



Sorption Potential of Soluble Phosphorus and Rhodamine WT onto Gravel Media from a Mature Constructed Wetland

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Abstract

The results of this study highlight the sorption potential of mature wetland-bed gravel and attached biomass for soluble phosphorus in trout farm effluents, the effectiveness of conventional lab studies in predicting field results, and the potential pitfalls associated with the use of RWT as conservative tracer. Gravel media extracted from a pilot-scale, horizontal subsurface flow (HSSF) wetland was evaluated for its phosphorus sorption affinity and influence on Rhodamine WT (RWT) transport. After receiving effluent from a flow-through trout farm for about one year, the gravel media exhibited moderate removals of soluble phosphorus ($\text{PO}_4\text{-P}$) in batch and column sorption experiments. Partition coefficients (K_d) from batch sorption tests ranged from 45-90 mL/g, with lower initial concentrations resulting in increased K_d values. Low (60 mL/min) and high (165 mL/min) flow column experiments resulted in partition coefficients of 0.9 and 0.8 mL/g, respectively. Removal of $\text{PO}_4\text{-P}$ from initial solutions was also generally greater in batch experiments with 69-82% removal, as compared to 40-50% removal for column experiments. The conservative nature of RWT in subsurface media has been called into question by many authors. Tracer response curves from tests conducted in a pilot-scale HSSF wetland exhibited elongated tails and dual peaks, in addition to mean tracer retention times far exceeding the theoretical value. Laboratory column testing of RWT and the more conservative NaCl tracer indicated that RWT was more reactive within the wetland media, even with good mass recovery (96%). First moment analysis revealed that the mean retention times were 4.6 and 7.4 hours for NaCl and RWT tracers, respectively, in the lab testing.

Keywords: Constructed Wetlands; Phosphorus; Rhodamine WT

Abbreviations: HSSF: Horizontal Subsurface Flow; RWT: Rhodamine WT; SSF: Subsurface Flow; FWS: Free Water Surface; HRT: Hydraulic Residence Times; HPLC: High Performance Liquid Chromatography; PDA: Photo Diode Array.

Introduction

Constructed wetlands have emerged as a popular wastewater treatment option worldwide, harnessing the

natural treatment processes in one of the most biologically active ecosystems [1-3]. Horizontal subsurface flow (HSSF) constructed wetlands are particularly of interest since the lack of an exposed water surface minimizes both animal and human impact on the wetlands, and vice versa. Additionally, this does not make them prone to becoming mosquito breeding grounds, which is especially important for systems treating effluent from single-family homes or small neighborhoods. Unlike free water surface wetlands which rely largely on the

sediment-water interface and suspended material for sorption sites, HSSF wetland media offer a myriad of additional potential sites. In HSSF wetlands, sorption becomes a particularly important removal mechanism for many wastewater parameters, yet can also interfere with certain tracers [4-6].

Eutrophication is of great concern in the Chesapeake Bay Watershed area where poor water quality and low populations of many species of fish and shellfish are being reported [7]. Established in December of 2010, the regulations for Total Maximum Daily Load on the Chesapeake Bay stipulate reductions in major sources of nitrogen, phosphorus, and sediment for the 64,000 square mile watershed. Phosphorus is often found to be the growth-limiting nutrient in freshwater systems, and annual loadings will have to be reduced by 24% within the watershed by 2025 [7]. HSSF wetlands are an economical means of controlling effluent phosphorus loadings from agriculture, aquaculture, and other industrial sources. Energy utilization was found to be less than $0.1 \text{ kW}\cdot\text{h}/\text{m}^3$ for subsurface flow wetlands, lower than facultative lagoons and the activated sludge process.

Results from many studies indicate that substrate sorption is the most significant factor in the removal of phosphorus in constructed wetland systems [6,8-10]. Sorption is highly dependent on the mineral properties of the aggregate; e.g., higher calcium content favors precipitation reactions, iron and aluminum oxides enhance phosphorus retention, and finer particles provide increased surface area, allowing for more sorption sites for phosphorus [5,10,11]. Laboratory sorption studies have been conducted with a variety of media intended for use in subsurface flow (SSF) constructed wetlands, including limestone, sand, gravel, topsoil, and other artificial materials [6,8,9,12,13]. Wetland beds are predominantly composed of either gravel or sand, as these materials allow for increased hydraulic conductivities and are widely available.

Though additional sorption sites within HSSF wetlands promote enhanced phosphorus removal, water tracers, such as Rhodamine WT (RWT), have exhibited increased reactivity with substrates and attached biomass in SSF wetlands, in comparison to free water surface (FWS) wetlands, leading to lower mass recoveries. Low mass recoveries, primarily due to sorption, cannot always be associated with poor hydraulic data. Experiments with simultaneous injections of multiple tracers in FWS wetlands have yielded mean hydraulic residence times (HRT) between 4-10% of the conservative agents, lithium and bromide [14,15]. Pang, et al. [16] determined that the shape and position of breakthrough curves for RWT and chloride were nearly identical in alluvial gravel aquifers.

In a controlled dispersion study, Ríos, et al. [17] found very similar recovery and shape of residence time distribution curves for lithium and RWT tracers. In the same report RWT recovery values were in excess of 75% in HSSF wetlands with HRTs between 41 and 54 hours.

