# MOBILIZATION OF PHOSPHORUS FROM EAST CREEK IN THE CHESAPEAKE BAY WATERSHED

By

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## ABSTRACT

Nutrients released to open waters may cause deterioration of water quality and impact aquatic ecosystems. Although a significant fraction of phosphorus (P) entering rivers is retained in riverine sediments, fluctuation of physiochemical and biological conditions may promote remobilization of the retained P and its removal to open waters. This study aimed to quantify the amount of inorganic phosphorus (Pi) that can be released from the selected sections of East Creek in the Chesapeake Bay watershed. Environmental parameters chosen to identify Pi release included changes in biological activity, redox potential, pH, salinity, and temperature. Pi released from sediment spiked with *Shewanella putrefaciens* CN32 varied from 1.42 μmol/g along the lower reaches of the creek to 5.08 μmol/g near the agricultural headwaters. Changing the ambient water column pH (7.4) to acidic (6.0) condition resulted in the retention of dissolved Pi while changing to basic (9.0) condition promoted release of Pi. Similarly, increase in salinity and temperature also resulted in Pi release. Pi flux at the sediment-water interface from field simulated experiments varied from 14.8 μmol/$m^2$–hr along lower reaches to 48.6 μmol/$m^2$–hr near agricultural headwaters. High Pi release in the upstream section of the creek was consistent with the high sediment and water column Pi near the agricultural field. Statistical analyses indicated that the enhanced biological activity and pH were the most sensitive parameters affecting Pi release from the sediment. A regression equation developed to approximate the amount of Pi release in the worst case scenario in East Creek was 0.30 μmol/g at the salinity of 15 PSU, 0.38 μmol/g at 25 $^oC$, 0.83 μmol/g at pH 9.5, and 3.10 μmol/g under enhanced biological condition. These results collectively suggest that the remobilization of P from the East Creek could be a new source to be exported to the Chesapeake Bay.

# Chapter 1

# INTRODUCTION

The Chesapeake Bay is the largest estuary in United States. It has a 167,000 $km^2$ watershed which covers parts of the District of Colombia, New York, Pennsylvania, Delaware, Maryland, Virginia, and West Virginia. Causes and extent of eutrophication in the Chesapeake Bay have been a major research focus and have resulted in several key findings (e.g., Boesch et al., 2001; Hagy et al., 2004; Kemp et al., 2005). Increase in nitrogen and phosphorus input to the Chesapeake Bay has caused eutrophication and resulted in phytoplankton blooms (Officer et al., 1984; Nixon, 1995; Jordan et al., 1991). Phytoplankton blooms have several detrimental effects. For example, when phytoplankton dies and sinks to the bottom of water bodies, their decomposition process consumes oxygen and deprives the bottom water of oxygen, creating hypoxic conditions. These conditions may lead to the ecosystem damage such as the loss of aquatic biodiversity and submerged vegetation, and alteration of food webs (Officer et al., 1984; Seehausen et al., 1997). These detrimental effects led to the multi-state Chesapeake Bay agreement that seeks to limit nutrient discharge to the Chesapeake Bay (Chesapeake Bay Agreement, 1987, 2000).

Phosphorus (P) is an essential element for all life forms. Therefore, its availability may impact primary production along with species distribution, ecosystem structure, and its function. P release from land to open water may be contributed from anthropogenic sources such as fertilizer, sewage, livestock, and industries (Carpenter et al., 1998), or from natural sources such as weathering, soil erosion, and atmospheric deposition from aerosols (Föllmi, 1996; Graham and Duce, 1979). Because of the low stoichiometric need of P among other major nutrients (106C: 16N: 1P; Redfield,

1958), small amounts of P addition could cause severe impacts on water quality in the receiving catchments or groundwater aquifers, and could promote eutrophication. For example, a series of studies in Lake Washington, Lake Erie, and Ashumet Pond have found P as a limiting nutrient for eutrophication (Edmondson, 1970; Schindler, 1977; Boyce et al., 1987; Schneider, 1997; Correll, 1999) as with a vast majority of surface waters (Wetzel et al., 1983). Similarly, long term variability of the dissolved inorganic nitrogen (DIN): dissolved inorganic phosphate (DIP) ratios in the Chesapeake Bay indicates that P is the limiting nutrient in the Bay for all seasons except in mid-summer (Figure 1; Prasad et al., 2010). One of the reasons for the nutrient enrichment in the bay watershed is the net import of nutrients from other regions of the country. For example, net anthropogenic P input (NAPI) in counties in the Chesapeake Bay region varies between 0.02 to 78.46 kg P /hr–yr (Russell et al., 2008), with higher NAPI values contributed by agricultural lands and developed land covers. About 10% of the total NAPI in the watershed is released to the Chesapeake Bay (Russell et al., 2008).

P exists in dissolved, colloidal, and particulate phases and both in inorganic and organic forms in river waters. Studies have shown that sedimentary P may be released due to the fluctuation in physiochemical parameters such as redox potential, pH, temperature (Kim et al., 2003), salinity (Jordan et al., 2008), and biological activity (Hupfer et al., 1995a; Jaisi et al., 2008, 2011). Physicochemical and biological parameters have often been combined in the past to identify Pi release (e.g., Jiang et al., 2008; Kim et al., 2003), but the roles of each parameter and their sensitivity under environmental fluctuations have been rarely studied. East Creek is one major nutrient release hotspot in the Chesapeake Bay watershed because of its high soil P content and proximity to the bay. Because the mobilization of P from East Creek sediments could be exported to the bay, it is important to understand the P mobilization potential of East Creek sediments due to the effect of individual physiochemical parameters and biological activities.

This study quantifies inorganic phosphate (Pi) release from the sediment-water interface during fluctuation of biogeochemical processes. These analyses were performed in sediment and water samples collected from the East Creek. Specific research objectives were:

- To quantify Pi release from sediments during fluctuation in physiochemical parameters (salinity, pH, temperature, and redox potential) and biological activities.
- To estimate the Pi flux from the sediment-water interface.
- To understand the seasonal variability of Pi and compare its relationship to near and far agricultural field Pi concentration.

A detailed understanding of different mechanisms that promotes the release of P from sediments is important to identify how P concentration in rivers may vary over time. This information will improve our understanding of sediment-nutrient interaction and help better quantify P mobilization potential from sediments. Results from these studies could be used to determine how natural environments may be manipulated to limit P mobilization or adjust appropriate nutrient management strategies to limit P release to the Chesapeake Bay.

# Chapter 2

# FORMS AND PHASES OF PHOSPHORUS IN SEDIMENTS AND WATER COLUMNS

## 2.1 Sedimentary Phosphorus Phases

Phosphorus (P) exists in a wide variety of chemical forms in natural waters and sediments. Depending on the bond P makes with metal (M) ions as M–O–P or with carbon (C) as C–P or C–O–P, P compounds are classified as inorganic or organic compounds, respectively. The P oxyanion can accommodate a variety of metal ions such as Ca, Fe, Mg, and Al or form condensed phase P such as pyrophosphate and polyphosphate. Organic P encompasses a series of compounds such as nucleic acids, phospholipids, sugar P, inositol phosphate, phosphoproteins, and phosphoamides (Meybeck, 1993).

P in sediments may involve chemical and biological reactions that may lead to the remobilization of P. During these processes, P may partition into different sediment phases including precipitated as amorphous P or crystalline minerals or sorbed to a variety of minerals such as clays, metal oxides, and hydroxides and to organic complexes or taken up by biota. Sequential chemical extraction methods have been widely used to separate and quantify different P phases in sediments (Psenner et al., 1988; Ruttenberg, 1992; Hupfer et al., 1995b). Depending on the nature and mineralogical composition of sediments, extraction methods have been revised accordingly. For example, the Ruttenburg (1992) method is the most commonly used method for marine and coastal sediments while the Psenner et al (1988) method is widely used for river and lake sediments. The targeted P phases in major sequential extraction methods include loosely sorbed, bound to and/or co-precipitated with

Fe/Mn/Al oxides, and precipitated crystalline minerals such as apatite (Hupfer et al., 1995b).

Several past studies have employed sequential extraction methods to identify the effect of salinity, nature of sediment P, authigenic and detrital Pi sources (Jordan et al., 2008; Hartzell et al., 2010; Hartzell and Jordan, 2012; Jaisi and Blake, 2010). For example, sequential extraction method was used to identify the effect of salinity on burial and release of P in the Patuxent River where iron oxide-bound P was found to be most dominant phase (Jordan et al., 2008). In Lake Sempach of Central Switzerland, sequential extraction was used to characterize various P species where NaOH extractable P was found to be the most dominant phase (Hupfer et al., 1995b). Similarly, sequential extraction used to characterize authigenic and detrital P phases in the sedimentary pool in Peru margin where authigenic P was the largest fraction of total P (Jaisi and Blake, 2010).

**2.2 Study Area and Sediment and Water Sampling**

East Creek is located in a small watershed in Somerset county in MD (Figure 3a). The East Creek watershed covers an area of ~26 miles and consists primarily of agricultural farmlands. Because of its proximity to the Chesapeake Bay and the highest recorded soil P index in the Chesapeake Bay watershed (Coale and Layton, 1999), this watershed is considered as the the hotspot of nutrient release.

To identify spatial variation of P in river sediment and water in East Creek, a closely spaced (~ 0.5 miles) sampling plan was adopted (Figure 3b). Sediment samples were collected from five major sites (A, F, H, K, and L) using a core sampler. Sediment cores were collected using a vacuum suction technique whereby a large piston inside a 3 inch tube was pulled that allowed gradually moving up of sediment during plunging the sampler into the sediment without disturbing the sediment structure and releasing sediment porewater. A total of 2–3 sediment cores each ~25–35 cm long and 7.6 cm in diameter were collected. Similarly, water samples were collected from 12 sites (A, B, C, D, E, F, G, H, I, J, K, and L) in 3.5 L polyethylene

bottles. Sampling was conducted in different seasons (May 25, July 10, September 30, 2012, and May 27, 2013). Sampling was done in coordination with the University of Maryland.

### 2.3 Sample Processing

All cores were kept in sealed liners and placed in ice upon retrieval. They were kept frozen before processing. Each core was sliced into 2 cm vertical depth intervals and pore water was extracted by centrifugation. The sliced core was then freeze dried and upper 2 cm depth dried sediment was ground, passed through a 212 micron sieve size, and used in experiments.

### 2.4 Methods and Materials

#### 2.4.1 Phosphorus phases in sediments

Sediment cores collected from five sites (A, F, H, K, and L) in East Creek (Figure 3b) were sequentially extracted to quantify $NaHCO_3$ and oxalic acid extractable Pi in sediments, following the method described in Hedley et al. (1984) and Poulton and Canfield (2005), respectively. Briefly, 0.2 g of sediment was first extracted with 20 mL of 0.5 M $NaHCO_3$ (sediment solution ratio of 1:100). The suspension was shaken for 16 hours and then centrifuged to separate extracted Pi (note that dissolved Pi refers to that present in solution and particles size $\leq$ 0.05 µm particles in this thesis, unless noted otherwise). The sediment residue was further washed with de-ionized (DI) water by shaking for 2 hours. The $NaHCO_3$ and water extracted solutions were combined before measuring Pi in this phase. The sediment residue after the $NaHCO_3$ extraction step was then extracted with 20 mL oxalic acid (0.2 M ammonium oxalate and 0.17 M oxalic acid; pH 3.2) by shaking the suspension for 6 hours. The suspension was then centrifuged to remove supernatant and followed by 2–hour water wash as before. The extracted solutions for these two Pi phases were measured by using ICP-OES (inductively coupled plasma optical emission spectrometry) at the Soil Testing Laboratory at the University of Delaware.

### 2.4.2 Particulate and dissolved phosphate in the water column

Seasonal variation of dissolved Pi in the water column was measured from all sites. To further identify particulate Pi in the water column, different size fractions (0.02, 0.05, 0.1 and 0.4 μm) of the suspended sediments from selected sites were separated by centrifugation. Pi concentrations were measured by using the phosphomolybdate blue method (Murphy and Riley, 1962).

### 2.4.3 Sediment mineralogy

Variation in sediment mineralogical composition along the water flow direction was studied by using X-ray diffraction. To identify any potential relationship of water column Pi with that of sediment at the sediment-water interface, shallow sediments (0–2 cm depth) from selected sites (A, F, H, K, and L sites, Figure 3b) were chosen for this analysis. Sediment cores were freeze dried, ground thoroughly, and size separated (≤ 212 μm). XRD data were obtained in powedered samples at Pacific Northwest National Laboratory (PNNL), WA by using a Philips MPD instrument with Cu Kα radiation ($\lambda$ =1.54056 Å).

## 2.5 Results and Discussion

### 2.5.1 Phosphorus phases in the sediment

Pi phases in the sediment showed that $NaHCO_3$ extractable Pi was 1.69 μmol/g at site A, and 5.90 μmol/g at site L (Table 1). Similarly, oxalic acid extractable Pi varied from 1.69 μmol/g at site A to 7.77 μmol/g at site L. Both $NaHCO_3$ and oxalic acid extractable Pi phases were high along the upper reaches of the creek and normally decreased towards the lower reaches of the creek (Table 1). This is most possibly due to release of Pi from agricultural fields in the upstream reaches and dilution in the downstream reaches. The dilution is most possibly caused by rainfall, selective sorption to sediments, groundwater input, and tides.

In Patuxent River sediments, loosely sorbed and iron oxide–bound Pi were found to be 43.9–55.3 and 1.39–2.82 μmol/g, respectively (Jordan et al., 2008).

Although these authors used $MgCl_2$ and diothionite citrate bicarbonate (DCB) extraction to quantify these two Pi phases, Patuxent River sediments contain an insignificant amount of loosely sorbed Pi. The high $NaHCO_3$ extractable Pi phase in East Creek sediments suggests that this Pi phase plays a more active role in P retention and mobilization in this site.

### 2.5.2 Particulate and dissolved phosphate in the water column

Particulate Pi in the water column in sites K and L was high in all size fractions (<0.02, < 0.05, < 0.1, and < 0.4 μm; Figure 4). It varied between 11.44 and 20.76 μM at site L but decreased to 0.53–0.56 μM at site H. High particulate Pi in site L may partly be related to the manure pile in the agricultural field during the sampling time. Effect of agriculture is expected to be high in site L because it was located at the drain ditch of a large agriculture field (Figure 3b). The trend of particulate Pi along the river flow direction is similar to that of sediment Pi and consistent with the interpretation of release of particulate Pi from the farms in the upstream.

