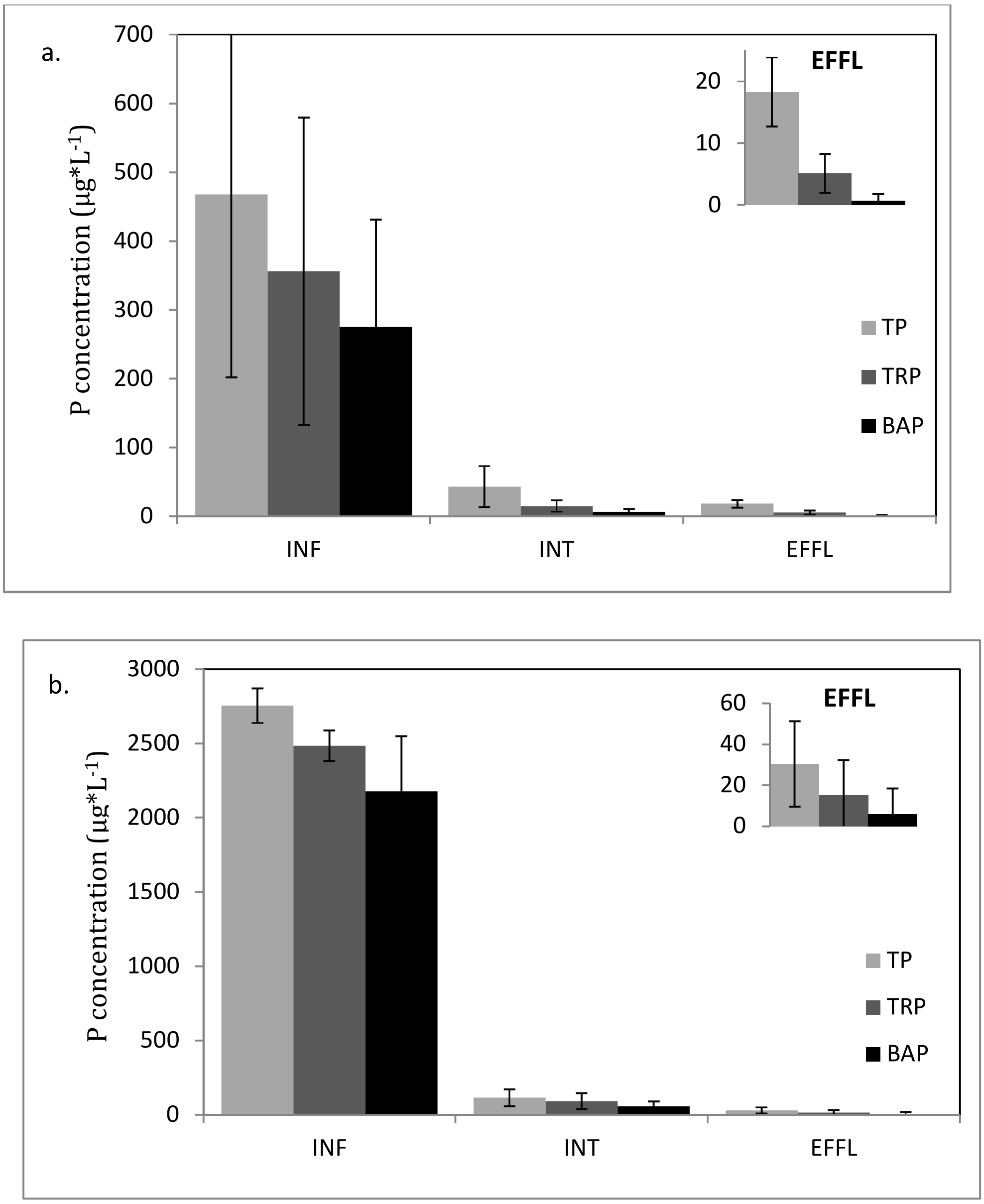


Figure 4. TP,TRP,BAP concentration profiles for the samples
(when the pilot plant was under summer (a) and winter (b) operation. INF-Influent, INT-Intermediate Effluent, EFFL-Final Effluent, Error bars represent standard deviations.)

Winter Operation

The TP in the INF sample (post primary and secondary treatment) averaged 2750 µg·L^{-1} during winter operation. The TP removal efficiency in the pilot processes for the first alum addition was approximately 96% TP while the second alum addition with filtration process removed around 74% from the intermediate effluent. 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 ± 17µg·L^{-1} in the final effluent. The bioassay reveals that average BAP for influent sample was similar to TP with a value of 2180 µg·L^{-1}. However, BAP 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} of this bioavailable.

Summer Operation

Because alum was added after secondary treatment in the summer, TP was reduced compared to winter samples by a factor of 5 (i.e., to 468 µg·L^{-1} for influent TP sample) and by a factor of 2 from (18 µg·L^{-1}) for the EFFL TP (Figure 4b). The TP in the influent samples (post primary and secondary treatment) ranged widely as did the concentration of the different fractions as shown in Figure 4b. After the alum addition in secondary wastewater treatment, the second alum addition in the pilot plant reduced TP concentration 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. The BAP concentration dropped off 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.

Relationship between %BAP and P removal level

How the percent of the TP that was bioavailable (calculated as dividing BAP by TP) varied with different P removal levels was assessed (Figure 5). 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 variation in BAP bioassay results is larger especially at very low BAP values.

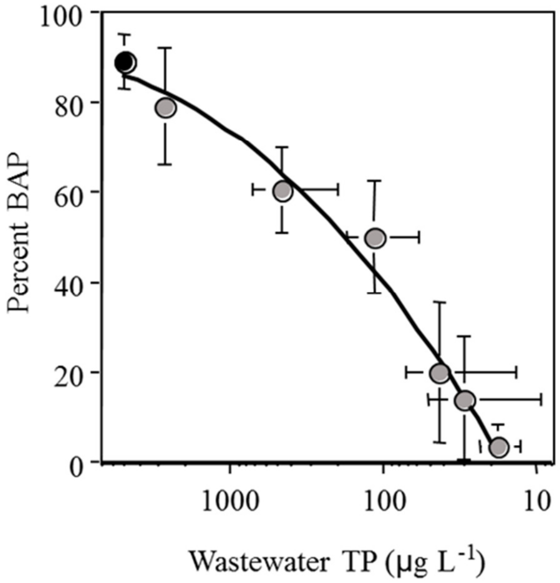


Figure 5 %BAP and TP relationship. Error bars represent standard deviations.

The %BAP vs. TP regression model we derived for the overall alum treatment process is shown as below:

%BAP = -12.19*log $(TP)^2$ + 92.03*log(TP) + 94.17; $r^2 = 0.98$, n = 7, MSE = 10.3%

BAP and 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 Figure 6. 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 > 0.99). The ratio of BAP and TRP declined as the P removal decreased 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.

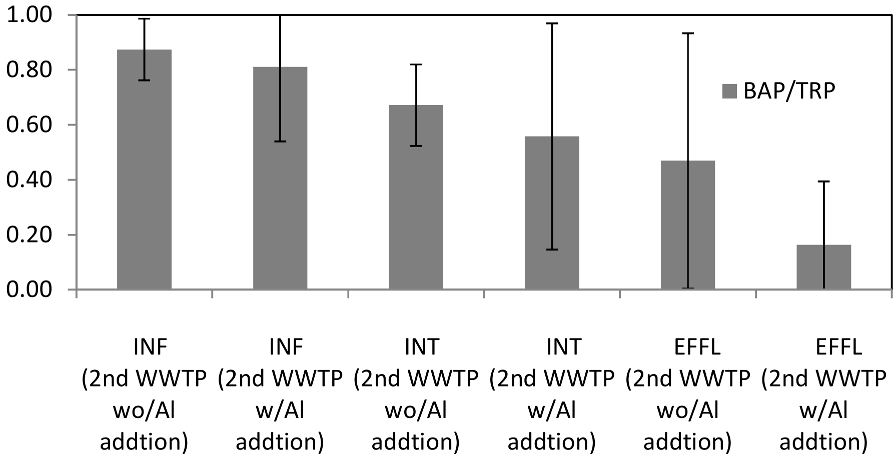


Figure 6. BAP/TRP for six different sample types.
(INF-Influent, INT-Intermediate Effluent, EFFL-Final Effluent. Error bars represent standard deviations.)

Discussion

The phosphorus fraction 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 the lowest levels (20 $\mu g \cdot L^{-1}$) for any WWTP facility that we are aware of in the United States.

The P concentration profile for the winter operation followed the same pattern as the summer condition albeit with a higher initial concentration and the concentrations of two phases are interlaid with each other. Without alum addition in secondary treatment, the pilot plant was able to reduce the TP to 30 $\mu g \cdot L^{-1}$ with only 6 $\mu g \cdot L^{-1}$ BAP. From a sustainability perspective, these results suggest it might be possible to implement plant operation without alum addition in secondary WWTP. It is interesting that the results also show the percentage of TRP (%TRP) relative to TP declined from ≈ 80% in the influent to ≈30% in the final effluent. The decreases in %TRP and %BAP indicate that not only was TP reduced to very low values but also the composition of the P was changed markedly.

Both the quantity of P as well as the availability of P in the environment is critical to the issue of eutrophication. In both the winter and summer scenarios BAP was reduced 99% with only ≈ 6 $\mu g \cdot L^{-1}$ and 1 $\mu g \cdot L^{-1}$ left in the final effluent for winter and summer scenario, respectively. This suggests that most of P forms, which can readily stimulate algal growth, have been effectively sequestered by this alum based 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 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 appears 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 averaged at 0.44, which suggests that TRP could be used in place of BAP as a conservative measure of the eutrophication potential of alum treated wastewater effluent. These results suggest that the 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.

Furthermore, although TRP is generally assumed to be mostly bioavailable for algal growth, our results indicated that BAP was only around half of TRP. This suggests that there is a large portion of TRP which can’t be utilized for algal growth. Thus it is necessary to analyze the actual P species transfer during the bioassay. 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 (21). 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 polymerised P rather than dissolved orthophosphate (8, 11). 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 (13). If water column or sediment dissolved oxygen concentration decline below 2 mg L^{-1}, some of this immobilized P will be released to the water column (13).

Recent studies have shown organic phosphorus is the dominate component of the non-reactive phosphorus which is estimated by subtracting reactive P from the TP concentration (22-24). Several studies have shown phytoplankton can utilize some forms of dissolved organic P in the absence of inorganic P (25, 26). For example, it is shown that nucleotides were the most readily utilizable of the combined phosphorus compounds investigated (27). 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 sensitive to pH and dissolved oxygen (5). This suggests the change in %BAP is caused by alum precipitation converting soluble reactive phosphorus into particulate Al-bound phosphorus. Also, particulate organic P is relatively stable and only bioavailable after protracted sedimentary digenesis (13).

