PHOSPHORUS TRANSPORT IN THE BRONX RIVER: QUALITATIVE AND QUANTITATIVE ANALYSIS

By

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A dissertation submitted to the Graduate Faculty in Earth and Environmental Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

2011

This manuscript has been read and accepted for the Graduate Faculty in Earth and Environmental Sciences in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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Abstract

PHOSPHORUS TRANSPORT IN THE BRONX RIVER: QUALITATIVE AND QUANTITATIVE ANALYSIS

By
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Phosphorus (P) is the primary limiting nutrient for algal growth in freshwater systems. Excessive P from external inputs and release from sediments could accelerate primary productivity leading to eutrophication in the water column, and consequently degrading water quality. The objectives of this study were to predict P bioavailability and estimate spatial and temporal variations in P transport in the Bronx River, New York, USA. The Bronx River originates from the Westchester Davis Brook and Kensico Dam, flowing south through Westchester County (WC) and Bronx to the estuary area where it joins the East River. The total length is about 20 miles. There are more than 100 stormwater and other discharges that flow to the river along the entire length from Westchester to the East River. The upper part of the river is freshwater, while the lower is saline. The overall goal of this research is to provide data that can help develop policies to control P runoff to the river, including regulation of P inputs from lawn fertilizers. It is hoped this data can be shared meaningfully among the United States Environmental Protection Agency (USEPA), Department of Environmental Protection (DEP), Department of Environmental Conservation (DEC), Bronx River Alliance to make the river meet the fishable/swimmable goal of the Clean Water Act.

The ^{31}P-NMR spectra showed that the dominant P species in Bronx River bed sediments were orthophosphate monoester, and lesser phosphate diesters and pyrophosphates (pyro-P). The P compounds were mostly glycerophosphate (GlyP), nucleoside monophosphates

(NMP), and polynucleotides (PolyN). A few sites showed a small amount of dihydroxyacetone phosphate (DHAP), inosine monophosphate (IMP), and pyrophosphates (pyro-P). The P sorption capacity of the bed sediments of the Bronx River was very high, and the maximum values of P sorption maximum (S_{max}) was 476 mg kg^{-1}, equilibrium P concentration (EPC_0) was 0.73 mg L^{-1}, and originally sorbed P (S_0) was 65.6 mg kg^{-1}. Sediments could potentially release P into the water column as the soluble reactive P (SRP) in the water column drops below EPC_0 under changing hydro-climatic conditions such as the changes in pH, redox etc. Correlation analysis showed that S_{max} was correlated with poorly crystalline and amorphous Fe, Al, acid-extractable Ca and Mg, and organic matter suggesting their influence on P sorption capacity of the sediments. Similarly, the bed sediments contained various P pools, and rank order was: HCl-P > NaOH-P > $NaHCO_3$-P > residueP, and the relative proportion of 3.7: 2.0: 1.4: 1 in 2006 sediment; HCl-P > NaOH-P > residue-P > $NaHCO_3$-P, with their relative proportion of 27.8: 6.2: 2.7: 1 in sediment collected in 2007. The sediments P mineralization studies that were conducted under flooding conditions for 0, 7, 15, 30 d showed changes in the size of the P pools, indicating variations in microbial activities during incubation period. Moreover, enzyme incubation studies showed that phosphodiesterase (PDEase) hydrolyzed up to 82% of OP. Native phosphatases (NPase) hydrolyzed substantial amount of OP (up to 76%) when incubated at 37ºC, indicating that OP could be hydrolyzed under favorable temperature, and there is a potential threat to river ecosystems if global rise in temperature continues. Water samples from the river showed that the SRP concentrations were higher than EPA standard of 15 µg L^{-1} (SRP_{max}=221 µg L^{-1}, SRP_{ave}=68 µg L^{-1}), and TP concentrations were in a substantial

amount (TP_{max}=1,113 µg L^{-1}, TP_{ave}=438 µg L^{-1}) compared with other rivers such as the Garonne River in southern France. The hydrolyzed OP by NPase was up to 100% when incubated at 37ºC indicating potential threat to water quality under changing hydro-climatic conditions. It is indicative that anthropogenic inputs such as the P fertilizer runoff from garden and golf course, sewer overflows from Hunts Point WWTP, raw sewer discharge along the river (e.g., from Yonkers), CSOs, land use changes, as well as hydro-climatic changes may cause the spatial and temporal variations on P transport in the Bronx River.

Acknowledgement

I give my deepest gratitude to my Ph.D. dissertation advisor, Professor Hari K. Pant, who introduced me to the field of phosphorus transport in freshwater systems, and whose guidance, encouragement, and support propelled me through this achievement. I would like to thank my committee, Professor Frank Buonaiuto and Professor Gary Hemming for their suggestions, advice and time on how to accomplish this research.

This dissertation project was funded by the Academic Affairs of the City University of New York (CUNY), and Professional Staff Congress-the City University of New York. I acknowledge Dr. Joseph Rachlin of Department of Biological Sciences, Lehman College, CUNY for his help on sampling in the Bronx River and sites selection. I thank Dr. Edward Kennelly and Ph.D. student James Lyles of Phytochemistry Laboratory, Department of Biological Sciences, Lehman College, CUNY for their help on NMR analysis. I also appreciate the suggestions provided by Dr. Michael Blumenstein, the director of NMR Lab, Chemistry Department, Hunter College, CUNY on NMR parameters.

I acknowledge help from Dr. Richard Shaw, USDA-NRCS NYC Soil Survey, USDA. I am grateful to scientists Margaret Falk and Jessica Arcate, Horticulture Department, New York Botanical Garden (NYBG), for their help in sample collection at site 10 inside NYBG. I acknowledge the invaluable help from Dr. Lex Van Geen and Dr. Zhongqi Cheng, as well as Dr. Gary Hemming of School of Earth and Environmental Science, Queens College in analyzing metal ions with ICP-MS at Lamont-Doherty Earth Observatory Core Lab in Palisades, New York. I appreciate my classmate Ph.D. student, Andrew Maroko's help on GIS mapping for Bronx River study area. I appreciate the

tireless support on interlibrary loan journal articles and books from Lehman College librarian Eugene Laper.

I thank Dr. Neil Phillips for the teaching opportunity from Bronx Community College Chemistry Department environmental science divisions. Also, thanks for the teaching opportunities from Hunter College- Geography Department, Lehman College- Environmental Geographical and Geological Sciences Department, Queens College- School of Earth and Environmental Science, and York College- Earth and Physical Science Department. I appreciate the opportunity working as research assistant at Dr. Pant's Environmental Research Laboratory in Lehman College-CUNY.

I thank for CUNY Graduate Center (GC) Earth and Environmental Sciences (EES) Ph.D. program Executive Officer, Dr. Yehuda Klein's encouragement and fellowship support during my Ph.D. study. I also appreciate assistant program officer Ms. Lina McClain's help and support.

Most importantly, I give the utmost thanks to my best friend Glen Harrison, Ph.D., geography, a great geographer working at Oak Ridge National Laboratory National Transportation Research Center, Knoxville, TN, for the greatest friendship, for the essential support and sponsor on my Ph.D. study. My Ph.D. dissertation is dedicated to my friend Glen Harrison.

Thanks for the encouragement, support, field trips and love from my best friend Peter Lowenstein during my Ph.D. study, (both of us got M.A. from East Carolina University Geography Department in 2002); Peter is a city planner working for City Development, Las Vegas, NV. Also, thanks to my swimming coach Peter Kiernan, Lehman College

Apex aquatics manager for his athletic training on swimming and 2009 ING NYC 26.2 mi marathon, giving me so much energy on phosphorus research.

Dedication

I dedicate this dissertation to my friend Glen Harrison, whose essential support and sponsor have provided me with the courage and strength to achieve this intellectual milestone.

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Chapter 1 General Introduction

1.1 Background

1.1.1 Phosphorus

Phosphorus (P) is an essential element for plant growth, and plants mainly take up inorganic phosphorus, thus organic phosphorus has to be hydrolyzed to an inorganic P to be bioavailable (Stevenson, 1986; Pant and Warman, 2000; He et al, 2004; Hunt et al., 2005; Adam et al., 2007). Moreover, P is often the primary limiting nutrient controlling algal blooms in aquatic and semi-aquatic systems (House et al, 1995a; Pant and Reddy, 2001a; Pant et al., 2002b; Barlow et al, 2004; Lake et al., 2007). The P bioavailability is the criteria for assessing the eutrophication potential in rivers and streams (Maynard et al., 2009). Some studies have been conducted to assess the qualitative and quantitative losses of P to surface and ground waters from agricultural fields. However, P transport from urban/suburban catchments to aquatic systems is not well understood.

Nutrient loadings have resulted in alternation of plant and microbial communities, increased productivity, and nutrient accumulation in many freshwater and coastal water bodies. Several physical, chemical, and biological transformations in sediments could result in breakdown of plant detritus and sediment organic matter, consequently exporting dissolved organic P (DOP) and particulate organic P (POP) to surface water through runoff, and DOP to groundwater via limited leaching. The rates and duration of organic matter accumulations are critical determinants of how P cycle functions within an ecological unit of catchments. It is, therefore, important to conduct in-depth study on P transport within an urban water body to help improve the water quality in urban area. Currently there are no clear answers for better estimations of bioavailability of P compounds that are found in sediments or freshwaters.

Enzymatic hydrolysis and ^{31}Phosphorus Nuclear Magnetic Resonance Spectroscopy (^{31}P NMR) have been used to predict bioavailabilty of P compounds with some success. The ^{31}P NMR is a less destructive method, i.e., it does not alter much of P compounds, thus helps to obtain, especially relative compositions of various P compounds, while enzymatic hydrolysis together with mineralization studies provide overall potential bioavailability of organic P. The goal of this proposed research is to conduct ^{31}P NMR, enzyme hydrolysis, and P mineralization studies, and correlate data from these different experimentations to derive best possible way to estimate P bioavailability in the sediment and surface water of the Bronx River. Successful completion of this proposed project will profoundly aid on the formulation of sediment and water quality management strategies for the Bronx River, as well as the rivers of the comparable region of the world. Moreover, this study may contribute to devise strategies that would increase resilience of such ecosystem from possible detrimental effects of hydro-climatic changes might be brought upon by global warming, e.g., global rise in temperature, changes in pH, ionic strength, redox conditions, etc.

Eutrophication is a major problem in the Bronx River of New York City, NY, which constitutes both freshwater and estuarine water systems. Algal blooms and oxygen depletion within the river have degraded water quality, endangered fishing, and limited recreation use. The Bronx River (~20 miles long) originates from Davis Brook and Kensico Dam in Westchester County, NY, runs through the Bronx Borough, and flows into the East River Estuary near Hunts Point, Bronx, NY. Bronx River water has been classified by the State of New York for use as primary and secondary contact recreation and fishing. However, water degradation is endangering fish habitat and water quality, which may compromise public health, as pollutants are bio-accumulated through consumption of fish, and swimming in the river. The studies of forms and bioavailability

of organic P, and factors regulating them in the Bronx River will not only provide most needed knowledge to ensure continuous acceptable water quality in a river that nourishes many aspects of New York City, the Financial Capital of the World, but also help assess the critical role urban land-use plays in water quality management.

1.1.2 Phosphorus in the Environment

Phosphorus is an essential element for plant growth; plants only take up inorganic P; thus, organic P has to be hydrolyzed to inorganic P prior to be taken up (He et al, 2004; Pant and Warman, 2000; Pant et al, 1994a, 1994b). Phosphorus is a primary limiting nutrient in freshwater and can also be in saline water bodies (House et al, 1995b; Pant and Reddy, 2001b; Pant et al, 2002a; Barlow et al, 2004; Banaszuk and Wysocka-Czubaszek, 2005).

The general P cycle (Fig.1-1) between soils, plants, and microorganisms include the processes of uptaking of soil P by plants, recycling through return of plant and animal residues, biological turnover through mineralization-immobilization, fixation reactions at clay and oxide surfaces, and solubilization of mineral phosphates through the activities of microorganisms. Nearly all the P consumed by plants is returned to the soil in plant and animal residues, and P losses of soil occur by leaching and erosion (Stevenson, 1986). Phosphorus reserves are mostly in the ocean sediments ($840{,}000*10^{12}$ kg), terrestrial soils (96 to $160*10^{12}$ kg), dissolved inorganic P in the ocean ($80*10^{12}$ kg), mineable rock (such as apatite, of $19*10^{12}$kg) and biomass ($2.7*10^{12}$kg; terrestrial biota is $2.6*10^{12}$ kg, and marine biota of $0.050\text{-}0.12*10^{12}$ kg) (Stevenson, 1986). An overview of P cycling on a global cycle (Fig. 1) shows that P reached to the ocean and deposited as ocean sediment and removed from the cycle, unless there is geologic uplift and P could come back to the land again; human beings activity such as mining of P, P fertilizer application, release

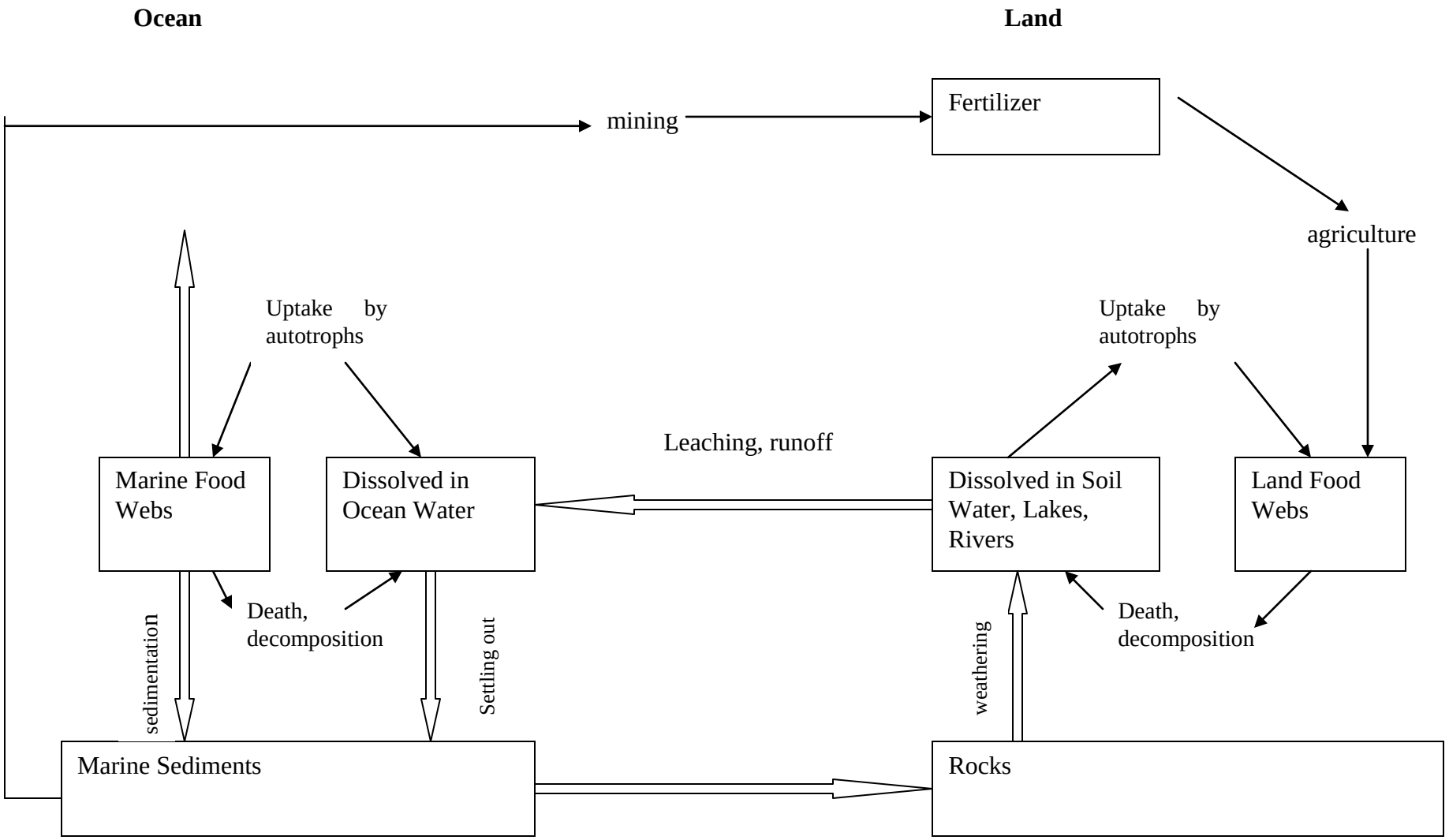


Figure 1-1 Phosphorus cycle (adapted from Miller, 2005)

of P to the environment by industrial and domestic effluents, intervened in the P cycle (Stevenson and Cole, 1999).

Anthropogenic factors result in eutrophication, consequently to algal bloom and oxygen depletion (Adam et al., 2007), as well as they degrade water quality in freshwater systems, such as rivers, lakes, wetlands, and saline water systems, e.g., estuaries, that are harmful to people's health from being used as drinking water or swimming or fishing (Pant et al., 2004; Iqbal et al., 2006).

1.1.3 Phosphorus transport in waters and sediments

Phosphorus transport in rivers affects ecology on marine and freshwater algae (House et al., 1995b). Sediment and nutrient loads in a river are important indicators of water pollution. Nutrients such as nitrogen (N) and P runoff from agricultural field to surface waters accelerate eutrophication process, resulting eutrophic algal blooms (Quilbe et al., 2006; Li et al., 2007). Phosphorus loss by subsurface drainage systems might be caused by P transport through macropores in loamy soils. Phosphorus loss in drainage water depends on groundwater level and soil type (Grant et al., 1996). High nutrient P resulted in high chlorophyll α concentrations, high turbidity, low dissolved oxygen concentration due to algae decomposition, bad smell from H_2S, and fish kills, degraded water quality, therefore, a reduction in P loading could reduce algal blooms, increased dissolved oxygen levels, eliminate the odors from the algal decomposition (Rodriguez et al., 2008).

Phosphorus transport in the river varies with season in the Narew Anastomosing River; NE Poland, P concentration changed during different season: P was retained within the river system during the high flows in winter and spring, whereas in summer P is released to water column and

sediment pore waters from river bed sediments because of the high temperature and low redox potential, and this internal loading provides additional bioavailable P to the downstream (Banaszuk and Wysocka-Czubaszek, 2005). Anthropogenic activities, such as nutrient loading, also lead to eutrophication in estuary and coastal waters worldwide (Neto et al., 2007; Arhondistisis et al., 2007; Pearl et al., 2003; Pearl et al., 2006). Nutrient loading in coastal waters accelerated estuarine and coastal eutrophication that are also affected by climatic changes on water such as tropical storms, hurricanes, flooding, droughts, wet periods, and El Nino vs. La Nina years (Pearl, et al., 2003; 2006). Hurricanes Dennis, Floyd and Irene in fall, 1999 caused a 100-500-yr flood in east coast of North Carolina, accumulated large amount of sediments that brought nutrient load and decreased the salinity. Human impacts precipitated phytoplankton's growth, causing pollution (Pearl, et al., 2006).

The interaction of P with suspended sediments and bed sediments determines the SRP concentration in the water column. Phosphorus and other nutrients, contaminants transport by fine sediments were related with sediment source (Walling, 2005). House et al (1995b) used outdoor and laboratory experiments methods to compare SRP interactions in both suspended and bed sediments (House et al, 1995b). High suspended sediments concentration indicates that they make an important contribution to the SRP transport (House et al, 1995b). Phosphorus in surface sediments increases in spring and summer, and decreases in the autumn and winter; this seasonal changes of P concentration had been studied at the river Wey in southern England. The bed-sediments either uptake or release P; this is associated with SRP (House and Denison, 1998). Dissolved inorganic P interacts with sediments and is involved in different precipitation/dissolution reactions, for instance, with calcium carbonate/phosphate and iron/aluminum oxide minerals (House and Denison, 1998).