Dissolved Pi concentrations in the water column showed a very similar trend as that of particulate Pi with high Pi in sites J, K, and L (Figure 5). Comparing seasonal effects, for example at L site near the farm, Pi concentration was found to be highest in May (39.29 μM) and started to decrease in July (27.36 μM), and then further decreased in September (23.25 μM). High Pi at this site coincides with the timing of manure application on the farm. However, increased biological activity and faster reaction kinetics at high temperature in summer months may also have caused high Pi concentration in the water column. Increase in biological activity has been previously found to promote release of Pi from sediments (Jiang, 2008; Hupfer et al., 1995a; Jaisi et al., 2008). Pi concentration at the export point (site A) to the Chesapeake Bay was about 4 times higher than in the Bay (Prasad et al., 2010) indicating net Pi export from East Creek.

### 2.5.3 Sediment mineralogy

Mineralogical composition of sediment, identifed from X-Ray diffraction, revealed that quartz, albite, illite, orthoclase, anorthosite, and chlorite were major minerals in the sites studied (Figure 6a,b). Ferrihydrite (FeOOH and $Fe_{9.5}O_{14}(OH)_{0.5}$), goethite, and pyrite were present as Fe containing minerals, but their quantity was very low. P bearing minerals were not detected by XRD possibly due to low concentration below the detection limit.

Semi-quantitative analysis of Fe containing minerals did not show significant differences among sites. For example, goethite and pyrite content were quite similar but ferrihydrite ($Fe_{9.5}O_{14}(OH)_{0.5}$) content was slightly higher in site H than in site A sediment (Figure 6a, b).

## 2.6 Summary and Conclusions

East Creek sediments contain quartz, albite, illite, chlorite, anorthosite, and orthoclase. Ferrihydrite, goethite, and pyrite are major Fe bearing minerals and fluorapatite and hydroxyapatite were likely phosphate bearing minerals, their identity is not fully confirmed. $NaHCO_3$ and oxalic acid extractable, particulate, and dissolved Pi concentrations were high along the upper reaches of the creek near the agricultural field. Even though the Pi concentration decreased by a log order from the headwaters to the export region to the Chesapeake Bay, it was still 4 times higher than in the bay. Therefore, there is a net export of Pi to the bay.

# Chapter 3
# PHOSPHORUS MOBILIZATION FROM SEDIMENTS

Phosphorus (P) dynamics within the sediment after burial are complex and involve its redistribution and transformation within the sediment column. P can be preferentially remineralized from both particulate and dissolved organic matter (Loh and Bauer, 2000). These processes may remobilize a significant amount of sediment–bound P to the water column. For example, more than 50% of the total P was found to be remobilized to the overlying water in the Chesapeake Bay (Boynton et al., 1995).

Exchange of P between the sediment and water column is the major component of P cycling at the sediment-water interface. A variety of physical, chemical, and biological processes (Table 7) and their relative dominance in a particular environment control the extent of P release (Pettersson et al., 1988). Major physicochemical processes include desorption, dissolution, ligand exchange, and hydrolysis (Yang et al., 2010; Table 7). Similarly, enzymatic hydrolysis, cell decomposition, and other chemical changes due to biological activities (such as pH and Eh) are major biological processes for P release.

## 3.1 Effect of Changes in Physicochemical Processes and Biological Activities on Phosphate Remobilization from the Sediment

### 3.1.1 Effect of pH

P in aqueous solution exists as $H_2PO_4^-$, $HPO_4^{2-}$, or $PO_4^{3-}$ ions with following pKa:

$$[H_3PO_4^-]=[H^+]+[H_2PO_4^-] \quad pKa_1=10^{-2.3} \quad (1)$$

$$[H_2PO_4^-]=[H^+]+[HPO_4^{2-}] \quad pKa_2= 10^{-7.2} \quad (2)$$

$$[HPO_4^{2-}]=[H^+]+[PO_4^{3-}] \quad pKa_3=10^{-12.1} \quad (3)$$

Because $PO_4^{3-}$ deprotonates with increasing pH and increases its net negative charge, this effect brings about major changes in P speciation in sediment and water columns. Most minerals have point zero charge (PZC) between 5–8 and when pH > PZC, their surface is negatively charged and results in net electrostatic repulsion of sorbed P (Stumm and Morgan, 1981; Sparks, 2003). Pi can also be remobilized to the water column due to ligand exchange with $SO_4^{2-}$, $OH^-$ and $Cl^-$ (Boström, 1988). For example, increase in pH, such as that due to mixing of freshwater with saline water, results in the remobilization of P originally bound to iron oxides. Similarly, increase in pH to as high as 9.5, for example in poorly buffered tidal freshwater such as that due to high photosynthesis, promotes the remobilization of P from sediments (Seitzinger, 1991). pH also controls the solubility of minerals. Therefore mineral dissolution and precipitation and changes in their surface properties including electrostatic charge affect P speciation.

### 3.1.2 Effect of salinity

Changes in salinity result in changes in ionic strength. Increase in competing ion concentration to a particle surface may decrease the surface charge on the particle by the shielding effect (Gardner et al., 1991; Grace et al., 1997). For example, anions such as $Cl^-$ and $SO_4^{2-}$ decrease the isoelectric point of the various Fe and Al oxide minerals that have point of zero charge between 5.6 to 9.0 (Sparks, 2003). Competitive sorption of these ions and Pi on available sorption sites on mineral surfaces may also promote the remobilization of sorbed Pi (Stumm and Morgan, 1981).

Salinity indirectly affects the chemical form of P that is eventually buried in the sediment. For example, P can be buried as sorbed species or co-precipitated with Fe(III) minerals in freshwater while P is remobilized in brackish water undergoing sulfate reduction because Fe(II) is consumed as sulfides (Gächter and Müller, 2003).

Increase in pore water salinity in the Patuxent River sediments has been found to result in a decrease of iron oxide-bound Pi, increase in organic P (Po), and decrease in total sediment particulate Pi (Jordan et al., 2008).

### 3.1.3 Effect of temperature

Temperature fluctuation has many direct and indirect impacts on Pi remobilization. At low temperature, reaction kinetics is generally slow due to which geochemical systems at low temperature may not be in equilibrium. Kinetic energy of a reaction is high at high temperature and may overcome the energy barrier for the reaction (Stumm and Morgan, 1981). To a point, increase in temperature also stimulates microbial activities. Increase in temperature has been positively correlated with the Pi remobilization from the sediments (Jiang et. al., 2008). For example, Pi remobilization rate was found to be increased by a factor of five when the temperature was increased from 2 to 25–35 ºC range (Figure 7) (Kim et al., 2003). However, the observed effect often results from a combination of physicochemical parameters that are directly and indirectly changed due to the change in temperature.

### 3.1.4 Effect of changes in microbial activities

Microorganisms participate in different biogeochemical processes, such as P cycling in sediment, and therefore can play an important role in the release of sediment P through i) decomposition of organic P compounds, ii) removal of polyphosphate stored inside cells (Hupfer et al., 1995a), and iii) decrease in dissolved oxygen in the pore water and lowering of redox potential, thereby promoting Fe(III) reduction (Lovley and Phillips, 1988) and release of iron oxide bound Pi. In sediment incubation experiments, Pi release rate was found to be increased from $4.2\times10^{-4}$ to $11\times10^{-4}$ moles/$m^2$–week when the aerobic condition is switched to anoxic (Figure 8a, b) (Kim et al., 2003).

Pi release during sediment organic matter decomposition depends on the type of dissolved organic P (DOP) and enzymes available for cleaving C–O–P bonds of the

organic matter. Release of up to 50–90 % of bacterially bound P due to grazing can be obtained when bacteria are carbon limited (Jürgens and Güde, 1990). Based on kind of bacterial population, < 20 % Pi was remobilized when bacteria was C limited. This effect is more pronounced in the summer season when dissolved Pi is low after the spring algal bloom (Jürgens and Güde, 1990). In the deeper water column of the Chesapeake Bay where anoxic conditions last for a long time, P release from sediment has been found to be high (Cowan and Boynton, 1996; Kemp et al., 2005). Reductive dissolution of Fe(III) oxides in anoxic conditions and release of P is a common process that leads to an increase of dissolved P in pore water as well as in the water column (e.g., Kemp et al., 2005).

### 3.2. Experimental Methods

#### 3.2.1 Effect of physicochemical parameters on phosphate mobilization from river sediments

The physicochemical parameters chosen in this study included pH, salinity, and temperature because these parameters vary with environmental conditions and their variation affects P retention to and mobilization from soils and sediments (Hupfer et al., 1995a; Kim et al., 2003; Jordan et al., 2008).

Before running experiments, all sediment and water samples were processed as discussed in sections 2.2 and 2.3, and were sterilized by autoclaving. River waters were subsequently filtered through 0.45 μm filter syringe to remove particulate matter. All experiments were performed at sediment: water ratio of 1:50. To identify Pi release due to sediment dilution, sediment-water suspension at the targeted experimental solid: solution ratio was first equilibrated for 24 hours before starting an experiment. Ranges of parameters chosen for salinity, pH, and temperature experiments were based on the expected fluctuations in the field.

**Effect of salinity**

To understand the effect of salinity on Pi release from East Creek sediments, salinity of 2, 4, 8, 15, 25, and 33 mM NaCl were chosen in this study. Salinity of the natural water may fluctuate between these values in river and saline waters. Sample pre-processing procedure before running experiment was the same as described in section 3.1.1. Replicate experiments were run for each salinity treatment. Throughout the experiment, all the experimental tubes were shaken at 169 RPM in a shaker. Aliquots were taken from each tube in 0, 8, 24, 48, 96, 192, and 288 hours after the start of experiment. All samples were then centrifuged for 73 min. at 13,800 RCF and Pi concentration in the supernatants was measured using the colorimetric method (Murphy and Riley, 1962).

**Effect of pH**

The effect of pH fluctuation on Pi release was studied in sediments from five sites (A, F, H, K, and L, Figure 3) using an automatic titrator. The titrator was used to maintain constant pH throughout the experiment so that any change in pH due to buffering capacity of the released or removed Pi to and from solution (Stumm and Morgan, 1996) could be avoided. Experiments were run at three pH values (6.0, 6.8, and 9.0), a range expected to vary in natural environments. During the epxerimental runs, pHs 6.0 and 6.8 were maintained by using 0.1 N HCl and 9.0 by using 0.05 N NaOH as titrating reagents. A magnetic stir bar was used throughout the experiment to mix the suspension. Aliquots were taken from each experimental reactor and processed for Pi measurement as described in section 3.2.1.1.

**Effect of temperature**

To understand the effect of temperature variation on Pi release, sterilized sediments (prepared as described in section 3.1.1) were incubated at 4, 10, 22(±0.5), and 30 $^{o}$C. Replicate experiments were run for each temperature. Throughout the

experiment, all experimental tubes were hand shaken three times a day. Sample processing and Pi measurement were performed as described in section 3.2.1.1

### 3.2.2 Effect of change in biological activities

To study the effect of biological activity on Pi release, intact core sediments were incubated with and without a bacterial spike. *Shewanella putrefaciens* CN32, a facultative anaerobe, was used to enhance the biological activity and to decrease the redox potential. *S. putrefaciens* CN32 was chosen for this study because it switches from oxygen to Fe(III) as terminal electron acceptor and has been used as a model bacterium to study Fe(III) reduction (Lovley and Philips, 1988; Jaisi et al., 2005, 2008; Roden, 2006).

Water samples were first filtered through 0.45 μm filter syringes before running experiments. All experiments were performed at sediment: water ratio of 1:50 (i.e., 1.2 g in 60 mL) after 24 hours of sediment water suspension equilibrium. Two sets of experiments were run to estimate Pi release from sediments as a result of enhanced biological activity (CN32 cells added) and natural incubation (sediment alone). 20 mM lactic acid (pH 7.0±0.2) was used as electron donor in all experiments. The control experiment included sterilized sediment and water samples without cells.

Enhanced biological activity experiments included adding freshly grown CN32 cells ($8.0\times10^7$–$1.3\times10^8$ cells/mL, final concentration). CN32 was first grown in a minimal nutrient broth for 24 hours in a water bath (37 $^oC$, 60 RPM), pelletized (centrifuged 15 min. at 5,000 RPM) and washed in a bicarbonate buffer [2 g/L $NaHCO_3$ with 0.1 g/L KCl, pH 7.2(±0.2)]. Sterile condition was maintained during the handling of culture media and bacteria. All experiments (spiked, natural incubation, and control) were conducted inside an anaerobic glove box (Coy Laboratory Products Inc., WI). Aliquots were taken from each experimental tube at selected sampling time points for Pi and Fe(II) measurements. Aliquots taken for Fe(II) measurement were first acidified (0.5 mL sample with 0.5 mL 1 N HCl) and reacted for 24 hrs inside the glove box in order to avoid re-oxidation of Fe(II) to

Fe(III). Samples collected both for Pi and Fe(II) measurements were then centrifuged for 73 min. at 13,800 RCF. Supernatants were then separated to measure Pi and Fe(II) concentrations. Over the course of the experiment, 0.1 mL of sediment-cell suspension was removed from the experimental tubes, diluted, and plated on agar plates (Difco$^{TM}$ minimal agar Davis) to monitor changes in cell concentration. The number of colony forming units (CFU) was visually counted.

### 3.2.3 Phosphate flux at the sediment-water interface

Pi flux from the sediment-water interface was measured in experiments simulated to field conditions using intact sediment cores and on-site water following a slightly modified approach originally used by Cowan and Boynton (1996). The flux experiments were performed on cores retrieved from A, F, H, K, and L sites (Figure 3b) by adding 200 mL of river water collected from the same site on the top of intact cores and spiking with freshly prepared *S. putrefaciens* CN32 cells (as in section 3.2.2) at $8.8\times10^{7}$–$3.1\times10^{8}$ cells/mL and 20 mM lactic acid (pH 7.0±0.2). To inhibit the growth of algae, experimental cores were wrapped with aluminum foil. All experiments were run inside an anaerobic glove box.

Aliquots were taken from the water column during selected sampling time points for Pi and Fe(II) measurements. The suspension was centrifuged to separate into four size fractions, namely 0.02, 0.1, and 0.4 μm, to identify Pi concentration in these particulate size fractions. The aliquots taken for Fe(II) measurement were processed as described in section 3.2.2. During each sampling time, 0.1 mL of sediment-cell suspension was also removed to monitor changes in number of viable cells (as described in section 3.2.2).

## 3.3 Measurements and Data Analysis

Pi concentrations in the samples collected after running different sets of experiments were measured using the phosphomolybdate blue method (Murphy and Riley, 1962). Similarly, the Ferrozine assay (Stookey, 1970) was used for Fe (II)

measurement. Pi and Fe (II) concentrations were measured in Spectrophotometer UV/Vis at an absorbance of 883 nm and 562 nm, respectively.