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 the 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 very low %BAP values.

Further studies need to be carried out in order to identify the species of P which are not bioavailable in this alum treated wastewater effluent. It would be of interest to analyze the bio-availability of certain 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. All these experiments would enable a better understanding of bio-availability 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.

6. City of Coeur d’Alene

First, it should be noted that the TP, TRP and BAP results we analyzed for the Blue Water Filtration effluent (*i.e.,* CCE) were reasonably consistent in all cases (Table 4). However, in several cases one or more samples did not fit the pattern suggested by the others. For example, four of the influent samples for the Coeur d'Alene pilot plant (*i.e.,* CCI) had TP concentrations that averaged 690 ± 50 µg L^{-1} (± 1 SD), while a fifth sample had a value of 960 µg TP L^{-1}. This outlier was also evident in the corresponding CCI TRP samples, i.e., 620 ± 50 µg L^{-1} for four samples, and 820 µg L^{-1} for the other. Similarly, four of the Zenon Membrane Filter effluent samples (*i.e.,* CTE) fell within a moderately consistent range, i.e., 28 ± 18 µg TP L^{-1}, while a fifth sample had a concentration of 550 µg L^{-1}. The same problem was evident for the TRP and BAP results for these same CTE samples. In the case of the influent samples from the MBR to the Zenon Membrane system (*i.e.,* CMI), three TP samples averaged 5360 ± 140 µg L^{-1}, and two averaged 8860 ± 210 µg L^{-1}, with similar variability for the corresponding TRP and BAP data. Finally, the effluent samples from the MBR to the Zenon Membrane system (*i.e.,* CME) varied considerably without an obvious central tendency (*e.g.,* TP ranged from 35 to 7260 µg L^{-1}). These highly variable results are almost certainly because the Cd'A Pilot Plant was in the process of ramping up and was not stable for all processes. But this high variably makes it challenging to distinguish what levels of P removal these plants were capable of versus what they actually achieved.

Table 4 City of Coeur d’Alene Overall removal Performance

Table 4a City of Coeur d’Alene Overall TP removal Performance ($\mu g \cdot L^{-1}$)

	TP								
	1	2	3	4	5	AVE	SD	Outliers	Optimal performance
	5/13	6/10	6/25	7/15	8/10				
CCI	956	662	648	688	758	742	127		
CCE	24	35	41	35	15	30	11		
CTE	27	**545**$^{\times}$	53	20	11	131	232	2	28±18
CMI	5227	**8715**$^{\times}$	**9009**$^{\times}$	5506	5344	6760	1924	2,3	5359±140
CME	261	**7264**$^{\times}$	**3203**$^{\times}$	94	35	2171	3143	2,3	130±117

Table 4b City of Coeur d'Alene Overall TRP removal Performance (µg·L^{-1})

TRP									
	1	2	3	4	5				Optimal performance
	5/13	6/10	6/25	7/15	8/10	AVE	SD	Outliers	
CCI	818	646	616	549	661	658	99		
CCE	9	21	24	19	3	15	9		
CTE	18	**517**$^{\times}$	38	9	2	117	224	2	17±16
CMI	4289	**4394**$^{\times}$	**6841**$^{\times}$	4176	4115	4763	1167	2,3	4193±88
CME	235	**6732**$^{\times}$	**2853**$^{\times}$	58	15	1979	2913	2,3	103±117

Table 4c City of Coeur d'Alene Overall BAP removal Performance (µg·L^{-1})

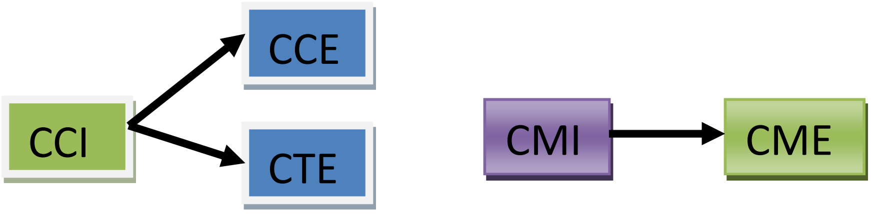
BAP									
	1	2	3	4	5				Optimal performance
	5/13	6/10	6/25	7/15	8/10	AVE	SD	Outliers	
CCI	351	517	345	608	673	499	149		
CCE	1	4	4	1	2	2	1		
CTE	8	**158**$^{\times}$	2	1	2	34	69	2	3±3
CMI	3034	**4127**$^{\times}$	**5075**$^{\times}$	3107	2910	3651	933	2,3	3017±99
CME	5	**2364**$^{\times}$	**2075**$^{\times}$	25	8	895	1213	2,3	13±11

$^{\times}$ The marked P concentrations were assumed to be outliers due to unstable operation based on their TP values. The average values in expected performance were calculated without consideration of the outliers.

Sampling

Coeur d'Alene pilot plant utilized several different processes to generate the samples we processed. In the first process the influent to advanced P removal process consisted of the effluent from the conventional treatment plant's secondary effluent with alum addition before secondary clarifiers, this "influent" to the pilot plant was labeled CCI. The water was fed into a Tertiary membrane filter (TMF) and Continuous up flow media filter, i.e., a Blue Water Continuous Upflow filtration, Iron sand filter. This advanced effluent was labeled CCE. Water from the CCI was also processed using a MBR - Zenon Membrane Filter, and was labeled CTE. Primary effluent from the Coeur d'Alene conventional plant (with no chemical addition ahead of primary settling) was also used as influent to a MBR – Zenon Membrane Bio Reactor system, and was labeled CMI. Final effluents from MBR – Zenon Membrane Bio Reactor system was labeled CME. The simplified diagram of the treatment process of the City of Coeur d'Alene is shown in Figure 7.

Five sets of samples were collected from five different treatment processes within the City of Coeur d’Alene pilot plant from May 13th 2010 to August 10th 2010. Two sets (i.e., July 25th and August 20th) were grab samples and the others were composite samples.



CCI--Coeur d'Alene Influent to Tertiary membrane filter (TMF) & Continuous up flow media filter (CUMF – same as blue water), the influent is the same as the plants secondary effluent with alum addition before secondary clarifiers.

CCE-- Coeur d'Alene Effluent from CUMF - Blue Water Continuous Upflow filtration, Iron sand filter

CTE-- Coeur d'Alene Effluent from TMF – Zenon Membrane Filter

CMI--Coeur d'Alene Influent to MBR – Zenon Membrane Bio Reactor system, MBR influent is the same as primary effluent (No chemical addition ahead of the primaries) Influent

CME--Coeur d'Alene Effluent from MBR – Zenon Membrane Bio Reactor system

Figure 7 City of Coeur d’Alene treatment process

Results

CCI

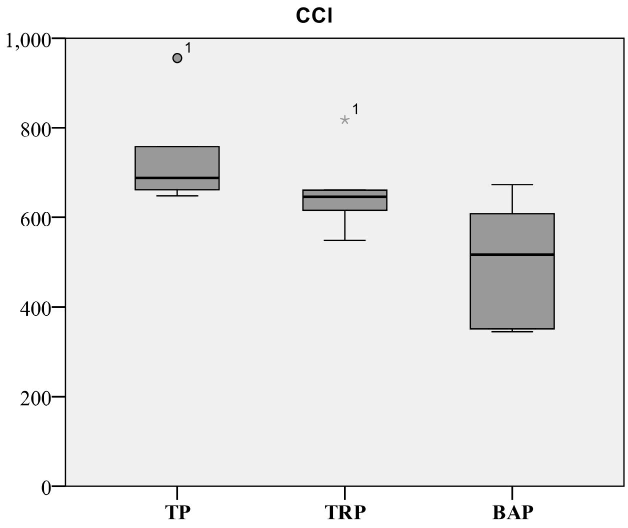


Figure 8 P concentrations in Coeur d'Alene Influent to Blue water and Zenon Membrane Filter.

The TP in the influent sample to the pilot plant (i.e., post primary and secondary treatment) to the Blue water and Zenon Membrane filtration averaged 742 ± 127 $\mu g \cdot L^{-1}$. The average TRP concentration (658 μg·L^{-1}) was similar to TP with the May 13th sample (TP = 956 μg·L^{-1}, TRP = 818 μg·L^{-1}) indicated as a statistical outlier. The bioavailable P averaged 499 ± 149 μg·L^{-1}), *i.e.*, 69% of TP. Hence, in the CCI sample, most of the phosphorus was available to algae.

CCE&CTE

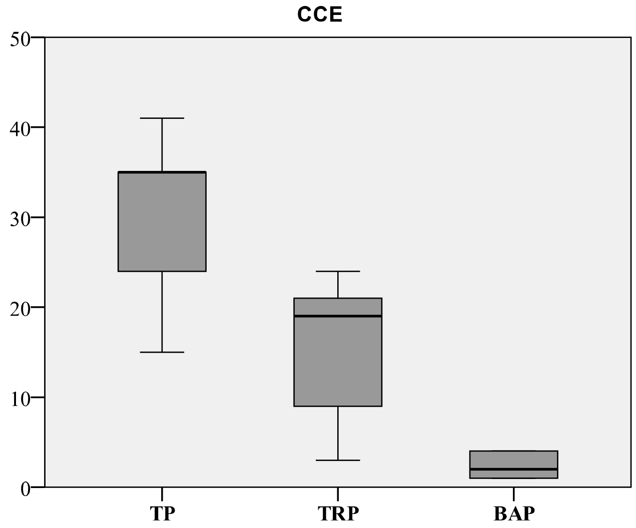


Figure 9 P concentrations in Coeur d'Alene Effluent from CUMF - Blue Water Continuous Upflow filtration, Iron sand filter

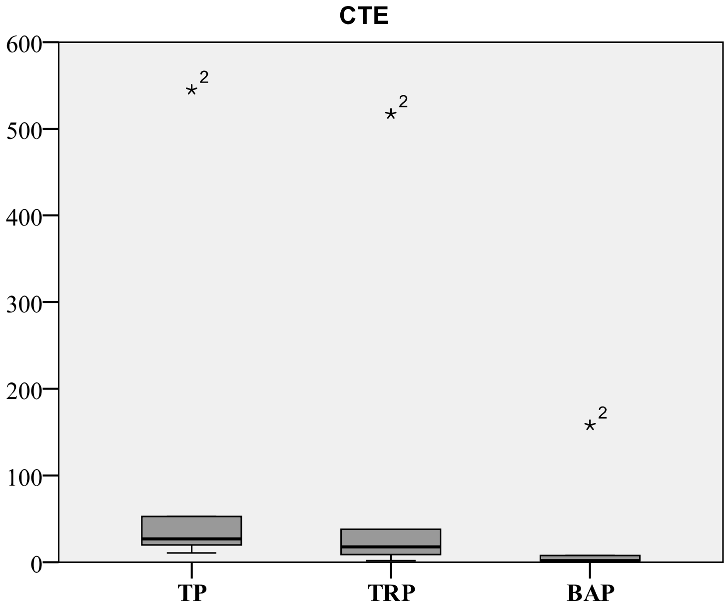


Figure 10 P concentrations in Coeur d'Alene Effluent from TMF – Zenon Membrane Filter

After Blue Water treatment, the TP of the CCE sample was reduced from 742 µg·L^{-1} to 30 µg·L^{-1}. The final effluent had a TRP concentration of 15 ± 9 µg·L^{-1}, and the algal bioassays indicated that the average BAP for the CCE samples was only 2 ± 1 µg·L^{-1}.