Most study focused on suspended sediments associated with transport, and the research by House et al (1995b) is focused on the kinetics of the interaction of P with river bed sediments. The reducing condition is associated with the siderite in the sediment ($FeCO_3$). Phosphorus export from irrigated land can be uptake by the bed sediments in surface drains. The P solute was transported by advection and dispersion in the streams (Barlow et al., 2004). The landscape control P output from the stream during stormflow, and baseflow stream sediments control P output (McDowell and Sharpley, 2002). Ditch sediments and stream play important roles of the ecosystem in nutrient and contaminant concentrations in surface water. Sediments removal and deposition will change P transport in drainage ditches. During severe storms, sediment P interacts with water column P in drainage ditches within agricultural watersheds (Smith et al, 2006). However, the correlation between sediment/pollutants concentration and streamflow may not strong enough to make any solid conclusion (Quilbe et al., 2006). Usually sediment absorb P till EPC_0 is achieved, but this is not applied to biotic processes because microbal processes can uptake up to 43% of P (Smith et al., 2006). Dense vegetation could help remove orthophosphate associated with suspended sediments within stormwater (Deletic and Fletcher, 2006). Grass swales and filter strips help remove sediments from urban stormwater runoff (Deletic and Fletcher, 2006).

1.1.4 Phosphorus removal

Phosphorus could be removed by biotic and abiotic processes from the water column. The dissolution of Ca bound P, hydrolysis of Fe/Al bound P, and the mineralization of organic P processes could be enhanced by flooded soil conditions during initial period (Pant and Reddy, 2003). According to the research of Atchafalyaya River input and fluxes by Perez et al (2003),

the high velocity northerly winds with frontal passage could also increase the total P (TP) concentrations in the water column. Removal of nutrients has greater variability than removal of total suspended solids (TSS).

Phosphorus removal is crucial for maintaining water quality for drinking supply. A case in point, the New Croton reservoir in Westchester County, NY, provides about 10-12% of New York City water supply system; it faces serious phosphorus pollution reaching the level of 17.2 μg l^{-1} that is higher than EPA criterion value for phosphorus of maximum of 15 μg l^{-1}. In summer and fall, the reservoir suffered phosphorus-induced algae blooms and reduce dissolved oxygen in the bottom waters due to increased bacteria ingesting dead algae, resulting in poor water taste, odor and color. Catskill offer higher quality of waters, and all seven source water reservoirs from both Catskill/Delaware and Croton portion of the watershed serve as a source of unfiltered drinking water (Spitzer, 2006). Phosphorus promotes algae bloom, resulting in poor water taste, oxygen depletion, odor and color, increased heavy metals of iron and manganese, plus organic carbon in the chlorine-based disinfection of waters caused health problems (Spitzer, 2006). The high levels of P leads algae bloom, specifically blue-green algae or cyanobacteria; the water turns to green color, and after algae dead and sink to the bottom of the Croton reservoir's water column.

Aluminum sulfate (Alum), which had been widely used to treat municipal sewage before discharge and had been added to eutrophic lakes for P removal, was used to remove soluble reactive phosphorus from the Salton Sea, however the product floc, $Al(OH)_3$, an amorphous aluminum hydroxide solid, from the reaction of alum with water, resulted in P desorption and P become bioavailable, thus canceling out the benefit of alum on P removal (Rodriguez et al., 2008). Wetland restoration and construction have been used for water quality management, which were sinks of P and other pollutants from non-point pollution (Jordan et al., 2003).

Storm water treatment areas (STAs) can transport P to the water column until the systems reaches equilibrium (Pant and Reddy, 2003). The construction of wetland is effective or not for P removal is related to P flux potential of soils. Strom water treatment areas could be effective only EPC_0 is maintained at certain level. The internal loading of P could decrease the wetland effectiveness. Some restoration strategies were made from the study on the Silver Lake of Iowa including dredging the sediments, applying buffer stripes and reduced application of P (Iqbal et al., 2006). P removal can also be affected by carbon: the carbon sources are used for nitrification process and the organic matter could affect P removal negatively by blocking the adsorption sites and competing adsorption sites with phosphates (Vohla et al., 2007). Other removal methods such as chemical amendments, establishing vegetation communities or flushing could also help on P removal in soils (Pant and Reddy, 2003).

1.1.5 Phosphorus and global warming

As the hydro-climatic changes occur as a consequence of global warming, corresponding changes in pH and redox status and subsequent variations in mineralizations of organic P to inorganic P do occur.

The increasing temperatures affected the mineralization of nitrogen (N), phosphorus (P) and carbon (C); more carbon dioxide (CO_2) was released when higher nutrients including nitrogen N, P, C available under higher temperatures from increased consumption of soil carbon, and this contributed to the climate change, global warming (Rinnan et al., 2007).

Organic phosphorus in the sludge could result eutrophication if it was applied as fertilizer to agricultural field; the sewage sludge treatment by mineralization of OP to IP and the extraction of IP was helpful to reduce the artificial fertilizer application and recycle of nutrients from

sludge to farmland (Johansson et al., 2007). The adsorption of phosphate by calcite was increased with increasing temperature (Griffin & Jurinak, 1973).

1.2 Hypothesis, research questions and objectives

1.2.1 Hypothesis

This proposed research is going to assess the qualitative and quantitative transport of P to the Bronx River including the river's upper freshwater and lower estuarine sections. The specific research hypotheses of this project are as follows:

1. Different organic and inorganic P compounds flow to the Bronx River by anthropogenic factors including fertilizer application, combined overflows (CSOs), raw sewer discharge from upstream, sewer overflows from wastewater treatment in Hunts Point Waste Water Treatment Plant (WWTP), and stormwater runoff.
2. Differential hydrolysis and mineralization of various P compounds occur under different pH and temperature as affected by hydro-climatic changes.
3. Various P compounds transport to the river results in increased P bioavailability, in turn, algal blooms, oxygen depletion, and subsequent degradation of water quality in the river.
4. There are exchange of P between sediment and water column in the river though adsorption and desorption process

1.2.2 Research questions

Based on the above hypotheses, this project will answer the following specific research questions:

1. What types of P compounds are being transported by suspended sediments in the Bronx River, and what is the bioavailability of those P compounds?
2. What are the spatial and temporal variations of P transport along the transects of the Bronx River?
3. Does the P sorption phenomenon between sediments and the water column play a significant role in maintaining the bioavailable P pool?

1.2.3 Objectives

To answer above mentioned questions, the following specific tasks would be performed:

(1) Qualitative and quantitative analyses: Chemical extraction of sediments will be conducted to determine different P pools including labile, readily available, moderately labile, and non-labile P pools. Moreover, ^{31}P NMR will be used to identify and quantify various P compounds to better estimate P availability status.

(2) Similarly, soluble inorganic P enters solid or liquid phase through sorption/desorption processes. Thus, the sorption and desorption of P in and out of sediments/suspended sediments maintain equilibrium P concentration (EPC_0) in solutions, the P concentration at which neither adsorption nor desorption occurs. Thus, P sorption parameters will be determined to estimate the potential internal loading of P from sediments to the water column.

(3) Phosphodiesterases (PDEase) that hydrolyze phosphodiesters, are known to exist in surface waters and soil/sediments. Thus, this study will determine the potential native phosphatases (NPase) and PDEase-induced hydrolysis of organic P (OP) in the sediments.

(4) Moreover, microorganisms in water columns and soils/sediments may play varying roles such as release or uptake of P depending on temperature. Therefore, sediments would be incubated at 37ºC to determine the mineralization potentials of water and sediment P in the Bronx River.

(5) The physical-chemical characteristics, P pool and NPase hydrolysis of OP in water sample will be analyzed, in order to predict the inherit enzymatically hydrolysable P, its bioavailbility, and the potential threat on river water quality and ecosystems when temperature increases.

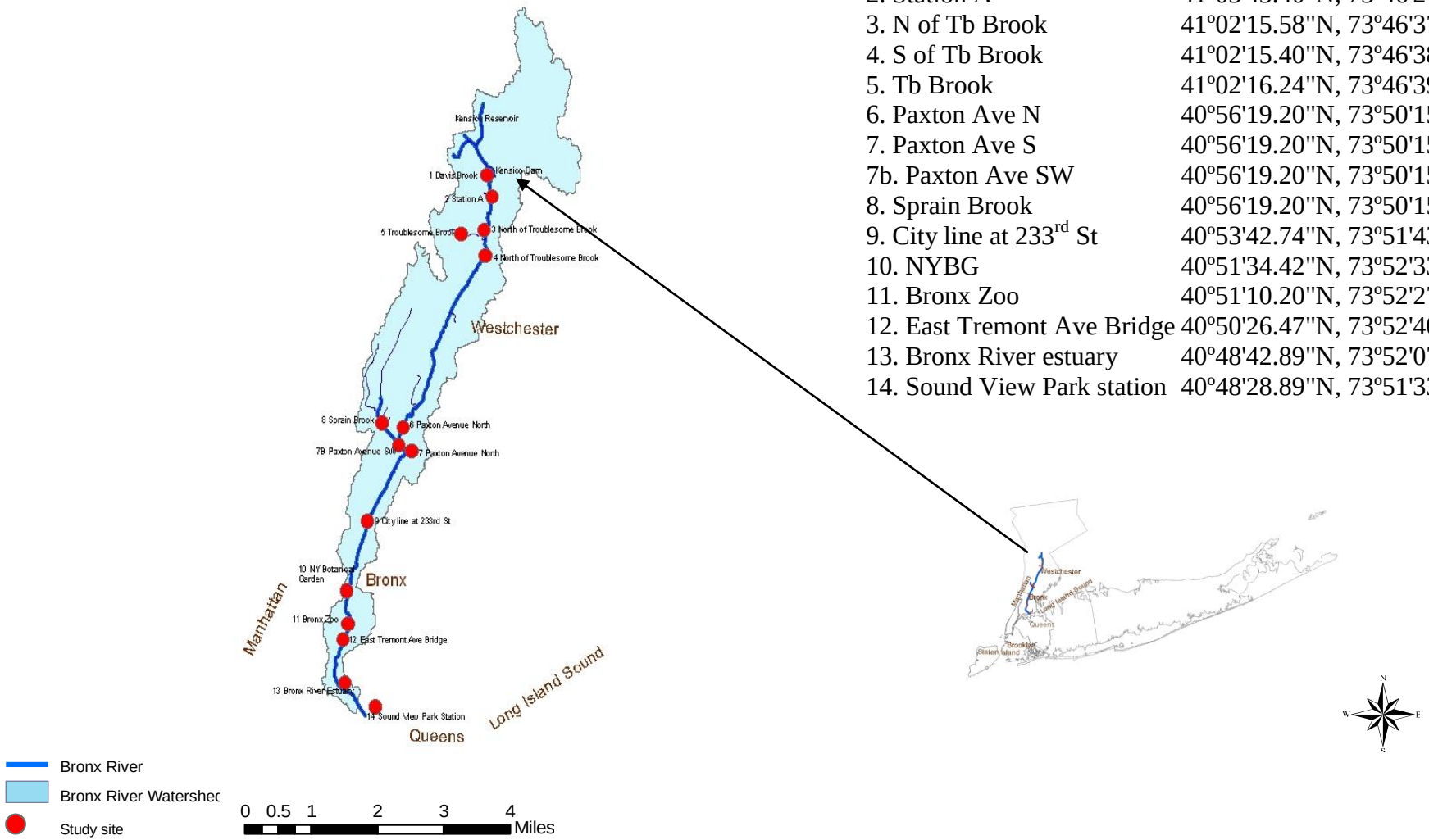
1.3 Study area and sampling sites

The Bronx River, originates from Westchester Davis Brook and Kensico Dam, flows though the Bronx, all the way to the East River estuary area (Fig. 1-2). The total length is about 26 miles (32 km). The Bronx River includes two watersheds: Westchester County watershed of 23,020 acres, and the Bronx watershed of 5,110 acres. The Bronx watershed drained to the Bronx River in NYC is altered to 4,163 acres currently. The sewers of 2,657 acres combined with the five combined sewer overflows (CSOs) discharge to the river in the saline reach. The Bronx River freshwater is used for primary and secondary contact recreation and finishing as the classification by the State of New York. There are more than 100 storm water and other discharges flow to the river along the entire length from Westchester to the East River. The Hunts Point WWTP services this area (Bronx River, 2001).

The river was divided into four portions while determining the sampling sites, which are geographically different: First, the Westchester portion from Davis Brook and Kensico Dam to the City line at Nereid Avenue; second, from the City line to East Gun Hill Road that is

dominated by residential and the Woodlawn Cemetery is on the west side of the Bronx River; third, from the Bronx Park to East 180th Street at East Tremont Avenue where the boundary of freshwater and saline water, including New York Botanical Garden and the Bronx Zoo, and the park area is around 700-acre including dense trees and other vegetations on both sides of the river; fourth, from 180th street to the estuary, where is a mix of parkland, industries and residential, the meat whole sale market and the Hunts Point WWTP were located at the estuary, facing Sound View Park (Bronx River, 2001), the New York City Fish Market was moved from the old location at Fulton Street to Hunts Point beside the meat market in November 2005. The first three sections, from the origin to East Tremont Avenue Bridge, are freshwater systems; while from East Tremont Avenue Bridge to the estuary is the saline water system.

The natural freshwater sources of the Bronx River are from Westchester County, and no freshwater sources to the river in New York City. The freshwater upstream discharge is 42.7 cubic feet per seconds (csf) measured at the USGS monitoring gage at Bronxville, NY, with a total drainage area of 26.5 square miles upstream of this gage (Bronx River, 2001). There are large forest areas, along with tidal marshes besides the upper Bronx River in the Bronx River valley, and many trout in the fresh water systems. There are different vegetations and migratory birds in the saline water systems (Bronx River, 2001). Water quality of the both fresh and saline water systems was not in compliance with the standards such as on dissolved oxygen and fecal coliforms level in the past, perhaps to date. The fresh water system of the Bronx River is from the City Line to the East Tremont Avenue Bridge, which is classified as Class B waters by New York State, and is used for primary and secondary fishing and contact recreation such as swimming. Both dissolved oxygen and coliform standards were not complied with the standards.



1. Davis Brook	41º04'23.63"N, 73º46'20.04"W
2. Station A	41º03'43.40"N, 73º46'24.80"W
3. N of Tb Brook	41º02'15.58"N, 73º46'37.99"W
4. S of Tb Brook	41º02'15.40"N, 73º46'38.39"W
5. Tb Brook	41º02'16.24"N, 73º46'39.58"W
6. Paxton Ave N	40º56'19.20"N, 73º50'15.11"W
7. Paxton Ave S	40º56'19.20"N, 73º50'15.11"W
7b. Paxton Ave SW	40º56'19.20"N, 73º50'15.11"W
8. Sprain Brook	40º56'19.20"N, 73º50'15.11"W
9. City line at 233rd St	40º53'42.74"N, 73º51'43.77"W
10. NYBG	40º51'34.42"N, 73º52'33.70"W
11. Bronx Zoo	40º51'10.20"N, 73º52'27.45"W
12. East Tremont Ave Bridge	40º50'26.47"N, 73º52'40.95"W
13. Bronx River estuary	40º48'42.89"N, 73º52'07.30"W
14. Sound View Park station	40º48'28.89"N, 73º51'33.67"W

Figure. 1-2 The Bronx River Study Area and 15 sampling sites along the river

The fecal coliform level was higher than the standard of 200MPN/100ml, and the dissolved oxygen was less than 4.0 mg/l (Bronx River, 2001). The freshwater quality was degraded by the storm water runoff from the Bronx River Parkway and surrounding areas, from upstream sources in Westchester County, and from the Wildlife Conservation Society's (WCS) Bronx Zoo (Bronx River, 2001). The saline water system from the East Tremont Avenue Bridge to the mouth at estuary was classified as Class I water that being used as secondary contact recreation and fishing. The coliform level is more than the standard of 2,000 MPN/100ml, and the dissolved oxygen was less than 4.0 mg/l; both were in non-compliance with the regulated standards. The saline water was also contaminated in the past by the combined sewer overflows (when the sewage water volume exceeded the sewage water treatment plant's holding volume), stormwater runoff, pollutants from Westchester County, and potentially from the East River (Bronx River, 2001). Recently, a New York State judge also ordered the City of Yonkers to pay more than $1 million in sewage fines for the discharge of raw sewage into the Bronx River (Gannon, 2006).

There were 15 bed sediment sampling sites and 14 water sampling sites (Fig.1-2) selected along the Bronx River from the origin at Davis Brook all the way to the estuary. These sites are representing the phosphorus transport in the Bronx River, from the freshwater to the saline zones. The 15 sites are: 1. Davis Brook, Valhalla, which is the headwaters of the Bronx River; 2. Station A, on Virginia Road; 3-5 Troublesome Brook merged into Bronx River includes (3. North of Troublesome Brook; 4. South of Troublesome Brook; 5. Troublesome Brook); 6-8 Sprain Brook merged into the Bronx River includes (6. Paxton Ave North, 7. Paxton Ave South, 7B. Paxton Ave Southwest (7 and 7B had the same water sample), 8.Sprain Brook); 9. 233rd Street City Line between Westchester and Bronx; 10. New York Botanical Garden (at old snuff mill); 11. Bronx Zoo (south of Mistubish waterfall, close to Gate B Bronxdale Parking lot); 12.

East Tremont Ave Bridge (East Tremont Ave and Boston Road, beside West Farm Market and Bronx Art Center, the boundary between fresh water and saline water); 13.Bronx River estuary (old water testing station at Sound View Park, facing meat market); 14.Sound View Park Station (1,000 yards south of site 13). The first 12 sites are in freshwater bodies, and sites 13 and 14 are in saline water estuary area. Site 1 Davis Brook is the headwaters of the Bronx River, the Sprain Brook and the Troublesome Brook are two major tributaries in Westchester County; the potential P sources could be fertilizer runoff from Botanical Garden and animal manures from the Bronx Zoo. The two estuary sites 13 and 14 are located in Sound View Park, facing the meat whole sale market, the fish whole sale market that was moved to this location from Fulton street at Lower Manhattan on Nov, 2005, and Hunts Point WWTP; the potential pollution from meat and fish market, the sewer overflows from Hunts Point WWSP, and the storm water run off were degraded water quality in the estuary area.

1.4 General methodology

1.4.1 Water and sediment sampling

Representative sediment samples were collected in the Bronx River from the origin at Davis Brook to the Sound View Park estuary at 15 sites (Fig. 1-2) in July/August 2006 and 2007. Each site was located with a Global Position System (GPS) unit, and the coordinates are provided (Table 1-1). A Core Sampler (diameter 8 cm; length 17 cm) was used to obtain the bed sediments. The sediment samples were sealed in gallon zipper bags. Water samples were also collected in the Bronx River from Davis Brook to estuary at 14 sites (not including 7B Paxton Ave Southwest because the water is the same as site 7 Paxton Ave South) using water bottles. Both the sediment and water samples were transported to Environmental Laboratory of

Table 1-1 Locations and geographic coordinates of sampling sites along the Bronx River, Bronx, NY

Site#	Location	Latitude(North)	Longitude(West)	Distance to Headwater (mi)
1	Davis Brook, Valhalla	41º04'23.63"N	73º46'20.04"W	0
2	Station A (Virginia Rd)	41º03'43.40"N	73º46'24.80"W	1
3	North of Troublesome Brook	41º02'15.58"N	73º46'37.99"W	8
4	South of Troublesome Brook	41º02'15.40"N	73º46'38.39"W	8
5	Troublesome Brook	41º02'16.24"N	73º46'39.58"W	8
6	Paxton Ave North	40º56'19.20"N	73º50'15.11"W	10
7	Paxton Ave South	40º56'19.20"N	73º50'15.11"W	10
7B	Paxton Ave Southwest	40º56'19.20"N	73º50'15.11"W	10
8	Sprain Brook	40º56'19.20"N	73º50'15.11"W	10
9	233rd St City Line (between Westchester and Bronx)	40º53'42.74"N	73º51'43.77"W	14
10	New York Botanical Garden (old snuff mill)	40º51'34.42"N	73º52'33.70"W	16
11	Bronx Zoo (south of Mitsubishi waterfall, north of Gate B, Bronxdale Parking lot)	40º51'10.20"N	73º52'27.45"W	17
12	East Tremont Ave Bridge (East Tremont Ave& Boston Rd)	40º50'26.47"N	73º52'40.95"W	18
13	Bronx River Estuary (old Sound View Park water testing station, facing meat and fish wholesale markets)	40º48'42.89"N	73º52'07.30"W	19
14	Sound View Park Station	40º48'28.89"N	73º51'33.67"W	20

Department of Environmental, Geographic and Geological Sciences at Lehman College of The City University of New York at the end of each sampling day, and stored at 4ºC in a Fisher Scientific Isotemp Laboratory Refrigerator until further experimentation. The sediment samples were immediately homogenized and saved in waterproof double-track zipper bags (10.2 x 15.2 cm; made by Fisher Scientific Co., USA), and stored at 4ºC until they were used for further analysis. A portion of each homogenized sediment sample was dried at 70°C for 72 h, thereafter, finely ground and used for selected physico-chemical analysis (House and Denison, 2002; Wang and Pant, 2009). The fresh water samples were analyzed of EC, pH, SRP and TP in 28days after sampling. The water samples were also put in 25 ml vials and frozen for future analysis.