Pi and Fe concentration data generated from experiments were analyzed using the GLM (General Linear Model) procedure of statistical software, SAS 9.2 (SAS Institute Inc., NC). The data were analyzed with one-way ANOVA model at a 0.05 level of significance. Regression equations were developed for each physico-chemical and biological parameter to estimate Pi mobilization from East Creek during normal (existing field conditions) and extreme conditions (defined as the worst condition for maximum Pi release).

### 3.4 Results and Discussion

All Pi and Fe (II) release mentioned in the results below refers to 288 hours of reaction time.

#### 3.4.1. Effect of changes in salinity on phosphate release

Pi release increased significantly ($P<0.05$) with an increase in salinity in samples from at all sites (Table 3; Figures 9 and 10). Pi release was most pronounced at salinity ≥15 mM NaCl which is usually the case when fresh water mixes with the saltwater. The highest amount of Pi was found to be released from site L (1.28 µmol/g) and lowest from site A (0.15 µmol/g) for all ranges of salinity tested in this study. In control experiments, Pi release was very low, 0.02 µmol/g at site A and 0.33 µmol/g at site L. Salinity alone contributed 0.12 µmol/g Pi from site A and 0.89 µmol/g Pi from site L. High Pi release from upper reaches of the creek was consistent with the higher sediment Pi concentrations near agriculatural field sites compared to the lower reaches of the creek (Table 1). Ambient salinity measured in sites K and L was 5.55 and 4.38 PSU (practical salinity units), respectively. It increased downstream and reached 8.99 at site A. Adding 15 mM NaCl corresponds to the increase in salinity by ~4 times at K, and L sites, and ~2.7 times at A, F, and H sites. Higher increase in salinity at sites K and L contributed to a higher release of Pi

compared to A, F, and H (Table 3; Figures 9 and 10). Pi release during the increase in salinity should be resulted from competitive sorption where Pi sorbed to mineral surfaces, e.g., $NaHCO_3$ extractable Pi, are removed by $Cl^-$ (from addition of NaCl).

Several field studies have found out that the salinity affects on Pi desorption and release. For example, the amount of Pi sorbed has been found to decrease from freshwater to salt-water sites of the Cooper River, SC (Sundareshwar and Morris, 1999) where salinity change (0.07 to 20 g/L) promoted increase in Pi release by 16 times. Another study in the same river found out that the pore water Pi varied from 1.0 (± 0.2) µM in fresh water, 0.8 (±0.2) µM in brackish water, and 13.5 (±0.25) µM in salt marsh (Paludan and Morris, 1999). In the Patuxent River in the Chesapeake Bay watershed, increase in salinity from 0 to 8 PSU resulted in the increase of dissolved Pi from 20 to 70 µmol/L while dissolved Fe decreased from 500 to < 50 µmol/L (Jordan et al., 2008). Pi release due to the effect of increase in salinity in East Creek sediment is lower than that found these studies. However, Pi release in East Creek discussed here is solely due to the changes in salinity without concomitant changes in other effects such as microbial activities. These results, in general, conclude that the increase in salinity mobilizes Pi from river sediments.

### 3.4.2. Effect of changes in pH on phosphate release

Effect of pH on P mobilization/retention was quite different in different pH values chosen in this study. When the ambient water column pH (7.47±0.19) was changed to 9.0, Pi release from the sediment increased significantly at all sites (Table 4; Figures 11 and 12). Pi release from site L was highest (1.20 µmol/g) and from site A (0.50 µmol/g) was lowest at pH 9.0. In general, Pi release increased by fourfold due to the increase in pH from pH 7.4(±0.19) to 9.0. When the ambient pH was decreased to 6.0, dissolved Pi, however, re-sorbed to mineral surfaces resulting in a net increase in sediment Pi, for example, by 0.059 µmol/g at site A and 0.517 µmol/g at site L. High Pi release in the upper reaches of the creek was consistent with the high Pi concentration in water column and sediment the near agricultural field (Table 1,

Figure 5). Pi release from sediments at high pH might be derived from $NaHCO_3$ extractable Pi because of increase in negative surface charge of mineral surface. 18-30 % of $NaHCO_3$ Pi was extracted when pH was increased from 7.4 to 9.0.

Increase in Pi release from East Creek sediments due to increase in pH was consistent with results from previous studies (Jensen and Anderson, 1992; Kim et al., 2003; Fisher and Wood, 2004). For example, the Pi release rate in Jamsil submerged dam area in the Han River, Korea doubled when pH was increased from 7 to 13 (Kim et al., 2003). Pi release increased by threefold when pH was increased from 8.1 to 9.7 in Lake Kvind, Netherland (Jensen and Anderson, 1992). Similarly, simulated laboratory experimental results have found that the Pi release rate increased by twofold when ambient pH of 8.1 was elevated to 10 in Upper Klamath Lake, Oregon (Fisher and Wood, 2004). Therefore, pH is one of the most important parameters affecting Pi release from sediments.

### 3.4.3. Effect of changes in temperature on phosphate release

When the sediment temperature was increased from room temperature (22±0.5 $^{o}$C) to 30 $^{o}$C, Pi release increased significantly from 0.14 to 0.21 µmol/g along the lower reaches and 0.59 to 0.81 µmol/g along the upper reaches of the creek (Table 5; Figures 13 and 14). Similarly, Pi release was 0.32 µmol/g at site K, 0.27 µmol/g at site H, and 0.25 µmol/g at site F (Table 5) when temperature was increased to 30 $^{o}$C. Temperature alone contributed 0.06 µmol/g at site A and 0.22 µmol/g at site L. $NaHCO_3$ extractable Pi may have been released from sediments during temperature elevated conditions.

Temperature has been found to contribute about 70% of the seasonal variation in sediment Pi release from Danish Lakes (Jensen and Anderson, 1992). This however, included other effects that changed with season and temperature. Maximum Pi release was found to be 2.1 µmol/L in Lake Kvind, 2.6 µmol/L in Lake Vaeng, 6.3 µmol/L in Lake Arreskov, and 8.4 µmol/L in Lake Sobygard during summer when pH increased from 7.5 to 10.5 (Jensen and Anderson, 1992). When temperature was

increased from 2 to 25−30 $^{o}$C in Chungpyung Lake, Han River, Korea, Pi release rate increased by fivefold at pH 8 at dissolved oxygen (DO) of 0.5 mg/L (Kim et al., 2003). Compared to these results, Pi release due to the effect of temperature in East Creek is low (Table 5; Figure 13 and 14). One of the reasons for the difference is possibly due to the different starting temperature, pH, and Pi concentrations in East Creek water from these sites. Similarly, this study focused on individual physiochemical effects such as temperature without including any secondary effects due to elevated temperature such as increase in bacterial activities and changes in pH. Overall, data from this study suggest that the elevation of temperature can enhance Pi release from sediments but temperature alone does not mobilize a significant amount of Pi compared to other parameters (see sections 3.4.2 and 3.4.4).

### 3.4.4 Effect of changes in biological activities on phosphate release

Pi release in *S. putrefaciens* CN32 spiked experiments was significantly higher at all sites compared to that of non-spiked experiments (Table 6; Figures 15 and 16). *S. putrefaciens* CN32 utilizes oxygen as an electron acceptor first, and then switches to Fe(III) when oxygen is not present. Consumption of dissolved oxygen by CN32 leads to more reducing conditions in water and sediment columns that result in the reductive dissolution of Fe(III) oxides (Lovley and Philips, 1988), and thus release of iron oxide bound Pi. Results from this study show a higher Pi release when the amount of Fe(II) is higher. For example, in the CN32 spiked condition, 5.08 µmol/g Pi and 130 µmol/g Fe(II) was released at site L. In control experiment, Pi release was 0.60 µmol/g at site A and 1.21 µmol/g at site L. Enhanced biological condition alone contributed 0.88 µmol/g Pi release at site A and 3.88 µmol/g at site L. Cell concentration in the spiked experiments decreased only within about a log order at the end of experiment (i.e. 288 hours) (Figure 15f). Comparing among sites, Pi release from site L was the highest (5.08 µmol/g), and site A was the lowest (1.48 µmol/g). Relatively higher Pi release at the upper reaches of the Creek than that along the lower reaches is similar to the

results found with physicochemical parametric tests, and is positively correlated with high water column and sediment bound Pi.

The extent of Fe(III) reduction by *S. putrefaciencs* CN32 was found to be higher along the upper reaches than the lower reaches of the creek (Figures 17 and 18). For example, Fe(II) release (due to Fe(III) reduction) was 40 µmol/g at site A and 130 µmol/g at site L. The higher Pi released from site H samples may be due to the fact that water samples used in the experiment were not pre-filtered (Figures 15 and 16). Although Fe(II) and Pi release are positively correlated and therefore are coupled, the extent of Pi release was proportionally much higher compared to the Fe(II) produced in all sites. Calculating P:Fe ratio on aliquots sampled showed that the ratio decreased after 48 hours of experiment suggesting lag in Fe(III) reduction (Figure 20). This change in ratio could have resulted from initial bacterial uptake of $NaHCO_3$ extractable Pi during when bacteria respire with oxygen as terminal electron acceptor. Once the dissolved oxygen decreased and Fe(III) reduction started, certain amount of oxalic acid extractable Pi could have remobilized. Preference of loosely sorbed Pi in sediments containing different P phases by bacteria during aerobic growth was confirmed by Jaisi et al. (2011). Separate Pi measurements performed with acidified samples (co-measured from those prepared for Fe(II) measurement) correlate well with oxalic acid extractable Pi. For example, Pi concentration in 0.5 N HCl extracted solution (see section 3.2.4) was 1.89 µmol/g at site A and 6.54 µmol/g at site L. Pi and Fe(II) concentration in natural incubation (non-sterile) and control (sterile) experiments was low but did not show any significant difference ($P<0.05$) (Table 6), the reason for this is unclear, but it is likely that the experimental conditions chosen for controlled laboratory experiments might not be favorable for the growth of native microorganisms as evidenced from no viable cell growth on agar plates. An alternative explanation could be that Pi released by a consortium of native microorganisms could have been taken up by other microorganisms resulting in no net increase in solution Pi.

Fe(III) reducing bacteria have been found to effectively mobilize sediment P

from different environments. For example, GS-15, a Fe(III) reducing bacterium isolated from Potomac River sediments increased the amount of Fe(II) produced by 1.5 to 7.0 times during 14 days of incubation (Lovely and Phillips, 1988). Similarly, Pi release rate at Jamsil submerged dam, Han River, Korea was found to be 2.5 times faster in anoxic conditions than that in oxic conditions (Kim et al., 2003). In Lake Sempach, Switzerland, sedimentary bacteria depleted dissolved Pi from 150 to 0.01–0.1 μmol/L partially aerobic medium (Gächter et al., 1988)). However, in anoxic conditions, 14–25 % of Pi was released. When the sediment suspension was spiked with FeOOH mineral precipitates, reductive dissolution of >50 % FeOOH simultaneously increased Pi concentration (Gächter et al., 1988). These results suggest strong coupling of Fe(III) reduction and Pi release. Fe(II) release in East Creek increased 4 to 10 times and Pi release promoted due to Fe(III) reduction increased by 4 to 15 times. Semi-quantitative analyses of sediments before and after incubation from site A and H showed a slight decrease in Fe(III) containing sediments, particularly ferrihydrite (Figures 6 and 21). These results confirm that enhanced biological activity is a significant parameter affecting Pi release from sediments.

### 3.4.5 Phosphate flux from the sediment-water interface

Pi flux at the sediment-water interface from the intact cores was significantly high at all sites. For example, Pi flux from sites K and L was high (53.4 and 48.6 μmol/$m^2$–hr, respectively) and from site A was low (14.8 μmol/$m^2$–hr) (Figure 19). There was a systematic decrease in Pi flux along the river flow direction. Higher Pi flux in the upper reaches of the creek is consistent with the higher sediment and water column Pi concentration at sites near the agricultural field than those along the lower reaches of the creek (Table 1 and Figure 5). Assuming top 0.5 cm of the intact sediment core as the reactive surface, Pi flux from the intact cores is 0.41 μmol/g at site A and 1.36 μmol/g at site L. P flux from intact core was significantly low compared to that of sediment in suspension during enhanced biological activity (Table 6; Figures 15 and 16). Sediment in suspension is thoroughly mixed so when bacteria

consume dissolved oxygen, condition may be reducing in whole suspension. But in intact cores, there is water column standing above the core so when bacteria consumes dissolved oxygen in the water column, there may still be oxygen at the sediment water interface due to which condition may be less reducing and less Pi may have been released.

Separation of suspended particles in the water column into 0.02, 0.1, and 0.4 μm size fractions and measuring Pi concentration showed Pi was high in finer particles (Figure 19). For example, Pi flux for size fraction <0.02 μm was between 9.3 to 37.0 μmol/$m^2$–hr, while it was only 10.7–48.4 μmol/$m^2$–hr for <0.4 μm. Particulate P might be originated from the resuspension of sediments at the sediment-water interface during the experiment. Pi concentration in small size fraction of sediments was higher than lager size fraction consistent with those from field samples.

Previous studies on Pi flux from sediments in the Chesapeake Bay watershed have found comparable results. For example, the Pi flux varied between –23 to 25 μmol/$m^2$–hr with an average of 5 μmol/$m^2$–hr at pH levels <~9.2 and 60 μmol/$m^2$–hr in pH >10 in Potomac River sediment (Bailey et al., 2006). Pi flux from Potomac estuary sediments, however, was high (<25 μmoles/$m^2$–hr) when the overlying pH was 9.0 or less (Seitzinger et al., 1991). At sites located in the algal bloom zone of the estuary, Pi flux from sediments was even higher (between 31 to 37 μmol/$m^2$–hr at pH 9.5). Similarly, Pi flux from Murderkill estuary, DE sediment was between –22.4 to 62.2 μmol/$m^2$–hr (Banta et al., 2010). Sediment-water oxygen and nutrient fluxes studied in sediments from North, middle, and South regions of the Chesapeake Bay showed average Pi flux between -16.5 μmol/$m^2$–hr and 148 μmol/$m^2$–hr (Cowan and Boynton, 1996).

Analyzing water column Pi and sediment Pi, preferably Pi in sequentially extracted sediments, could provide a quantitative estimate of Pi flux. However, this information is not present in many studies mentioned above. Unpublished results in our lab shows that the Chesapeake Bay sediments have very low exchangeable Pi

compared to that in the East Creek. Water column Pi in the Chesapeake Bay varies with season, could reach to ~5 μmol/L in summer months near the sediment-water interface (Kemp et al., 2005). It is likely that relatively high water column Pi in East Creek (2.8 –23 μM, Figure 5) and high $NaHCO_3$ extractable Pi could have resulted in high Pi flux. Comparative results of Pi flux in different regions of the Chesapeake Bay watershed show that the East Creek could potentially mobilize higher Pi than other rivers.