Zenon membrane filtration following conventional treatment reduced TP to 28 µg·L^{-1} (if one ignores the June 10th outlier). The TRP and BAP concentrations were 17 ± 16 µg·L^{-1} and 3 ± 3µg·L^{-1} respectively, in the CTE samples (again ignoring one outlier).

CMI

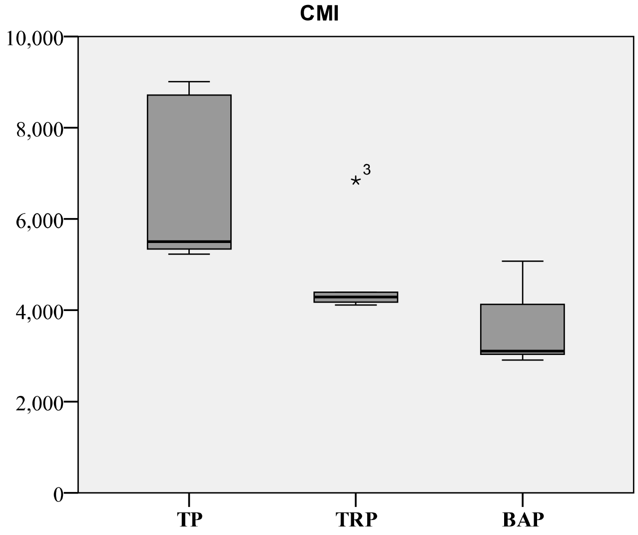


Figure 11 P concentrations in Coeur d'Alene Influent to MBR – Zenon Membrane Bio Reactor system.

The TP concentrations in the Coeur d'Alene influent to the membrane bioreactor system were much higher than the influent to the membrane and blue water systems. The average TP concentration was 6760 ± 1920 µg·L^{-1} with 4760 ± 1170 µg·L^{-1} as TRP. The algal bioassays indicated the bioavailable P was 3650 ± 930 µg·L^{-1}, i.e., 50-60% of the TP concentration.

CME

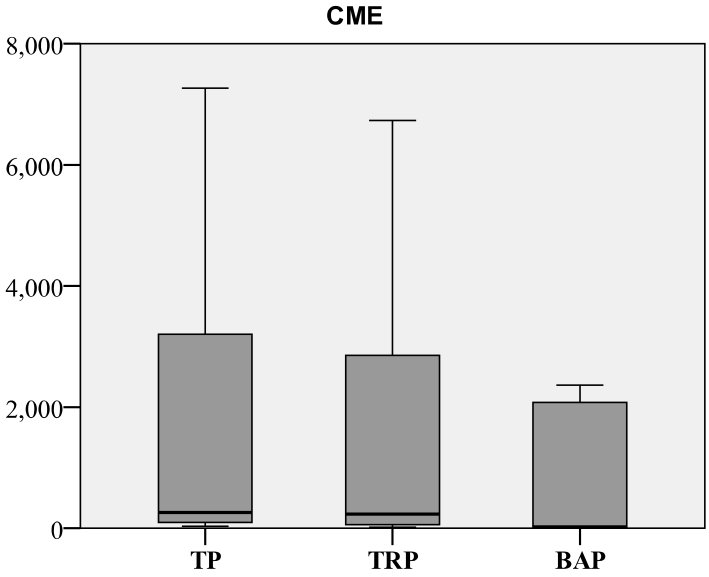


Figure 12 P concentrations in Coeur d'Alene Effluent from MBR – Zenon Membrane Bio Reactor system

Presumably, because the operation of the MBR system was still in a development phase, highly variable values were obtained. In particular, the P concentrations on June 10th and June 25th were much higher than those for other dates. TP concentration were around 7000 µg ·L^{-1} and 3000 µg·L^{-1} on these days, respectively. However, when the MBR was presumably operating as intended, the TP mean for the other three sets of samples was markedly decreased in the final CME to only 130 ± 120 µg·L^{-1} with 103 ± 120 µg·L^{-1} as TRP. The algal bioassays showed the BAP for the effluent sample was reduced to 13 ± 11 µg·L^{-1}, for the "optimal" condition.

Overall Performance Summary

Conclusions

As we can see from Tables 4a-4c, there was high variation associated with the P concentrations for most samples, especially the samples from CTE and CME. From the QA/QC data, it was shown that the average analytical coefficients of variation (CV) were in the range of 2%-3% for TP and TRP. Although, the CVs in our BAP estimates were higher (*i.e.,* ± 7%) after taking into account sample replication. All CV outliers for BAP were caused by the mean BAP for that specific sample (etc., CTE and CME) approaching the analytical limits for this bioassay.

P Removal Efficiency

From Table 1, it can be seen that although there were some unexpected values for the membrane and MBR systems, if we only consider the dates with optimal results, the overall system operation performance was quite encouraging. The TP removal efficiency was approximately 96% in the blue water and membrane treatment processes while the MBR removed around 98% from a much higher influent concentration.

%TRP vs. %BAP

Also, the percent of the TP that reacted with acid molybdate reagents (%TRP) and the percent that was bioavailable to algae (%BAP) varied with different P removal techniques as summarized in Figure 8.

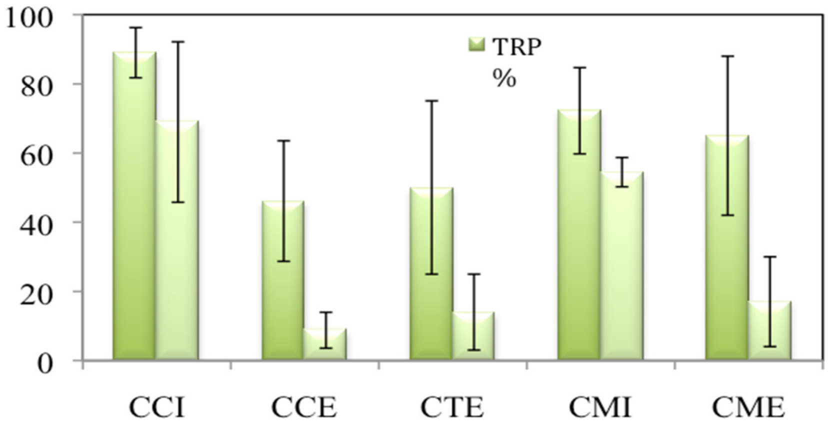


Figure 13 Comparison of %TRP and %BAP in City of Coeur d'Alene.
(The data under unstable conditions have been excluded.)

Prior to any treatment, the influent to the pilot plant with Blue Water and Zenon Membrane systems had an average %TRP of 89 ± 7% and %BAP of 69 ± 23%. After Blue water treatment, the percent P which reacted with molybdate was reduced to 46% while only 9% was bioavailable. Similarly, the %TRP and %BAP were reduced to 50 ± 25% and 14 ± 11%, respectively, after membrane treatment. In the MBR system, the percentage of BAP decreased from 55 ± 4% in the influent to 17 ± 13% in the effluent. This suggests that the forms of P which most readily stimulate algal growth, were effectively sequestered by these P removal processes. Furthermore, it is indicated in the Figure 13 that the %BAP was consistently lower than %TRP which is in agreement with the results obtained from the Spokane WWTP pilot plant. This result in a different plant using different technologies suggests that TRP could be used in place of BAP as a conservative measure of the eutrophication potential of highly treated wastewater effluents.

In sum, when operating as designed, the three technologies employed in this pilot plant were quite effective in P removal. Although this result is encouraging, the instability of operation reflected in the highly variable results indicated that more samples from the whole process, especially the effluent from the membrane treatment system, are needed to establish more conclusive outcomes.

7. Post Fall

The overall P removal efficiency of Post Falls WWTP using biological nutrient removal technology was around 90%. However, it should be noted that the effluent samples varied considerably without an obvious central tendency (Table 5). For example, TP ranged from 176 to 1024 μg·L^{-1} with two TP samples averaging 175 ± 2 μg·L^{-1}, and the rest averaging 752 ± 334 μg·L^{-1}. The corresponding TRP and BAP data showed similar variability. Considered relative to the consistent replication within samples as shown in the QA/QC section, these highly variable results suggest the Post Falls WWTP was still in a developmental phase. This variation for the effluent samples makes it challenging to distinguish what levels of P removal this plant was capable of versus what they actually achieved.

Table 5 Overall P removal Performance (μg·L^{-1})

		1	2	3	4	5	AVE	SD	Outliers	Optimal performance
		5/13	6/10	6/25	7/15	8/10				
TP	PFI	5527	8444	7844	6816	6478	7022	1148		
	PFE	176	**852**$^{\times}$	174	379	**1024**$^{\times}$	521	395	2,5	243±118
TRP	PFI	4980	6173	5489	5236	5032	5382	485		
	PFE	72	**652**$^{\times}$	73	279	**788**$^{\times}$	373	332	2,5	141±119
BAP	PFI	3432	3269	2973	3290	4020	3397	386		
	PFE	58	**561**$^{\times}$	64	241	**839**$^{\times}$	352	340	2,5	121±104
%TRP	PFI	90	73	70	77	78	78	8		
	PFE	41	**76**$^{\times}$	42	74	**77**	62	19	2,5	52±19
%BAP	PFI	62	39	38	48	62	50	12		
	PFE	33	**66**$^{\times}$	37	64	**82**$^{\times}$	56	21	2,5	44±17

$^{\times}$The marked P concentrations were classified as outliers based on these TP values. The average values in bracket were calculated without the consideration of the unexpected value.

Sampling

Five sets of samples were collected from five different treatment processes within Post Falls WWTP from May 13th 2010 to August 10th 2010. All five sets of samples were composite samples. The simplified diagram of the treatment process of the Post Falls WWTP is shown in Figure 14. Based on the information from the plant, the influent is the raw municipal sewage as it enters the treatment plant at the headwork prior to any screening or treatment. Sewage sources include residential, commercial and industrial facilities, with at least 80% from residential. The effluent from this facility is the final product of biological P removal treatment process prior to discharge to the Spokane River, and after all treatment steps have been completed.