1.4.2 Physico-chemical analysis of the water samples

1.4.2.1 EC and pH of water samples

Electrical Conductivity (EC) and pH combination electrodes were used to determine the EC and pH of the water samples. Put 25 ml water sample from each site in a 50 ml beaker, and then the pH and EC were measured.

1.4.2.2 Soluble reactive phosphorus in water samples

The water samples collected from the 14 study sites in the Bronx River were analyzed for SRP using automated ascorbic method (ESS Method 310.1; USEPA, 1992): the color reagents were combined with four stock solution A- sulfuric acid solution 4.9 N 50 ml, stock B-Ammonium molybdate solution 15 ml, stock C-Ascorbic acid solution 30 ml, and stock D-Antimony-tartrate solution 5 ml. The standard P solution was made of from potassium phosphate monobasic (KH_2PO_4) 100 mg l^{-1}, then diluted to several standards: 0, 10, 20, 30, 50, 80, 100, 200, 300, 500,

and 800 µg l^{-1}. The standard phosphorus solutions and the water samples each 4.2 ml was put in a small tube, then added the color reagent 0.8 ml into each tube for both water samples and P standards. After that, use Vertex mixture to mix each tube solution, left 20 min for color development. In automated ascorbic method, the ammonium molybdate and antimony potassium tartrate react in an acid medium with dilute solutions of orthophosphoate-phosphorus to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue-colored complex by ascorbic acid. The color is proportional to the P concentration (ESS Method 310.1; USEPA, 1992, Method 365.1; USEPA, 1983, and Method 365.4; USEPA, 2003). The water samples were analyzed for SRP by UV-2501PC UV-VIS Recording Spectrophotometer (Shimadzu Corporation) using ascorbic method at wavelength 880 nm (ESS Method 310.1; USEPA, 1992).

1.4.2.3 Total phosphorus in water samples by persulfate digestion

Aliquot 5ml of water sample was taken into a digestion tube and a scoop of (about 0.05 g) potassium persulfate-$K_2S_2O_8$ and 1 ml 11 N (or 5.5 M) H_2SO_4 was added to each tube (Fig. 1-3). Digested sample using digestion block at 160ºC for 2 h and the volume reduced to around 1-2 ml; then increased the temperature to 360ºC for 2 h. The standards were 0, 0.01, 0.02, 0.05, 0.07, 0.1, 0.2, mg l^{-1} P; took 5 ml standard; added 1 ml 11 N (5.5 M) H_2SO_4 plus a scoop of (about 0.05 g) $K_2S_2O_8$ to each tube, then digested together with the sample on the digestion block (Method 365.4; USEPA, 2003). After digestion, cool down the sample, and then added de-ionized distilled water diluting to 10 ml and analyzed at 880 nm using spectrophotometer with automatic ascorbic method as described previously. The color reagent was made by reducing the

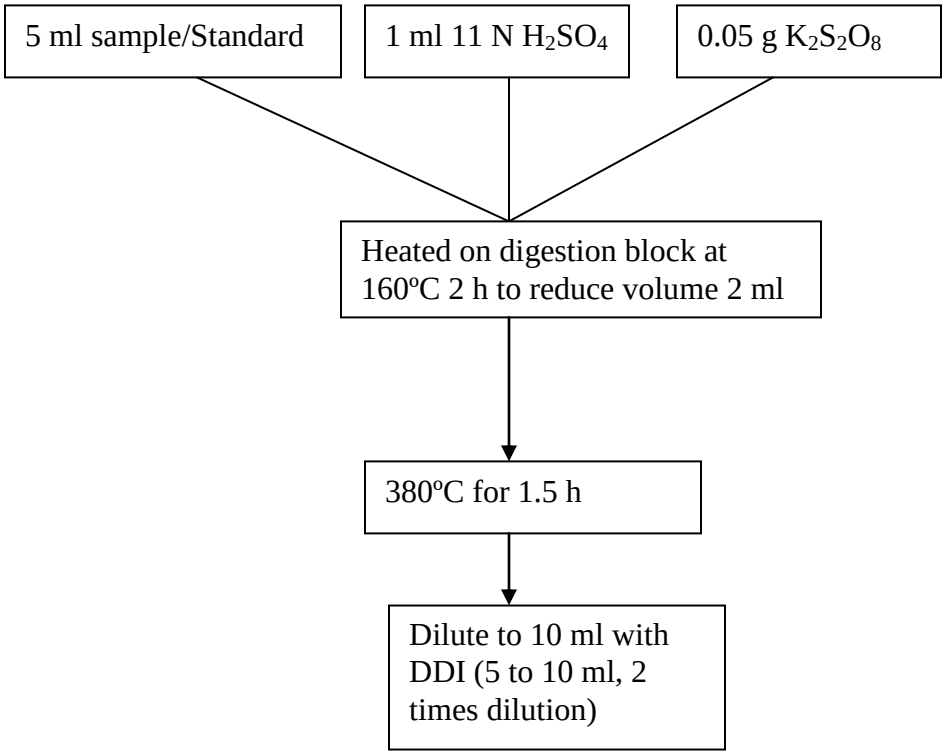


Figure 1-3 Schematic illustration of digestion of water samples for TP determination.

acid (4.9 N H_2SO_4) volume to half to increase the sensitivity of the color development of the digested standards and water samples.

1.4.3 Physico-chemical analysis of the bed sediments

1.4.3.1 EC and pH of sediments

Mettler Toledo InLab 730 Electrical Conductivity and pH combination electrodes were used to determine the EC and pH of sediments. 10 g sediment of each site was mixed well with 25 ml deionized distilled water (DDI H_2O) in a 50 ml centrifuge tube, shaking using a mechanical shaker at 230 l/m, 25 $\pm$ 2°C for 0.5 h, then put the mixed solution to a 50 ml beaker, and measured the pH and EC as described by Pant et al., 2002a.

1.4.3.2 Total phosphorus determination

Weighed 1 g finely-ground dry sediments into a 50-ml beaker, then the beakers were placed in a muffle furnace to combust the sediments at 550 ºC for 5 h (Andersen, 1976). Took the samples out after the furnace cooled down, and weighed the sample again, the weight loss was considered as total organic matter (OM). Thereafter, 20 ml 6 M HCl was added to the combusted samples (ash), and allowed the solution to evaporate slowly on a hotplate (Method 365.1;USEPA, 1983). When the residue was dry, the temperature on the Hot-plate was raised till the color turned to brown. Aliquot 2.25 ml 6 M HCl was added to the contents of the beakers once they were cooled. The solutions in the beakers were warmed up till the solution just started bubbling. Thereafter, the beakers were cool down, and the solutions were filtered into 100-ml volumetric flasks using Fisherbrand medium-fine filter paper with diameter of 12.5 cm, and the beaker were washed several times with DDI water. The sides of the filter papers were also rinsed several

times. The final volume of the each filtrate was made to 100 ml and shaken well. The filtrates were analyzed for SRP by UV-2501PC UV-VIS Recording Spectrophotometer (Shimadzu Corporation) using ascorbic method (ESS Method 310.1; USEPA, 1992).

1.4.3.3 Total organic matter determination

Took around 1g ground dry sediment into a 50-ml beaker, put in oven dry for 1 h, then weight and placed in a muffle furnace combustion at 550 ºC for 5 h (Andersen, 1976). Weighted the combusted sediment, the weight loss was considered as organic matter weight. The total organic matter (%) equals to the percentage of organic matter weight to total dry sediment weight (House and Denison, 1997).

1.4.3.4 Phosphorus fractionation

1.4.3.4.1 Sediment sequential extraction

The sequential extraction determines the P pool, which includes the readily available P, moderately available P and less available or stable P (Ivanoff et al., 1998). Sediment samples 5 g were extracted with 30 ml 0.5 M $NaHCO_3$ in an end-over-end shaker for 16 h. Sodium bicarbonate extracted the readily available P inorganic and organic P. The suspensions are centrifuged at 20ºC, 10,000 X *g* for 30 min. Decanted the liquid into the vials, and then weighted the $NaHCO_3$ residues. The supernatants were stored at 4°C for SRP and TP analysis. The residues were extracted with 30 ml 0.5 M NaOH in an end-over-end shaker for 16 h, and then the suspensions were centrifuged as described above. The supernatants were saved for TP analysis. The NaOH extracted moderately available P, mostly organic P. After that, DDI water was used to wash the sediments to remove NaOH prior to HCl extraction to avoid the acid neutralization.

Then the residues are extracted with 30 ml 1 M HCl for 3 h. Hydrochloric acid extracted less available or stable P; the remaining residues are mostly insoluble inorganic P forms such as apatite (Ivanoff et al., 1998). Thereafter, the extracts were centrifuged and decanted as described above, and the supernatants were saved for TP analysis. An automatic ascorbic acid method (ESS Method 310.1; USEPA, 1992) is used to determine the SRP and TP in the extracts as described for water samples. The difference between TP and SRP is considered as organic P (OP). Total P in the residues from the sequential extraction was determined by the modified ignition method by Anderson (1976) as described previously.

1.4.3.4.2 Total phosphorus determination for $NaHCO_3$ extraction

Total P in the supernatants was determined by persulfate digestion method (Method 365.4; USEPA, 2003).

Take 1 ml of the 0.5 M $NaHCO_3$ extraction supernatant into a digestion tube. Add a scoop about 0.05 g $K_2S_2O_8$, and 1 ml 11 N (or 5.5 M) H_2SO_4 to each tube. Digested sample using digestion block at 160ºC for one hour, and the volume reduced to around 1-2ml; then increased the temperature to 360ºC for 2 h. The standards were 0, 0.01, 0.02, 0.05, 0.07, 0.1, 0.2, 0.5, 0.7, 1, 2, 5 mg P l^{-1}.Took 5 ml standard; added 1 ml 0.5 M $NaHCO_3$ in order to keep the same matrix as the sample; then added 1 ml 11 N (5.5 M) H_2SO_4 plus a scoop about 0.05 g $K_2S_2O_8$ to each tube, then digested together with the sample on the digestion block. After digestion, cooled down the samples, and then added de-ionized water diluting to 10 ml and analyzed at 880 nm using spectrophotometer with automatic ascorbic method (the color reagent was made by reducing the volume of 4.9 N H_2SO_4 to half).

1.4.3.4.3 Total phosphorus determination for NaOH extraction

Total P in the filtrate was determined by persulfate method (Method 365.4; USEPA, 2003).

Took 0.25 ml of the NaOH extraction supernatant into a digestion tube. Added 5 ml DI water, a scroop about 0.05 g $K_2S_2O_8$, and 1 ml 11 N (or 5.5 M) H_2SO_4 to each tube. Digested sample using digestion block at 160ºC for one hour, and the volume reduced to around 1-2 ml; then increased the temperature to 360ºC for 2 h. The P standards were prepared in DI Water. The standards are 0, 0.01, 0.02, 0.05, 0.07, 0.1, 0.2, 0.5, 0.7, 1, 2, 5 P mg l^{-1}. Took 5 ml standard; added 0.25 ml 0.5 M NaOH in order to keep the same matrix as the sample, and then added 1ml 11 N (5.5 M) H_2SO_4 plus a scoop about 0.05 g potassium persulfate $K_2S_2O_8$ to each tube, then digested together with the sample on the digestion block. After digestion, cooled down the samples, and then added de-ionized water diluting to 10 ml and analyzed at 880 nm using spectrophotometer with automatic ascorbic method (the color reagent made reducing the volume of 4.9N H_2SO_4 to half).

1.4.4 Statistical analysis

All the experiments were carried out in triplicates and analyzed with SAS JMP software Version 7.0 (SAS Inc., 2008) using one-way analysis of variance (ANOVA). A Tukey's honestly significant difference (HSD) test was used for statistical differences at $p<0.05$ level. All the data were normally distributed ($p<0.01$) unless otherwise stated. Similarly, correlation coefficients were obtained using the Pearson's correlation (r) significant at the $p<0.05$ or $p<0.01$ level with SPSS Version.15.0 software (SPSS Inc., 2006). Regression analysis was performed using the SPSS 15.0 software, and JMP 7.0 fit model-standard least squares.

1.5 Significance of the study

This study estimates P bioavailability in the Bronx River, a fresh water system in New York City. The potential effects of local hydro-climatic changes on P availability in the river can be estimated. This may help to formulate water quality management strategies for the river, in turn, help to develop a model for nutrient transport in urban system. The results will be contributions to EPA, DEP, Bronx River Alliance for water quality monitoring and environmental policy making. Eutrophication is a major problem in the Bronx River. Algal blooms and oxygen depletion within the river have degraded water quality, endangered fishing, and limited recreation use. The Bronx River water has been classified by the State of New York for use as primary and secondary contact recreation and fishing, however, water degradation is endangering fish habitat and water quality, and compromising public health through consumption of fish, and swimming in the river. The studies of forms and bioavailability of organic P, and factors regulating them in the Bronx River will not only provide most needed knowledge to ensure continuous acceptable water quality in a river that nourishes many aspects of New York City, but also help to assess the critical role urban land-use plays in water quality management. As the goal of this proposed research is to conduct ^{31}P NMR, enzyme hydrolysis, and P mineralization studies, and correlate the data from these different experimentations to derive best possible way to estimate P bioavailability in the sediment and surface water of the Bronx River, successful completion of this proposed project will profoundly aid on the formulation of sediment and water quality management strategies for the Bronx River, as well as the rivers in the comparable part of the world. Moreover, this study may contribute to devise strategies that would increase resilience of such ecosystem from possible detrimental effects of

hydro-climatic changes might be brought upon by global warming, e.g., global rise in temperature, changes in pH, ionic strength, redox conditions, etc.

Chapter 2

Phosphorus Sorption Characteristics of the Bronx River Bed Sediments

Abstract

Phosphorus (P) is a major nutrient for plant growth, and it is often the primary limiting nutrient in freshwater ecosystems controlling algal blooms. The Bronx River of New York City, New York, United States includes freshwater and coastal water systems. The water quality of both fresh and saline water is lower than the standard levels designated by New York State, and classified as Class B and Class I waters, respectively. Algal blooms and oxygen depletion within the river have degraded the water quality, endangered fishing, and limited recreational use. The internal loading of P, an important bioavailability indicator in the Bronx River, is determined by the sorption processes and cycling of P between solid and liquid phases. The objectives of this study were to understand how P sorption characteristics affect the internal loading of P and the conditions that might give rise to a flux of P from sediment to water column, and to estimate the effects of physico-chemical properties of the sediments on P sorption parameters. Bed sediments were collected from 15 sites along the Bronx River, from the origin in Westchester Davis Brook, Kensico Dam through the Bronx to the Sound View Park estuary. Phosphorus sorption maximum (S_{max}) were significantly correlated with oxalate-extractable iron (Ox-Fe), aluminum (Ox-Al), acid-extractable calcium (HCl-Ca), magnesium (HCl-Mg), and total organic matter (OM), suggesting that not only metal ions affected P sorption characteristics, but OM also influenced the P sorption processes. This study also shows that originally sorbed P (S_0) was significantly correlated with Ox-Fe, Ox-Al, HCl-Mg, and OM. The extremely high values of the percentage of sorbed P

retained in sediments (>98% for all sites except the two estuary sites - site 13 of 88% and site 14 of 92%) suggest that a large flux of P to the water column from the sediments could potentially occur under changing hydro-climatic conditions, such as the changes in pH, ionic strength and redox conditions, which may in turn, creation of eutrophic conditions, and subsequent algal blooms.

2.1 Introduction

Plants can only take up inorganic P (IP). Organic P (OP) must be hydrolyzed to IP to be bioavailable (Stevenson, 1999; Hunt et al., 2005; Adam et al., 2007). Phosphorus is also a primary limiting nutrient for algal growth in freshwaters such as in river systems (Thomas, 1973; Carraz et al., 2008), and saline waters (House et al, 1995; Barlow et al., 2004; Lake et al., 2007), Anthropogenic factors contribute to eutrophication, leading to algae blooms and oxygen depletion (Quilbe et al., 2006; Adam et al., 2007; Li, et al., 2007), as well as degradation of water quality, and such degradation may be potentially harmful to human health, if the waters being used for drinking or recreation purposes (Huanxin et al., 1997; Iqbal et al., 2006). Anthropogenic inputs to rivers around urban areas, such as Washington, DC, are mostly derived from urban runoff, storm water, and industrial and domestic sewage, and such inputs are usually found in larger amounts than those generated from natural sources (Huanxin et al., 1997).

Soluble reactive P (SRP) strongly interacts with sediments, and is associated with Fe, Al oxides, and calcium carbonate/phosphate during the dissolution and precipitation processes (Fox, 1989). The IP and OP interact with sediments by adsorption, bonding with minerals and biological assimilation in cells, etc. (House and Denison, 2002).

Biological P uptake rates increased as OM increased in streams and rivers (Gregory, 1978), therefore, the rates and durations of OM accumulation in rivers sediments can be critical determinants of P transports in a river system.

The sorption and desorption of P from bed sediments and suspended sediments maintain water column equilibrium P concentration (EPC_0), the P concentration at which neither adsorption nor desorption occurs (Langmuir, 1997; Pant and Reddy, 2001). The EPC_0 affects biological processes, and the measurement of EPC_0 in sediments could indicate the direction of P flux (House and Denison, 1997). Thus, P sorption isotherms can be constructed to estimate the potential internal loading of P from sediments to the water column. Langmuir isotherms have been widely used for P sorption research (Nair et al., 1998). Phosphorus sorption can be affected by redox (Rhue and Harris, 1999), and the availability of amorphous and poorly crystalline Fe and Al. For instance, when Fe (III) is reduced to Fe (II) under anaerobic conditions, this releases the adsorbed P from sediments to the water column (Lake et al., 2007). The Al sorbed P can provide irreversible P sorptive capacity to sediments, whereas $Fe(OH)_3$ can be changed from Fe(III) to Fe (II) under reduced conditions, and subsequently release P (Lake et al., 2007). Thus, it is important to determine the P sorption characteristics of river bed sediments to gain insight on how the eutrophic conditions of the river change with the physico-chemical properties of both the bed sediments and the water column, as they are altered by anthropogenic influences and hydro-climatic changes.

The Bronx River watershed includes Westchester County (WC) and the Bronx in the New York City (NYC). The WC watershed is 23,020 acres, and in New York City, the topographical watershed is 5,110 acres. The Bronx River includes fresh and saline water.

The Bronx River from City Line (of the Bronx) to East Tremont Avenue Bridge is designated as Class B waters, and the lower Bronx River from East Tremont Avenue Bridge is classified as Class I waters, and they are used for secondary contact recreation and fishing regulated by New York State. Water quality of both fresh and saline water systems has a history of not meeting water quality standards, mainly as a result of excessive levels of dissolved oxygen and fecal coliform (Bronx River, 2001). Freshwater quality is degraded by stormwater runoff from the Bronx River Parkway and surrounding areas, upstream sources in WC, and the Wildlife Conservation Society (WCS) in the Bronx Zoo (Bronx River, 2001). The saline water was contaminated in the past by CSOs, sewer overflows (when sewage water volume exceeded the holding volume of sewage water treatment plants), as well as stormwater runoff, pollutants from WC, and pollutants from the East River (Bronx River, 2001). Recently, a New York State judge ordered the City of Yonkers, NY to pay more than $1 million in fines for the discharge of raw sewage into the Bronx River (Gannon, 2006).