### 3.4.6 Correlation between phosphate release and physiochemical parameters and biological activity

Correlation coefficients (R) calculated using the SAS program to understand sensitivity of each parameter to Pi release from the sediment showed that the Pi release was most significantly correlated with pH (R=0.84), and enhanced biological activity (R= 0.79) (Table 7). Similarly, R value for salinity and temperature was 0.50 and 0.52, respectively. Similarly, Fe(II) and enhanced biological activity were also correlated with each other (R=0.40). These results suggest that fluctuation in pH and biological activity could mobilize a higher amount of Pi than other parameters.

To compare the extent of Pi that could be mobilized, regression equations were developed for each parameter as follows:

*Salinity:* $Y=0.01X+0.17$ $(R^2=0.26)$…. (3)

*pH:* $Y=0.33X–2.30$ $(R^2=0.72)$… (4)

*Temperature:* $Y=0.01X+0.02$ $(R^2=0.23)$ …(5)

*Redox change:* $Y=0.87X–4.78$ $(R^2=0.63)$… (6)

*Y* in above equations refer to Pi release for the specified parameters and *X* is the value of the parameter, chosen to be normal (existing field condition) and extreme physiochemical and biological conditions (extreme conditions for high Pi release). Because the value of *X* is qualitatively defined, clarification is needed on the definition of extreme and normal cases chosen. For example, ambient water column pH values in the East Creek ranged between 7.20 and 7.62 (Table 8a) and salinity varied from

4.38 to 8.99 PSU. To define extreme conditions, river water pH was chosen to vary between 6.5 and 9.5, temperature between 0 and 25 $^{o}$C, and salinity from 4.0 to 19 PSU. When 'extreme' values of temperature, pH, and salinity were not available in East Creek, similar data for Patuxent River were used assuming that extreme cases are similar in the Patuxent River and East Creek because of their proximity. However, there are several differences among these two rivers (see above).

Using the regression equation developed above, at 'normal' salinity of East Creek, for example at site L, 0.21 µmol/g of Pi is released from the sediment (Table 8b). Similarly at normal pH, temperature, and biological activity conditions, 0.16, 0.25, and 1.21 µmol/g of Pi is released from site L sediment. In all cases, Pi release during normal conditions is high in the headwater region of the creek. When the conditions change to 'extreme', Pi release increases by 0.38 µmol/g at 19 PSU, 0.83 µmol/g at pH 9.5, 0.30 µmol/g at 25 $^{o}$C, and 3.10 µmol/g at CN32 cell spiked condition. During extreme low pH of 6.5, 0.15 µmol/g of Pi is sorbed back in the sediment. Except during extreme low pH events, fluctuations in other parameters such as salinity, temperature, and enhanced biological activities all cause large amount of Pi release from the sediment.

### 3.5 Summary and Conclusions

pH and enhanced biological activity were found to be the most sensitive parameters affecting Pi release in East Creek sediments. Comparing the amount of Pi released at the headwater and the mouth of river, effect of fluctuation in salinity, pH, temperature, and enhanced biological activity in the headwater region resulted in high Pi release in all cases. Because the headwater water in East Creek originates primarily from agricultural fields, it is likely that the Pi leached or washed from the fields contributed to the high Pi in the creek.

## SUMMARY AND CONCLUSIONS

East Creek sediments contain quartz, albite, illite, chlorite, orthoclase, and anorthite. Ferrihydrite, goethite, and pyrite are major iron bearing minerals in East Creek sediments. Fluorapatite and hydroxyapatite are suspected phosphate bearing minerals but their identity is not fully confirmed. Both sediment bound and water column Pi were high along the upper reaches of the creek near the agriculture field, but it gradually decreased down gradient possibly due to the dilution in different proportions from rainfall, selective sorption to sediments, groundwater input, and tides.

Controlled laboratory experiments performed to quantify the mobilization of P during fluctuation in physicochemical parameters and biological activities showed that the pH and biological activities were most sensitive parameters. For example, increase in pH from 7.4 (ambient pH) to 9.0 resulted in release of 0.5 to 1.20 μmol/g Pi. Pi released from sediment spiked with *Shewanella putrefaciens* CN32 was between 1.42 to 5.08 μmol/g. Changes in salinity varied from 5.10 to 8.65 PSU from agricultural area to the lower reaches of the creek. Pi release due to increase in salinity and temperature were <1.0 μmol/g. In field simulated Pi flux experiments, Pi release varied between 14.8 and 48.6 μmol/$m^2$–hr with higher Pi in the upstream section of the creek near agriculture field than that in the lower reaches of the river. This result is consistent with high particulate and dissolved Pi in water column and $NaHCO_3$ and oxalic acid extractable Pi in the sediment in upstream section of the creek. Amount of Pi that could be released during 'extreme conditions', defined as likely field condition in which Pi release is highest, calculated from regression equations developed from measured data was 0.83, 0.38, 0.30, and 3.10 μmol/g in pH, salinity, temperature, biological activities, respectively.

All parameters used to analyze Pi release from the study site indicated that Pi is being continuously mobilized from sediments and transported to the bay. Quantitative data generated in this study will be important to better understand sediment-nutrient interactions and resulting P mobilization. Pi mobilization data calculated for extreme conditions can be used as a guideline to develop better strategy to curb Pi release from agricultural regions or efficiently retaining Pi in the sediments.

## TABLES

Table 1 Sodium bicarbonate and oxalic acid extractable Pi in East Creek sediments.

| Site | $NaHCO_3$ extractable Pi, µmol/g | Oxalic acid extractable Pi, µmol/g |
|---|---|---|
| A | 1.69 | 1.28 |
| F | 2.29 | 1.89 |
| H | 2.27 | 3.50 |
| K | 5.35 | 4.52 |
| L | 5.90 | 7.77 |

Table 2: Mechanisms of phosphorus release and relevant geochemical processes (Yang et al., 2010).

| **Release Mechanisms** | **Factors** | **Relevant P forms in sediment** |
|---|---|---|
| Desorption (Boström et al., 1982) | 1. pH decrease induced P release (Boström et al., 1988)<br>2. Temperature elevation induced pH decrease (Lehtoranta, 2004)<br>3. Increase in $SO_4^-$ and $Cl^-$ can increase competition for $PO_4$ of sorption on Fe oxides (Stumm and Morgan, 1981) | Ca, Mg–P<br>Fe, Mn, Al–P |
| Dissolution (Boström et al., 1982) | 1. Microbial activity:<br>Inorganic or organic acid production (Ehrlich and Newman, 2008)<br>Chelators (e.g., gluconate and 2- ketogluconate (Banik and Dey, 1983)<br>Fe(III) reduction (Jansson, 1987)<br>$H_2S$ induced Fe(III) reduction (Sperber, 1958)<br>Solubility change by a drop in the pH due to the uptake of ammonium by fungi (Cochrane, 1958)<br>2. Redox change (reduction):<br>$NO_3$ enhanced increase of redox potential (Gatcher et al., 1988)<br>$SO_4$ reduction to form FeS or $FeS_2$ (Caraco et al., 1993)<br>3. Salinity and pH<br>Decrease sorption on Fe- oxides by increased salinity and reduced pH (Stumm and Morgan, 1981; Kitano et al., 1978) | Ca, Mg-P<br>Fe, Mn, Al–P |
| Ligand exchange (Boström et al., 1982) | 1. High pH promoted ligand exchange ($OH^-$ for $PO_4^-$) on iron-hydroxyl complexes (Cooke et al., 1993) | |
| Enzymatic hydrolysis (Boström et al, 1982) | 1. Microbial activity:<br>Mineralization (Marsden, 1989)<br>Temperature enhanced bacterial activity (Lehtoranta, 2004)<br>2. Decomposition produced reducing environment, enhances releases of P bound to Fe oxides (Nielsen and Andersen, 2003) | Organic-P<br>Fe, Mn–P |

Table 3 Pi release due to the increase in salinity and correlation results (note A, F, H, K, and L refer to study sites in East Creek; LSD, Pr, and F refer to least significant difference between the treatments, probability, and F-value resulting from statistical test to determine level of significance, respectively).

| Salinity | Pi release (μmol/g) | | | | |
|---|---|---|---|---|---|
| | Site A | Site F | Site H | Site K | Site L |
| Control | $0.02^{D}$ | $0.19^{E}$ | $0.15^{B}$ | $0.02^{F}$ | $0.33^{E}$ |
| 2mMNaCl | $0.02^{D}$ | $0.19^{E}$ | $0.21^{B}$ | $0.02^{F}$ | $0.41^{E}$ |
| 4mMNaCl | $0.05^{DC}$ | $0.20^{E}$ | $0.20^{B}$ | $0.13^{E}$ | $0.62^{D}$ |
| 8mMNaCl | $0.06^{DC}$ | $0.25^{D}$ | $0.18^{B}$ | $0.21^{D}$ | $0.80^{C}$ |
| 15mMNaCl | $0.09^{BC}$ | $0.41^{B}$ | $0.34^{A}$ | $0.42^{C}$ | $1.01^{B}$ |
| 25mMNaCl | $0.12^{BA}$ | $0.38^{C}$ | $0.35^{A}$ | $0.55^{B}$ | $1.30^{A}$ |
| 33mMNaCl | $0.15^{A}$ | $0.51^{A}$ | $0.34^{A}$ | $0.76^{A}$ | $1.28^{A}$ |
| | | | | | |
| $LSD_{(0.05)}$ | 0.05 | 0.03 | 0.07 | 0.06 | 0.10 |
| Pr > F *(0.05)* | 0.0067 | <0.0001 | 0.0021 | <0.0001 | <0.0001 |

Table 4 Release and sorption of Pi as a result of changes in pH of the sediment-water suspension from four sites in the East Creek. The ranges of pH chosen correspond to pH fluctuation in natural environments.

| pH | Pi release (μmol/g) | | | |
|---|---|---|---|---|
| | Site A | Site H | Site K | Site L |
| 6.0 | $-0.059^{C}$ | $-0.097^{B}$ | $-0.477^{A}$ | $-0.517^{C}$ |
| 6.8 | $-0.056^{B}$ | $-0.183^{C}$ | $-0.410^{B}$ | $-0.314^{B}$ |
| 9.0 | $0.503^{A}$ | $0.419^{A}$ | $0.972^{C}$ | $1.201^{A}$ |
| Prob>$F_{0.05}$ | <0.001 | <0.001 | <0.001 | <0.001 |

Table 5 Pi release due to increase in temperature and correlation results (note symbols as same it explained in Table 3).

| Temperature | Pi release (μmol/g) | | | | |
|---|---|---|---|---|---|
| | Site A | Site F | Site H | Site K | Site L |
| 4 $^{o}$C | -0.10$^{C}$ | 0.00$^{C}$ | 0.04$^{D}$ | 0.07$^{C}$ | 0.38$^{C}$ |
| 10 $^{o}$C | -0.06$^{BC}$ | 0.01$^{C}$ | 0.08$^{C}$ | 0.17$^{B}$ | 0.44$^{C}$ |
| 22.5 $^{o}$C | 0.14$^{BA}$ | 0.10$^{B}$ | 0.13$^{B}$ | 0.34$^{A}$ | 0.59$^{B}$ |
| 30 $^{o}$C | 0.21$^{A}$ | 0.25$^{A}$ | 0.27$^{A}$ | 0.32$^{A}$ | 0.82$^{A}$ |
| | | | | | |
| LSD $_{(0.05)}$ | 0.21 | 0.04 | 0.03 | 0.06 | 0.11 |
| Pr > F *(0.05)* | 0.04 | 0.001 | 0.001 | 0.003 | 0.005 |

Table 6 Effect of incubation and enhanced biological activities on Pi release from East Creek sediments (note symbols A, F, H, K, L, LSD, Pr and F are the same as described as in Table 3).

| Treatment | Pi release (μmol/g) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Site A | | Site F | | Site H | | Site K | | Site L | |
| | Pi | Fe(II) | Pi | Fe(II) | Pi | Fe(II) | Pi | Fe(II) | Pi | Fe(II) |
| Cell spiked | $1.48^{A}$ | $0.04^{A}$ | $2.19^{A}$ | $0.06^{A}$ | $3.79^{A}$ | $0.14^{A}$ | $3.55^{A}$ | $0.08^{A}$ | $5.08^{A}$ | $0.13^{A}$ |
| Control | $0.61^{B}$ | $0.01^{B}$ | $0.43^{B}$ | $0.04^{A}$ | $1.19^{B}$ | $0.12^{A}$ | $0.51^{B}$ | $0.03^{B}$ | $1.25^{B}$ | $0.04^{B}$ |
| Natural incubation | $0.60^{B}$ | $0.01^{B}$ | $0.43^{B}$ | $0.04^{A}$ | $0.93^{B}$ | $0.13^{A}$ | $0.52^{B}$ | $0.03^{B}$ | $1.21^{B}$ | $0.04^{B}$ |
| | | | | | | | | | | |
| $LSD_{0.05}$ | 0.15 | 0.00 | 0.20 | 0.04 | 1.17 | 0.06 | 0.12 | 0.02 | 0.14 | 0.00 |
| Pr > F *(0.05)* | 0.0034 | <0.001 | 0.001 | $0.36^{¥}$ | 0.021 | $0.55^{¥}$ | 0.00 | 0.02 | 0.00 | 0.00 |

Table 7 Correlation of Pi release and physiochemical parameters and biological activities.

| Parameters | Pi release R value | Fe(II) R value | Pi and Fe(II) R value |
|---|---|---|---|
| Salinity | 0.50 | - | - |
| pH | 0.84 | - | - |
| Temperature | 0.52 | - | - |
| Redox change | 0.79 | 0.40 | 0.66 |

Table 8a ‘Normal’ and ‘extreme’ physicochemical and biological conditions chosen to calculate Pi release in East Creek. Low and high for extreme conditions correspond to that for low and high Pi release.

| Parameters | Normal (ambient) field condition | Extreme low condition | Extreme high condition |
|---|---|---|---|
| pH | 7.4 | 6.5 | 9.5 |
| Temperature ($^{o}$C) | 21 $^{o}$C | 0 $^{o}$C | 25 $^{o}$C |
| Bacterial activity | Natural incubation | Control | Cell spiked |
| Salinity (PSU) | 5.1 (upper reaches);<br>8.6 (lower reaches) | - | 15 |

Table 8b Pi release during normal and extreme physicochemical and biological conditions. (Note that the Pi release at extreme low condition is zero because it is assumed that there are no natural microbial activities).

| Parameters | Normal condition (Pi, μmol/g) | Extreme high condition (Pi, μmol/g) | Extreme low condition (Pi, μmol/g) |
|---|---|---|---|
| pH | 0.17 | 0.83 | -0.15 |
| Temperature | 0.23 | 0.30 | 0.02 |
| Bacterial activity | 1.17 | 3.10 | 0 |
| Salinity | 0.29 | 0.38 | 0.24 |

## FIGURES

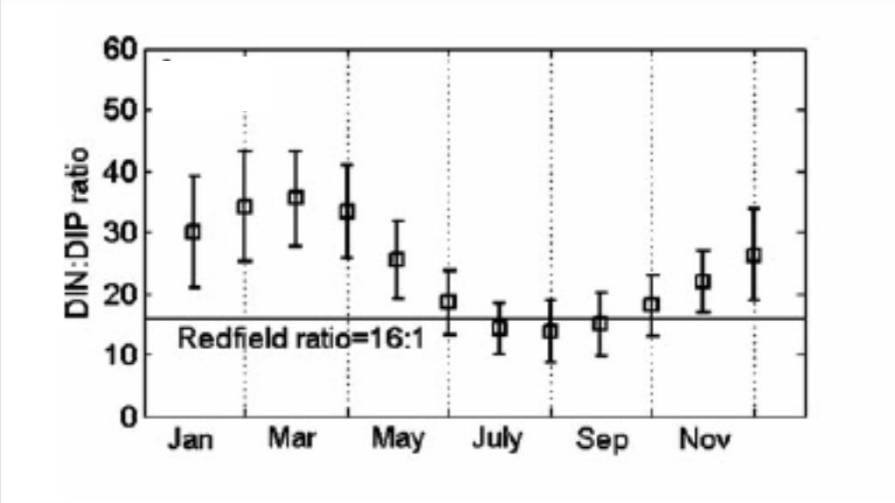


Figure 1 Monthly mean (1985-2008) of DIN: DIP ratio in the Chesapeake Bay (Prasad et al., 2010).