One of the main draws for tracer usage in subsurface wetlands is the identification of short-circuiting and flow stratification within a wetland cell. Short-circuiting and the development of preferential flow paths are especially of concern in colder climates, where ice formation has the potential to reduce the hydraulic efficiency of subsurface flow wetlands [18]. Flow stratification can often be deduced visually, by the presence of multiple peaks on a tracer response curve. Multiple peaks often indicate flow traveling through strata of varying hydraulic conductivity, arriving at different times in the effluent. Studies have determined RWT will chromatographically separate during experiments conducted with subsurface media [19,20], which can lead to different arrival times of the two RWT isomers. This can complicate the interpretation of tracer response curves and also artificially increase theoretical residence times. Sorption of RWT to attached biomass has been considered to be the main cause of low mass recovery in subsurface flow systems [17,21]. Therefore, it is important to analyze the conservative nature of a tracer after a constructed wetland has been operating long enough for biomass accumulation. It has been said that some degree of nonconservative transport is inevitable due to all solutes having varying propensity to react with the subsurface [19]. Thus, a case-by-case evaluation of the conservative nature of a tracer in subsurface media is important.

The objectives of this study were to:

- Characterize the sorption of phosphorus in gravel media utilized in a HSSF constructed wetland that had been operating for about one year.
- Investigate the utility of Rhodamine WT as a conservative tracer in gravel media, used in a HSSF constructed wetland, by conducting laboratory batch and column experiments.

Methods and Materials

Site Description

A pilot scale study was undertaken to evaluate the use of HSSF constructed wetlands for the treatment of effluent from a flow-through trout farm in southwest Virginia. Wetland basins were made 6.7 m long, 2.4 m wide, and 0.9 m deep and constructed of plywood covered with 60 mil EPDM liner. The wetland basins were filled with coarse (19-25 mm) gravel at the influent and effluent ends to promote even distribution of flow through the wetland.

The remainder of the wetland basins was filled with finer gravel (2-6 mm), with a porosity of 0.44 and a bulk density of 1.5 g/cm³. Both wetlands were left unplanted; however, diverse native flora quickly emerged in the first growing season of operation. The wetlands were subject to continuous loading from the trout facility, each receiving a flow of 1 L/s, on average. Influent phosphorus concentrations averaged 0.26 mg/L as TP, and 0.04 mg/L as ortho-PO₄-P (OP).

Wetland Bed Media Sampling and Physical Analysis

Wetland gravel was extracted from the center of the beds, 15 months after the start of operations. Following extraction, media was transported to Virginia Tech, and stored in a 6 °C refrigerator until employed in sorption experiments. Clean-bed gravel was used to determine porosity and bulk density. Porosity was analyzed using the water evaporation method.

Chemical Analysis

Phosphorus (OP) was analyzed using the Ascorbic Acid method (equivalent to USEPA method 365.2 and Standard Method 4500-P-E). Samples were analyzed on a HACH DR2800 spectrophotometer and diluted with nanopure water when concentrations exceeded the upper limit of the test.

Rhodamine WT (2.5% active ingredient) was acquired in two different lots from Cole Parmer (EW-00298-16). A new lot of RWT tracer was acquired for laboratory batch and column studies, whereas dye purchased for previous experiments was utilized in the field. Both lots of dye presumably retained similar analytical characteristics, such as fluorescence with a maximum absorbance of 550 nm and emission of 588 nm. Chemical constituents of each RWT lot were analyzed using high performance liquid chromatography (HPLC) according to methods proposed by Sutton, et al. [19], to assess their presumed uniformity. For each lot, a 50 mg/L (active ingredient) solution was prepared and analyzed with a HP 1090 Liquid Chromatograph with a Photo Diode Array (PDA) detector. Separation was achieved using a 250 x 4.6 mm column as the immobile phase, and a solution of 65% methanol and 35% 5x10⁻³ M phosphoric acid as the mobile phase. The UV spectra were used to analyze the two primary isomers of RWT [19].

Prior to fluorometer analysis, calibration curves were made to assure instrument (Turner Digital Fluorometer Model 450) accuracy and dye integrity. NaCl concentrations were analyzed using a handheld conductivity probe (YSI Model 85). Standards were made

with tap water, and a series of blanks were used to zero out background conductivities. A standard curve was constructed to correlate conductivity and NaCl concentration.

Field Tracer Study

Tracer studies were conducted on two occasions with Rhodamine WT (RWT) dye in the HSSF wetlands. A preliminary tracer experiment was performed to determine a satisfactory sampling scheme and tracer mass input. An impulse of tracer was introduced by pipetting 9 mL of concentrated dye (2.5%) into the baffled quiescent settling zone, staged prior to wetland distribution. Immediately after, the settling zone was stirred for a period of 5 minutes to ensure homogenous distribution into the wetland cell. For this preliminary experiment, samples were withdrawn from the effluent of the wetland cells every 10-20 minutes over the course of about 12 hours.

A second tracer experiment was performed approximately four months later, in the same wetland cells as the preliminary tracer study. Introduction of the tracer impulse was carried out in the same way as the preliminary study, with the exception that 10 mL of RWT tracer was injected, rather than 9 mL. Samples were taken every 15 minutes during the first five hours of the study to ensure that the tracer response curve was captured in sufficient detail between the head of the curve and the peak. Sampling frequency was reduced to every 75 minutes until the final sample was taken about 30 hours into the study. Overnight, autosamplers (ISCO 3700) were utilized to attain samples. Samples were transferred to amber glass vials the next morning to prevent possible sorption to the plastic bottles that were filled by the autosampler. Following the study, samples were transported back to a laboratory at Virginia Tech and brought to room temperature (23°C) prior to analysis.