The objectives of this study are to determine the P sorption characteristics of the bed sediments in the Bronx River, NYC, USA and to estimate the potential effects of cations Fe, Al, Mg, Ca, and OM on the P sorption capacity of the sediments. This in turn will give insights into transport and bioavailability of P in the Bronx River; estimate the P release from sediments to the water column and potential effect on water quality. It provides reference data for P application regulations, and the ecological restoration of the Bronx River.

2.2 Materials and methods

2.2.1 Physico-chemical analysis of the bed sediments

A Mettler Toledo InLab 730 conductivity electrode was used to determine the electrical conductivity (EC) and a Mettler Toledo InLab413 pH electrode was used to determine the pH of the sediments. Wet sediments (10 g) from each site were mixed well with 25 ml deionized distilled (DDI) water at a ratio of 1:2.5 in a 50 ml centrifuge tube, shaking using a mechanical shaker at 230 l/m, 25 $\pm$ 2°C for 0.5 h, then measured for EC and pH as described by Pant et al., 2002.

The wet sediments were sequentially extracted by 0.5M $NaHCO_3$, 0.5M NaOH, and 1M HCl (Ivanoff et al., 1998; Stone and English, 1993). The TP in the extracts from $NaHCO_3$ and NaOH was analyzed by persulfate digestion block method (Method 365.4; USEPA, 2003). The ascorbic acid method (ESS Method 310.1; USEPA, 1992) was used to determine the SRP and TP in the extracts. The difference between TP and SRP is considered as organic P (OP). The total OP equals to the sum of $NaHCO_3$-OP and NaOH-OP.

Total P was determined by the modified ignition method (Anderson, 1976), 1 g ground dried sediments were combusted at 550ºC in a muffle furnace for 5h and dissolved the ash in 6M HCl (Method 365.1; USEPA, 1983). The digested was analyzed for P using the ascorbic acid method (ESS Method 310.1; USEPA, 1992). The total organic matter (OM) was determined by ignition of finely ground dried sediments at 550°C for 5h (House and Denison, 1997).

To determine poorly crystalline Fe and Al, 0.5 g finely ground dry sediments were extracted with 30 ml 0.175M ammonium oxalate + 0.1M oxalic acid at a ratio of 1:60 and

by shaking for 4 h using an end-over-end mechanical shaker in the dark (as modified from Danen-Louwerse et al., 1993). The suspensions were then centrifuged at 10,000 *g* for 10 min and filtered through a Millipore 0.45-µm sterilized membrane filter in the dark. The filtrates were analyzed for oxalate-extractable Al, Fe, and oxalate-extractable P using an Axiom single collector high resolution magnetic sector (Thermo Elemental) inductively coupled argon plasma-mass spectrometer (ICP-MS).

Similarly, calcium Ca and Mg were determined by extracting 0.5 g finely-ground dry sediment with 30 ml of 1M HCl at a ratio of 1:60 and shaking on an end-over-end mechanical shaker for 4 h as described by Pant and Reddy (2001). The suspensions were then centrifuged at 10,000 *g* for 5 min and filtered through a 0.45µm membrane filter. The filtrates were analyzed for HCl-extractable Ca, Mg and P by ICP-MS.

2.2.2 Sorption experiments of phosphorus

To determine P sorption and desorption characteristics of the bed sediments under aerobic conditions, 5 g (wet) sediment was placed in a 50-ml centrifuge tube, and treated with 10 ml of solution containing 0, 0.1, 0.2, 0.5, 1, 2, 5, 10, 25, and 50, 100 mg P l^{-1} (using potassium phosphate monobasic, KH_2PO_4 as a source) prepared in 0.01 M potassium chloride (KCl) solution. The tubes were then shaken for a 24 h equilibration using an end-over-end mechanical shaker at 230 l/m**,** 25 $\pm$ 2°C. The equilibrated samples were centrifuged for 30 min at 20°C at 10,000 rpm, and filtered through a 0.45-µm membrane filter. The supernatants were acidified with a drop of concentrated H_2SO_4 and stored at 4°C for P absorption analysis. Soluble reactive P was measured in the supernatants using the automated ascorbic acid method, as described previously.

Phosphorus not recovered in solutions is considered as the amount adsorbed by the sediments. The sediment residues were weighted and equilibrated with 10 ml 0.01 M KCl solution using an end-over-end mechanical shaker for 24 h at 25 $\pm$ 2°C to continue the P desorption experiment. Thereafter, the equilibrated samples were centrifuged for 30 min at 10,000 rpm, and filtered through a 0.45-µm membrane filter. The filtrates were acidified with one drop of concentrated H_2SO_4, and analyzed for SRP, as described above.

2.2.3 Sorption parameter calculations

Phosphorus sorption energy (K_f), P sorption coefficient (K_d), S_0, S_{max} and EPC_0 were calculated using the Freundlich and Langmuir Isotherms (equations 1-5) and equations determined from batch experiments.

The modified Freundlich equation had been applied in this research:

$$S = K_f * C^n \quad (1)$$

$$LogS = n * LogC + LogK_f \quad (2)$$

S = P sorption total, mg kg^{-1}

$K_f = 10^{Log_{Kf}}$, P absorption energy, l kg^{-1}

n=a correction factor,

C=concentration of P after 24 h equilibration, mg l^{-1}

The modified Langmuir equation also had been used in this study:

$$\frac{C}{S} = \frac{1}{k * S_{max}} + \frac{C}{S_{max}} \quad (3)$$

$S=S'+S_0$, P sorption total, mg kg^{-1}

S'=P sorbed, the solid phase, mg kg^{-1}

S_0=originally sorbed P, on the solid phase, mg kg^{-1}

C=concentration of P after 24 h equilibration, mg l^{-1}

S_{max}=P sorption maximum, mg kg^{-1}

k, a constant related to the bonding strength, l mg^{-1}

$$EPC_0 = \frac{S_0}{K_d} \quad (4)$$

K_d=sorption coefficient, l kg^{-1}

S_0=initial or native sorbed P in sediment (mg kg^{-1})

EPC_0=an equilibrium P concentration (mg l^{-1}) is defined as the concentration of P in solution where no net sorption or desorption of P occurs, (Nair et al, 1998; Langmuir, 1997; Pant and Reddy, 2001)

The P retention was defined, and the ratio of P retention vs. P adsorption was also calculated:

$$P_r = P_{ad} - P_{de} \quad (5)$$

P_r=P retention, mg kg^{-1}

P_{ad}=P adsorbed in the sediment, mg kg^{-1}

P_{de}=P desorbed from the sediment to the solution, mg kg^{-1}

$\frac{P_r}{P_{ad}} * 100\%$ = the percentage of P retention to P adsorbed in the sediment, %

(Pant and Reddy, 2001).

2.2.4 Statistical analysis

Pearson's correlation coefficients (significant at $p<0.05$ or at $p<0.01$ level) were obtained using SPSS 15.0 software (SPSS Inc., 2006). Multiple linear regressions were performed by SPSS 15.0 at $p \leq 0.05$.

2.3 Results and discussion

2.3.1 Physico-chemical characteristics of the bed sediments at different geographical sections of the river

The EC of freshwater samples ranged from 66 μs cm^{-1} to 342 μs cm^{-1}. The two estuary sites had much higher EC at 1,917 and 4,290 μs cm^{-1} than the freshwater sites. There were no significant differences in sediment pH among the 15 sites, which ranged from 6.5 to 7.8, although the saline water sites 12, 13, and 14 had slightly higher pH than freshwater sites.

The OM ranged from 0.1 to 10.7%, and TP varied from 183 to 867 mg kg^{-1}. The OP varied from 51 to 480 mg kg^{-1}. The Ox-Al ranged from 128 mg kg^{-1} to 830 mg kg^{-1}. The Ox-Fe ranged from 1,752 mg kg^{-1} to 14,274 mg kg^{-1}. The oxalate-extractable P (Ox-P) was correlated with Ox-Al (r=0.836), which was stronger than that with Ox-Fe (r=0.644) indicating most of the Ox-P was associated Ox-Al. It is known that Al sorbed P is more stable than Fe-sorbed P because Al $(OH)_3$ is not affected by redox conditions, while $Fe(OH)_3$ could be changed from Fe(III) to Fe (II) under reduced conditions. Both HCl-Ca and HCl-Mg had the highest values on site 12, the boundary between fresh and saline water, followed by sites 13, 4, 5, and 3.

There are four geographical sections in the Bronx River from the origin at Davis Brook and Kensico Dam to the estuary. The first geographical section in WC includes two major tributaries that have the distinguishing characteristics. Site 4 at Troublesome Brook was a unique site among the freshwater sites; it had the highest TP, OP, Ox-P, OM, and Ox-Al among all sites. It also had high values of Ox-Fe, HCl-Ca, and HCl-Mg (Table 2-1). The strong correlation between Ox-Fe and Ox-Al (Table 2-3) indicated that Fe and Al

associated P were deposited in similar ways (Makris et al., 2005). Similarly, the strong correlation between HCl-Ca and HCl-Mg (Table 2-3) indicated that the Ca and Mg associated with P in the bed sediments were deposited in similar ways.

Total P had a significant positive correlation with OM ($P<0.001$). And, Ox-P also had significant positive correlation with OM ($P<0.001$) (Table 2-3), meaning that OM affected P sorption. Perhaps, OM associated amorphous and poorly crystalline Fe, and Al formed suitable conditions for P sorption (Pant and Reddy, 2001; Jordan et al., 2005). Geographically, P from the north upper river (site 3) and the Troublesome Brook (site 5) joined the Bronx River, flowing to the south, at site 4 (Figure 1-2), resulting in the P accumulation and deposition in the sediments of site 4.

Sprain Brook located south of Troublesome Brook, among the four sites (6, 7, 7b, and 8) at this tributary, site 7b had a much higher OM and OP than other sites (Table 2-1). A positive correlations ($p<0.01$) existed between OM and Ox-Fe, Ox-Al, and Ox-P (Table 3). Site 7b, located southwest of Sprain Brook, is closer to the tributary. The tributary flows from Sprain Brook to the Bronx River reaching 7b first, and then joins the Bronx River and reaches site 7. This might accumulate more P, resulting in higher TP at 7b than at sites 6, 7, and 8.

The second and third geographical regions of the Bronx River are from the City Line at Nereid Ave, site 9, 233rd Street to site 12, East Tremont Avenue Bridge, where saline

Table 2-1 Selected chemical properties of the Bronx River bed sediments (P sorption)

Site#	Location	Oxalate-extractable Al	Fe	P	Fe:Al	OM	HCl-extractable Ca	Mg	TP	OP	pH	EC
			mg kg^{-1}			%	mg kg^{-1}		mg kg^{-1}			($\mu s\ cm^{-1}$)
1	Davis Brook, Valhalla	188	2876	50	15.3	0.7	5314	2074	340	51	7.1	66
2	Station A	690	4880	141	7.1	3.3	2887	1495	430	191	6.8	298
3	North of Tb Brook	289	6122	88	21.2	1.9	5891	2293	460	143	6.7	133
4	South of Tb Brook	830	12015	361	14.5	10.7	11033	3896	867	480	6.5	342
5	Troublesome Brook	277	3039	60	11.0	0.1	10294	2654	183	57	7.4	220
6	Paxton Ave North	159	2251	67	14.2	0.1	3484	1256	319	44	7.5	158
7	Paxton Ave South	128	1752	44	13.7	0.3	1633	501	237	85	7.0	167
7B	Paxton Ave Southwest	282	4192	88	14.9	1.7	5053	2068	317	173	6.7	160
8	Sprain Brook	294	2532	95	8.6	1.0	5432	2075	231	52	7.0	103
9	233rd St City Line	215	2577	52	12.0	1.7	5796	2288	384	53	6.8	209
10	NY Botanical Garden	219	2460	73	11.2	3.7	3024	1161	258	131	7.0	108
11	Bronx Zoo	267	1812	64	6.8	3.2	3493	459	326	55	6.8	133
12	East Tremont Ave Bridge	416	14274	139	34.3	3.8	31464	5371	388	68	7.8	254
13	Bronx River estuary	463	12480	168	27.0	4.9	15440	4814	494	77	7.8	1917
14	Sound View Park Station	394	4783	262	12.2	3.6	5632	1874	862	270	7.8	4290

OM: total organic matter, %

TP: the total phosphorus in bed sediments from Anderson ignition method, mg kg^{-1}

EC: electrical conductivity, $\mu s\ cm^{-1}$

water meets freshwater. This section includes site 10 at the New York Botanical Garden (NYBG) and site 11 at the Bronx Zoo. From site 9 to site 12, there were minor changes in TP values. Sites 10 and 11 had relatively low TP values. Site 9 is located at the city line at 233rd Street, dominated by residential land use, thus, other than metal ions and OM, the occasional raw sewage discharge from Yonkers since 2004 might have added more P to this site (Gannon, 2006), resulting in higher TP than sites at NYBG and the Bronx Zoo. The Bronx Zoo, at site 11, did not have high Ox-Al, Ox-Fe, HCl-Ca, HCl-Mg, or OM values. Its TP was relatively lower than site 12, and higher than TP at site 10 at NYBG. This may indicate the animal waste from the Bronx Zoo as a possible P source. It has also been indicated as a source of P to the river previously (Bronx River, 2001). The OM and OP at site 10 were higher than at site 11, indicating that plants might uptake IP from the application of fertilizer in the garden, and convert IP to OP (Paul and Mayer, 2001). Site 12 had the highest Fe to Al mass ratio of 34.3 among the 15 sites, which indicated Ox-Fe associated P was dominant in the estuary boundary where the upper river freshwater and lower river saline water meet. Site 12 had much higher Ox-Al, Ox-Fe, HCl-Ca, and HCl-Mg values than sites 9, 10, and 11.

The fourth geographical segment includes site 13, the Bronx River estuary, and site 14, the Sound View Park Station, located at the Bronx River estuary in the saline water system. The Pr/Pad ratio (%) of site 13 (88%) was lower than site 14 (92%). This means that more P was retained at site 14, which resulted in a higher TP at site 14, even though site 13 had much higher Ox-Fe, Ox-Al, HCl-Ca, and HCl-Mg values than site 14. The Pr/Pad ratios for these two sites were much lower than the upper river sites, indicating the P retained by bed sediments in the estuary was less than the fresh water system. Site 14

had a much higher OP than site 13. Site 14 was located at the Sound View Park (Figure 1-2), the Bronx River estuary, where Bronx River joined the East River flowing to the Long Island Sound. The sediment depth at this station was very shallow; the bottom below the water was very rocky. However, the Hunts Point fish and meat wholesale markets and Hunts Point WWTP are on the other side of the river, geographically close to this site (around 0.5 mi). Both of these activities are potential P sources. Overall, the CSOs, the sewage overflow from Hunts Point WWTP (Paul and Meyer, 2001), and the pollutants potentially from the East River contributed to higher P in the estuary (Protopapas, 1999).

2.3.2. Phosphorus sorption characteristics of the bed sediments

The equilibrium P concentration values ($EPC_{0,}$ mg L^{-1}) of the sediments ranged from 0.02 at site 7 to 0.73 mg L^{-1} at site 7b (Table 2-2). Sediments from Site 14 had the second highest EPC_0, followed by those of sites 11, 13, (Fig. 2-1) and 4, and these high EPC_0 sites showed a higher risk of releasing P from the sediment to the river (Wang et al., 2009). The EPC_0 affected the P concentration in the river; sediments would desorb P if P concentration were less than sediment's EPC_0, and sediments would adsorb P if P concentration in the water column were more than EPC_0 (Kunishi et al., 1972; Wang et al., 2009). The P sorption maximum S_{max} (mg kg^{-1}) of the sediments ranged from 81mg kg^{-1} at site 4, South of Troublesome Brook, to 476 mg kg^{-1} at site 12, East Tremont Avenue Bridge (Table 2-2). The second highest S_{max} was at site 14, and followed by sites 8, 2, and 13 (Fig. 2-2).

Table 2-2 Phosphorus sorption characteristics of the river bed sediments

Site#	Location	K_d L kg^{-1}	S_0 mg kg^{-1}	EPC_0 mg L^{-1}	S_{max} mg kg^{-1}	k L mg^{-1}	K_f L kg^{-1}
1	Davis Brook, Valhalla	64	9.5	0.15	120	0.37	8.1
2	Station A	91	3.7	0.04	333	0.29	40.0
3	North of Tb Brook	71	19.9	0.28	244	0.36	47.4
4	South of Tb Brook	146	65.6	0.45	81	17.71	147.2
5	Troublesome Brook	14	2.0	0.15	167	0.18	10.1
6	Paxton Ave North	20	3.0	0.15	175	0.31	11.7
7	Paxton Ave South	15	0.3	0.02	192	0.12	7.6
7B	Paxton Ave Southwest	40	29.0	0.73	179	0.35	39.4
8	Sprain Brook	121	53.0	0.44	370	0.16	86.6
9	233rd St City Line	58	24.3	0.42	169	0.48	41.3
10	NY Botanical Garden	33	13.5	0.41	208	0.20	26.3
11	Bronx Zoo	23	13.2	0.58	120	0.23	20.9
12	East Tremont Ave Bridge	142	46.3	0.33	476	0.43	45.7
13	Bronx River estuary	56	30.7	0.54	333	0.20	49.4
14	Sound View Park Station	63	42.4	0.67	435	0.16	57.9

K_d: distribution coefficient, L kg^{-1}
S_0: P originally sorbed on the solid phase, mg kg^{-1}
EPC_0: equilibrium P concentration, mg L^{-1}
S_{max}: P sorption maximum, mg kg^{-1}
k: a constant related to the bonding strength, L mg^{-1}
K_f: P absorption energy, L kg^{-1}

Table 2-3 Pearson's correlation matrix (r) of sorption parameters and chemical characteristics of the Bronx River sediments

Parameter	S_{max}	EPC_0	S_0	K_f	K_d	k	Ox-P	TP	OM	Ox-Fe	Ox-Al	HCl-Ca	HCl-Mg
S_{max}	1												
EPC_0	-	1											
S_0	0.720**	0.599*	1										
K_f	0.719**	-	0.883**	1									
K_d	0.764**	-	0.795**	0.782	1								
k	-	-	0.569*	0.798**	0.526*	1							
Ox-P	0.805**	-	0.728**	0.826**	0.616*	0.756*	1						
TP	0.542*	-	0.608*	0.681**	-	0.621*	0.916**	1					
OM	0.546*	-	0.660**	0.795**	0.592*	0.821**	0.867**	0.752**	1				
Ox-Fe	0.673**	-	0.613*	0.543*	0.667**	-	0.644**	0.516*	0.689**	1			
Ox-Al	0.639*	-	0.534*	0.748**	0.683**	0.692**	0.836**	0.678**	0.832**	0.659**	1		
HCl-Ca	0.582*	-	-	-	0.548*	-	-	-	-	0.836**	-	1	
HCl-Mg	0.573*	-	0.600*	-	0.626*	-	-	-	-	0.915**	-	0.892**	1

* Correlation is significant at the 0.05 level (2-tailed), $p<0.05$

** Correlation is significant at the 0.01 level, $p<=0.01$

- no correlation

S_{max}: P sorption maximum, mg kg^{-1} (one outlier was removed)

EPC_0: equilibrium P concentration, mg L^{-1} (two outliers were removed)

S_0: P originally sorbed on the solid phase, mg kg^{-1}

K_f: P absorption energy, L kg^{-1}

K_d=sorption coefficient, L kg^{-1}

k: a constant related to the bonding strength, L mg^{-1}

Ox-P: Oxalate-extractable P, mg kg^{-1}

TP: the total phosphorus in bed sediments from Anderson ignition method, mg kg^{-1}

OM: total organic matter, %

Ox-Fe, oxalate-extractable Fe, mg kg^{-1}
Ox-Al, oxalate-extractable Al, mg kg^{-1}
HCl-Ca, HCl-extractable Ca, mg kg^{-1}
HCl-Mg, HCl-extractable Mg, mg kg^{-1}; TOC, total organic carbon (%)