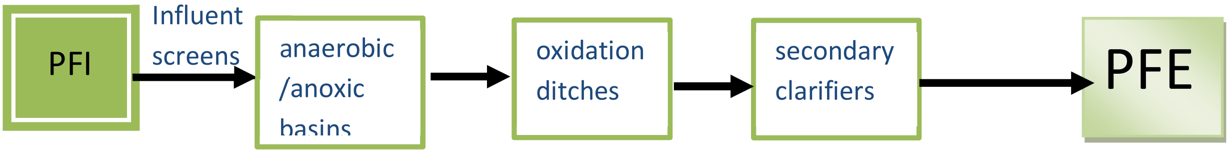


Figure 14 Post Falls treatment process

PFI--Post Falls Influent
PFE--Post Falls Effluent - Biological nutrient removal

Result

PFI

The TP in the influent samples ranged widely as did the concentration of the different fractions as shown in Figure 15. The influent P concentrations were relatively high with a mean TP concentration of around 7000 µg·L^{-1}. TRP averaged 5380 ± 490 µg·L^{-1}, which was 78% of the TP pool. The BAP bioassay result showed the BAP concentration was 3400 ± 390 µg·L^{-1}, which was approximately 50% of TP.

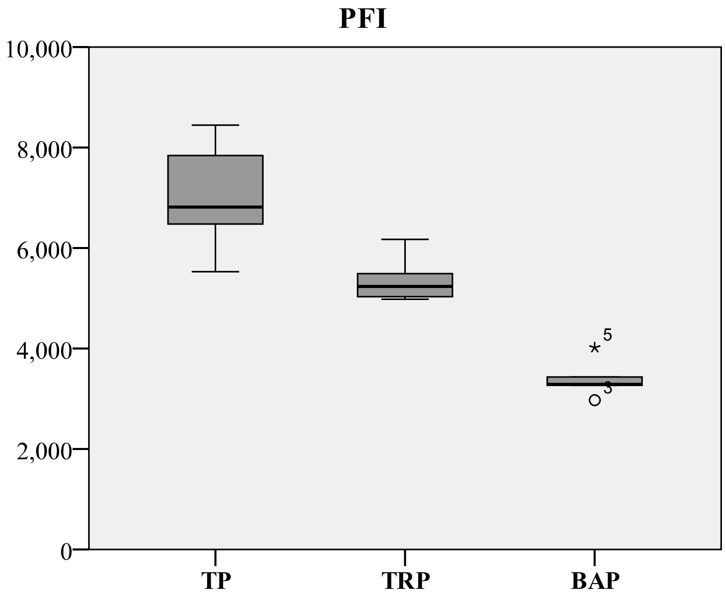


Figure 15 P concentrations in Post Falls Influent

PFE

In Figure 16, it is shown that the P concentrations for the PFE samples had huge variation which might due to pilot plant operational instability. Taking all the samples into consideration, the TP concentration was 520 µg·L^{-1} with a very large standard deviation of 400 µg·L^{-1}. However, if one excludes the June 10th and August 10th samples, the TP concentration averaged 243 µg·L^{-1}. Similarly, when ignoring apparent outliers, the TRP and BAP concentrations were 141 µg·L^{-1} and 121 µg·L^{-1}, respectively.

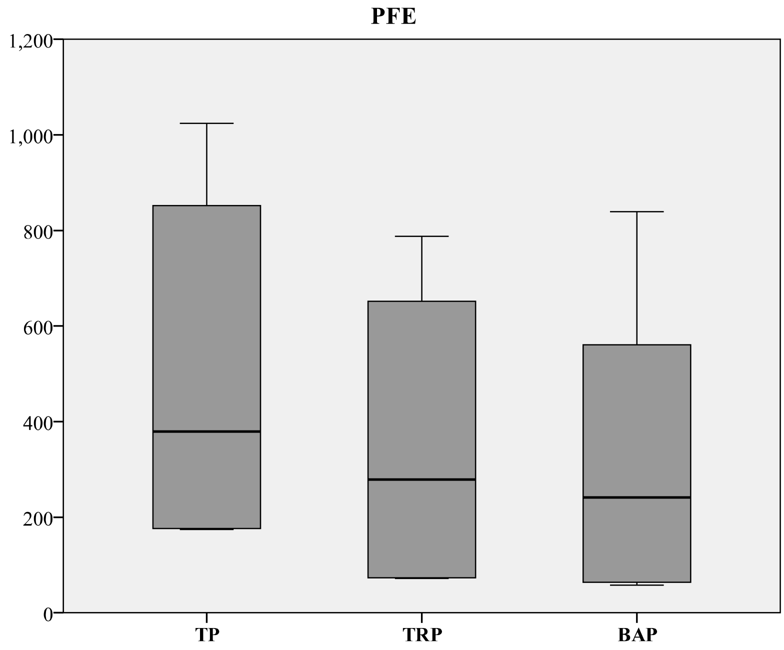


Figure 16 P concentrations in Post Falls Effluent

Overall P removal performance

Conclusions

From Table 5, it is clear that there was high variation associated with the P results for the PFE samples, e.g., TP ranged from 176 to 1024 µg·L^{-1}. In particular, two sets of samples showed much higher concentrations than the others (June 10th and August 10th). The TRP and BAP analysis also showed similar high values on these sampling days. When calculating the %TRP and %BAP based on TP, these three sets of samples also had clearly higher values than the others. These unexpected values suggest these results were caused by process variability in the WWTP as opposed to analytical uncertainties from our laboratory analyse.

P Removal Efficiency

Despite the variation in the effluent samples, the removal efficiency of the Post Falls plant was very high. After biological nutrient removal, the TP concentration in the influent was reduced 99% from around 7000 μg·L^{-1}. Thus, this facility was able to get the TP concentration down to 243 ± 118 μg·L^{-1} in the final effluent. Also, the TRP concentrations were reduced 99% and BAP concentration dropped off 98% to only 121 ± 104 μg BAP ·L^{-1} in the final effluent.

%TRP vs. %BAP

We also examined how the percent of the TP that can react with molybdate (%TRP) and the percentage of BAP (%BAP) varied after biological treatment (Figure 17).

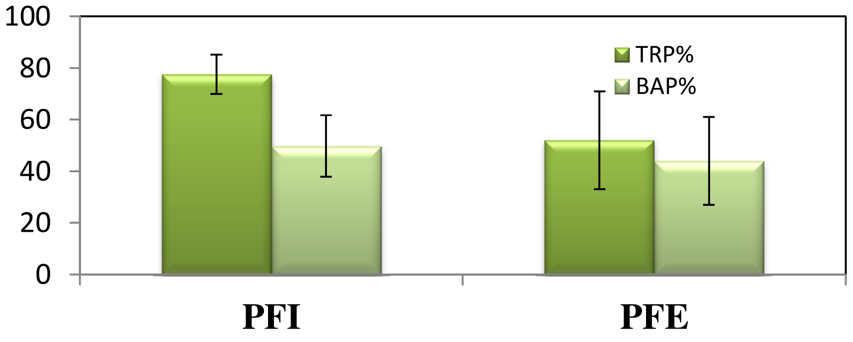


Figure 17 Comparison of %TRP and %BAP in Post Falls.
(The data under unstable condition have been excluded.)

Prior to any treatment, the influent to the plant had an average %TRP and %BAP of 78 ± 8% and 50 ± 12%, respectively. After biological nutrient treatment, the percentage of TP which reacted with molybdate was reduced to 52% while 44% was bioavailable. This suggests that even though there was some variation in the effluent results, the decreasing tendency of percentage of the P forms which react with chemical reagents and readily stimulate algal growth showed that both the quantity and the quality of phosphorus that promotes eutrophication was reduced. Furthermore, it was again confirmed in Figure 17 that the %BAP was consistently lower than %TRP which is in agreement with the result from the Spokane WWTP plant and other pilot plants. Thus, this result in a different plant applying different technologies, suggests that TRP can be used in place of BAP as a conservative measure of the eutrophication potential of wastewater effluents.

In conclusion, the biological nutrient removal technologies employed in this plant was quite effective for P removal as regard both quantity and quality. However, the high removal efficiency estimates were due in part to the exclusion of several outlier values. The instability of operation reflected in the result indicated that more samples from the whole process are needed to establish conclusive outcomes.

8. Liberty Lake

To assess the results from the Liberty Lake Sewer and Water District (LLSWD) WWTP, we need to first discuss the variability for the samples we processed from this facility. Although the overall P removal efficiency of LLSWD WWTP using biological nutrient removal technology was relatively high (≈ 90%), one set of effluent samples (LLE) that had almost 3 times higher concentration than other four sets of samples (Table 6). For example, the TP of five effluent samples fell within a moderately consistent range of 240 ± 53 (± 1SD) µg·L^{-1}, while a sixth sample had a concentration of 1070 µg·L^{-1}. The same problem was evident for the TRP and BAP results for this same LLE sample. Thus, with the consideration of the relatively consistent analytical replication within lab replicates as shown in the QA/QC section, these highly variable results suggest the LLSWD WWTP was not stable during the time the sixth sample were located.

Table 6 Overall P removal Performance (µg·L-1)

		1	2	3	4	5	6	AVE	SD	Outliers	Optimal performance
		4/15	5/13	6/10	6/25	7/15	8/10				
TP	LLI	6675	5490	6722	7430	6395	6733	6574	632		
	LLE	162	219	263	304	259	**1066**[×]	379	340	6	241±53
TRP	LLI	4814	4111	4783	5366	4484	5526	4847	531		
	LLE	84	208	152	188	171	**904**[×]	284	307	6	160±47
BAP	LLI	4046	3176	3529	3096	4751	3929	3755	621		
	LLE	51	96	141	126	161	**1034**[×]	268	377	6	115±43
%TRP	LLI	72	75	71	72	70	82	74	4		
	LLE	52	95	58	62	66	**85**[×]	70	17	6	66±17
%BAP	LLI	61	58	53	42	74	58	58	11		
	LLE	32	44	54	42	62	**97**[×]	55	23	6	47±12

[×] The marked P concentrations were classified as outliers based on their TP values. The average values in bracket were calculated without the consideration of the unexpected value.

Sampling

Six sets of samples were collected from the LLSWD WWTP from April 15th 2010 to August 10th 2010. All samples were composite samples. The simplified diagram of the treatment process of the LLSWD WWTP is shown in Figure 18. The plant is a 2 MGD design capacity activated sludge biological nitrogen and phosphorus removal facility.

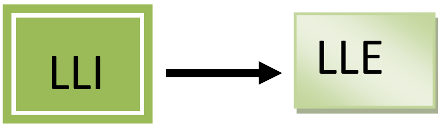


Figure 18 Libery Lake treatment process

LLI-- LLSWD WWTP - Influent
LLE— LLSWD WWTP - Effluent

(Note: we need a lot more detail for the treatment process, we feel only conventional biological treatment can't get TP from 7,000µg·L^{-1} INF down to 300 µg·L^{-1} in the effluent.)