Nominal detention time in the constructed wetlands (τ_n , hr) was calculated with equation 1.

$$\tau_n = \frac{nV_w}{Q} \quad (1)$$

where n = clean bed porosity of wetland media
 V_w = wetland volume (L)
 Q = flow rate (L/hr)

Tracer detention time (τ , hr) was calculated from tracer output data using first moment analysis, illustrated in equation 2.

$$\tau = \frac{1}{M_e} \int_0^{\infty} tQCdt \quad (2)$$

where M_e = Total mass of tracer recovered in the effluent (μg)

t = time (hr)

Q = flow rate (L/hr)

C = Tracer effluent concentration ($\mu\text{g/L}$)

The upper limit of the integration was originally truncated by temporal restraints imposed by sampling complications. Enough data were collected, however, to exponentially extrapolate beyond the final sampling point. Extension of the tracer response curve was described using an exponential decay function, as suggested by Pope, et al. and Wang & Jawitz [22,23].

$$C(t) = C_b e^{\left(\frac{t-t_b}{a}\right)} \quad (3)$$

where t_b = earliest time in regression (hr)

t = time (hr)

C_b = concentration at t_b ($\mu\text{g/L}$)

$-a^{-1}$ = slope of linear regression

Existing tail data, between hours 15.5 and 30.5, was plotted on a semi-log scale with linear regression being used to determine "a."

Batch Sorption Experiments

Batch-type phosphorus sorption experiments were conducted according to techniques outlined by the U.S. EPA [24]. Gravel samples were air-dried until constant weight and passed through a 2 mm sieve. Remaining sample was crushed, and passed through the 2 mm sieve until sufficient sample was obtained. Agitation intervals of 24, 48, and 72 hours indicated that 48 hours was sufficient equilibrium time. Soil to solution ratios of 1:40, 1:20, and 1:10 were initially used, which revealed that a 1:20 ratio was optimal. Water for batch testing was taken from the groundwater spring directly upstream of the trout farm to ensure the mineral properties of the water were the same as on-site. Five grams of sample were placed into 125 mL amber glass bottles containing solutions of 0, 0.5, 1, 1.5, 2, and 4 mg/L $\text{PO}_4\text{-P}$. Samples were then mixed on a rotary shaker for 48 hours at 21°C ($\pm 1^\circ\text{C}$). Whatman 934-AH grade glass microfiber filters were used to ensure only soluble reactive P was in the filtrate. Phosphorus not recovered in solutions was considered to be retained by the wetland bed media.

Batch sorption tests with Rhodamine WT (RWT) were carried out in a manner similar to the phosphorus batch experiments, with a 1:10 soil: solution ratio and 48 hour

equilibration time. The solutions contained RWT concentrations of 10, 50, and 100 $\mu\text{g/L}$. Sorption parameters were estimated using the Langmuir and Freundlich isotherms. The Langmuir expression is given in equation 4:

$$\frac{C_e}{q_e} = \frac{1}{q_{\max} \cdot b} + \frac{C}{q_{\max}} \quad (4)$$

where

q_{\max} = sorption maxima (mg/kg)

b = binding energy coefficient

C_e = equilibrium concentration in solution (mg/L)

q_e = equilibrium sorbed concentration (mg/kg)

The maximum sorption is calculated from the slope of the resultant curve. The Freundlich equation is provided in equations 5 and 6:

$$\frac{x}{m} = KC_e^{1/n} = \text{equilibrium sorbed concentration (mg/g)} \quad (5)$$

and

$$\log \frac{x}{m} = \log K + \frac{1}{n} \log C_e \quad (6)$$

where K = Freundlich adsorption constant ($\text{mg}^{1-n} \text{kg}^{-1} \text{L}^n$)

n = empirical constant related to binding intensity

x = mass of adsorbate (mg)

m = mass of adsorbent (mg)

C = equilibrium solution concentration (mg/L)

Partition coefficients were calculated according to the following equation:

$$K_d = \frac{V_w(C_0 - C_i)}{M_{\text{media}} \cdot C_i} \quad (7)$$

where K_d = partition coefficient (mL/g)

V_w = solution volume (ml)

C_0 = initial solution concentration (mg/L)

C_i = equilibrium solution concentration (mg/L)

M_{media} = mass of gravel media (g)

Column Sorption Experiments

Unlike in the laboratory batch tests, HSSF media was taken in its original state (not crushed) to investigate phosphorus and RWT sorption. Columns were constructed from PVC pipe, 15 cm in diameter and 0.76 m long. Water was pumped from a reservoir into the bottom of the column by a Fisher variable flow peristaltic pump (Model 3389). Minimal vinyl tubing was used in order to minimize sorption outside the column. Flow progressed up the column until it emerged from the media into a

small (< 1 cm deep) FWS, where it then cascaded into a collection reservoir. Step inputs of phosphorus and RWT were injected into empty (no media) columns to assess sorption onto column walls, etc. The study was conducted using tap water at room temperature ($22.0 \pm 2.0^\circ\text{C}$). Background phosphorus concentrations were zeroed out, and there was no interference for RWT measurements in the tap water.