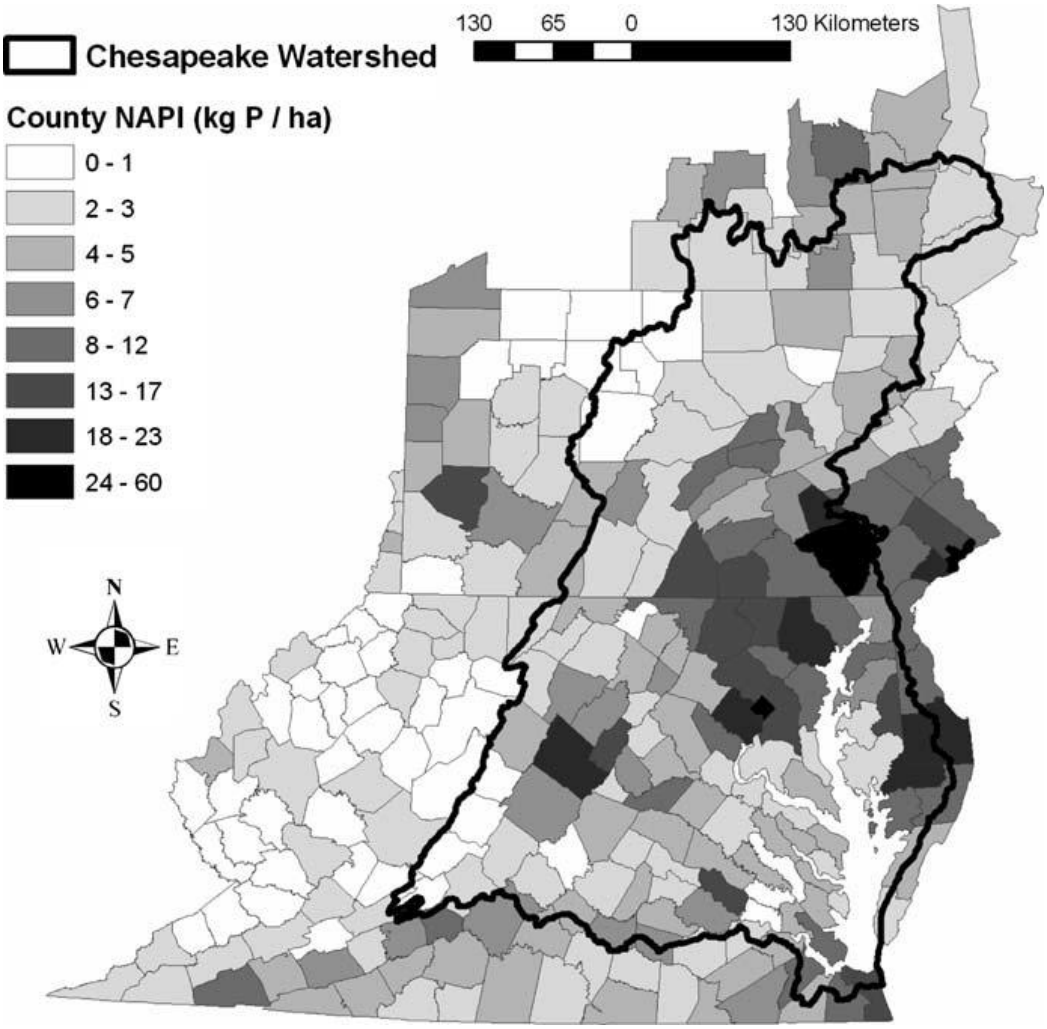


Figure 2 Net anthropogenic P input (NAPI) averaged for the years 1987, 1992, 1997, and 2002 in Mid-Atlantic region counties. Black outline is the boundary of the Chesapeake Bay watershed. Dark tone represents high NAPI (Russell et al., 2008)

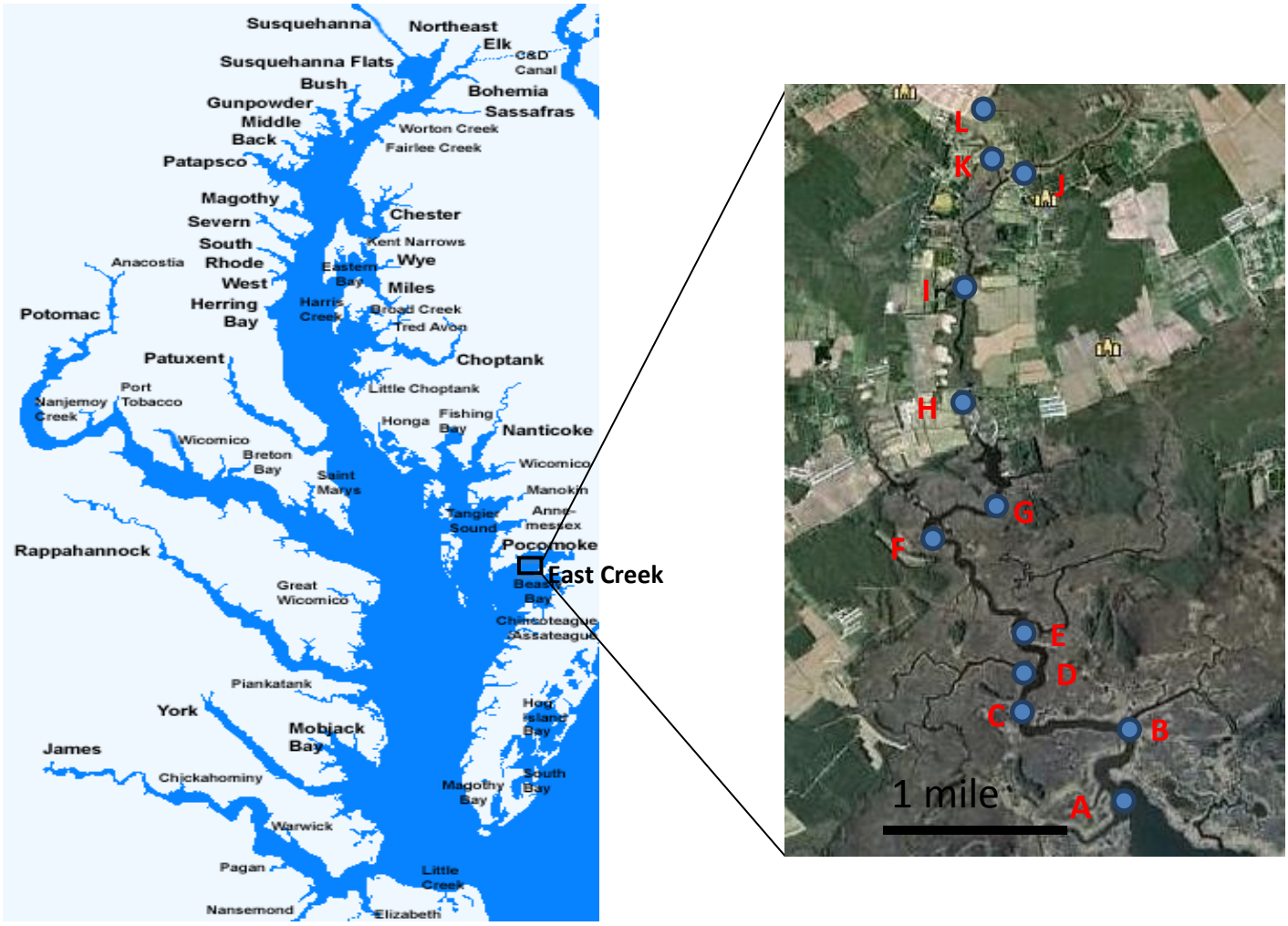


Figure 3 a) Map of Chesapeake Bay and the location of East Creek in the watershed, b) A major section of East Creek showing sites chosen for water and sediment sampling. Site A is located to the mouth of the river and L at the ditch of an agricultural farm.

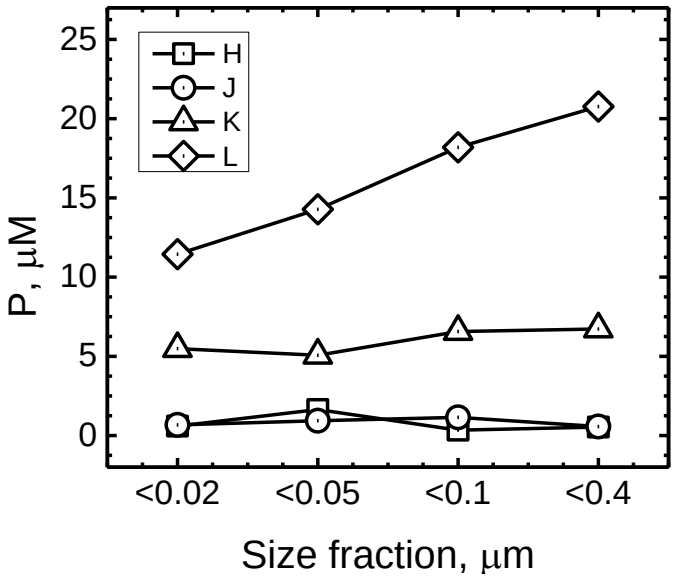


Figure 4 Variation of particulate Pi in the water column in four different sites. Size fractions <0.02, < 0.05, < 0.1, and < 0.4 μm were separated by centrifugation.

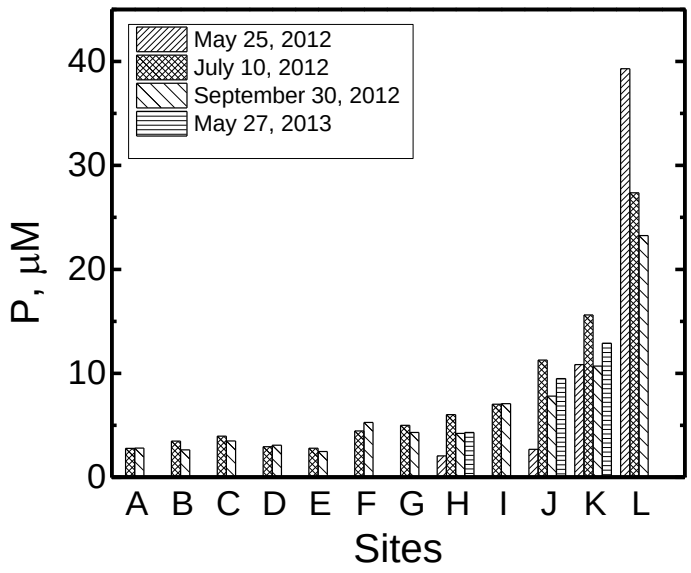


Figure 5 Seasonal changes in dissolved Pi concentrations in East Creek water column measured in May, July, and September 2012, and May 2013.

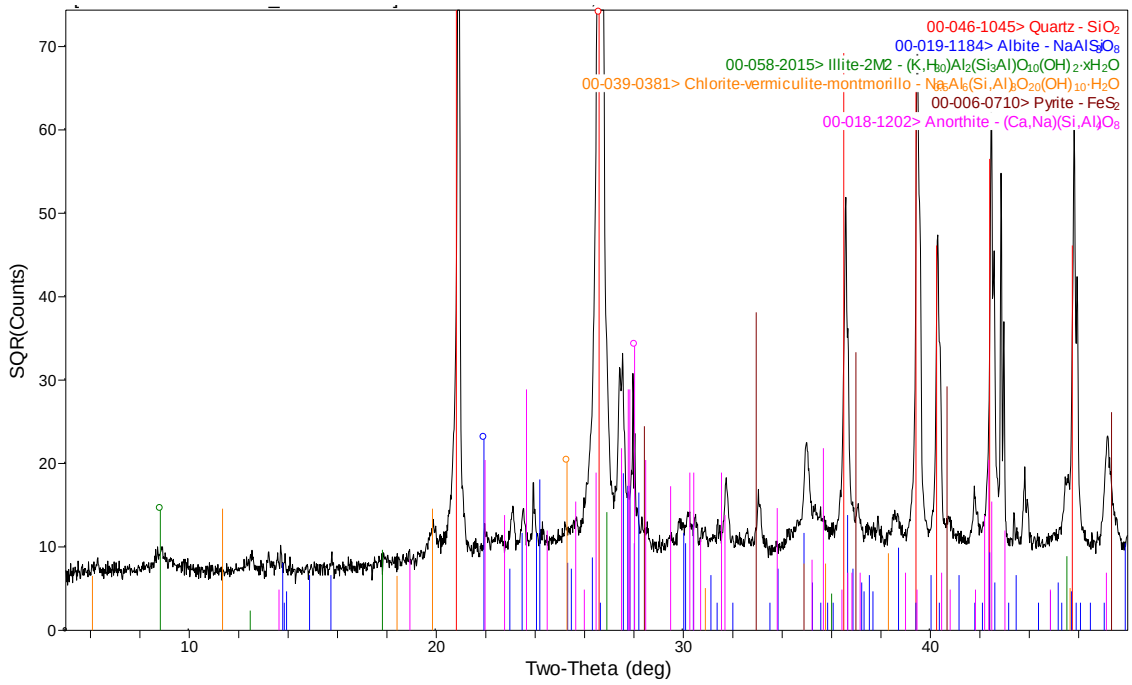


Figure 6a Mineralogical composition of sediment (0-2 cm) from site A (obtained by Tamas Varga at PNNL). Different color lines represent peaks of different minerals fitted to the measured spectra.