Result

LLI

The P concentrations in the influent sample were relatively high as shown in Figure 20. TP concentration was approximately 6570 ± 630 µg·L^{-1}. The TRP concentration (4850 ± 530 µg·L^{-1}) formed 74% of total phosphorus. The BAP bioassay indicated that the average BAP concentration was 3760 ± 620 µg·L^{-1}, which was approximately 60% of the TP.

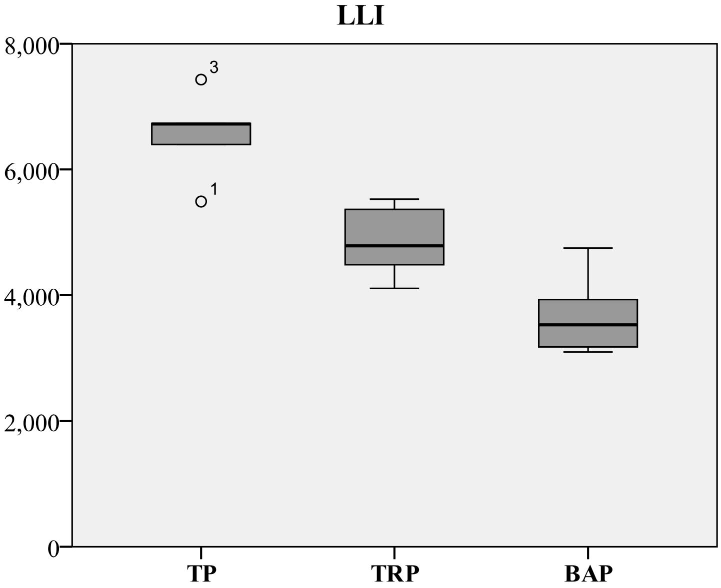


Figure 19 P concentrations in Liberty Lake Influent

LLE

The P concentration profiles in effluent from LLSWD WWTP are shown in Figure 20. The performance of P removal was relatively stable except for one outlier value collected on August 10th 2010. When ignoring this sample, the average TP concentration in the effluent was 241 ± 53 µg·L^{-1}. Similarly, excluding the outlier, the TRP and BAP concentrations averaged 160 ± 47 µg·L^{-1} and 115 ± 43 µg·L^{-1}, respectively.

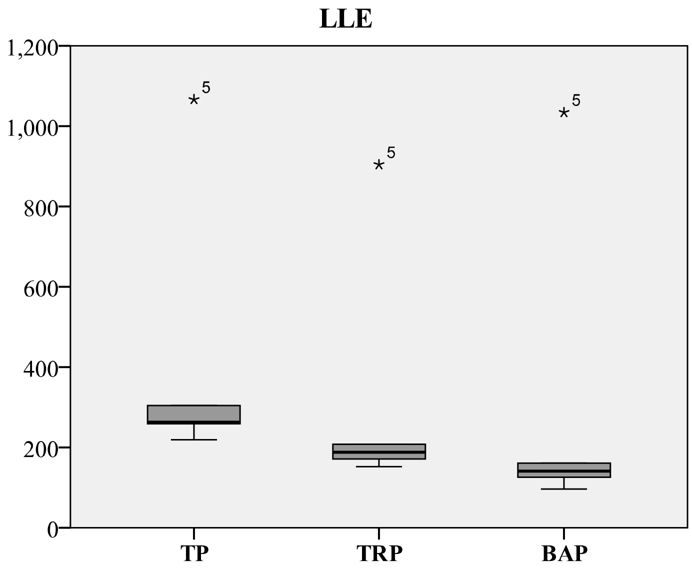


Figure 20 P concentrations in Liberty Lake Effluent
(5: the fifth sample on August 10th, 2010)

Overall P removal performance

Conclusions

As we can see from Table 6, the P concentrations for first five sets of samples had reasonable variation. However, the effluent TP on August 10th (1066 μg·L^{-1}) was almost 3 times higher than the average for the other samples. Furthermore, TRP and BAP analyse also showed similar high results for that sampling day. When calculating the %TRP and %BAP relative to TP, these values for the sixth set of samples was also clearly higher value than the others. This suggests these results were caused by treatment process variability rather than analytical uncertainties from the laboratory analysis.

P Removal Efficiency

For the five consistent samples, the removal efficiency for the Liberty Lake WWTP was high. After nutrient removal, the TP, TRP and BAP concentrations in the effluent were all reduced 96%. Thus, this pilot facility was able to get TP concentrations down to 241 ± 53 μg·L^{-1} with 115 ± 43 μg·L^{-1} bioavailable to algae in the final effluent.

%TRP vs. %BAP

The fraction of TP that reacted with acid-molybdate (%TRP) and the percentage of BAP (%BAP) was also calculated (Figure 21).

In the influent to the plant, %TRP and %BAP averaged 74 ± 4% and 58 ± 11%, respectively. After P removal, the percentage of TRP was reduced to 70% while 47% of the TP was bioavailable. Although these changes in the %TRP and %BAP were not significant, these results suggest a modest decreasing tendency of %TRP and %BAP suggesting some changes in the composition of the P pool in the effluent compared with the influent. We also showed in Figure 21 that the %BAP was consistently lower than %TRP, which was consistent with the results from the Spokane WWTP and other pilot plants, suggesting that TRP can be used in place of BAP as a conservative measure of the eutrophication potential of wastewater effluents.

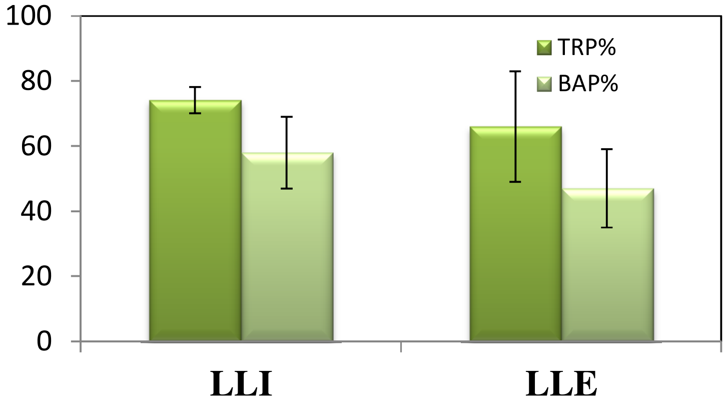


Figure 21 Comparison of %TRP and %BAP in LLSWD WWTP.
(The data under unstable condition have been excluded.)

In conclusion, the P removal technologies employed at this plant showed good P removal. However the one outlier in the effluent samples indicated that more samples from the whole process might be useful. Overall, the technologies employed in LLSWD WWTP efficiently reduced the quantity of P in the effluents, but our results suggest the quality of P in the effluents did not change markedly. The %BAP values obtained from the final effluents from this process were only somewhat lower than %BAP for the influents, and about a factor 5 higher than the %BAP values obtained for several of the other plants we tested (i.e., ≈ 10% of %BAP).

9. Hayden Area Regional Sewer Board

First, it should be noted that we didn't have the chance to obtain more than three samples from a specific processes from Hayden Area Regional Sewer Board (HARSB) at same time. Further only one effluent sample from Blue Water effluent was analyzed at June 25th, 2010. Also, in several cases one or more samples did not fit the pattern suggested by the others. For example, the three influent samples for the HARSB pilot plant (*i.e.,* HARSBI) had TP concentrations that ranged from 3740 μg L^{-1} to 7169 μg L^{-1}. Similarly, the four regular effluent samples (*i.e.,* HARSBEr) fell within a moderately large range, i.e., 1292 μg TP L^{-1} to 3575 μg TP L^{-1} with similar variability for the corresponding TRP and BAP data. These sample limitation make it impossible for us to conclusively assess the P removal performance for this facility.

Table 7 Overall P removal Performance (μg·L^{-1})

		1	2	3	4	AVE	SD
		5/13/2010	6/25/2010	7/15/2010	8/10/2010		
TP	HARASBI	3740		7169	6831	5913	1890
	HARASBEr	3575	2484	1649	1292	2250	1015
	HARASBEb		32			32	NA
TRP	HARASBI	3662		5362	5726	4916	1102
	HARASBEr	3448	2369	1571	997	2096	1062
	HARASBEb		26			26	NA
BAP	HARASBI	3382		2402	3560	3115	624
	HARASBEr	3169	1969	1255	1095	1872	944
	HARASBEb		7			7	NA
%TRP	HARASBI	98		75	84	86	12
	HARASBEr	96	95	95	77	91	9
	HARASBEb		81			81	NA
%BAP	HARASBI	90		33	52	59	29
	HARASBEr	89	79	76	85	82	6
	HARASBEb		23			23	NA

Sampling

Four sets of samples were collected from two different treatment process in Hayden Area Regional Sewer Board WWTP from May 13th 2010 to August 10th 2010. All four sets of samples were composite samples. The simplified diagram of the treatment process of the Hayden Area Regional Sewer Board wastewater treatment plant is shown in Figure 22. (Note: we don’t have a description of this plant from its operators yet.)

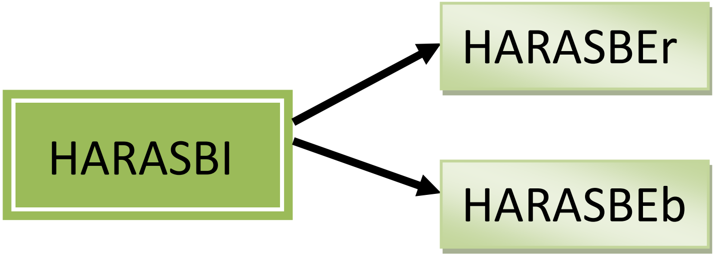


Figure 22 Hayden Area Regional Sewer Board treatment process

HARASBI--HARSB Influent
HARASBEr--HARSB Tertiary effluent - (Regular Effluent)
HARASBEb--HARSB Tertiary effluent - (Blue Water Effluent)

Result

HARASBI

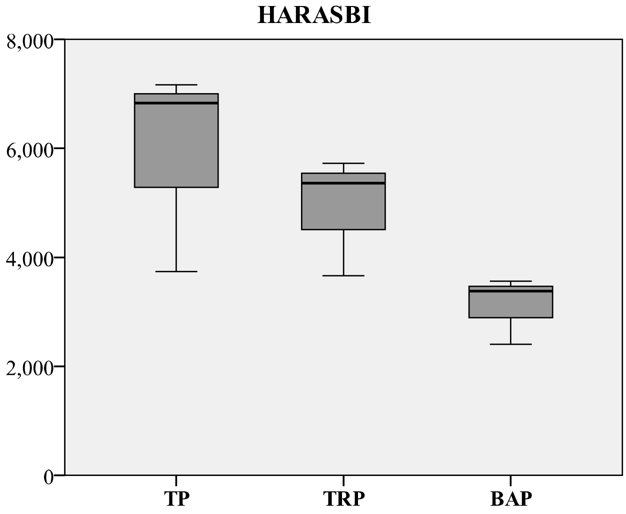


Figure 23 P concentrations in Hayden Area Regional Sewer Board Influent

The TP in the influent sample to the pilot plant averaged 5910 µg·L^{-1}. However, the standard deviation for these samples was quite large (SD = ± 1890 µg·L^{-1}). The average TRP concentration (4920 ± 1100 $\mu g \cdot L^{-1}$) was similar to TP. The bioavailable P averaged 3120 ± 620 $\mu g \cdot L^{-1}$ which was 59% of TP. Hence, in the HARSBI samples, most of the phosphorus was available to algae.