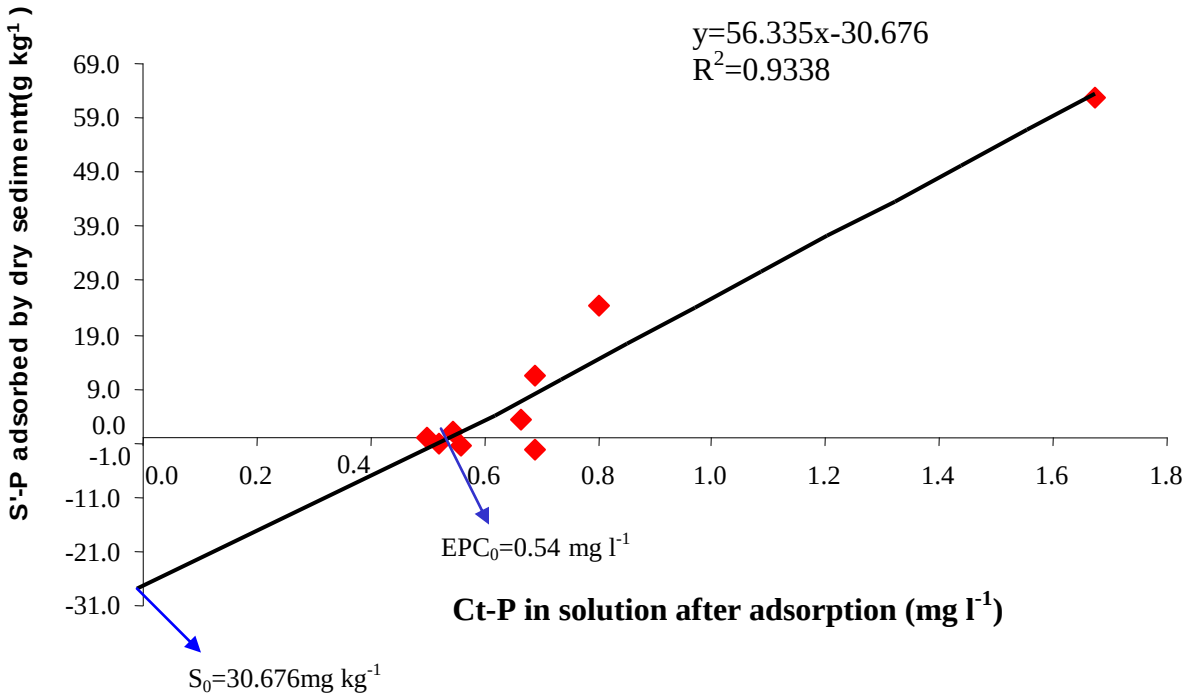


Figure 2-1 EPC_0 and S_0 calculation using Langmuir Isotherm example at Site 13, Bronx River estuary

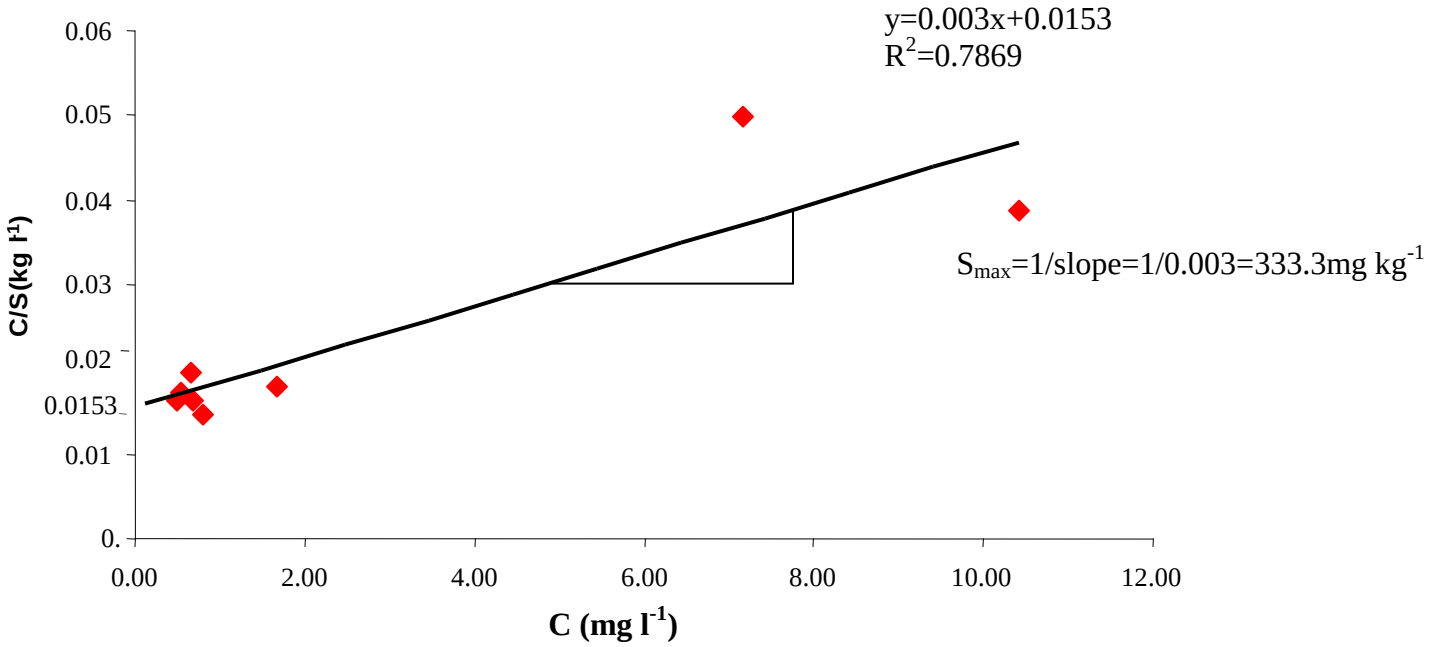


Figure 2-2 S_{max} calculation using Langmuir Isotherm at Site 13, Bronx River estuary

Originally sorbed P (S_0) values ranged from 0.3 to 65.6 mg kg^{-1}. The highest value appeared at site 4, followed by sites 8, 12, 14, and 13 (Table 2-2). The P absorption energy K_f (L kg^{-1}), from the Freundlich equation of the sediments ranged from 7.6 L kg^{-1} at site 7, Paxton Ave South, to 147.2 L kg^{-1} at site 4, South of Troublesome Brook. The ranking of sites in order of decreasing P absorption energy levels was as follows: site 4, site 8, site 14, site 13 (Fig. 2-3), and site 3. The sorption coefficient (K_d) ranged from 15 to 146 L kg^{-1}, and bonding strength (k) ranged from 0.12 to 0.48 L mg^{-1} for most of the sites, and it was 17.71 L mg^{-1} at site 4 that had much higher bonding strength than other sites.

Ratios of P retained to the P sorbed (P_r/P_{ad}), or the amounts of hysteretic P were above 98% for most of the sites, indicating retention of most of the sorbed P following the immediate desorption experiment. The two exceptional sites 13 and 14, located at the estuary including, had relatively lower P retained values. These results showed that a high percentage (>88%) of P was sorbed by the bed sediments. Sediments from most of the sites had high S_0, the initially adsorbed P, indicating that there is a potential to release P to the water column from the sediments due to changes in pH, ionic strength or redox conditions. The released P could cause subsequent eutrophication/algal blooms in the water column (Wang and Pant, 2010b).

Correlation coefficient (r) between S_{max} and Ox-Fe was 0.673 ($p<0.01$), and that between S_{max} and Ox-Al was 0.639 ($p<0.05$). The correlation coefficient between S_{max} and HCl-Ca was 0.582 (Table 2-3), and that between S_{max} and HCl-Mg was 0.573, both $p<0.05$. The results indicated that S_{max} was correlated with Ox-Fe, Ox-Al, HCl-Ca, and HCl-Mg, and the content of Al, Fe, Ca, or Mg ions and their associated P in the sediment has an impact

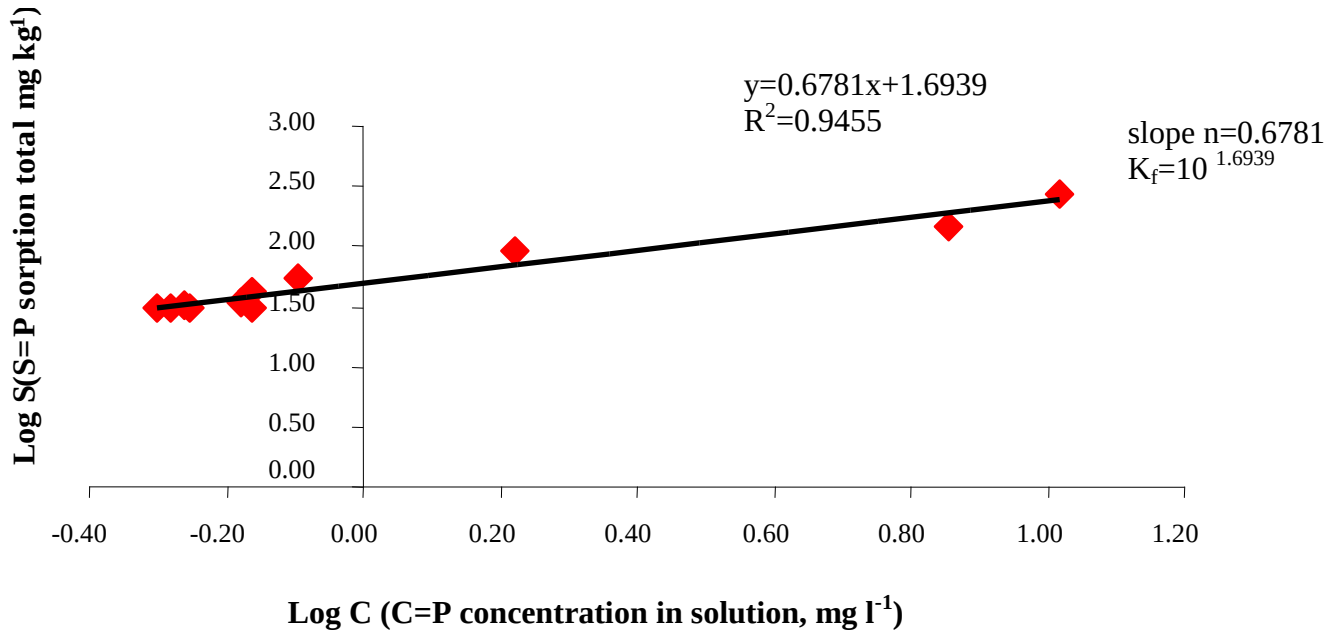


Figure 2-3 K_f calculation using Freundlich isotherm at Site 13, Bronx River estuary

on P sorption (Adam et al., 2006; Makris et al., 2005). Likewise, Fe played an important role in the P sorption by sediments (Eckerrot and Pettersson, 1993); and the S_{max} was affected by Ox-Fe since the bed sediments were under aerobic conditions during this study.

Site 12, East Tremont Ave Bridge, was the boundary between fresh and saline water. The sediments in this section had the maximum P sorption because there was the maximum amount of Ox-Fe, HCl-Ca, HCl-Mg, and a large amount of Ox-Al at this site. Because under aerobic conditions, Fe^{3+} binds P in a stable form, so as Al^{3+} binds P in an irreversible form without being affected from redox, as well as Ca^{2+}, and Mg^{2+} binds P in a relatively stable form (Wang and Pant, 2010b). Site 13, the Bronx River estuary, had the third highest Ox-Al value, and the second highest Ox-Fe, HCl-Ca, and HCl-Mg values, thus the bed sediments had a high S_{max}. Site 14, Sound View Park Station, had the second highest S_{max} value, and it had the second highest TP and Ox-P values as well. S_{max} was correlated with OM ($r=0.546$, $p<0.05$) indicating, perhaps, OM associated amorphous and poorly crystalline Fe and Al played a role in P sorption (Pant and Reddy, 2001). Multiple regression [S_{max} = 92.0 + 0.31 Ox-Al + 0.014 Ox-Fe; $R^2 = 0.56$; $p = 0.011$] indicated that 56% of variability in sediment S_{max} could be explained by amorphous and poorly crystalline Al and Fe content of the bed sediments (i.e., Ox-Al and Ox-Fe) (Wang and Pant, 2010b).

The EPC_0 was not highly correlated with the Ox-Al, Ox-Fe, HCl-Ca, or HCl-Mg values, and this indicates that EPC_0 was not controlled by these cations in the Bronx River. The S_0 was correlated with the Ox-Fe, Ox-Al, HCl-Mg and OM values (Table 2-3), indicating that these cations and associated OM affected P sorption at the initial stage of adsorption.

The P sorption energy (K_f) had a strong correlation with the Ox-Fe and Ox-Al. The strong correlations at the statistically significant level of $p<0.01$ between K_f and S_0 ($r=0.883$), K_f and S_{max} ($r=0.719$), K_f and OM ($r=0.795$), between k and OM ($r=0.821$), between OM and Ox-Fe ($r=0.689$), between OM and Ox-Al ($r=0.832$) indicate that Fe, Al, and OM were associated with P sorption (Wang and Pant, 2009), and that OM-associated Fe, Al provided sites for P sorption (Jordan et al., 2005).

2.3.3 Sediments chemical and sorption properties compare with other studies

The Bronx River flows through Bronx borough in the NYC, an urbanized area. There is no major industry on the river. The Hunts Point WWTP is in the estuary area. Huaxin et al. (1997) reported that, the river sediments from the three major rivers or streams in the Washington, DC area (the Potomac and Anacostia Rivers and Rock Creek) had TP levels from 880 to 1,736 mg kg^{-1}, and OP levels from 0-580 mg kg^{-1} at 12 sites. The OM was from 3.8 to10.1%, and the water and sediment quality in the DC area is affected by CSOs, industrial and domestic sewers and WWTP overflows (Huaxin et al., 1997). Sediment TP of the Bronx River ranged from 183 to 867 mg kg^{-1}, and the OP varied from 51 to 480 mg kg^{-1}; the OM ranged from 0.1 to 10.7%. The average values of TP (406 mg kg^{-1}), OP (129 mg kg^{-1}), and OM (2.7 %) in the Bronx River sediment were all lower than the sediment in rivers and streams in the DC area with average values of TP (1,276 mg kg^{-1}), OP (233 mg kg^{-1}), and OM (6%). Since the P in sediments in rivers and streams around Washington, DC is mostly derived from urban runoff, storm water, and industrial and domestic sewage, those anthropogenic inputs were higher than from natural sources (Huaxin et al., 1997). There is no industry in the Bronx River watershed, which is a

mostly natural and residential area, so that it had lower TP, OP, and OM values than the rivers in Washington, DC area where P is derived partically from industrial sewage.

In the agricultural drainage ditches in the Cedar Creek sub-watershed of the St. Joseph River watershed in northeast Indiana, the average TP value was 45 mg kg^{-1}, Ox-Al was 103 mg kg^{-1}, and Ox-Fe was 1,033 mg kg^{-1}. All of these values are much lower than the Bronx River. However the OM was higher (4.4%) in the ditches (Smith, 2009). The rural and urban environment comparison in UK also indicates similar pattern. The rural Swale River sediment had lower TP (138<294 mg kg^{-1}) and OP (51<111 mg kg^{-1}) levels than that of urbanized and industrialized Aire and Calder in the middle and lower parts of the river (Owens and Walling, 2002). Rivers in urban areas usually have more P sources than rural waterways although variations can occur depending on the watershed characteristics.

Daliao River watershed in China is a mixed urban, industry and agriculture area. The average sorption parameters at six sites along the Daliao River in China were: EPC_0 0.05-0.54 mg L^{-1}, TP 479-1,202 mg kg^{-1}, S_{max} 204 to 714 mg kg^{-1}, k 0.41 - 2.65 L mg^{-1}. While the average parameters in the Bronx River, NY, USA were: EPC_0 0.02-0.73 mg L^{-1}, TP 183-867 mg kg^{-1}, S_{max} 81 - 476 mg kg^{-1}, k 0.12 - 17.71 L mg^{-1}. The major difference between the Daliao River and the Bronx River was that there was no significant correlation between k and S_{max} in the Bronx River bed sediment, while k and S_{max} were correlated and in a reverse pattern from upstream to downstream of Daoliao River (Lin et al., 2009). Since k is related to the sorption process, it may or may not be correlated with S_{max}, depending on sediment characteristics.

2.4 Conclusions

It is indicative that bed sediments may store a large amount of P corresponding to the contents of amorphous and poorly crystalline Al and Fe, and acid-extractable Ca and Mg in the sediments, indicating that changes in redox and pH could cause release of massive amount of stored P from the sediments, in turn, degradation in water quality. The S_{max} was affected by Ox-Fe the most since the bed sediments were under aerobic conditions during P sorption experiments, i.e., there were more Fe^{3+} sites available in the sediments for P to be sorbed. The goal of this project was to provide data that can help develop policies to control P runoff to the river, including regulation of P inputs from lawn fertilizers. It is hoped that the results of this research can be shared among the United States Environmental Protection Agency (USEPA), New York City Department of Environmental Protection (NYC DEP), New York State Department of Environmental Conservation (NYS DEC), and the Bronx River Alliance to make the river meet the fishable/swimmable goal of the Clean Water Act. It is important to be able to predict P bioavailability under changing hydro-climatic conditions because it can result in changes in redox conditions, in turn, release of, especially Fe-sorbed P and subsequent algal blooms, oxygen depletion and water quality degradation. The S_{max} and EPC_0 data could be used for the estimation of bioavailable P in the Bronx River. Water quality improvements in the Bronx River could be an excellent example for other urban water systems. The raw sewage discharge and storm water runoffs from surrounding areas have degraded the water quality in the Bronx River, thus, this research may provide needed data to the public with the hope that the Bronx River ecology can be restored.

Chapter 3

Identification of Organic Phosphorus Compounds in the Bronx River Bed Sediments by Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy

Abstract

Sediment characteristics influence the distribution and bioavailability of phosphorus (P) in rivers and lakes. The objectives of this study were to identify P compounds in sediments collected from 15 sites along the Bronx River to get insights on nutrient transport for management of highly variable and modified ecosystems such as the Bronx River. The Nuclear Magnetic Resonance (NMR) spectra showed that the dominant P species in Bronx River bed sediments are orthophosphate monoester, and lesser phosphate diesters and pyrophosphates (pyro-P). The P compounds were mostly glycerophosphate (GlyP), nucleoside monophosphates (NMP), and polynucleotides (PolyN). A few sites showed a small amount of dihydroxyacetone phosphate (DHAP), inosine monophosphate (IMP), and pyrophosphates (pyro-P). By allowing a downstream comparison of P compound variations along the Bronx River, this study provides a step toward improving water quality in an urban river system such as New York City (NYC), and helps to asses the bioavailability of P, in turn, design estuary habitat restoration projects in comparable region of the world.

3.1 Introduction

Phosphorus is a major nutrient for plants growth, and it is a primary limiting nutrient in the freshwater systems (Ahlgren et al., 2005; Zhang et al., 2008). Phosphorus is critical for all organisms; however too much P in aquatic system causes eutrophication and too

little P limits plant growth (Schindler, 1978). The mineralization of organic P (OP) to inorganic P (IP) directly affects P bioavailability in river systems. The identification of P compounds is essential for qualitative and quantitative research into nutrient studies in the Bronx River bed sediments.

Bioavailable P (BAP) is composed of dissolved P (DP) and particulate P (PP) (Sharpley and Rekolainen, 1997). Phosphorus can be found in DNA, RNA, membrane phospholipids, and a variety of compounds (Wetzel, 1999). Microbial metabolism influences P mobilization by biochemical mobilization from inositol phosphates, by bacterial influences, and by changes in pH, redox, OP solubility and enzymatic hydrolysis potential (Wetzel, 1999). Microorganisms can either release P from their cells, or immobilize P when carbon is available as a P sink in the soil or sediments (Bunemann et al., 2008a and b). In general, diester-P is easier to be hydrolyzed than monoster-P because Diester-P is more labile than monoester-P (Taranto et al., 2000).

Chromatography and Nuclear Magnetic Resonance Spectroscopy (NMR) are used for OP and IP studies in freshwater systems (Newman and Robinson, 1999), and natural sediment extracts (Ahlgren et al., 2007). Nuclear Magnetic Resonance is an instrumental technique that measures the energies required to 'flip' a nuclear spin orientation in a magnetic field to determine the number, type, and relative positions of certain atoms in a molecule. Any element with a nuclear spin such as ^{13}C, ^{17}O, ^{19}F, ^{31}P, will produce a NMR signal (Bulter, 2003; Pavia et al., 2003; Lambert and Mazzola, 2004).