Columns were constructed according to guidelines published by Relyea [25]. In order to prevent spiked solute inputs from peaking in fewer pore volumes than might be expected, it was recommended that pore-water velocity be large enough to minimize the effects of diffusion in laboratory columns. Low flow phosphorus and RWT tests were performed at an average flow of 60 mL/min, corresponding to a pore-water velocity of $1.2 \cdot 10^{-2}$ cm/s, above the minimum criteria published by Relyea [25]. High flow experiments, only performed with phosphorus, were conducted at 165 mL/min.

To minimize the impact of high pore-water velocities on the effective pore volume, Relyea [25] suggested column length be greater than, or equal to, four column diameters. The ratio of column length to width used in this experiment was 5:1 to ensure compliance with these guidelines. Lastly, to avoid velocity effects such as channeling or radial velocity gradient, it was proposed that the column diameter be 30-40 times the particle diameter of solids used to pack the column. Though sieve analysis was not performed in this study, the fraction of media less than or equal to 5 mm in size fits these specifications for a column 152 mm in diameter.

Step inputs of phosphorus were pumped continuously from a reservoir containing a KH_2PO_4 solution. Phosphorus concentrations were 2 mg/L $\text{PO}_4\text{-P}$ for both the low and high flow experiments. Effluent samples were filtered through Whatman 934-AH grade glass microfiber filters prior to analysis to quantify the soluble fraction of phosphorus. For a step input, retardation factors (R) can easily be calculated by setting 'R' equal to the number of pore-volumes required to reach 50% of the influent concentration [26]. This estimation, however, assumes symmetrical, sigmoidal breakthrough curves and sorption equilibrium. Retardation factors for this study were calculated according to equation 8, based on the conservation of mass principle [26].

$$R = \int_0^{p_{\max}} (1 - F) dp \quad (8)$$

where F = normalized breakthrough curve for step input

p_{\max} = pore volumes consumed when equilibrium conditions prevail

For this technique, the area above the normalized breakthrough curve was analyzed using Microcal Origin 8.5.1.

Tracers were pulsed into the column by temporarily switching the pump intake line into a volumetric cylinder containing 61 mL of tracer solution. The tracer solution contained 1.83 mg of RWT (active ingredient) and 1,530 mg of NaCl. The integration of RWT and NaCl was evaluated for possible interferences, and none were detected for the purposes of this study. The mean residence time for a pulse input was calculated according to the following equation [27].

$$t_{\text{pulse}} = \frac{\int_{t_{\min}}^{t_{\max}} t C_i dt}{\int_{t_{\min}}^{t_{\max}} C_i dt} \quad (9)$$

where t_{pulse} = mean residence time for a pulse input (hr)

t_{\min} = beginning of breakthrough curve (hr)

C_i = tracer concentration (mg/L)

Velocity of contaminant transport was then calculated using the calculated mean residence time and column length as the distance traveled. The retardation factor (R_f , unitless) was then calculated as follows:

$$R_f = \frac{v_p}{v_c} \quad (10)$$

where v_p = pore-water velocity (cm/s)

v_c = velocity of contaminant (cm/s)

The pore-water velocity was estimated by the velocity of a non-adsorbing tracer (NaCl). For tracer column experiments, the 1D transport equation was applied as follows:

$$D_L \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} = \frac{\partial C}{\partial t} \quad (11)$$

where v_p = pore-water velocity (cm/s)

v_c = velocity of contaminant (cm/s)

By applying boundary conditions appropriate in column experiments, Lenda and Zuber [28] created a normalized solution:

$$C(t) = \frac{M}{Qt_0} \frac{1}{\sqrt{4\pi P_D (t/t_0)^3}} \exp\left[-\frac{(1-t/t_0)^2}{4P_D t/t_0}\right] \quad (12)$$

where Q = column flow rate (L/hour)
 M = mass of tracer injected (mg or μg)
 t_0 = parameter used in the calculation of the effective porosity
 P_D = Peclet number

Two unknown parameters (T_0 , P_D) were solved using breakthrough curve data and MS Excel solver to minimize the sum of squared error. This normalized transport equation was utilized to model tracer response curves for NaCl and RWT, and to calculate mass recoveries and mean retention time from first moment analysis.

Results

Parameter	C_i (mg/L $\text{PO}_4\text{-P}$)	Soil: Solution Ratio	V_{solution} (ml)	T_{eq} (hr)	Temperature (oc)	PH	C_{eq} (mg/L $\text{PO}_4\text{-P}$)	K_d (mL/g)	Removal (%)
PO_4	3.8					9.2	1.17	44.7	69
PO_4	2.0					9.2	0.5	62.3	76
PO_4	1.5	1:20	100	48	21±1	9.1	0.34	69.7	78
PO_4	1.0					9.0	0.18	90.0	82
PO_4	0.5					8.9	0.12*	70.0	78
(ug/L RWT)						(ug/L RWT)			
RWT	105					8.0	90.05	1.6	14
RWT	50	1:10	100	48	21±1	8.0	45	1.0	9
RWT	10					8.0	10.05	0.4	4

Table 1: Summary of batch sorption parameters.

An asterisk (*) indicates that equilibrium concentration fell below method detection limit (0.15 mg/L $\text{PO}_4\text{-P}$)
 C_i = initial concentration, T_{eq} = equilibrium time, C_{eq} = equilibrium concentration, K_d = Partition Coefficient.