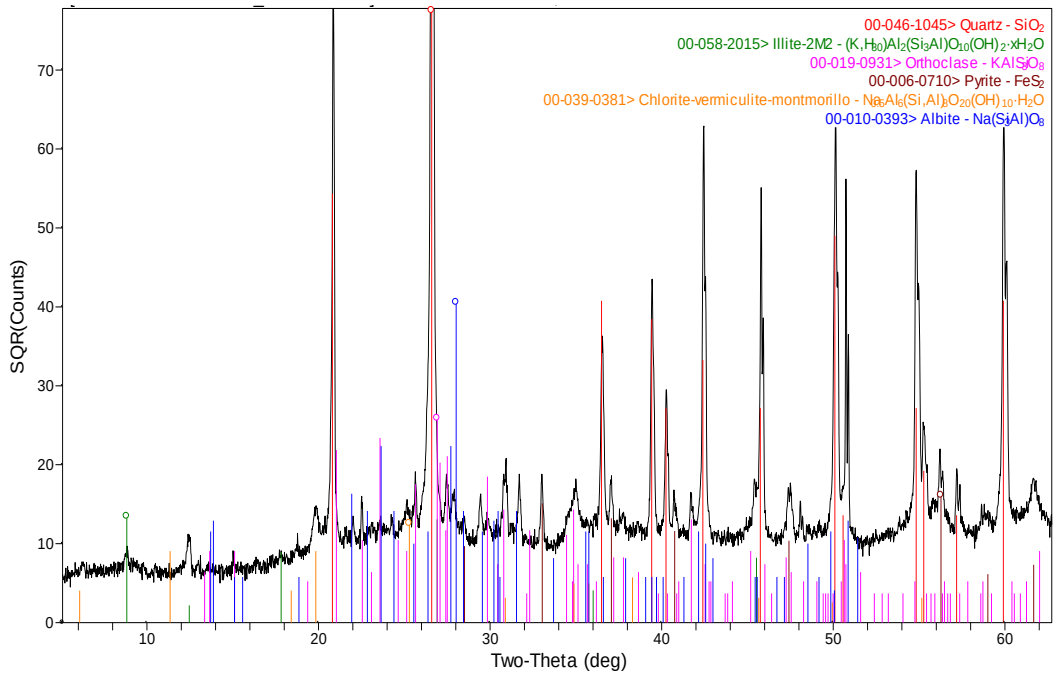


Figure 6b Mineralogical composition of sediment (0-2 cm) from site H (obtained by Tamas Varga at PNNL). Different color lines represent peaks of different minerals fitted to the measured spectra.

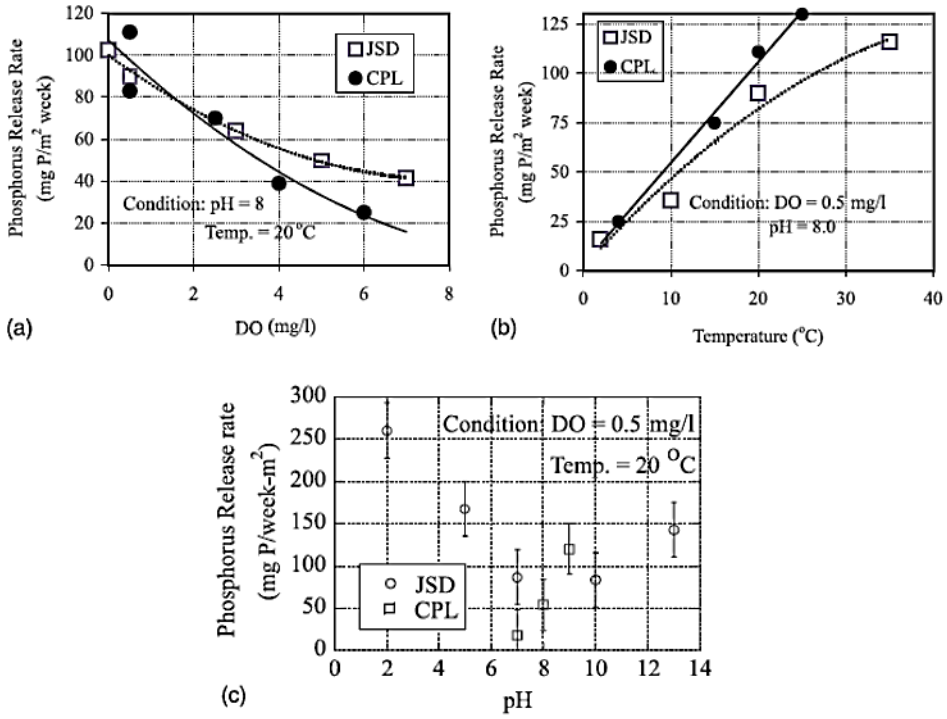


Figure 7 Rate of Pi release as a function of changes in DO (a) temperature (b), and pH (c). CPL and JSD refer to Chungpyung and Jamsil submerged dams in Korea, respectively (Kim et al., 2003).

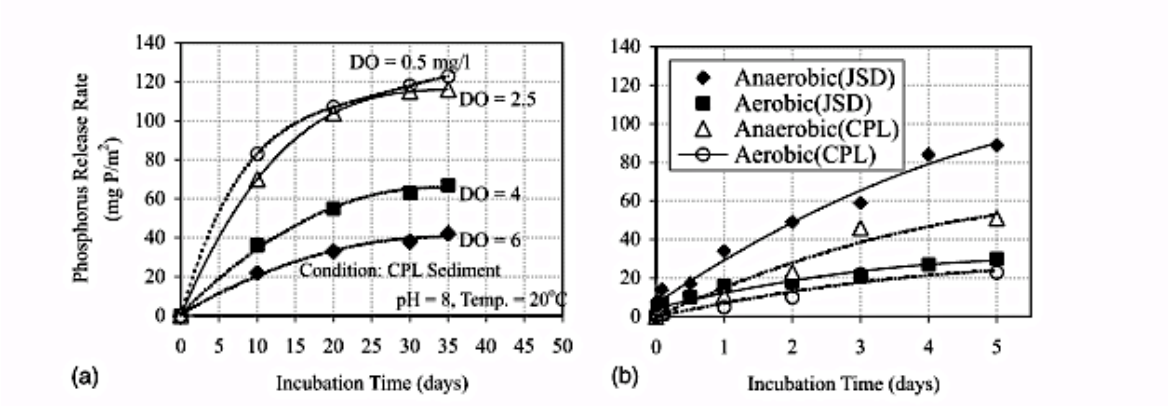


Figure 8 Cumulative changes in Pi release rates with incubation time for (a) different DO and (b) aerobic (DO = 6 mg/L) and anaerobic (DO <0.5 mg/L) conditions. CPL and JSD refer to Chungpyung and Jamsil submerged dam in Korea, respectively (Kim et al., 2003).

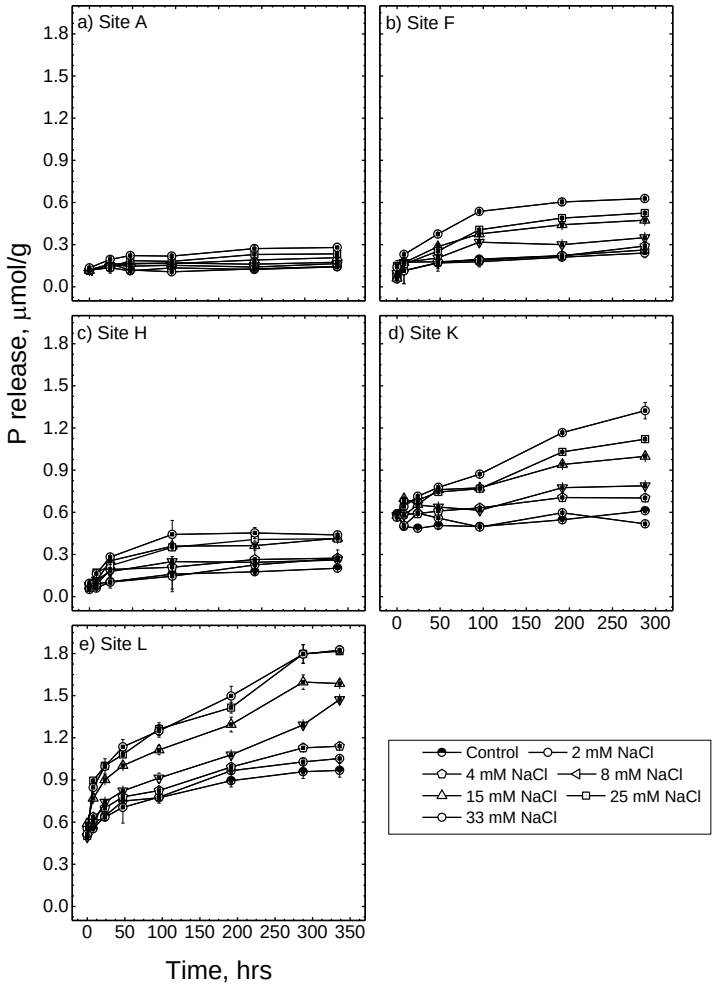


Figure 9 Effect of salinity on Pi release from East Creek sediments, a) site A, b) site F, c) site H, d) site K, and e) site L. Site L is closest to the agricultural field and site A opens to the mouth of the Chesapeake Bay. Control refers to autoclaved sediment and water from the specific site without any salt added.

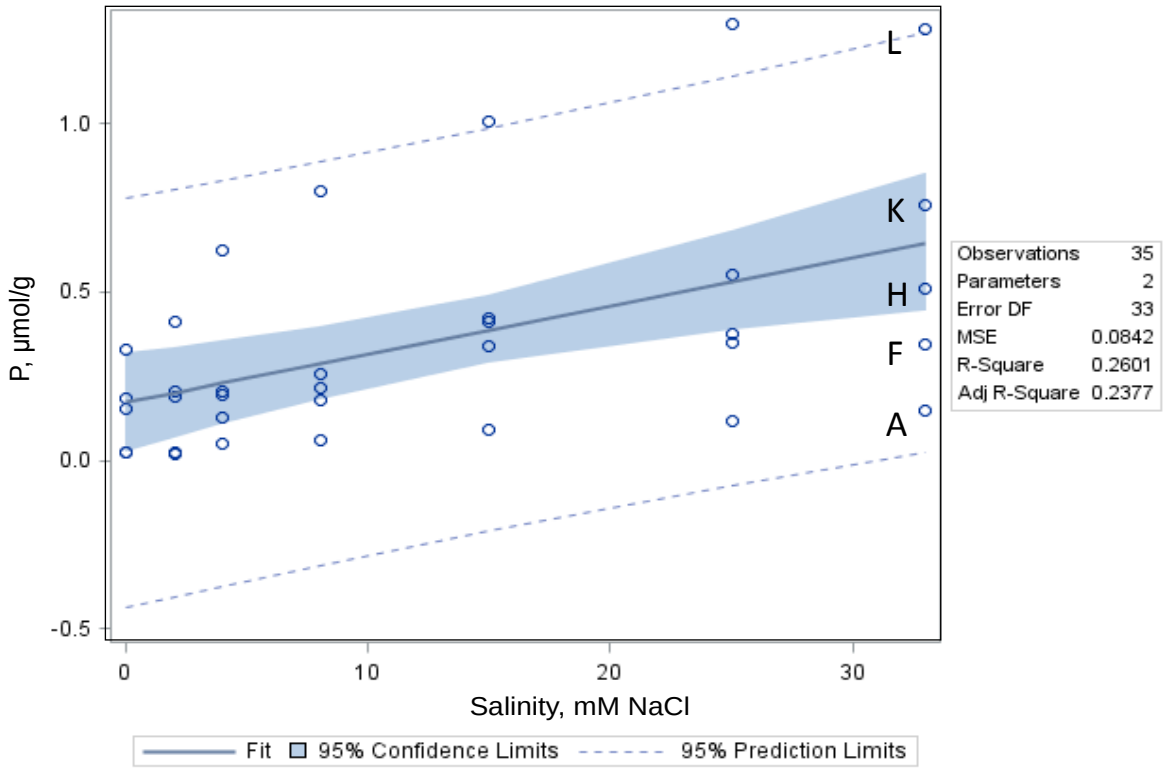


Figure 10 Regression plot for the effect of salinity on Pi release from sediments indicated in the figure 9. Pi data at the end of the experiments (288 hrs) were used for the calculation.

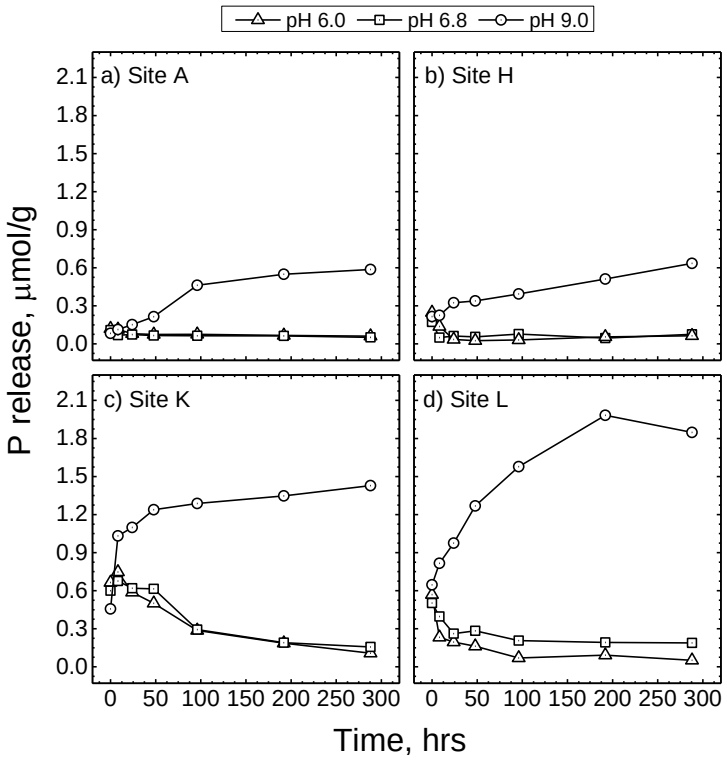


Figure 11 Effect of pH on release and sorption of Pi in sediments from five sites. Ambient pH of the water samples was 7.4 (±0.2).