After extraction and gel filtration, identifications of both IP and OP compounds using ^{31}P-NMR are reported by Gadian et al (1979) and Pant et al (2002). Both DP and PP could be identified by ^{31}P-NMR (Cade-Menun et al., 2006). The ^{31}P-NMR spectroscopy

has advantages compared with chromatographic and other procedures, as it can be used to identify multiple P compounds simultaneously (Turner, 2004). All P compounds could be analyzed by NMR because ^{31}P is the only naturally occurring stable P isotope (Cade-Menun, 2005). The ^{31}P-NMR has been used to identify P in sediments from rivers, lakes, oceans, and estuaries, in sewage sludge and animal manure, in soil and other environments (Turner, 2004; Cade-Menun, 2005; Bartoszek, et al., 2008). Similarly, ^{31}P NMR spectrometry has been used to identify P compounds in water systems both temporally and spatially, including IP such as mono or diprotonated orthophosphate and OP such as phosphonates, pyrophosphate, polyphosphate, phosphate monoesters and diesters (Worsfold et al., 2008).

Soil and sediment samples are typically extracted by NaOH (Pant et al., 1999; Ahlgern et al., 2005), and then centrifuged before gel filtration/fractionation. Similarly, algae samples can be analyzed by solid-state ^{31}P-NMR, as well as by extracting with sodium hydroxide (NaOH) and ethylenediaminetetraacetic acid (EDTA), and finally analyzed by solution ^{31}P-NMR (Cade-Menun, 2005). However, NaOH could affect P determination, altering relative P compositions during sample concentration. Different extractants have been used to analyze P by ^{31}P-NMR spectroscopy such as 0.5 M NaOH, 0.5M NaOH and 0.1 M EDTA in a 1:1 mix (Cade-Menun and Preston, 1996; Cade-Menun, et al., 2002), and 0.25 M NaOH plus 0.05 M EDTA (Turner et al., 2005). The better way to get more accurate result of soil samples is to remove NaOH before analysis with ^{31}P-NMR. However, most researchers still use NaOH and EDTA extraction, a better procedure for P determinations needs to be considered (Pant et al, 1999). Cade-Menun (2005) also mentioned that if extraction is altering the P forms in samples in any way, such as by

hydrolysis, this still needs further exploration. The chemical shifts from ^{31}P-NMR would change with pH because protonation of most of the P compounds is controlled by pH, especially for orthophosphate (Cade-Menun, 2005). The IP and OP ratio was varied under different pH (Bedrock et al., 1995).

There are seven P groups including orthophosphate (Ortho-P); orthophosphate monoesters (Mono-P); orthophosphate diesters -deoxyribonucleic acid P (DNA-P), P lipids (Lipid-P), and teichoic acid P (Teichoic-P); pyrophosphate (pyro-P), and polyphosphate (Poly-P) (Ahlgren et al., 2007). The major P forms in soil include monoesters, diesters, pyrophosphate, polyposphate and phosphonates (Cade-Menum, 2005; Bunemann et al., 2008a and b). The P compounds from ^{31}P-NMR signals generally fall between +25 and –25 ppm, i.e., phosphnates at 20 ppm; orthophosphate at 5-7 ppm (Cade-Menun, 2005) or 5.4-6.6 ppm (Bunemann et al., 2008a); orthophosphate monoesters at 3-6 ppm (Cade-Menun, 2005) or 3.4-5.4 ppm (Bunemann et al., 2008a); orthophosphate diesters at 2.5 to –1 ppm (Cade-Menun, 2005) or 0.4 to -0.8 ppm (Bunemann et al., 2008a); pyrophosphate at –4 to –5 ppm (Cade-Menun, 2005) or –4.5 to –5.5 ppm (Bunemann et al., 2008a); and polyphosphate at –20 ppm (Cade-Menun, 2005).

NMR offers an accurate and non-destructive method to identify and quantify P compounds (Ahlgren et al., 2006 and 2007). Thus, to better understand P stability in bed sediments and P availability in the water column, this study tried to identify P compositions in the sediments. Many studies have been done for soil P compound identification, but only limited data is available on the P species to predict the P bioavailability in urban river system including fresh and estuarine waters. Therefore, the objectives of this study were to identify P compounds in the Bronx River bed sediments

to predict P bioavailbility in the Bronx River to aid to control P sources and provide needed data for future regulation of land use along the river parkway and surrounding areas to reduce P runoff to the river.

3.2 Materials and methods

3.2.1 Sediment physico-chemical analysis

A Mettler Toledo InLab 730 conductivity electrode was used to determine the electrical conductivity (EC) and a Mettler Toledo InLab413 pH electrode was used to determine the pH of the sediments. Total P in the sediments was determined by the modified ignition method (Anderson, 1976), i.e., 1g finely ground dry sediments were combusted at 550ºC in a muffle furnace for 5h, and dissolved in 6M HCl followed by hot plate digestion (Method 365.1, USEPA, 1983). The NaOH-P was determined by persulfate digestion method using Digestion Block (Method 365.4, USEPA, 2003). After the digestion process, both TP and NaOH-P values were determined using automated ascorbic acid method (ESS Method 310.1, USEPA, 1992). The total organic matter (OM) was determined by weight loss from combustion of ground dry sediment at 550°C in a muffle furnace for 5h (Pant and Reddy, 2001; Schumacher, B.A., 2002).

3.2.2 Phosphorus speciation by ^{31}P-NMR identification

To identify P compounds in the sediments collected at the 15 sites in 2006 and 2007, wet sediment sample 40 g was extracted twice with 80 ml 0.4 M NaOH, each for 4 h by shaking in an end-over-end shaker at 25$\pm$2ºC, 230 l/min. The suspensions were centrifuged at 21$\pm$2ºC, 10,000 X *g* for 30 min after each extraction. The supernatants

were combined and concentrated 10 times from100 ml to 10 ml using a Vacuum Rotary Evaporator (R-205, BUCHI) at 25ºC. Then 0.6 ml concentrated extracts were put in a 0.5 mm diameter Wilmad NMR tube, added one drop of D_2O to lock the signal (Ahlgern et al., 2005; Cade-Menun et al., 2006; Teleman et al., 1999), and then the solution was scanned at 121.494 MHz (Ahlgern et al., 2005) on a BRUKER 300 UltraShield ^{31}P NMR for 12-17 h (Ahlgern et al., 2005) using a 90º pulse with a 0.5 sec relaxation delay and an acquisition time of 0.67 sec. The spectra were processed using Bruker TOPSIN 2.0 software. The P compounds were identified using an external standard of 85% phosphoric acid (H_3PO_4) (Ahlgern et al., 2005; Cade-Menun et al., 2006; Pant et al., 1999; Pant and Reddy, 2001), as well as by referencing the sample peaks with the peak of internal standard, namely, pyrophosphate. The internal standard was prepared in a ratio of 30:1 with 0.6 ml samples spiked with 0.02 ml 10,000 mg l^{-1} sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H2O$) (Gadian et al., 1979; Pant et al., 1999; Pant et al., 2002).

3.3 Results and discussion

In 2006, the EC of wet sediment samples ranged from 66 μs cm^{-1} at site 1 to 342 μs cm^{-1} at site 4 (Table 3-1). The two estuary sites 13 (1,917 μs cm^{-1}) and 14 (4,290 μs cm^{-1}) had much higher EC. In 2007, EC values ranged from 9 to 62 μs cm^{-1} in freshwater sites, and estuary sites 13 and 14 had 277 and 363 μs cm^{-1}, respectively; both were much lower than those in 2006. The pH among the 15 sediment sites ranged from 6.5 to 7.8 in 2006, and from 7.1 to 8.3 in 2007 (Table 3-1). Except for Site 12, all sites had slightly higher pH in 2007. The OM ranged from 0.1 to 10.7% in 2006, and from 0.3 to 16.7% in 2007. The

Table 3-1. Selected physico-chemical characteristics of the bed sediments (NMR)

Site #	Location	NaOH-P		TP		OM		pH		EC	
		% of TP		mg kg^{-1}		%				µs cm^{-1}	
		2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
1	Davis Brook, Valhalla	30 def	15 de	492 cdef	306 b	0.7	1.0	7.07	7.17	66	17
2	Station A	45 abcd	18 cde	703 c	219 b	3.3	0.5	6.79	7.46	298	9
3	North of Tb Brook	29 def	20 cde	506 cde	396 b	1.9	1.2	6.70	7.37	133	12
4	South of Tb Brook	59 a	21 cde	1563 a	311 b	10.7	1.1	6.50	7.27	342	14
5	Troublesome Brook (Tb)	24 f	14 ef	398 ef	318 b	0.1	0.3	7.40	7.88	220	34
6	Paxton Ave North	42 abcde	20 cde	288 f	393 b	0.1	0.8	7.48	7.68	158	60
7	Paxton Ave South	27 ef	43 b	312 ef	735 b	0.3	3.5	6.97	7.13	167	62
7b	Paxton Ave Southwest	47 abc	21 cde	523 cde	335 b	1.7	1.0	6.74	7.43	160	14
8	Sprain Brook	52 ab	26 cd	479 def	239 b	1.0	1.1	7.04	7.76	103	10
9	233rd St City Line	39 bcdef	18 cde	477 def	257 b	1.7	0.6	6.82	7.67	209	10
10	NY Botanical Garden	39 bcdef	16 cde	470 def	255 b	3.7	0.3	6.97	8.20	108	12
11	Bronx Zoo	29 def	57 a	376 ef	1051 b	3.2	6.7	6.83	7.06	133	23
12	East Tremont Ave Bridge	34 cdef	3 f	473 def	5360 a	3.8	1.1	7.78	7.14	254	13
13	Bronx River estuary	43 abcde	28 c	663 cd	621 b	4.9	16.7	7.75	8.24	1917	277
14	Sound View Park Station	45 abcd	45 b	974 b	1106 b	3.6	3.4	7.78	8.26	4290	363

% of TP: the percentage of TP (NaOH extracted P is considered as organic P)
TP: the total phosphorus in bed sediments from Anderson ignition method, mg kg^{-1}
OM: total organic matter, %
Levels not connected by the same letter are significantly different as determined by Tukey-Kramer's mean comparison
EC: electrical conductivity, µs cm^{-1}

TP ranged from 288 to 1563 mg kg^{-1} in 2006, and from 219 to 5360 mg kg^{-1} in 2007. The percentages of NaOH-P compositions were higher in 2006 compared to 2007 except for sites 7 and 11. Most of the P compounds found in the Bronx River bed sediments (Table 3-2) were ortho-mono-P as interpreted from the resonances, which ranged from 3.0 to 5.6 ppm that represented the characteristics of orthophosphoate monoester (Nanny et al., 1997; Pant et al., 1999; Teleman, et al., 1999; Cade-Menun, 2005; Liu et al., 2009). Few sites had diester phosphates (2 to -3 ppm) and pyrophosphate (-3 to -8 ppm) (Nanny et al., 1997; Cade-Menun, 2005; Bartoszek et al., 2008).

3.3.1 P compounds identified in bed sediments collected in 2006

The major P compound for almost each site other than site 4 and 7b was GlyP; the relative composition of GlyP was ranged from 95-100% (Table 3-2). Site 4 had DHAP as a major peak of 95%; site 7b had the NMP as a major P compound of 96%. There were small percentages of NMP and PolyN from a trace amount to 4% in some of the sites: NMP was found at sites 2, 4, 7b and 14; PolyN was in sites 2, 3, 6, 7b, 10, 12 and 13. The resonances of DHAP, GlyP, NMP and PolyN spreading from 3.0 to 6.4 ppm representing the characteristics of orthophosphate monoester (Pant et al., 1999; Cade-Menun, 2005). As expected small percentage (1-2%) of IMP was found at sites 2, 4, 6, 7b, 10 and 12 because it is a relatively unstable P compound, thus it is more labile and easily mineralized than monoester-P (Pant et al., 1999; Taranto et al., 2000; Cade-Menun, 2005). The orthophosphate diesters (2.5 to –1 ppm) that are found in nucleic acids of oranismic remains were also detected. Although pyro-P is related to IP (Liu et al., 2009),

Table 3-2. Relative composition of organic P compounds as estimated from ^{31}P-NMR spectra of the bed sediments collected in 2006 and 2007

Site	DHAP 6.2±0.3 ppm		GlyP 5.3±0.3 ppm		NMP 4.6±0.2 ppm		PolyN 3.5±0.5 ppm		IMP -1.5±1 ppm		pyro-P -5.1±0.7 ppm	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
1	-	-	100	100	-	-	-	-	-	-	-	-
2	-	-	95	100	trace	-	trace	-	trace	-	-	-
3			98	98			trace	trace			trace	trace
4	95	-	2	98	2	-		2	trace	-	-	-
5	-	-	100	99	-	-	-	-	-	-	-	trace
6	-	-	96	100	-	-	3	-	trace	-	-	-
7	-	-	100	98	-	-	-	trace	-	trace	-	-
7b	-	-	-	99	96	-	2	-	trace	-	trace	trace
8	-	-	100	99	-	-	-	trace	-	-	-	-
9	-	-	100	98	-	-	-	-	-	-	-	2
10	-	-	97	100	-	-	trace	-	trace	-	trace	-
11	-	10	100	90	-	-	-	-	-	-	-	-
12	-	-	96	100	-	-	2	-	2	-	-	-
13	-	-	99	98	-	trace	trace	-	-	-	-	trace
14	-	-	96	100	4	-	-	-	-	-	-	-

DHAP=dihydroxyacetone phosphate
GlyP=glycerophophate
NMP=nucleoside monophosphates
PolyN=polynucleotides
IMP=inosine monophosphate
pyro-P=pyrophosphates.
Trace: ≤1.5% (of relative composition)

trace amount of pyro-P was also detected at sites 3, 7b and 10 in NaOH-P fraction, perhaps, representing an occluded P within OM/matrices.

The headwater at Valhalla site 1 had one major peak of GlyP for 100%. Close to the headwater, the east side of Virginia Road on the Bronx River Parkway South (BX Pkwy S) at site 2 had NMP, PolyN and IMP in trace amounts other than major peak of GlyP (Table3-2, Fig.3-1). The Troublesome Brook (TB) tributary including site 3-5 showed three different patterns. The north of TB at site 3 had trace amounts of PolyN and pyro-P other than GlyP. The pyro-P could possibly come from the fertilizer runoff from golf courses and lawns along the BX Pkwy in Westchester; the phosphate from the fertilizer may have been converted to pyro-P, which is considered as IP (Stutter et al., 2008; Liu et al., 2009), perhaps, due to precipitation process by amorphous and poorly crystalline forms of Al, Fe (Pant and Reddy, 2001). The major P compound of the TB tributary at site 4 was DHAP, while GlyP, NMP and IMP were detected from a trace amount to 2% (Fig. 3-2). This site (site 4) had the highest proportion of NaOH-P, and the greatest amounts of TP and OM among the 15 sites. Also, the TB tributary is very narrow, just behind WC courthouse north of the parking lot, the water flows very slowly in this tributary, thus, indicative of high accumulation of OM at varying stages of decomposition (Wang and Pant, 2009). At the south of Tb Brook site 5, GlyP was the only P compound indicating high microbial activities.

The Sprain Brook (SB) tributary is another major tributary of the Bronx River, which is located further south downstream from TB tributary, flows through the city of Yonkers residential area by the BX River Pkwy North (N) exit to village of Bronxville. The north of SB at site 6 had GlyP as major P compound, followed by PolyN and IMP in a trace

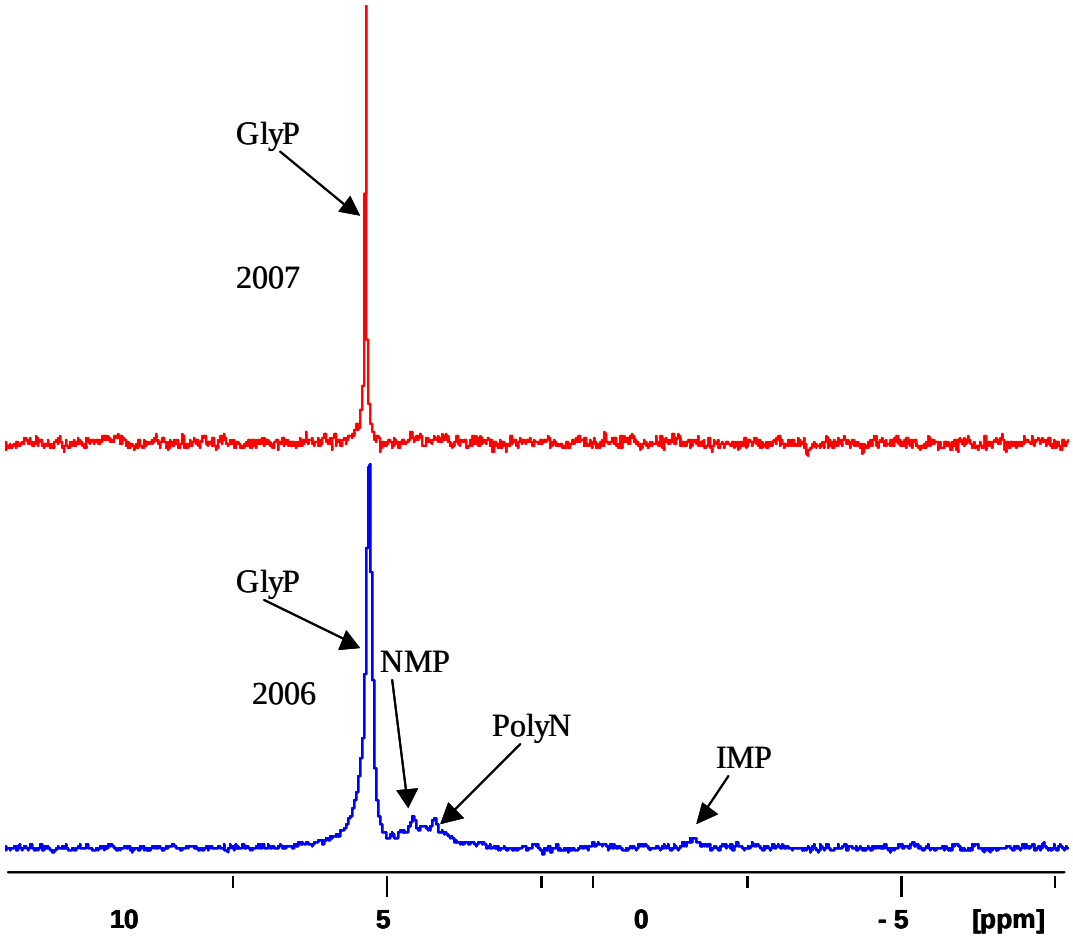


Figure 3-1 ^{31}P-NMR spectra of NaOH-extract of sediments from Site 2, Station A, collected in 2006 and 2007.