HARASBEr

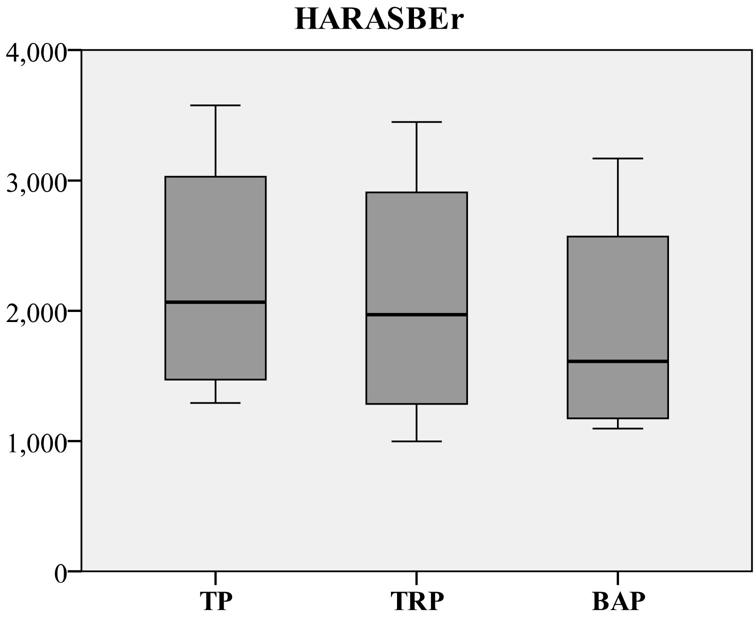


Figure 24 P concentrations in HARSB Tertiary effluent (Regular Effluent)

When assessing the results obtained from regular effluent, the variation was quite large. In particular, the P concentrations on May 13th and June 25th were higher than those for other dates. TP concentration were around 3580 $\mu g \cdot L^{-1}$ and 2480 $\mu g \cdot L^{-1}$ on these days, respectively. The TRP of four samples averaged 2100 $\mu g \cdot L^{-1}$. The algal bioassays showed the BAP for the effluent sample was reduced to 1870 ± 940 $\mu g \cdot L^{-1}$. The result for the regular effluent samples showed the regular P removal process reduced around 60% of TP from influent. However, the large variation and small sample size made it hard to establish conclusive assessment on its removal performance.

HARASBEb

After Blue Water treatment, a single sample indicated the TP of the HARSBEb effluent sample was reduced to 32 $\mu g \cdot L^{-1}$. This effluent had a TRP concentration of 26 $\mu g \cdot L^{-1}$, and the algal bioassay indicated that the BAP for the HARSBEb sample was only 7 $\mu g \cdot L^{-1}$.

Overall Performance Summary

Conclusions

As we can see from Tables 7, there was high variation associated with the P concentrations for most samples which was compounded by a small sample size.

P Removal Efficiency

From Table 7, since we only have one effluent sample from Blue Water system, but for this sample the P removal performance was encouraging. The TP removal efficiency was over 99% for the Blue Water processes for a much high influent concentration.

%TRP vs. %BAP

The percent of the TP that reacted with acid molybdate reagents (%TRP) and the percent that was bioavailable to algae (%BAP) varied with different P removal techniques as summarized in Figure 25.

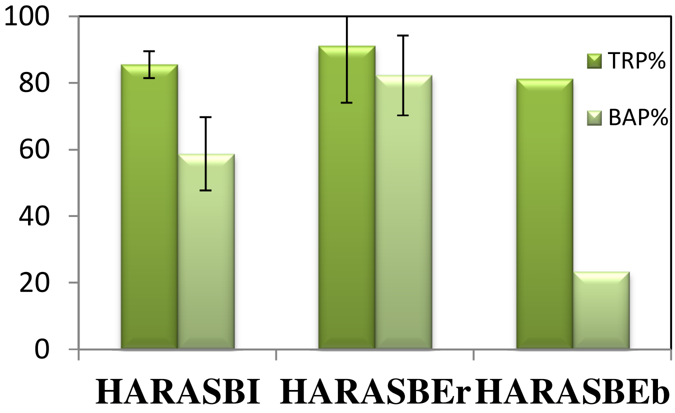


Figure 25 Comparison of %TRP and %BAP in Hayden Area Regional Sewer Board influent and effluent

In the Blue Water filtration system, the percentage of BAP decreased from 59 ± 29% in the influent to 23% in the effluent. This suggests that the forms of P which most readily stimulate algal growth were effectively sequestered by this process. In sum, the very small sample we processed for Blue Water effluent was encouraging, but the sample size available for this pilot plant was too small to draw firm conclusions.

10.Inland Empire Paper

The effluent that we have tested from the Inland Empire Paper Company (IEP) treatment process had quite variable total phosphorus (TP), i.e. 20-210 µg TP/L, but the availability of the P in this effluent to support algal growth as determined in the classic algal growth bioassay was also quite low, i.e. 9 ± 8% of TP (Table. 8). In fact, the %BAP estimate may even be an over-estimate of the true bioavailability of the P in the IEP effluent because the size distribution of the particles in the IEP samples at the end of the algal bioassay experiments was not consistent with the expected size distribution of the algae used in these experiments, nor with the size distribution of particles actually measured for all other effluents tested during this series of experiments. In particular, particles in the size range expected for algae were 3-5 µm in size, whereas the most prevalent size for particles in the IEP samples was 6-8 µm (Figure 26).

Table 8 P concentrations in effluent from Inland Empire Paper (µg·L^{-1})

Inland Empire Paper (Effluent)										
	9/10/09	9/24/09	10/8/09	11/19/09	12/3/09	3/4/10	4/15/10	6/10/10	AVE	STDEV
TP	111	211	35	45	33	18	23	21	62	67.3
TRP	23	27	6	7	6	5	8	11	12	8.4
BAP	4	2	0	3	9	2	2	4	3	2.6
TRP%	21	13	19	16	18	25	37	53	25	13.5
BAP%	4	1	0	7	26	9	8	19	9	8.8

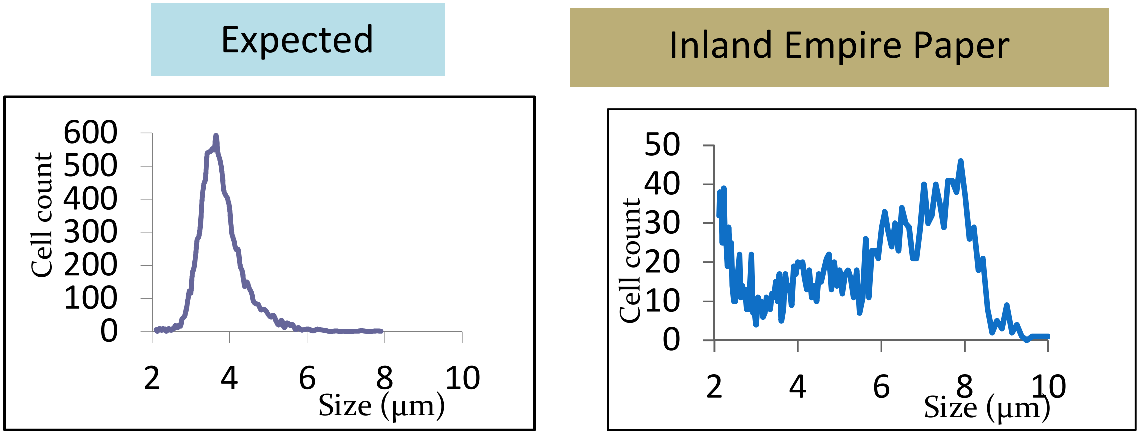


Figure 26 the size distribution of putative algal particles at the end of our BAP bioassay experiments. (These results show the expected Selenastrum size distribution in the left hand panel and the particle size distribution for the experiments using IEP effluent in the right hand panel.)

The low %BAP estimated for the samples could be because the IEP effluent inhibits algal growth or because the P in this effluent is bound up with organic complexes. The effluents of paper and pulp mills typically have very high concentrations of humic and tannic substances (28, 29). These compounds can have two impacts on phytoplankton, i.e. they can sequester P making it biologically unavailable for phytoplankton (30) or they can inhibit the growth of certain phytoplankton groups (31). In particular, past research has shown some humic substances may preferentially inhibit the growth of cyanobacteria, which are usually the greatest water quality concern (32). Conversely, humic substances can complex phosphorus by itself and/or with iron flocs making it biologically unavailable (32). It has also been reported that lakes with high humic content generally have less phytoplankton than expected based on their P concentrations (32). Humic-iron-phosphorus complexes may be degraded by UV radiation, possibly changing the availability of the associated P (31, 32).

Sampling

Process water from IEP manufacturing systems are treated in a state-of-the-art facility consisting of a group of unit operations and processes performing Primary Solids Settling, Microbiological Treatment, Secondary Solids Settling, Chemical Precipitation and Filtration, sludge dewatering and energy recovery. IEP commissioned a 1.0 MGD advanced tertiary treatment system in August, 2007 for low-level phosphorus removal as a proactive commitment to the dissolved oxygen water quality improvement plan for Lake Spokane. The tertiary treatment system is a multi-media filtration technology that incorporates a pre-stage tube settler stage for enhanced solids removal. The first stage, Tube Section, combines the functions of mixing, sludge blanket flocculation, and solids removal utilizing inclined settling tubes. The second stage, Adsorption Clarifier, utilizes packed bed buoyant media that combines the functions of additional mixing, contact flocculation, and solids removal. The Adsorption Clarifier “polishes” and conditions any remaining solids prior to the stream entering the final filter. This multi-barrier clarification system provides well-conditioned clarified water to the third stage, Mixed Media filter, consisting of anthracite, silica sand and high density sand. Flow is upward through the Tube Section, upward through the Adsorption Clarifier and downward through the Mixed Media filter.

Ten samples were collected from IEP from Sept. 10th, 2009 to June 10th, 2010. One sample was from the influent to IEP's full-scale tertiary treatment system (Trident HS), and the other nine were collected from the effluent of this tertiary treatment system.