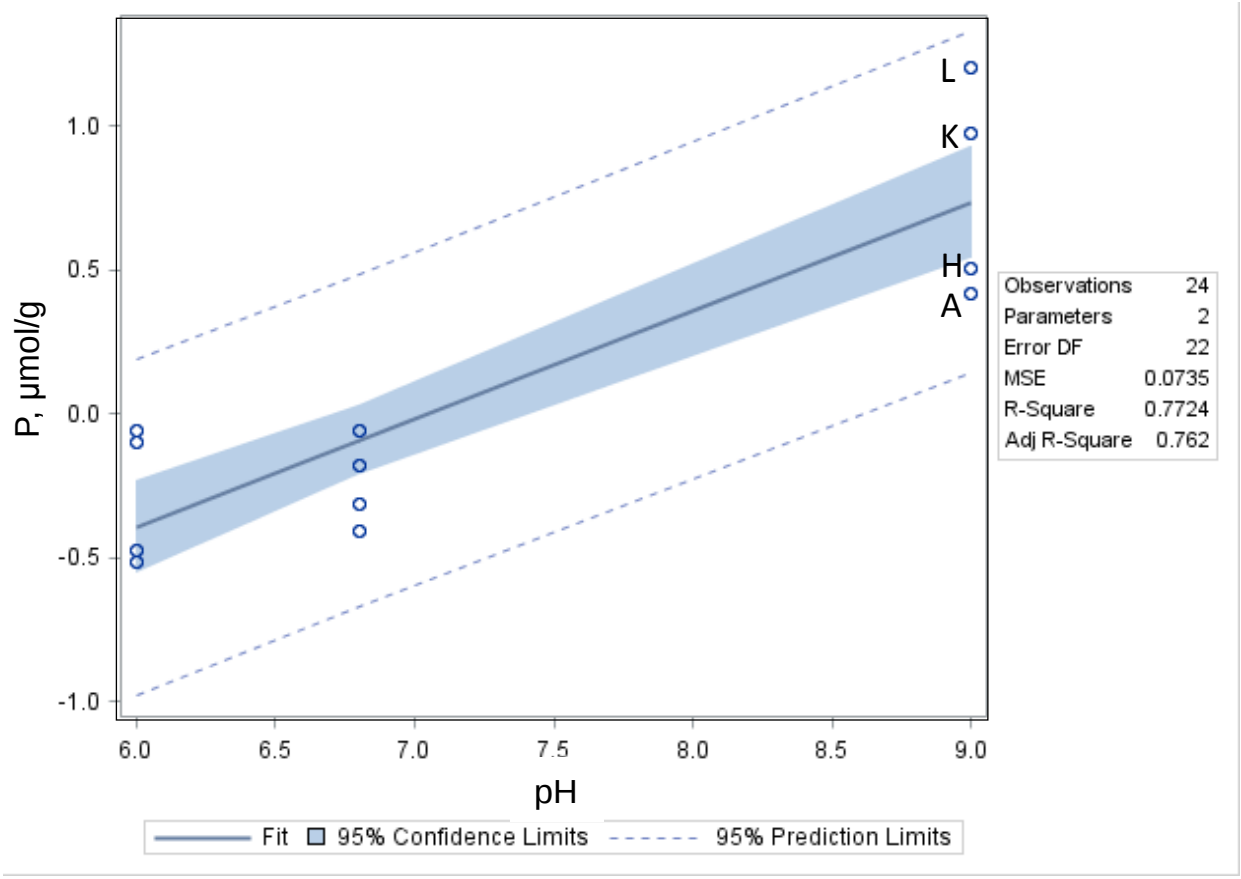


Figure 12 Regression plot for the effect of pH on Pi release from East Creek sediments. Data from last time points (in Figure 11) were used for the regression.

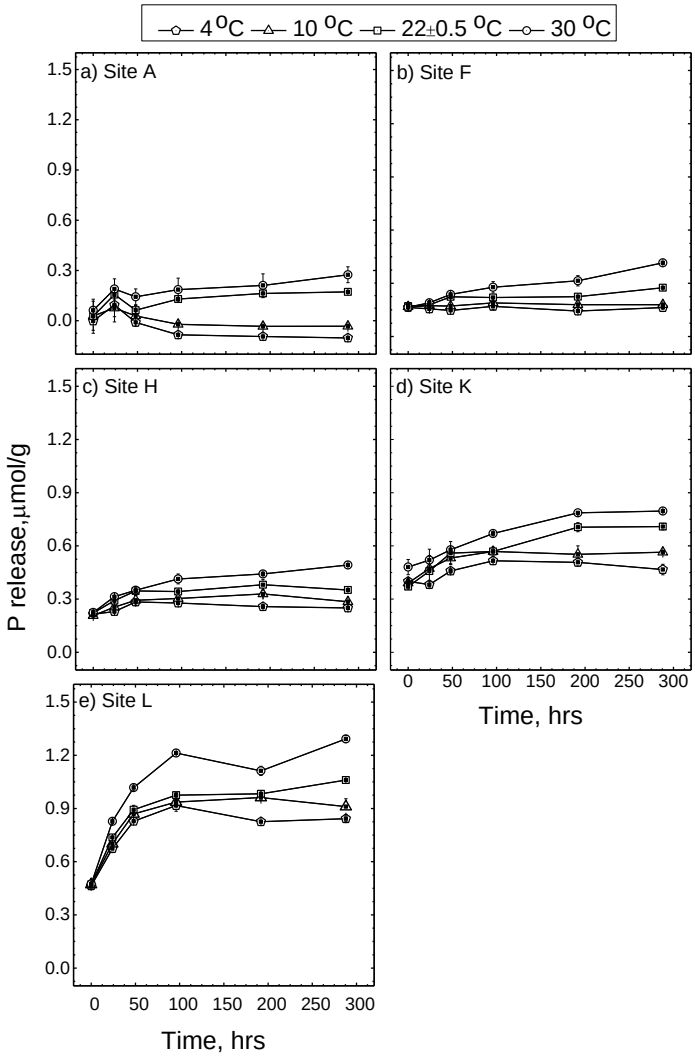


Figure 13 Effect of temperature on Pi release from selected sediments from the site indicated. Sediments from 0-2 cm depth were used for this analsysis. Ambient water column temperature was 19± (0.5) $^{o}$C.

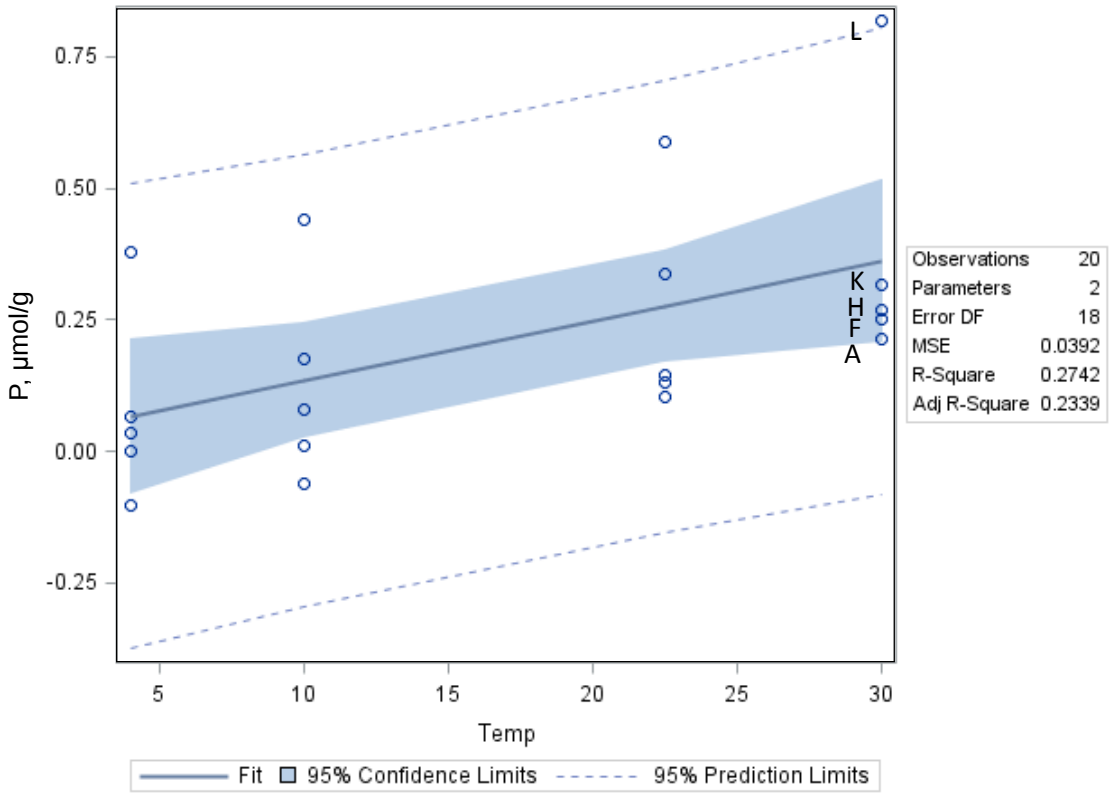


Figure 14 Regression plot for effect of temperature on Pi release (data from Figure 13).

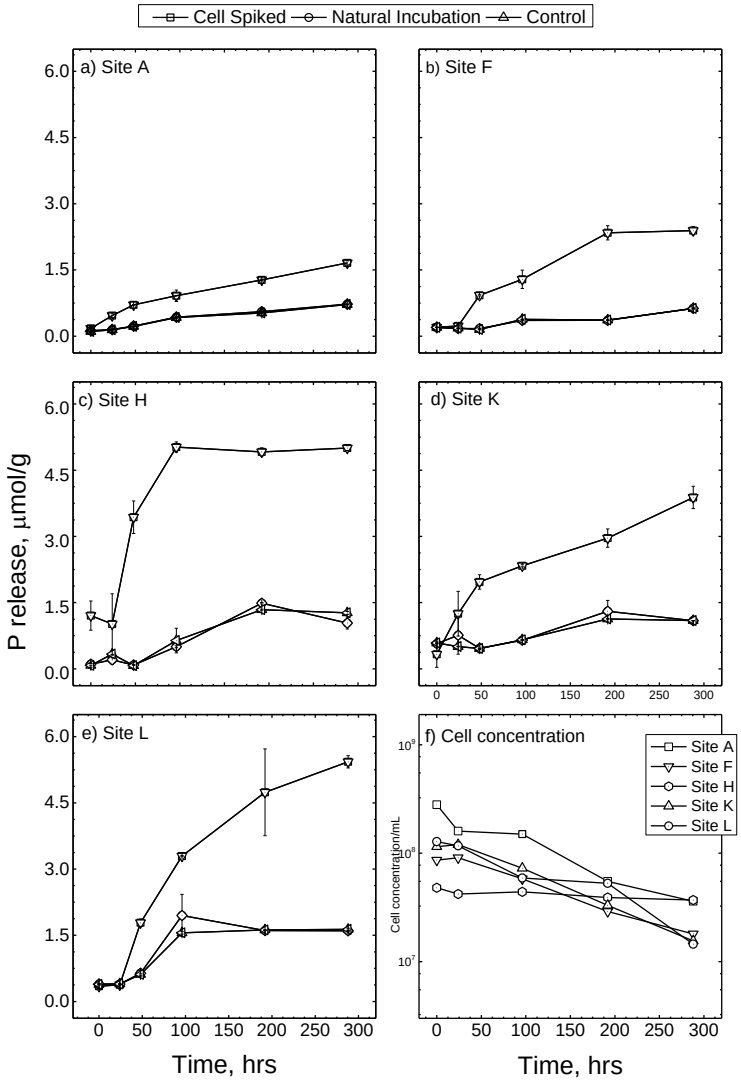


Figure 15 Role of biological activity on Pi release from a) site A, b) site F, c) site H, d) site K, and e) site L. Figure f) shows the changes in cell concentration in the spiked cell experiment. No growing cells were detected in the natural incubation. Sediments and water samples for control experiments were sterilized by autoclaving.

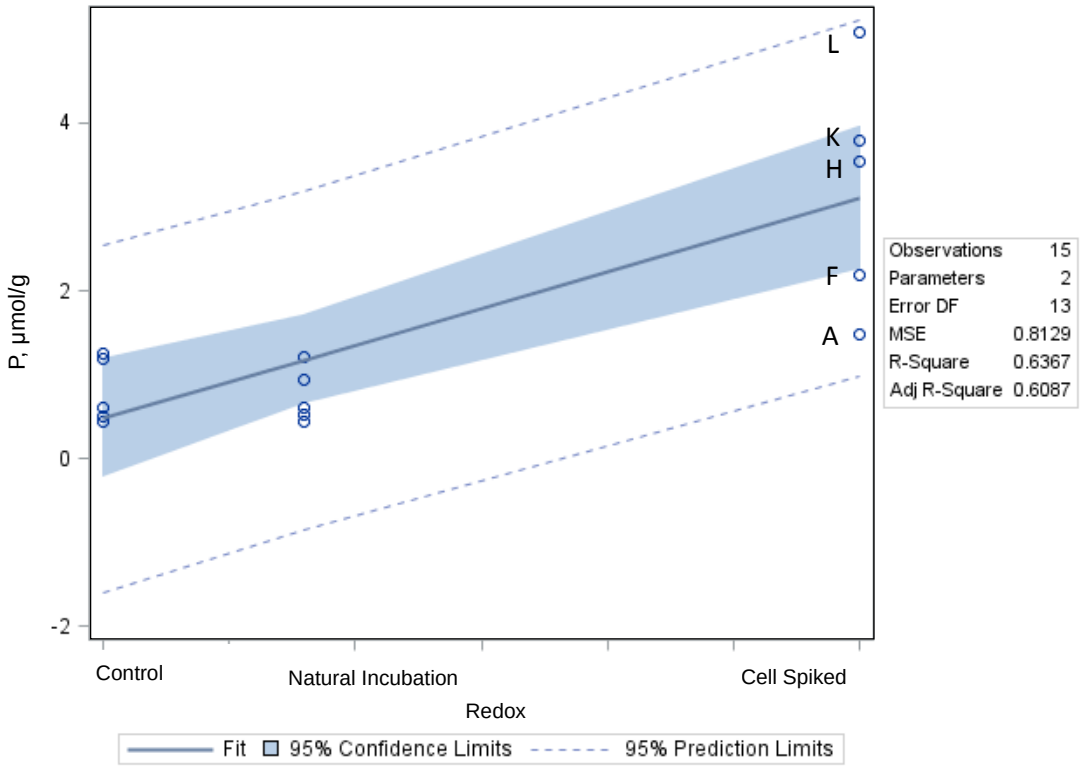


Figure 16 Regression of Pi release due to the enhanced biological activity obtained from 288 hrs incubation (data in Figure 15).