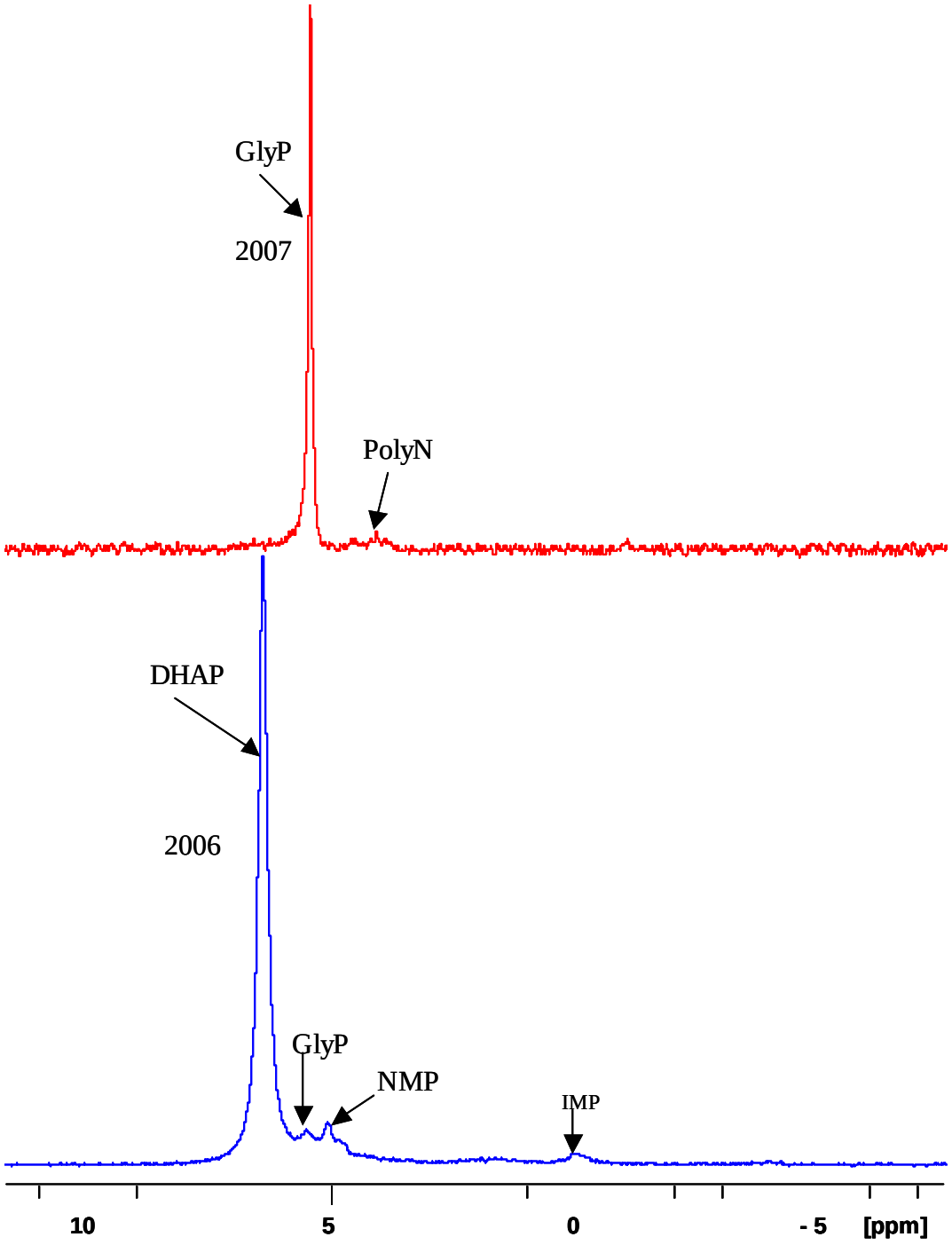


Fig. 3-2 ^{31}P-NMR spectra of NaOH-extract of sediments from Site 4, South of Troublesome Brook, collected in 2006 and 2007

amount. The south of SB at site 7 had only GlyP. The southwest of SB at site 7b (Fig.3-3) had a different composition pattern: NMP as a major peak, PolyN 2% plus IMP and pryo-P in trace amounts. This site also had relatively high NaOH-P composition that associated with OM (Pant and Reddy, 2001; Wang and Pant, 2009). The SB tributary at site 8 had GlyP only, and the NaOH-P was fairly high in this site, too. The SB tributary was wider and had higher flow rate compared with TB tributary at site 5. In 2006, New York State Department of Environmental Conservation (DEC) had issued one million dollars fines for city of Yonkers because of raw sewage discharged into the tributary in 2004 and possibly through 2006 (Gannon, 2006). Thus, the raw sewage discharges may have added various OP and IP compunds to the bed sediments at these sites.

The site 9 is located at 233rd Street, Nereid Ave, the city line boundary between WC and the Bronx borough of New York City (NYC), showed GlyP as only P compound. In the Bronx Park area, the New York Botanical Garden (NYBG)- site 10 had polyN, IMP, and pyro-P in trace amounts other than a major peak of GlyP. Again here, the pyro-P is likely to be associated with the P fertilizer application in the garden (Stutter et al., 2008; Liu et al., 2009). South of NYBG, the Bronx Zoo at site 11 only had GlyP. At site 12, the boundary between fresh and saline water at East Tremont Avenue (Ave.) Bridge, polyN and IMP of about 2% each other than major peak of GlyP were detected indicating rapid P mobilization. It is known that the diester forms of soils and sediments OP are more readily mineralized and more labile than monoesters (Taranto et al., 2000; Makarov et al., 2002).

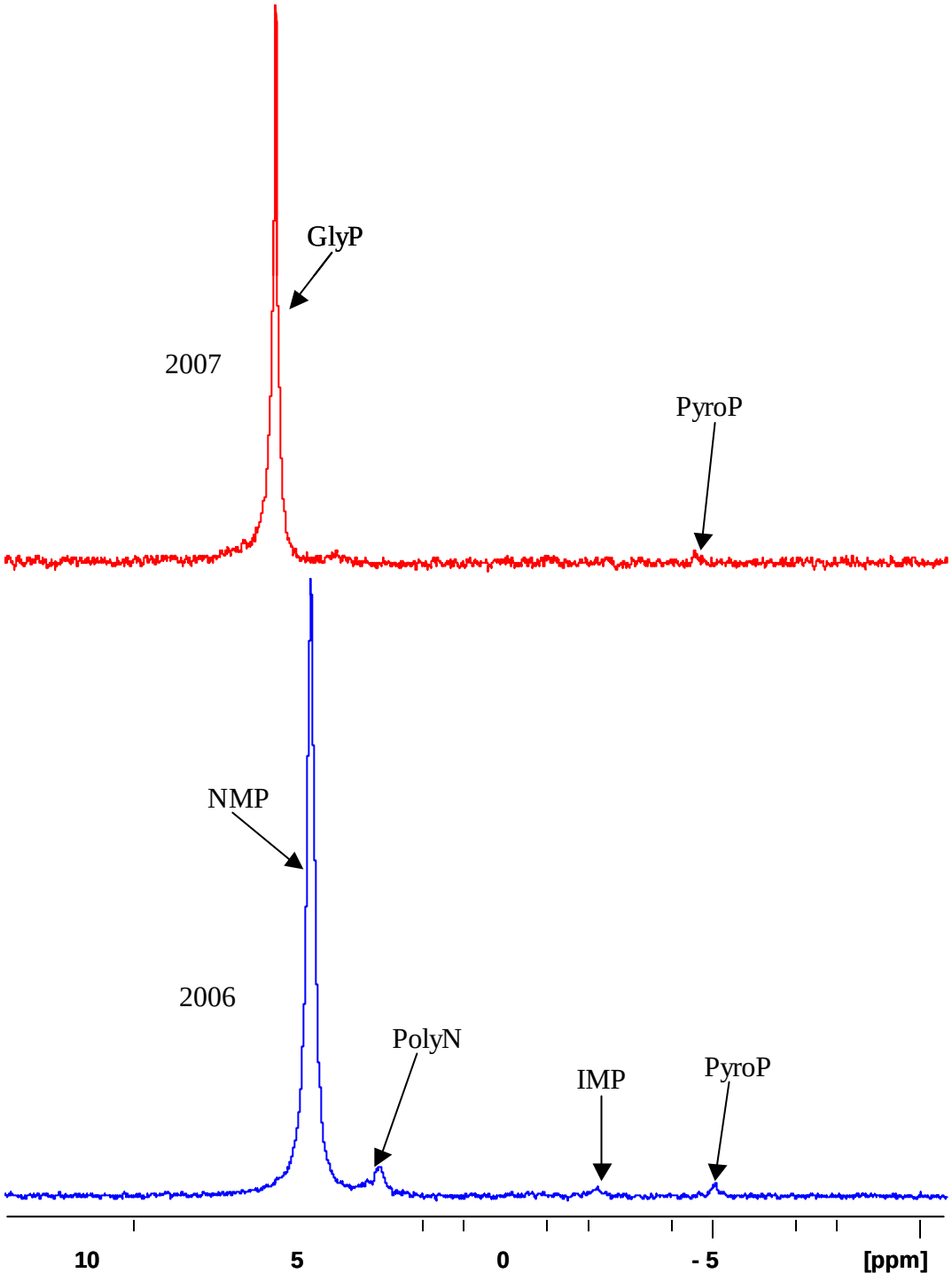


Fig. 3-3 ^{31}P-NMR spectra of NaOH-extract of sediments from Site 7b, Paxton Ave Southwest, collected in 2006 and 2007

The two estuary sites were located south of site 12. There was PolyN in a trace amount at site 13 other than GlyP, and site 14 had NMP about 4% besides GlyP. The NaOH-P distribution, TP and OM at estuary sites 13 and 14 were relatively higher than most of the other sites (Table 3-1) because of accumulation of OM, perhaps, caused by higher salinity, as well as due to sewer overflows from Hunts Point WWTP and pollutants from the East River (Protopapas, 1999).

3.3.2 P compounds identified in sediments collected in 2007 and the temporal variations from 2006 data

In sediments collected during 2007, GlyP was the dominant P compound at all sites (Table 3-2). Site 11 had a small peak of DHAP in 10%. There were four sites -3, 4, 7 and 8 had PolyN for about 1-2%. The other P compounds that only appeared at one or a few sites in trace amounts were NMP, IMP, and pyro-P: NMP at site 13 and IMP at site7 in a trace amount. There were four sites 3, 5, 7b, 9 and 13, which had pyro-P from a trace amount to 2%. Overall, the P compounds found at the TB and SB tributary area, the city line, the Bronx Zoo, and estuary area, were very different with other sites.

At site 2 (Fig.3-1), GlyP was of ~ 95% and trace amounts of NMP, PolyN and IMP in 2006, while GlyP was of 100% in 2007; TP decreased from 703 to 219 mg kg^{-1}, and the proportion of NaOH-P dropped from 45% to 18% from 2006 to 2007 (Table 3-1), indicating the high temporal variation in P dynamic within a short time frame, perhaps, due to corresponding microbial activity, i.e., mineralization of OP to IP and consequent increase in P availability for plants uptake (Pant and Reddy, 2001; Pant et al., 2002; Wang and Pant, 2009).

In the TB tributaries site 3, there was not much change in the P composition over the studied period, while the sediments at site 4 had GlyP of 98% and a trace amount of PolyN (Fig. 3-2), instead of DHAP of 95%, GlyP, NMP and IMP in trace amounts in 2006; the NaOH-P in 2006 was 59% much higher than in 2007 of 21%; the OM in 2006 was much higher than that of 2007 (10.7% vs 1.1%), perhaps, indicating the OM associated P dynamic in the sediments (Pant and Reddy, 2001; Pant et al., 2002; Wang and Pant, 2009). The trace amount of pyro-P at site 5 in 2007 might be associated to the fertilizer runoffs from Westchester that moving down stream from site 3.

The SB tributary sites 2007 data had a different pattern compared with TB tributary sites. Site 6 had 100% of GlyP in 2007, and a small amount of PolyN and traces of IMP in 2006 (Table 3-2). In site 7, there were a major peak of GlyP (98%), and two traces of PolyN and IMP in 2007, while GlyP was about 100% in 2006. This is probably related with the higher proportion of NaOH-P composition and OM in 2007 than those in 2006 (Table 3-1). Site 7b (Fig.3-3) had altered P compounds in 2007 of 99% GlyP and pyro-P in a trace amount from 2006 of NMP of 98%, PolyN of 2%, IMP and pyro-P in trace amounts, while the NaOH-P% and OM were higher in 2006; site 8 Sprain Brook almost remained the same in the two years except for there was adding up a trace peak of PolyN in 2007. The land use was not changed significantly in SB tributary. The OM mineralization by microorganisms could possibly be the reason (Newman and Robinson, 1999; Wetzel, 1999; Pant and Reddy, 2001). The Yonkers raw sewage spill could possibly affect SB tributary area more in 2006 than 2007 (Gannon, 2006; Nicolai, 2006; Wang and Pant, 2009). However the differential variations from site 6-8 still need further exploration.

At the city line at site 9 in 2007, the sediments had pyro-P in a trace amount (Table 3-2). The NaOH-P, TP and OM decreased from 2006 to 2007 (Table 3-1). It is not known whether the IP addition was related to the construction at Woodlawn Metro-North train station in summer 2007, moreover, it could be related to the associated microbial activity changes (Pant and Reddy, 2001; Pant et al., 2002), or caused by the fertilizer application upstream (Stutter et al., 2008; Liu et al., 2009; Wang and Pant, 2009).

Site 10 NYBG in 2007 had only the GlyP of 100%, while traces of PolyN, IMP, pryo-P besides GlyP were detected in 2006. The NaOH-P, TP and OM was decreased from 2006 to 2007 (Table 3-1), which could be related to fertilizer regulation changes, and the increasing microbial activity such that the OP was mineralized to IP ready for plant uptake and P redistribution (Hedley et al., 1982; Pant and Reddy, 2001; Wang and Pant, 2009). Site 11, the Bronx Zoo changed from GlyP of 100% in 2006 to DHAP 10% and GlyP 90% in 2007, possibly caused by higher NaOH-P, TP and OM content in 2007, and this was possibly related with animal manure management from Wildlife Conservation Society (WCS) in the Bronx Zoo. In 2007, site 12 also had different P species compositions; PolyN and IMP were not seen in 2007, while GlyP remained the major P compound. There was no gas spill found during July 2007 sample collection time unlike in 2006, which might have enhanced the microbial activity (Wang and Pant, 2009).

The estuary site 13 had the same GlyP as a major P compound in 2006 and 2007; the traces of PolyN in 2006 changed to NMP and pyro-P in 2007; even though the NaOH-P, TP had higher values in 2006, the OM was much higher in 2007 almost 3.4 times of OM in 2006. Site 14 showed only GlyP (100%) in 2007 and about 4% of NMP besides GlyP in 2006; NaOH-P had no difference between 2006 and 2007; TP was slightly higher in

2007 and OM were slightly higher in 2006. The Hunts Point WWTP sewer overflow, CSOs in estuary area and pollutants from the East River (Protopapas, 1999), fishing, recreation, and other anthropogenic factors could possibly explain the temporal variations in P composition in sediments at the estuary area (Wang and Pant, 2009).

3.4 Conclusion

The major P compounds were GlyP, NMP and PolyN, with a few sites had DHAP, IMP, and pyro-P. The tributaries, the NYBG, the Bronx Zoo, the fresh and saline water boundaries and the estuaries showed the most distinguished characteristics from other sites, caused by OM content variation, microbial mineralization, raw sewer discharge, fertilizer application, manure management, storm water runoff, CSOs, and pollutants from the East River. The temporal variation between the 2006 and 2007 samples indicates that land use, spills, and local hydro-climatic changes altered P compounds. These data allows P characterization in sediments and gives an understanding of P transport in an urban river system, thereby having implications on formulating P application legislation along the river parkways to improve urban river water quality and restore ecology in the comparable region of the world.

Chapter 4

Assessments of Potential Phospohrus Mineralization in the Bronx River Bed Sediments

Abstract

Sediment characteristics influence the distribution and bioavailability of phosphorus (P) in river sediments. In this study, we analyzed different P fractions in the sediments of the Bronx River, the New York City, NY using sequential extraction. The results showed that the average P pool rank order was HCl-P > NaOH-P > $NaHCO_3$-P > residueP, and their relative proportions were 3.7: 2.0: 0.4: 1 in sediment collected in 2006, while HCl-P > NaOH-P > residue-P > $NaHCO_3$-P, with their relative proportion of 27.8: 6.2: 2.7:1 in sediments obtained in 2007. The strong correlation between microbial P and organic P, along with the changes in microbial P over time indicate that much of the organic P in the river bed sediments is potentially bioavailable. The sediment transport, deposition, assimilation, the exchange of P between sediments and water columns, the land use changes, raw sewer discharge, gas spill, construction, fertilizer application, etc., as well as the hydro-climatic changes could result in the spatial and temporal variations in P bioavailability in the river bed sediments. The estimations of P pools and their bioavailability in river bed sediments could help determine the spatial and temporal variations in P transport and impacts of land use on water quality, in turn, help regulate P in the river's watershed.

4.1 Introduction:

Phosphorus is a limiting nutrient for primary production in aquatic ecosystems (Ahlgren et al., 2005; Fytinaos and Kotzakioti, 2005; Zhang et al., 2008), however excessive P

from external inputs and release from sediments could accelerate primary productivity, leading to eutrophication in the water column (Pollock and Meyer, 2001; Wetzel, 2001; Bai et al., 2009). The P bioavailability in sediment is affected by P speciation, P transport and exchange processes in river (Barbanti et al., 1994). Phosphorus pool gives the pattern of P distribution, and clarifies the amount of inorganic P (IP) and organic P (OP) in total P (TP), giving a quantitative analysis of sedimentary P. Sequential extraction determines various P pools in sediments (Barbanti et al., 1994). Sequential extraction with $NaHCO_3$, NaOH and HCl (Ivanoff et al., 1998; Zhang et al., 2008), and other extraction methods (Stone and English, 1993; Williams et al., 1976) had been widely used for P pool predictions that are critical for P bioavailability estimation (Fytianos and Kotzakioti, 2005). Sediment sequential extraction had also been used for estimating land use influences on the P distribution, and its availability in sediments (Castillo and Wright, 2008).

Organic P components are composed of easily decomposable OP such as nucleic acids, phospolipids, sugar phosphoates etc., and slowly decomposable OP such as inositol phosphates-phytin etc. (Reddy et al., 1999). The P content bounded with Fe, Al oxides and hydroxides increased with decreasing particle size, in small sized particles, such as clay minerals (Kaolinite, pyrophylite) that could enhance P adsorption resulting in P adsorption increased with decreasing particle size (Stone and English, 1993; Stone and Mudroch, 1989). For example: the Fe, Al ions associated organic matter in silty, clay sediments bounded P (especially organic associated P) tighter than sandy sediments (Bostrom et al., 1982). The sediments with lower pH tend to have higher fractions associated with Fe, Al-hydrous oxides and organic matter, whereas the sediments with

higher pH tend to have more HCl-P associated with Ca like apatite (McDowell and Sharpley, 2003).

P in river could affect overall water quality and ecosystem (Thomann et al., 1985). The P sources in river include both organic and inorganic forms in point and diffuse source input. The diffuse sources include fertilizer application and other landuse activities, the point sources include sewage treatment works and combined sewer overflows (CSOs) (Owens and Walling, 2002). In urban environment, the wastewater treatment plant (WWTP) effluent and CSO discharges increased dissolved and particulate organic carbon and P concentrations during storms (McConnell, 1980; Kronvang et al., 1997). The spatial differences along the river are to some degrees affected by anthropogenically (Carman et al., 2000). The P content variations in the sediments relate with land use, and other catchments characteristics (Owens and Walling, 2002). Assessments of spatial and temporal variations of P distribution in river bed sediments crucial to control diffuse pollution sources and improve water quality (Stutter et al., 2008). The objectives of this study were to determine the spatial and temporal variations on P fractionation and mineralization in the bed sediments, as well as to estimate the bioavailability of P in the water column from the Bronx River bed sediments.

4.2 Materials and methods

4.2.1 Sediment physico-chemical analysis

A Mettler Toledo InLab 730 Conductivity Electrode was used to determine the electrical conductivity (EC). Also, a Mettler Toledo InLab 413 pH electrode was used to determine the pH of the sediments (at sediment to water ratio of 1:2.5). Total P in the sediments was

determined by the modified ignition method (Andersen, 1976): 1g finely ground dry sediments were combusted at 550ºC in a muffle furnace for 5 h, and dissolved in 6 M HCl followed by a hot plate digestion (Method 365.1, USEPA, 1983). The organic matter (OM) in the sediments was determined by ignition of finely ground dry sediments at 550°C for 5 h (Pant and Reddy, 2001a; House and Denison, 1997; Schumacher, B.A., 2002).