Result

Influent

The one influent sample was collected on June 10th, 2010 from IEP's tertiary treatment system. This sample had a TRP concentration (570 $\mu g \cdot L^{-1}$) which was close to its TP concentration (604 $\mu g \cdot L^{-1}$), i.e., 94% of TP. The BAP result showed the BAP concentration was 321 $\mu g \cdot L^{-1}$, i.e., ≈50-60% of TP. For this influent sample the bioassay results had a particle size distribution which showed the expected range (3-5 µm) for our test algae. Thus the phosphorus in this influent sample was partially bioavailable to algae growth.

Table 9 P concentrations in influent from IEP tertiary treatment system ($\mu g \cdot L^{-1}$)

IEP (Influent)	
	6/10/10
TP	604
TRP	570
BAP	321
BAP%	53
TRP%	94

Effluent

The P concentration results for the effluent samples are shown in Figure 27 and Table 9. The P concentration of effluents from the IEP pilot plant were quite varible. The average TP concentration was 68 ± 66 $\mu g \cdot L^{-1}$. The TRP and BAP concentrations were relatively low and averaged 13 ± 9 $\mu g \cdot L^{-1}$ and 4 ± 3 $\mu g \cdot L^{-1}$, respectively. As discussed in the introduction, the low BAP estimates might have several causes. Regards our initial results suggest this effluent is a poor substrate for phytoplankton growth.

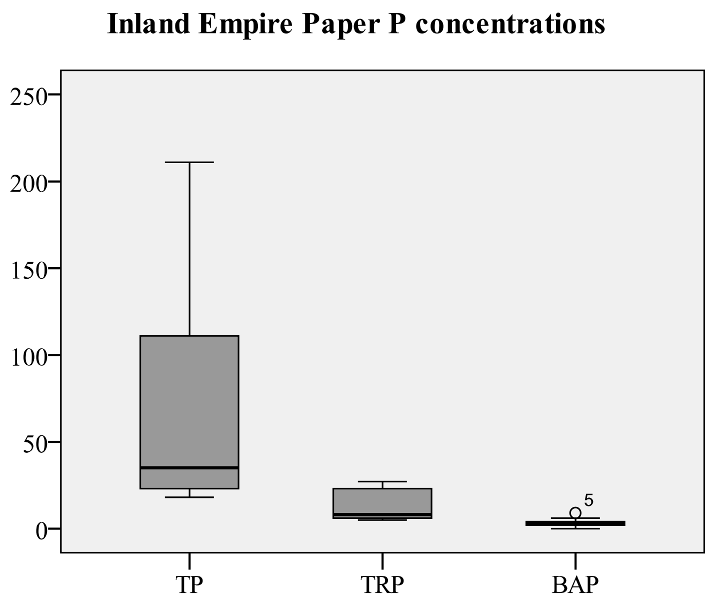


Figure 27 P concentrations in Effluent in IEP's tertiary treatment system

Overall P removal performance

Despite the variation in the effluent samples, and if one merely considers the result for the one influent sample, the removal efficiency of the Inland Empire Paper tertiary treatment system appears to be relatively high. After tertiary treatment, the TP concentration in the influent was reduced by 89%, to an average TP to 68 µg·L^{-1} in the effluent. Overall, the TRP concentrations were reduced 98% and BAP declined 99% to only 4 µg·L^{-1} BAP in the final effluent.

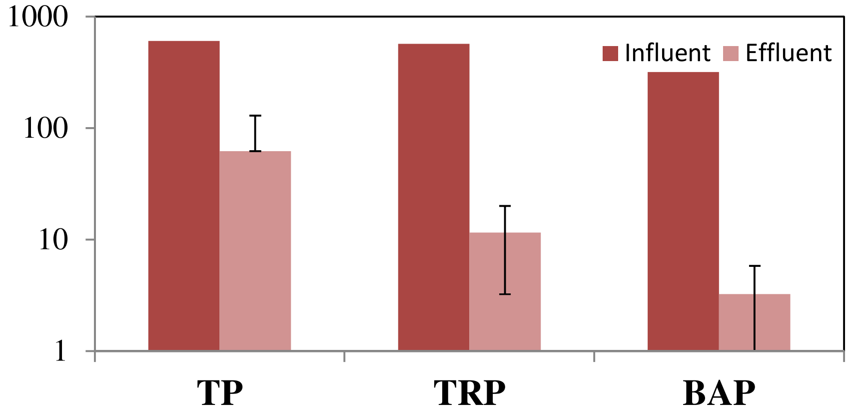


Figure 28 Comparison of P concentrations in Influent and Effluent in IEP.
(Note: Y axis is in logarithmic scale.)

%TRP & %BAP

Also, we calculated how the fraction of TP that reacts with the acid-molybdate reagents (%TRP) and the percentage BAP (%BAP) changed after phosphorus removal (Figure 29).

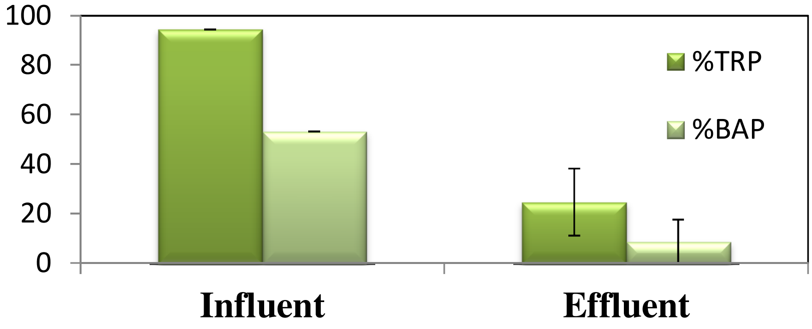


Figure 29 Comparison of %TRP and %BAP

In the influent sample, 94% of the TP were "reactive" and 54% could be used by algae. After tertiary treatment, the percentage of P which reacted with acid-molybdate declined to 25%, and only 9% was bioavailable. Although there were uncertainties associated with the effluent BAP results, the low percentages of TRP and BAP indicated that the tertiary treatment process reduced the P forms that are more likely to cause eutrophication problems. Furthermore, it is noteworthy that the %BAP was consistently lower than %TRP which is consistent with the result from other treatment processes.

In conclusion, our bioassay probably over-estimated the absolute %BAP for the IEP effluents because the particle counter we used like misidentified some flocs as algal cells. This misidentification problem could be resolved if we used algal chlorophyll concentration as opposed to cell counts as our experimental outcome measure. (In general, the cell count method is much more sensitive, but due to the flocs in the IEP samples, this was not the best method in this particular case.) Also, it is plausible that the low %BAP for the IEP effluents could have been due to two different causes as discussed in the report. If the low growth in IEP effluent is due to algal growth inhibition, it might suggest IEP effluents may have a beneficial impact on the phytoplankton species composition of receiving water bodies by creating less favorable conditions for nuisance cyanobacteria. If the %BAP of the IEP effluents is low because the P is sequestered in humic-iron complexes, it suggests the behavior of these complexes in Long Lake will have an important impact on whether this P may become remobilized to a bioavailable form after it is discharged. To differentiate these two causes, different experimental design will need to be used.

11.Spokane River

Sampling

Seven sets of samples were collected from the Spokane River between August 28th 2009 and March 4th 2010, six of which were collected in the river downstream of Nine Mile Falls Dam. The seventh sample set was collected from the Spokane River at the Washington-Idaho state line. Four samples were collected at Nine Mile Falls when the City of Spokane Riverside Park Water Reclamation Facility (RPWRF) was under a summer operation scenario, and two samples (Nov. 19th, 2009 to Dec. 3rd, 2010) were collected during the winter scenario. Since there were concerns about the "upstream" condition of the river, one sample was collected upstream of the RPWRF discharge at State Line on March 4th, 2010.

Result

Table 10 Spokane River P concentrations Result

Spokane River Result

		Summer	*Winter*	*State line*
TP	AVE	30	63	11
	SD	5.3	4.4	NA$^{\times}$
TRP	AVE	14	52	4
	SD	3.0	4.6	NA$^{\times}$
BAP	AVE	4	5	0
	SD	2.3	5.3	NA$^{\times}$

$^{\times}$NA: not applicable.

Spokane River - 9 Mile P concentration

Table 11 Spokane River - 9 Mile P concentrations Result

Spokane River P concentrations ($\mu g \cdot L^{-1}$)

	8/27/09	**9/10/09**	**9/24/09**	**10/8/09**	**11/19/09**	**12/3/09**	**AVE**	**STDEV**
TP	38	29	29	25	60	67	41	17.9
TRP	18	15	12	11	49	55	27	19.9
BAP	7	3	2	2	1	8	4	3.0
TRP%	47	53	43	43	81	83	58	18.6
BAP%	18	12	7	10	1	13	10	5.8

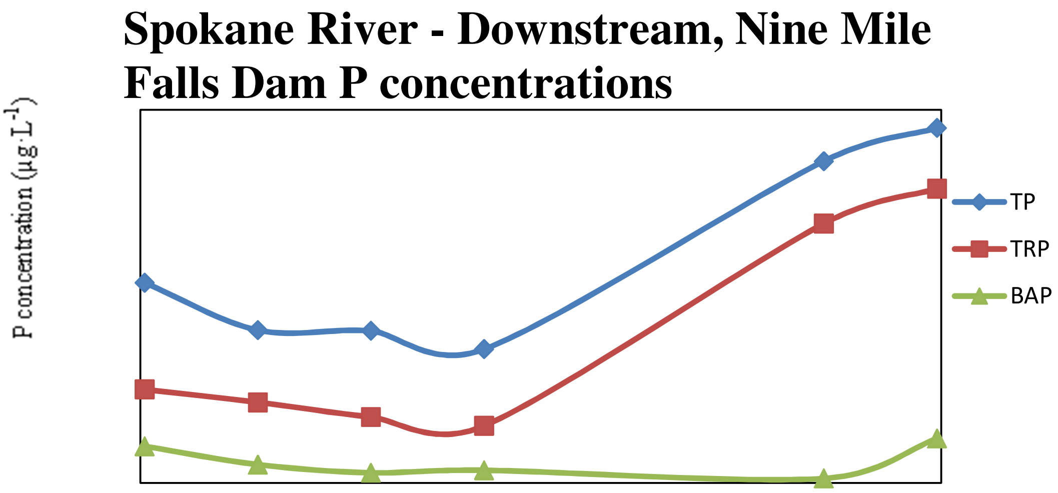


Figure 30 P concentrations in Spokane River – 9 Mile regarding the sampling date

From Table 10 and Figure 30, it is clear that the samples collected when the City of Spokane RPWRF was under its summer operation scenario had lower concentrations than the Spokane River samples collected when Spokane RPWRF ceased alum addition after secondary treatment in the winter. Because there is large difference between the summer and winter scenarios, the two situations will be discussed separately.