Phosphorus Column Sorption Tests

Decreased hydraulic loading was found to have a positive impact on phosphate reductions within the column. For a flow rate of 165 mL/min (pore-water velocity of 2.1 cm/min), soluble phosphorus concentrations in the outflow reached approximately 60% of the inflow concentrations after 6.4 pore volumes. A flow rate of 60 mL/min (pore-water velocity of 0.7 cm/min) resulted in equilibrium effluent concentrations of about 50% of inflow concentrations after 6.61 pore volumes. Flow-cell experiments were modeled with exponential decay functions, illustrated in Figure 1. Retardation coefficients were 3.79 and 4.13, for high and low-flow experiments, respectively. Partition coefficients were 0.8 for the high flow experiment and 0.9 for the low flow experiment.

Parameter	Input	C_i (mg/L $\text{PO}_4\text{-P}$)	Q_{avg} (mL/min)	t_{eq} (hr)	t_{eq} (pore)	Temperature (oc)	PH	C_{eq} or C_{max} (mg/L $\text{PO}_4\text{-P}$)	R	K_d (mL/g)
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Phosphorus Batch Sorption Experiments

Sorption of phosphorus to the gravel media and attached biomass was well modeled by both the Langmuir and Freundlich isotherms. The Langmuir isotherm generated a sorption capacity of 93.2 mg/Kg and a binding strength of 1.07 L/mg. The Freundlich constant was 47.4 ($\text{mg}^{1-n} \text{kg}^{-1} \text{L}^n$), with an empirical constant of 1.60. Correlation coefficients were 0.99 and 1 for the Freundlich and Langmuir isotherms, respectively. Percent removal of orthophosphate was inversely related to initial concentration (i.e., removals decreased with increasing initial concentration). Data for the initial solution of 0.5 mg/L $\text{PO}_4\text{-P}$ were removed from isotherm construction because equilibrium concentrations were below detection limits. Results from batch testing are summarized in Table 1.

Results from the phosphorus column sorption experiments are summarized in Table 2.

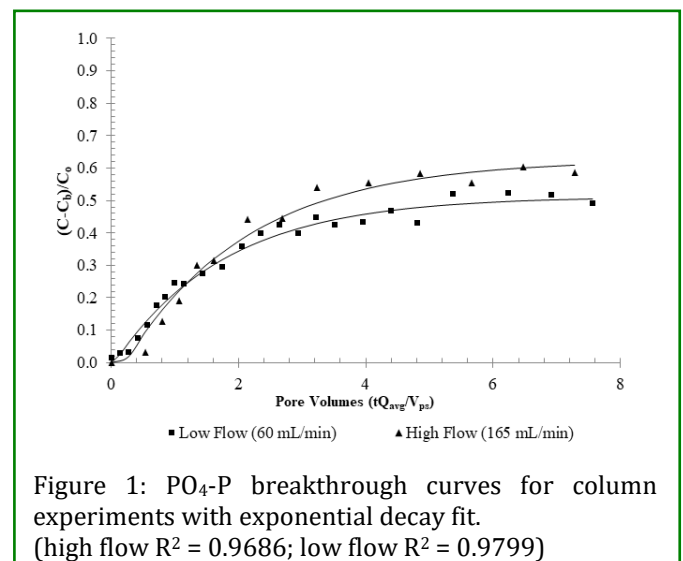


Figure 1: $\text{PO}_4\text{-P}$ breakthrough curves for column experiments with exponential decay fit. (high flow $R^2 = 0.9686$; low flow $R^2 = 0.9799$)

		P)			volumes)		p)			
PO ₄ (165mL/min)	Step	2	165	4.0	6.4	21±1	7.9	1.23	3.79	0.9
PO ₄ (60mL/min)	Step	2	60	10.5	6.6		8.0	0.23	4.13	0.8
(µg/L RWT)			Q _{avg}	t _b (10%)	t _b (90%) (hr)	Temperature	PH	(µg/LRWT)	R	
RWT	Pulse	N/A	50	1.9	16.0	21±1	8.0	0.15	17.4	0.24
(mg/L NaCl)			Q _{avg}	t _b (10%)	t _b (90%) (hr)	Temperature	PH	(mg/L NaCl)	R	
NaCl	Pulse	N/A	50	1.5	6.6	21±1	8.0	0.00	N/A	N/A

Table 2: Summary of flow-through column parameters.

C_i = initial concentration, Q_{avg} = average flow rate, t_{eq} = equilibrium time, t_b (%) = time in which % mass recovered, C_{eq} = equilibrium concentration, C_{max} = peak concentration, R = Retardation coefficient, K_d = Partition Coefficient.

Separation of RWT Constituents

The separation of RWT constituents by HPLC analysis was primarily performed to determine whether isomer composition varied among the two different lots of tracer-grade RWT. A newer lot, acquired immediately prior to column and batch testing, exhibited a UV spectra very similar to that presented by Sutton, et al. [19]. The UV spectra of the older lot used for the field study, was starkly different than that of the new lot. Only two constituents were detectable in the chromatogram, with significant absorbance recorded by a single constituent, detected in the eluent between 12 and 14 minutes. It is unknown at this time, whether RWT degradation or changes in chemical formulation was responsible for differences in UV absorption, or whether these differences were responsible for dissimilar solute transport.