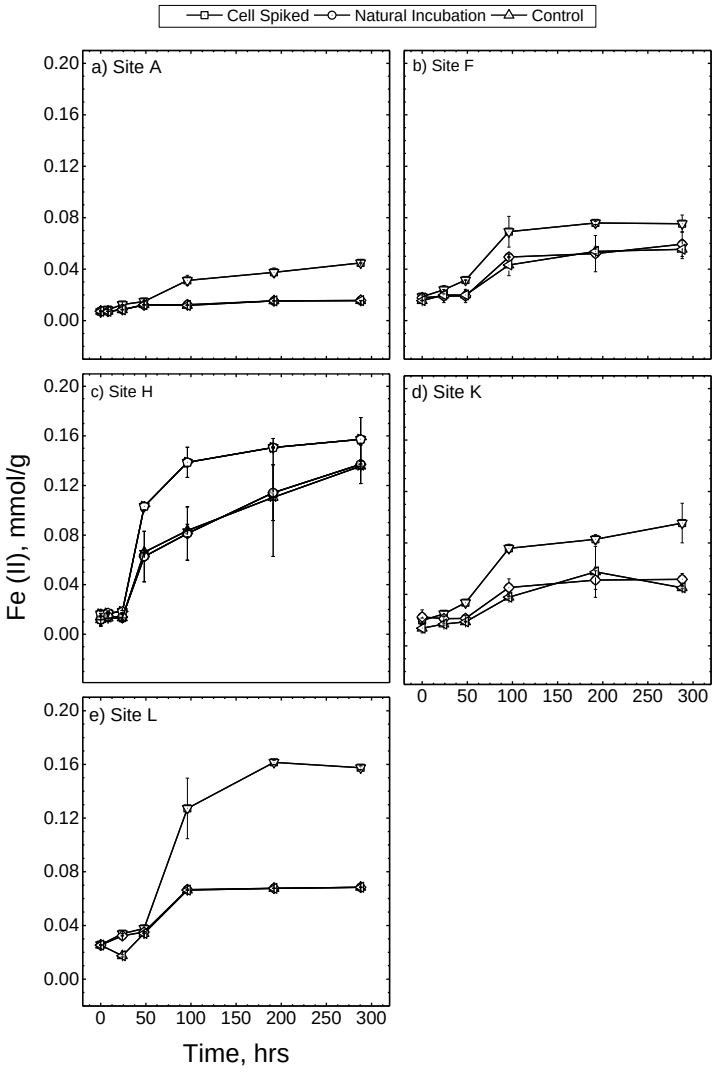


Figure 17 Effect of enhanced biological activity on Fe(II) release at a) site A, b) site F, c) site H, d) site K, and e) site L.

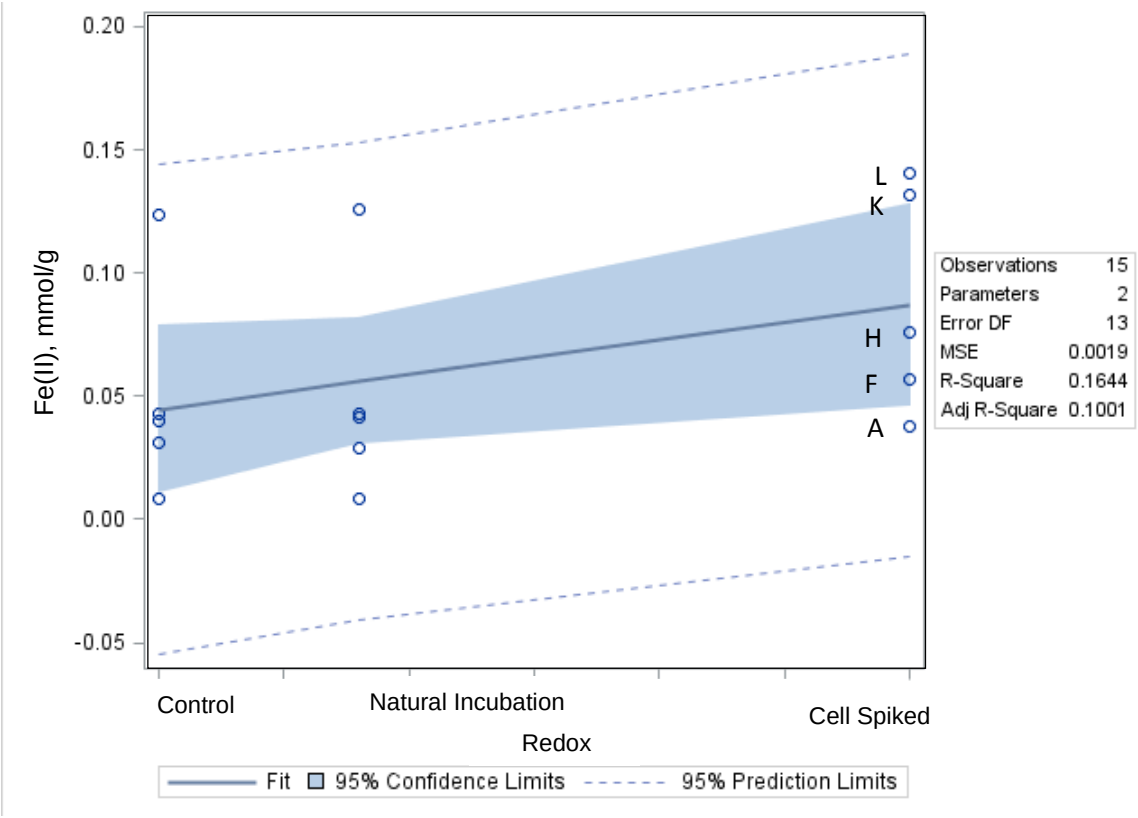


Figure 18 Regression plot of Fe(II) produced from enhanced biological activity.

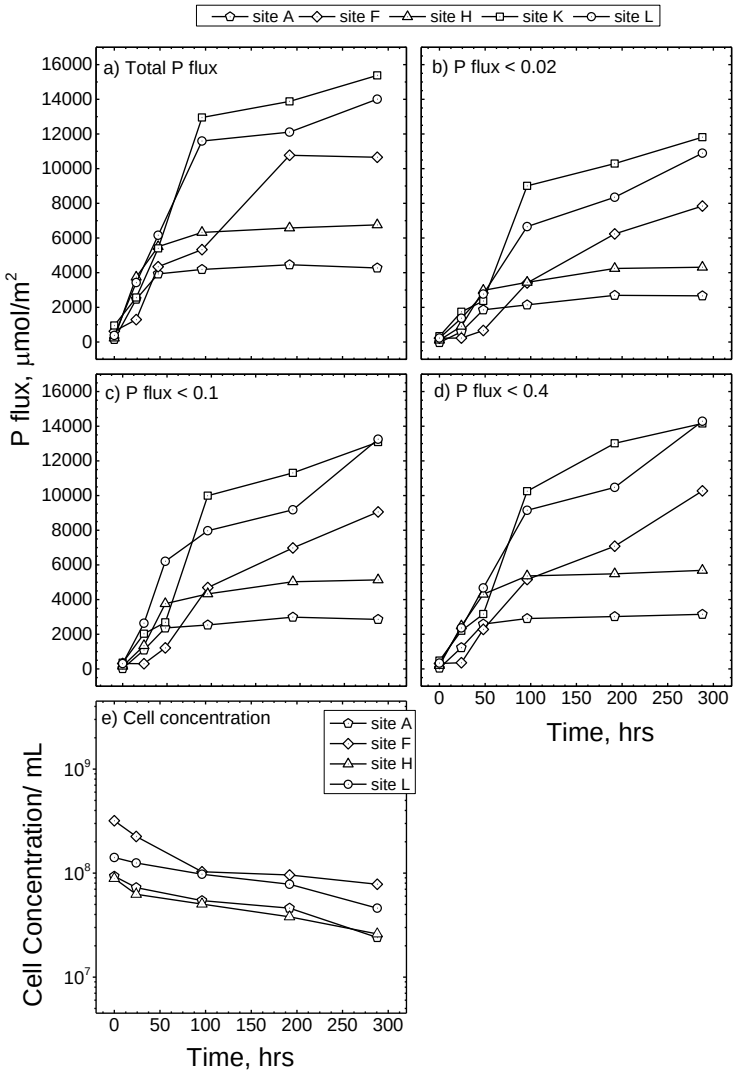


Figure 19 Total (a) and size fractioned (b-d) Pi flux at the sediment-water interface and changes in cell concentration during the flux experiment (e).

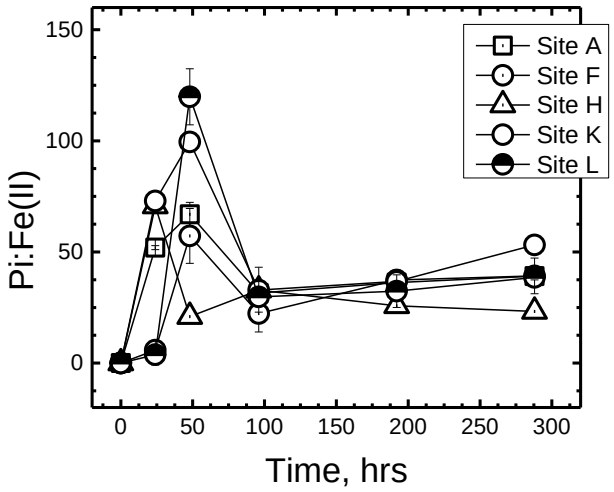


Figure 20 Ratio of Pi:Fe(II) in *Shewanella putrefaciens* CN32 spiked conditions.

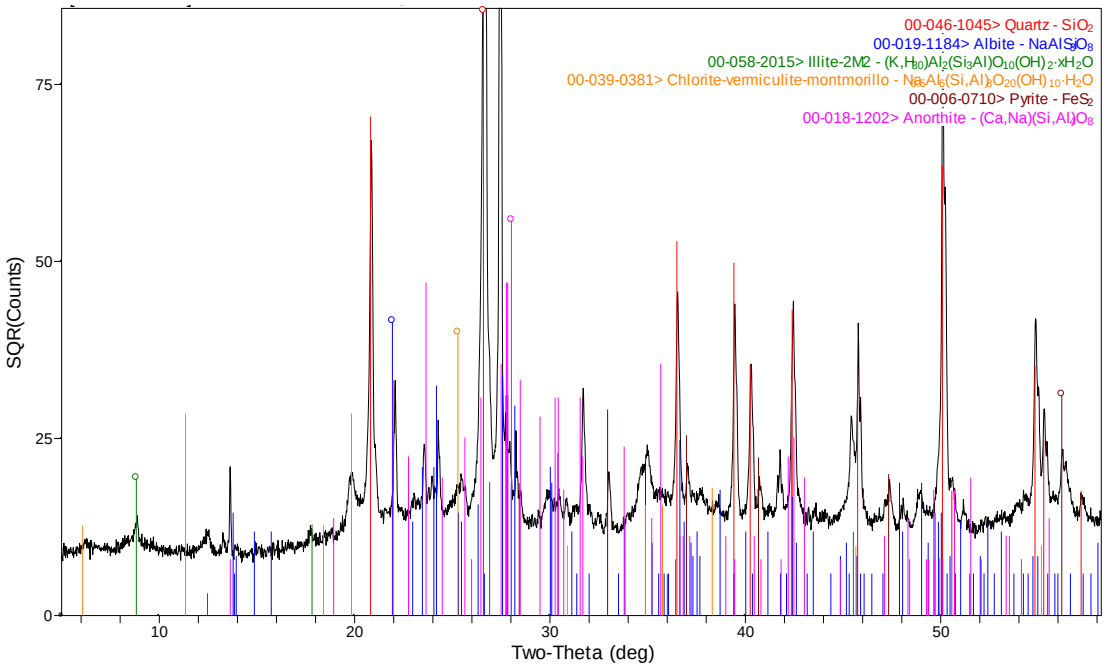


Figure 21a Mineral composition in site A after incubation with *S. putrefaciens* CN32. Changes in mineralogy after incubation were insignificant (compared with figure 6a).

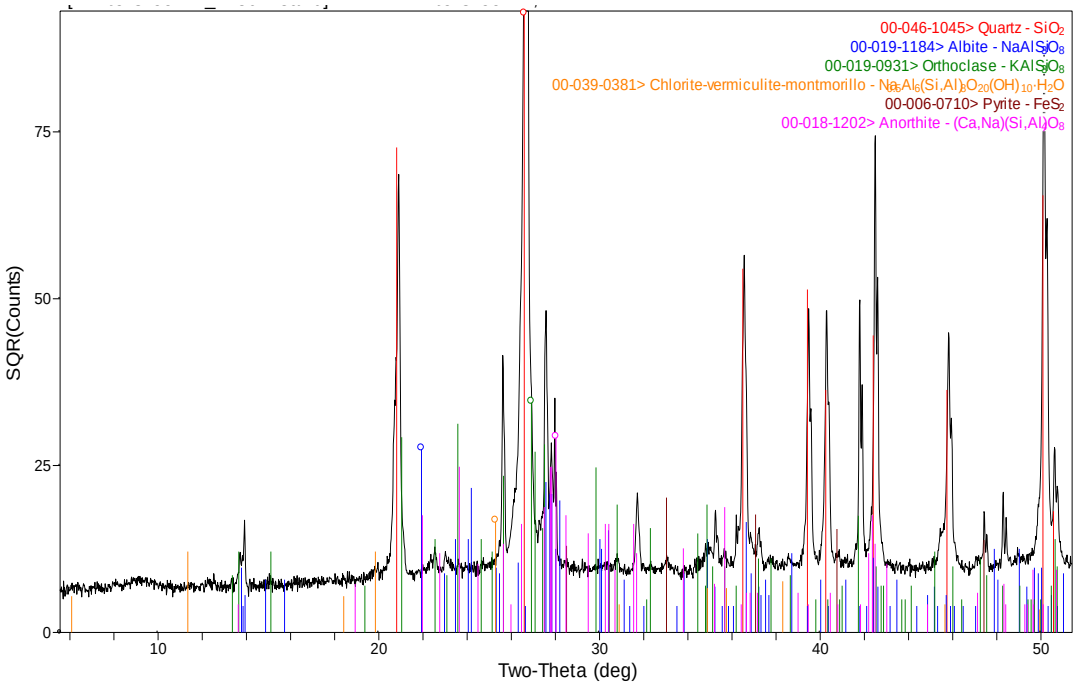


Figure 21b Mineral composition in site H after incubation with *S. putrefaciens* CN32. Changes in mineralogy after incubation were insignificant (compared with figure 6b).

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