Summer Scenario

Because alum was added after secondary treatment during the summer, compared to winter samples, the effluent TP was reduced by a factor 5 for the City of Spokane RPWRF. Thus the TP in the river samples from the summer also had lower values (≈ 30 µg·L^{-1}), with a relatively small standard deviation (SD = ± 5 µg·L^{-1}). The average TRP concentration (14 µg·L^{-1}) was approximately half of TP, and the P pool that was bioavailable averaged 4 ± 2 µg·L^{-1}) which was only 13% of TP. Hence, in the Spokane River samples, the algal bioassays indicated that most of the phosphorus was unavailable to algae.

Winter Scenario

The Spokane River sample collected at Nine Mile Fallsduring the winter had considerably higher concentrations than the summer samples. This was probably caused in part by the cessation of alum addition after secondary treatment at the RPWRF. The average TP concentration was 63 ± 4 µg·L^{-1} with 52 ± 5 µg·L^{-1} as TRP. The algal bioassays indicated the bioavailable P was 5 ± 5 µg·L^{-1} which was < 10% of TP.

Thus, the Spokane River TP concentration was twice as high in the winter. This suggests that there was a noticeable effect from seasonal cessation of alum treatment in at the RPWRF in the river. However, our study didn't consider other factors that might have contributed to the increase of P concentrations in the river, such as watershed loading to the river and changes in stream flow.

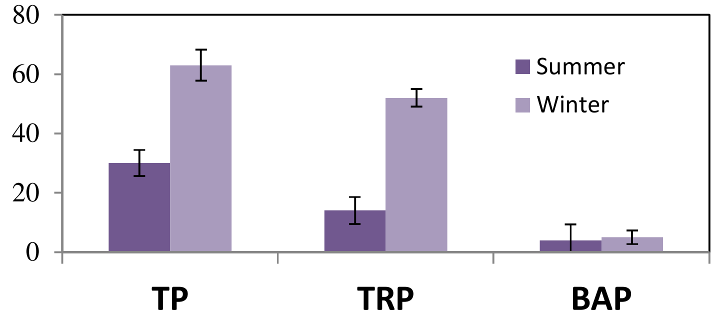


Figure 31 Comparison of P concentrations in Spokane River between the summer and winter

State Line (Washington – Idaho)

To address the concern about the "upstream" situation in the river, we collected a single sample on March 4th, 2010 from the Spokane River at State Line. The results were given in Table 10. The TP and TRP concentrations were much lower than the Nine Mile Falls samples collected earlier (Table 12), i.e., 11 µg·L^{-1} and 4 µg·L^{-1}, respectively. The bioassays indicated that the concentration of P that was bioavailable was lower than the analytical limit for this method.

Table 12 Spokane River – State Line P concentrations Result

Spokane River Upstream	
TP (µg·L^{-1})	11
TRP (µg·L^{-1})	4
BAP (µg·L^{-1})	Non detect
TRP%	39
BAP%	1

Discussion

Analytical Uncertainties

From the QA/QC data, it was indicated that the average coefficients of variation (CVs) were in the range of ± 2-3% for TP and TRP. The coefficient of variation for our BAP estimates was higher (CV = ± 7%), our low BAP estimate could have been caused by other limiting nutrients since the bioassay analysis tested the raw sample. This result could also be due to most of the bioavailable phosphorus already having been used up by algae or other plants before our river samples were collected.

%TRP & %BAP

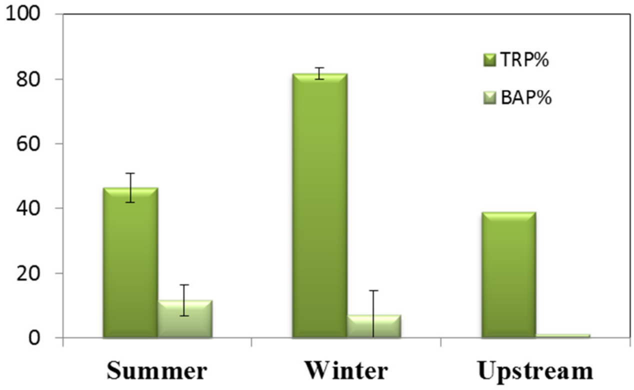


Figure 32 Comparison of %TRP and %BAP in Spokane River

As shown in Figure 32, the samples in the winter showed a higher percentage of TRP. This might be a consequence of the increase in TP concentrations in the river due to changes in weather or an increase P loading from the City of Spokane RPWRF.

Conclusions

As shown in the results section, the algal bioassays indicated the bioavailable phosphorus in the Spokane River sample was very low. However, this low BAP estimate could be caused by limiting nutrients other than phosphorus since we used 100% river water in our bioassays. In most cases for other sample types (*e.g.,* Spokane RPWRF), it was necessary to at least partially dilute our samples with P-free synthetic media. In these cases it was possible to rule out other types of nutrient limitation (e.g., N or micronutrient) because the synthetic media is replete with every conceivable nutrient except P. Further research where we dilute the samples with P-free media to saturate with all other nutrients might be warranted to confirm the low %BAP values for the Spokane River samples. In this way, we can test whether the sample has other limiting nutrient. Also, more upstream samples would be needed to establish more conclusive outcomes for the river water characteristics upstream of the RPWRF discharge.

12. Exclusive Summary

In our study, we used algal bioassays to determine the BAP of effluents from the main WWTPs discharges to the Spokane River. Spokane pilot plant used multiple alum additions. We tested how the percent BAP (%BAP) varied with different P removal levels using an algal growth bioassay methodology. The Spokane pilot plant reduced total P concentrations from ≈ 3 mg L^{-1} in the influent to 19 ± 4 (± SD) µg 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 ± 13%, and after three steps of alum based removal the %BAP averaged 7 ± 4%. Thus, this alum based P removal process was very effective at sequestering the P forms that most readily stimulate algal growth. Based on data collected at different steps along the Spokane pilot plant 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 critical 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. Our results show the final BAP of the effluent was only ≈ 50% of the "reactive" P concentration by counting all the effluent samples we had from the WWTP. This suggested it might be possible to implement TRP as a conservative measure of BAP.

We also tested the samples from other WWTPs (City of Coeur d'Alene, Post Fall, Liberty Lake, Hayden Area Regional Sewer Board), industrial wastewater effluent from Inland Empire Paper Company, and surface water samples from the Spokane River. A comparison of the effluent results is presented in Figure 33.

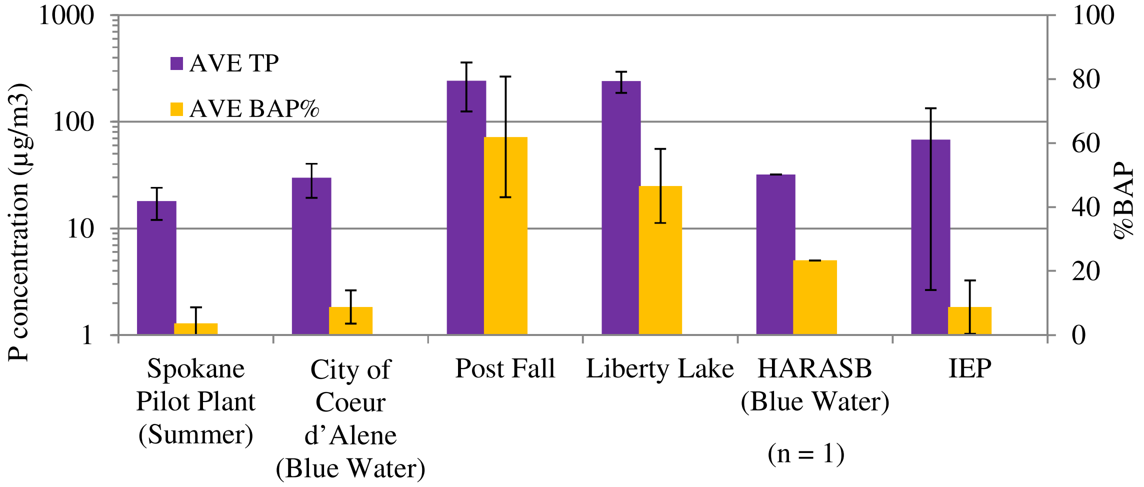


Figure 33 Comparison of %BAP and TP from different effluent.

For City of Coeur d'Alene plant, the most efficient technology for P removal was Blue Water with 96% removal efficiency when treating wastewater that had previously undergone considerable treatment. The algal bioassays indicated that the average %BAP for the Blue Water effluent samples was 14 ± 11% with only 2 ± 1 µg·L^{-1} BAP. For the Post Falls WWTP, considerable variation for the effluent P concentrations was observed in our analyses. However, despite this variation, the overall P removal efficiency of Post Falls WWTP using biological nutrient removal technology was ≈ 90%. When ignoring apparent outliers, the TP concentration in effluent was 520 µg·L^{-1}, whereas the raw influent samples for this WWTP averaged 6570. In Liberty Lake Sewer and Water District (LLSWD) WWTP the average TP concentration was 241 ± 53 µg·L^{-1} (with one outlier removed), with 47% of TP bioavailable. Only one sample was obtained from the Hayden Area Regional Sewer Board WWTP Blue Water process. This single sample indicated the Blue Water process reduced the P concentration from 5910 µg·L^{-1} to 32 µg·L^{-1} with only 7 µg·L^{-1} being BAP in the bioassay. Although this sample suggested good removal efficiency, the very small sample size available for this pilot plant made it very hard to reach firm conclusions.

For the IEP samples, the algal bioassays indicated only 9% of TP in the final effluent was BAP. The flocs observed in the sample suggested that we might have over-estimated the %BAP for the IEP effluents because the particle counter used for analyses likely misidentified some flocs as algal cells. The possible solution for the similar situation in the future will be analyzing the algal chlorophyll concentration as our experimental outcome measure. Also the low %BAP estimated for the samples could be caused by two different mechanisms: the effluent might inhibit algal growth or the P in this effluent may be bound up with organic complexes. A different experimental protocol (which has already been developed and validated in the Brett lab) is needed to distinguish between these mechanisms. In Spokane River samples, the very low %BAP may be due to limiting nutrients other than phosphorus since we used 100% river water in our bioassays. This could be tested by rerunning bioassays for these samples after diluting with 50% P-free synthetic media.

Very encouraging %BAP reduction tendencies from influent to effluent were seen for several treatment plants. Also %BAP was consistently lower than %TRP which is in agreement with the results obtained from the Spokane WWTP pilot plant. This finding suggests it may be possible to apply TRP as a surrogate measure of BAP. In general, future experiments should attempt to determine why BAP was consistently lower than TRP for many of the samples we processed.

13.Acknowledgements

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