Field Tracer Tests

Preliminary tracer tests resulted in residence time distribution (RTD) curves that were asymmetrical in nature, with a sharp rise followed by an extended tail. Dual peaks were evident in the RTD (Figure 2) for both HSSF Wetland Cells 1 and 2, though more pronounced in Wetland Cell 2. Effluent concentrations peaked near the nominal detention time of each wetland (2.9 hours). Wetland Cell 1 peaked after 3.2 hours at a concentration of 7.3 µg/L, and Cell 2 peaked after 2.8 hours at a concentration of 6.1 µg/L. A second tracer experiment on HSSF Wetland Cell 1 did not indicate any signs of this dual peak phenomenon in the RTD (Figure 3). Mean tracer retention time, as calculated from the method of moments, was nearly four times greater than nominal retention time at 11.2 hours. Mass recovery of the RWT tracer was 67%.

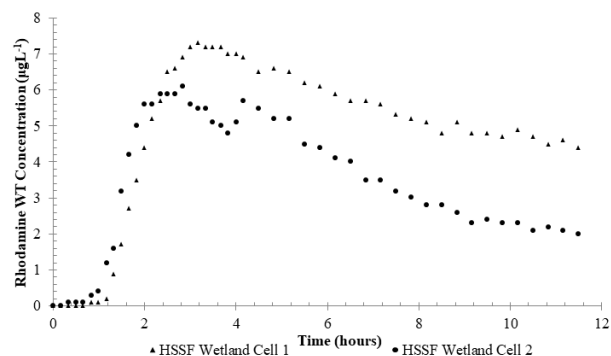


Figure 2: Tracer response curve from RWT impulse addition (9 mL) to HSSF Wetland Cells 1 and 2. (November, 2010). Data truncated at 11.8 hours due to temporal restraints. Mass Recovery of Cells 1 and 2 were 66% and 49%, respectively.

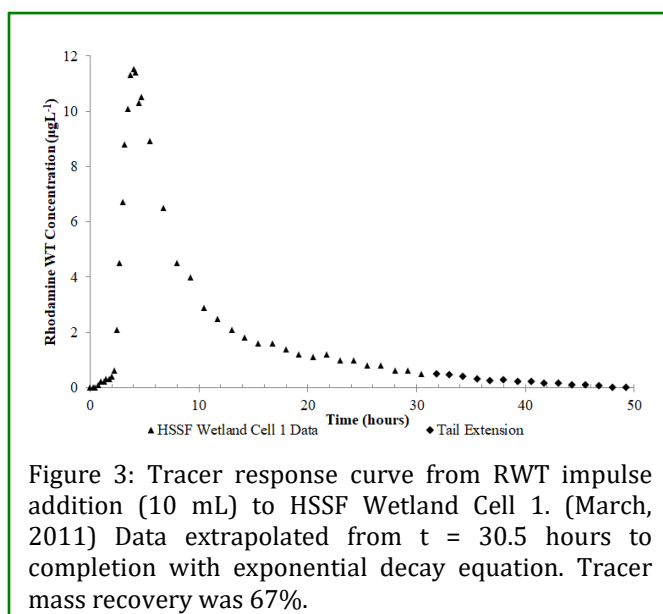


Figure 3: Tracer response curve from RWT impulse addition (10 mL) to HSSF Wetland Cell 1. (March, 2011) Data extrapolated from $t = 30.5$ hours to completion with exponential decay equation. Tracer mass recovery was 67%.

Laboratory Tracer Experiments

RWT exhibited a direct relationship between sorption losses and initial concentration. Percent removals of RWT

from initial solutions of 10 μ g/L, 50 μ g/L, and 100 μ g/L at equilibrium were 4.0%, 9.4%, and 14.0%, respectively. Parameters from the RWT column sorption experiments are summarized in Table 2.

Tracer response curves from the column experiment (Figure 4) indicated that RWT was more reactive within the wetland media than NaCl. The NaCl tracer peaked after approximately 0.8 pore volumes had passed through the column, reaching a maximum concentration of 129 mg/L. correspondingly, peak RWT was measured after 1.1 pore volumes at a concentration of 80.8 μ g/L. Mean residence times, using first moment analysis, were 4.2 and 7.4hr for NaCl and RWT, respectively. Tracer response curves indicated that 10% mass recovery occurred at 1.5 hours for NaCl and 1.9 hours for RWT. The difference between tracer break through was more pronounced towards the tail of each curve, with 90% breakthrough at 6.6 hours for NaCl, and 16.6 hours for RWT. Using the ratio of the velocity of the conservative tracer, NaCl, to the velocity of RWT, a retardation factor of 1.4 was calculated for RWT. A Péclet number of 0.24 was calculated graphically from NaCl tracer response curve data using equation 12. Mass recoveries were satisfactory, with 111% recovery of NaCl, and 96% recovery of RWT tracer. Blank column tests, conducted to assess sorption onto column material, revealed that 97% of influent orthophosphate and 94% of RWT were detected when the effluent concentration reached steady state.

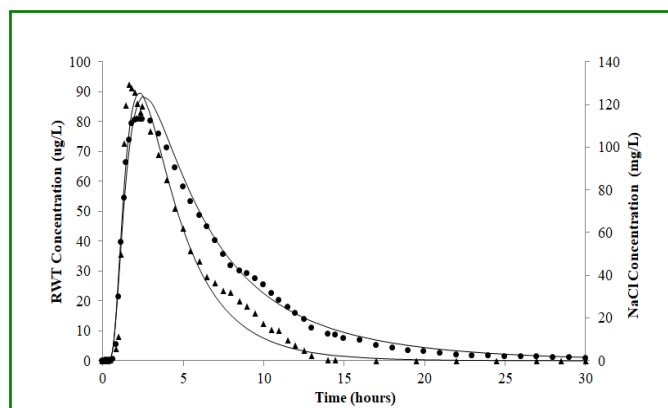


Figure 4: Tracer response curves for RWT and NaCl pulse injections. Curves illustrate 1-D transport equation proposed by Lenda and Zuber (1970). Mass recoveries for RWT and NaCl were 96% and 111%, respectively.

Discussion

Phosphorus Sorption Experiments

Sorption capacities of gravel substrates, determined from batch experiments using the Langmuir isotherm, vary tremendously throughout the literature. The value determined in this experiment, 93.2 mg/Kg, exceeded the maxima (25.8-47.5 mg/Kg) determined by Mann and Bavor [8] for gravel employed in constructed wetland systems. Cui, et al. [6] determined a sorption capacity of 494 mg/Kg for gravel substrate, clearly demonstrating more sorption potential for gravel. In their experiments, initial phosphorus standards were made in 0.01 M KCl solution, to serve as a supporting electrolyte. For comparison, a 0.01 M KCl solution is equivalent to a conductivity of 1,412 μ S/cm at 25°C, whereas conductivities in this study were about 200 μ S/cm. The effects of supporting electrolyte solutions are also more pronounced at higher equilibrium phosphorus concentrations using initial phosphorus concentrations of 100-500mg/L [6,29]. Sorption maxima of gravel media in this experiment was comparable to that determined from a sampling of sands used in subsurface flow constructed reed beds in Denmark [10]. In the Denmark tests, sands characterized as having high hydraulic conductivities (>200 m/day) had phosphorus sorption capacities of 49-86 mg/Kg.

Gravel is limited in its ability to sorb phosphorus, in comparison to other substrates utilized in constructed wetlands. A survey of nine substrate materials for constructed wetlands revealed that gravel had the second lowest phosphorus sorption capacity [6]. Superior sorption appears to be related to higher concentrations of exchangeable Ca and Mg in basic wetlands, and Al and Fe in acidic wetlands. This is because under basic conditions, soluble phosphorus can react with Ca to form hydroxyapatite, which can precipitate out of solution [30]. When receiving acidic effluent, substrates high in Al and Fe content are believed to chemically adsorb phosphate ions onto the surfaces of hydrous oxides of Fe and Al by ligand exchange [1,31]. Phosphate ions can further be immobilized by chemical precipitation reactions involving metallic cations such as Fe, Al, Ca, or Mg, especially at higher concentrations of either metallic cations or phosphate [32].

It is important to note that a higher maximum P sorption capacity for a given substrate does not ensure lower effluent phosphorus concentrations, since the equilibrium phosphorus concentration (concentration at which no net sorption or desorption occurs) can increase with sorption maxima [13]. The sorption capacity is also limited in that it represents the maximum for fast, reversible sorption, whereas constructed wetlands often process phosphorus through fast and slow sorption processes. Desorption from rapid sorption sites, induced from dilution of the solution, is predominantly from rapid sorption sites [33].

McGechan and Lewis [33] note that the extent to which slow sorption processes progress will impact the amount of sorbed phosphorus available for desorption. Therefore the impact of certain processes, including desorption, cannot be directly associated with sorption maxima. Sorption capacities do however, remain effective indicators of a substrate's affinity for a sorbate, and serve as an important basis of comparison among potential wetland media.

Column sorption experiments indicated a much smaller partition coefficient for $\text{PO}_4\text{-P}$, in comparison to the batch sorption experiments. Batch experiments with an initial concentration of 2 mg/L $\text{PO}_4\text{-P}$ generated a partition coefficient of 62 mL/g, whereas flow-through tests resulted in a partition coefficient of 0.8-0.9 mL/g. Phosphorus was 76% removed from solution in the aforementioned batch test, while removals were 40-50% in columns operated at low and high flows, respectively. Flow-through experiments reflect both hydrodynamic conditions and substrate composition in the field more closely than batch sorption tests. Additionally, unlike batch experiments, column tests can more closely mimic field conditions by producing slow movements through the porous medium, which limit contact-time with the sediment matrix [34]. This is partially offset by the incorporation of phosphorus onto biomass and plant litter. Sorption and storage in biomass are processes subject to saturation and are not believed to contribute to long term removal [35]. Soil adsorption was found to play a major factor in controlling long-term phosphorus sequestration in wetlands.

Tracer Studies

Tracer response curves from studies performed in a pilot-scale HSSF constructed wetland revealed features indicative of non-conservative transport. Dual peaks were clearly visible in HSSF Wetland Cell 2 at hours 2.8 and 4.2, while all RWT tracer response curves featured elongated tails. There are multiple reasons why a tracer response curves can exhibit such elements. Chromatographic separation of RWT isomers, as suggested by Shiao, et al., Sutton, et al., Vasudevan, et al. [4,19,20], is believed to account for some non-conservative behavior. Sutton, et al. [19] that chromatographic separation of RWT's two isomers is one plausible reason for dual peaks. Isomer 1 sorbs to a lesser extent and reaches equilibrium sorption an order of magnitude faster than isomer 2. Thus, the flow of isomer 2 has the potential to be retarded in subsurface media, and can therefore arrive in the effluent of a HSSF wetland at a later time than isomer 1.

Chromatographic separation of RWT's two isomers complicates the interpretation of multiple flow paths, as

multiple flow paths will also produce distinctive peaks. For instance, clogging in the inlet region of a HSSF constructed wetland can result in surface flow until hydraulic conductivity at the surface is large enough to allow water to percolate into the wetland cell. This short-circuiting flow will arrive at the outlet quicker than flow retarded from traveling entirely through the lower hydraulic conductivities of the subsurface. Knowles, et al. [36] documented this scenario with a tracer study and reported that the subsurface flow path to arrive 8 hours later than the short-circuiting flow path. The lack of distinct multiple peaks in HSSF Wetland Cell 1 for both the first and second tracer tests indicate that wetland heterogeneity was more likely than chromatographic separation to have been the cause of multiple peaks in Wetland Cell 2. The elongation of the tracer response tail, as indicated by the presence of tracer in the effluent nearly 50 hours into the second tracer study, is believed to be attributed to a mixture of transient storage and reversible sorption. The 33% of tracer mass not recovered in the second field study was mainly attributed to sorption.

The transport of RWT within the gravel media was further confirmed to be non-conservative in laboratory column testing. A retardation factor of 1.74 was calculated using NaCl as a non-reactive tracer. The retardation factor does not decipher the degree to which surface adsorption, absorption, precipitation, or other mechanisms interfere with contaminant transport. Both Giraldo, et al. and Ríos, et al. [17,21] have indicated sorption to attached biomass is the primary reason for RWT loss in subsurface wetland media field experiments. Lin, et al. [15] determined that 10% of RWT sorption was reversible. When sorption is reversible, wetland media temporarily adsorbs a fraction of influent RWT. When influent concentrations decrease, desorption occurs, releasing RWT into the effluent. Rhodamine WT was detectable in the column effluent until 34.1 pore volumes (69.5 hours) had passed. This is quite different than the 6.6 pore volumes (13.5 hours) required for the NaCl tracer to pass through the system. Though only 10% of RWT sorption may be reversible, reversible sorption can still greatly impact the shape of the tracer response curve. This is because a very small percentage of tracers mass can be responsible for a significant extension of the tracer tail. For instance, 90% of tracer mass was recovered within the first 17.4 hours of the column experiment, leaving just 6% to be recovered until hour 69.5, when the effluent concentration reached zero. Thus, even if reversible sorption was a fraction of the 4% not recovered, it can significantly inflate mean tracer residence times.

Upon first glance of the tracer response curve for RWT, it appeared that a change in inflection at approximately

hour 8 was due to the arrival of the less conservative isomer 2. Chromatographic separation of RWT isomers and heterogeneity of the subsurface are two potential causes for different arrival times of the tracer. Chromatographic separation is not believed to be the primary reason, as both tracers exhibited a similar change in inflection on the tail end of the tracer response curve. NaCl exhibited this change at hour 8, about one hour prior to RWT. The “bump” on the tracer response curve is likely due to non-homogeneous velocity profiles along the column cross-section, induced by the effect of higher drag associated with the inner circumference of the PVC column. The photodegradation and biodegradation of RWT were believed to be only minor contributors to RWT losses due to relatively short retention times [15].

Though the method of moments can exaggerate the mean detention time for tracer response curves featuring elongated tails, it is obvious that RWT transport was far more reactive than NaCl within the HSSF wetland media. Therefore, RWT did not serve as a truly conservative tracer in this study.

Conclusion

Gravel media employed in an HSSF constructed wetland has the potential to sorb moderate levels of soluble phosphorus. Though increased $\text{PO}_4\text{-P}$ concentration resulted in an increase of phosphorus associated with the solid phase, the percentage removal of phosphorus from solution decreased with increasing concentration. Removals of $\text{PO}_4\text{-P}$ from column experiments will resemble potential field removals more closely than batch experiments. Column experiments allow for the presence of hydrodynamic effects which mimic internal hydraulics within a HSSF constructed wetland to a greater degree than the 48 hour batch tests. Decreased pore-water velocity (increased retention time) was found to have a positive impact on $\text{PO}_4\text{-P}$ removals in laboratory column tests. The sorption experiments in this study indicated that the gravel media within a HSSF constructed wetland that had received relatively constant loadings of phosphorus for approximately 15 months, still had potential to remove considerable soluble phosphorus from wastewater.

An investigation into the potential of RWT to exhibit characteristics of a conservative tracer in gravel media with attached biomass indicated that RWT was more reactive than the salt tracer, NaCl. Mean detention times for RWT were significantly inflated due to an elongated tail of its response curve. This undesirable characteristic was exacerbated by the utilization of method of moments to calculate mean detention time. Therefore, one needs to

be cautious in using the results of RWT tracer studies to predict the fate of agents in subsurface systems. Even though RWT did not behave conservatively in this study, RWT can still be used qualitatively in subsurface flow tracer studies and perhaps quantitatively on a case-by-case basis, when transport is evaluated, and compared with the behavior of known conservative tracers.

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