4.2.2 Sequential extraction

Fractionation experiment intends to selectively extract discrete P pools with chemical extractants (Nair et al., 1995). The modified sequential extraction (Fig.4-1) determines the P pool, which includes the readily labile P, moderately available P and less available or stable P (Ivanoff et al., 1998; Hedley et al., 1982). Sediment samples 2 g were extracted with 30 ml 0.5 M $NaHCO_3$ in an end-over-end shaker for 16 h. The fraction extracted with 0.5 M $NaHCO_3$ consists mostly readily labile P including readily available IP and OP (Pant and Reddy, 2001a; Tchienkoua and Zech, 2003), and this pool of P represents bioavailable P to plants (Bowman and Cole, 1978). The suspensions were centrifuged at 20ºC, 10,000 X *g* for 30 min, and the supernatants were decanted into vials, then weighted the $NaHCO_3$ residues. The supernatants were stored at 4°C for soluble reactive P (SRP) and TP analysis. The residues were extracted with 30 ml 0.5 M NaOH in an end-over-end shaker for 16 h, and then the suspensions were centrifuged as

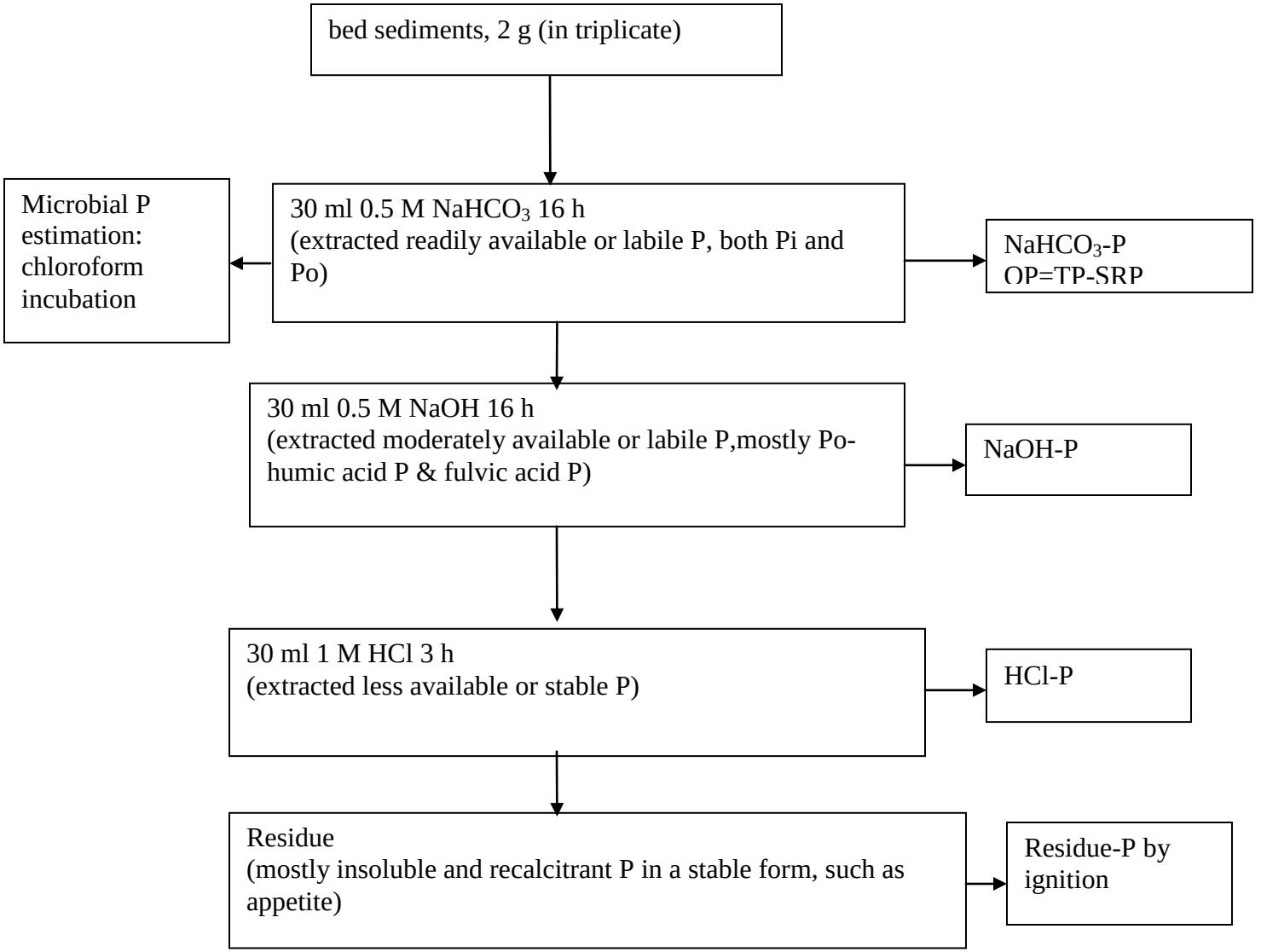


Figure 4-1 Flow chart of sediment phosphorus sequential extraction (based on Bostrom et al., 1982; Hedley et al., 1982; Ivanoff et al., 1998)

described above. The supernatants were saved for TP analysis. The NaOH extracted moderately available P including IP and OP, which is mostly associated with Al and Fe of organic matter (Barbanti et al., 1994; Tchienkoua and Zech, 2003). The NaOH-Pi associated with Fe and Al oxides and humic materials, which was not readily bioavailable (McDowell and Sharpley, 2003); the NaOH extracted OP was calculated by subtracting P measured in undigested extracts from digested extracts (Reddy et al., 1999). After that, DDI water was used to wash the sediments to remove NaOH prior to HCl extraction to avoid the acid neutralization. Then the residues are extracted with 30 ml 1 M HCl for 3 h. Hydrochloric acid extracted less available or stable P or moderately recalcitrant P (Zvomuya et al., 2006), the HCl-Pi associated with Ca/Mg, the apatite minerals (Barbanti et al., 1994; Tchienkoua and Zech, 2003); the remaining residues are mostly insoluble or recalcitrant stable inorganic P forms such as apatite (Bostrom et al., 1982; Nair et al., 1995; Stone and English, 1993). Thereafter, the extracts were centrifuged and decanted as described above, and the supernatants were saved for TP analysis.

In each fraction, the SRP was determined by automated ascorbic acid method (ESS Method 310.1; USEPA, 1992). The TP in the extracts from $NaHCO_3$ and NaOH was analyzed by persulfate digestion block method (Method 365.4; USEPA, 2003). The difference between TP and SRP is considered as OP. Total P in the residues from the sequential extraction was determined using the modified ignition method (Andersen, 1976) followed by Method 365.1 (USEPA, 1983): digestion with 1 M HCl after oven dry at 105ºC then ignition at 550ºC in furnace. In this study, the TP was the sum of $NaHCO_3$-P, NaOH-P, HCl-P and residue-P.

4.2.3 Microbial P

The microbial P was estimated by incubating 2 g wet sediment with 4 ml chloroform ($CHCl_3$) in a vial with an air-tight cap for 24 h. Afterward, the tubes were left open in a Fume Hood for 24 h to let the $CHCl_3$ evaporate; the $CHCl_3$-treated residues were extracted with 30 ml 0.5 M $NaHCO_3$ for 16 h as described previously. The difference in $NaHCO_3$-SRP of $CHCl_3$ treated sediments and that of untreated sediments was considered as microbial P (Hedley et al., 1982; Pant and Reddy, 2001a).

4.2.4 Phosphorus mineralization experiments

Wet sediments (2 g) were incubated under flooding condition at 37ºC for 7, 15, and 30 d, and extracted with $NaHCO_3$, NaOH, and HCl sequentially as mentioned previously. The changes in $NaHCO_3$-P, NaOH-P, HCl-P, residue-P in the sediments during incubations at 37ºC for 7, 15, 30 d from without incubation were to determine the extent of P mineralization at physiological/optimum temperature.

Wet sediments (2 g) were also incubated under flooding condition at 37ºC for 7, 15, and 30 d, and treated with $CHCl_3$ for 24 h, thereafter the sediments were extracted with 0.5 M $NaHCO_3$. The differences in microbial P were estimated as described previously, i.e., the differences in the amount of $NaHCO_3$-P between $CHCl_3$ treated and untreated sediments.

4.2.5 Statistical analysis

All the experiments were carried out in triplicates and analyzed with SAS JMP Version 7.0 (SAS Institute Inc., 2008) using one-way analysis of variance (ANOVA). A Tukey's HSD (honestly significant difference) test was used for statistical differences at $p<0.05$

level as described by Tao et al. (2008). Similarly, correlation coefficients were tested using Pearson's correlation (r) significant test at the $p<0.05$ or $p<0.01$ level using SPSS Version.15.0 (SPSS Inc., 2006). Also, regression analyses were performed using the SPSS 15.0 software.

4.3 Results and discussion

4.3.1 Physico-chemical properties

In 2006, the EC of wet sediment samples ranged from 66 μs cm^{-1} at site 1 to 342 μs cm^{-1} at site 4. The two estuary sites 13 (1,917 μs cm^{-1}) and 14 (4,290 μs cm^{-1}) had much higher EC. In 2007, EC values ranged from 9 to 62 μs cm^{-1} in freshwater sites, and estuary sites 13 and 14 had 277 and 363 μs cm^{-1}, respectively; both were much lower than those in 2006. The pH of the sediments among the 15 sites ranged from 6.5 to 7.8 in 2006, and from 7.1 to 8.3 in 2007. Except for Site 12, all sites had slightly higher pH in 2007. The OM ranged 0.1-10.7 % in 2006 and 0.3-16.7% in 2007.

The TP in the sediments ranged from 288 to 1563 mg kg^{-1} in 2006 (Fig. 4-2), and from 219 to 5360 mg kg^{-1} in 2007 (Fig. 4-3). The average TP in sediments was higher in 2007 than 2006. The significantly higher TP concentrations were at sites 2, 4 and14 in 2006 and at 7,11,12,13 and 14 in 2007 (Table 4-1). The TP content in these sediments is appeared to be related to sediment particle sizes; the higher the TP content, the finer and muddier the sediments as reported in the past studies such as Fytianos and Kotzakioti (2005) and Vervier et al. (2009). The sites that had decreased TP values in 2007 were: sites 1, 2, 3, 4, 5, 7b, 8, 9, 10, and 13, and the sites that had increased TP values in 2007 were: sites 6, 7, 11, 12, and 14. It is known that TP content in river sediments could vary

Table 4-1 The sizes of the various P pools in the sediments, which were collected in 2006 and 2007. Values in the same column with different letters are significantly different (Turkey's HSD test, $p<0.05$)

site	Microbial P		Readily labile P		Moderately labile P		Stable P		Nonlabile P		TP	
			$NaHCO_3$-P		NaOH-P		HCl-P		Residue-P			
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
1	-72 ab	1 d	81 cde	11 cd	64 c	34 e	242 cdef	217 b	104 abc	44 c	492 cdef	306 b
2	-56 ab	3 d	82 cde	7 d	233 b	31 e	240 cdef	144 b	147 a	37 c	703 c	219 b
3	-27 ab	8 d	55 e	16 cd	90 bc	62 e	335 bc	291 b	25 bc	27 c	506 cde	396 b
4	-154 c	13 d	272 a	14 cd	647 a	52 e	512 a	205 b	132 ab	40 c	1563 a	311 b
5	-15 a	-3 d	38 e	10 cd	57 c	31 e	244 cdef	219 b	60 abc	58 bc	398 ef	318 b
6	-32 ab	14 cd	63 de	14 cd	59 c	62 e	140 f	270 b	26 bc	47 c	288 f	393 b
7	-13 a	34 bcd	37 e	29 bc	49 c	288 c	207 def	342 b	18 c	76 bc	312 ef	735 b
7b	-81 b	7 d	109 bc	16 cd	136 bc	53 e	237 cdef	231 b	41 abc	34 c	523 cde	335 b
8	-78 b	4 d	108 bcd	14 cd	139 bc	48 e	186 def	146 b	46 abc	31 c	479 def	239 b
9	-56 ab	5 d	71 cde	9 cd	115 bc	35 e	220 def	178 b	70 abc	35 c	477 def	257 b
10	-84 b	5 d	115 bc	11 cd	70 c	22 e	266 cde	183 b	19 c	30 c	470 def	255 b
11	-39 ab	82 a	61 e	24 bcd	48 c	576 a	246 cdef	284 b	21 c	167 a	376 ef	1051 b
12	-33 ab	51 abc	57 e	40 b	103 bc	97 de	168 ef	5143 a	145 a	120 ab	473 def	5360 a
13	-79 b	6 d	130 a	18 cd	159 bc	154 d	280 cd	396 b	94 abc	53 bc	663 cd	621 b
14	-162 c	63 ab	239 a	84 a	195 bc	407 b	431 ab	565 b	108 abc	50 bc	974 b	1106 b
mean	-67	18	101	21	144	130	264	588	70	57	580	793
ratio			1.4	1	2	6.2	3.7	27.8	1	2.7		
rank			3	4	2	2	1	1	4	3		

Readily labile P: $NaHCO_3$-P, moderately labile P: NaOH-P, Stable P: HCl-P, Nonlabile P: Residue-P, TP: total P

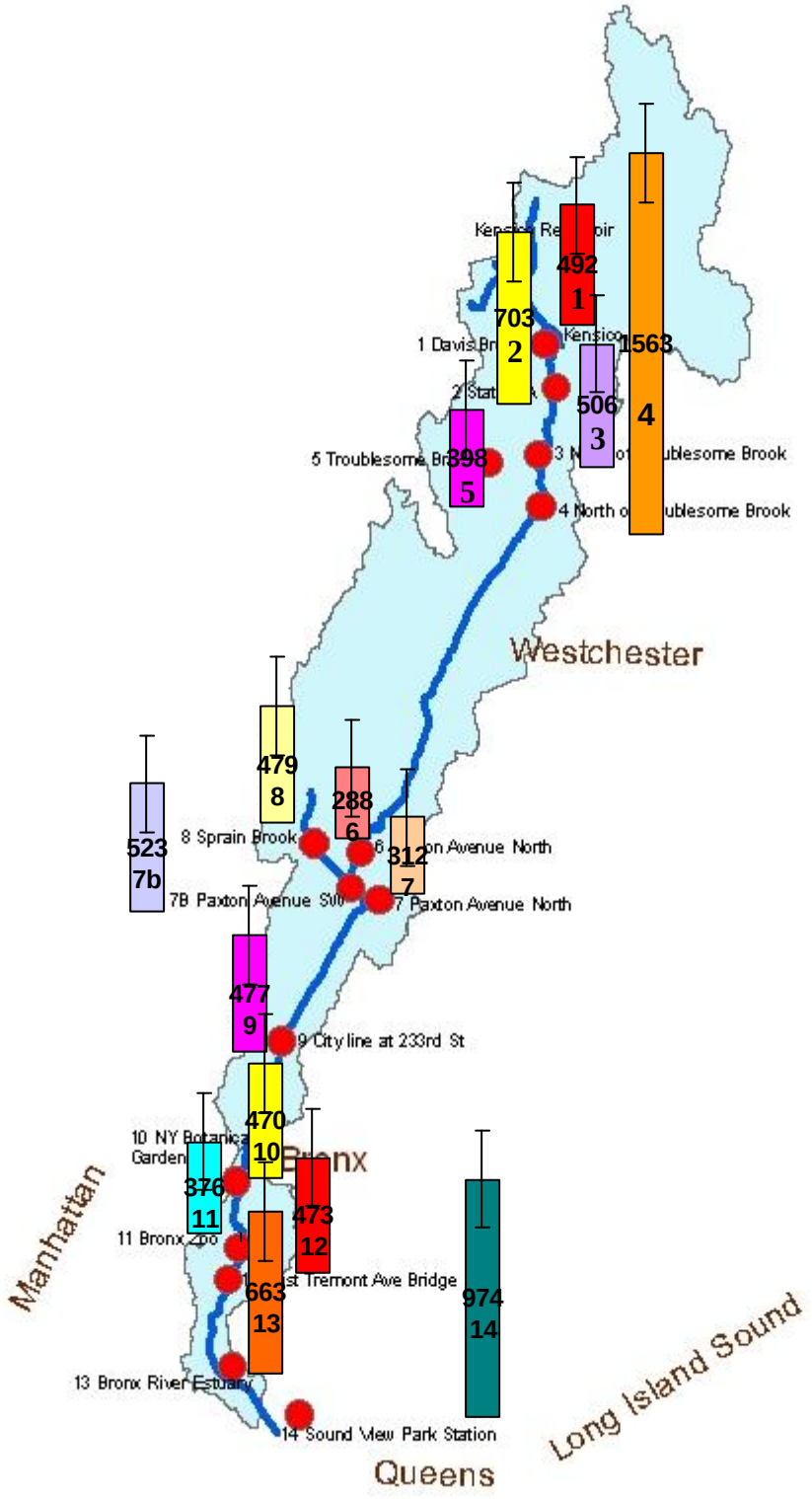


Figure 4-2 TP in sediment collected in 2006

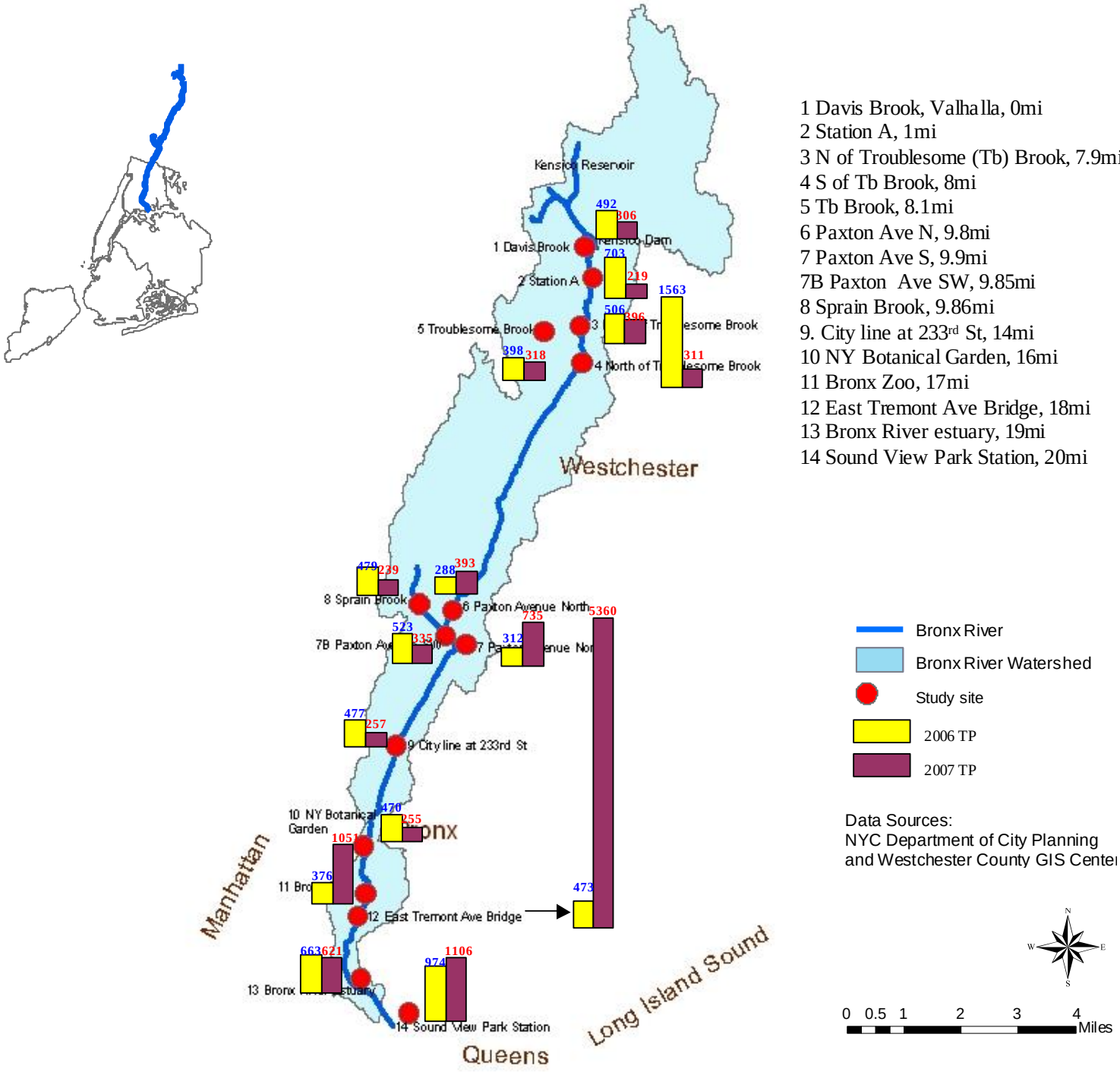


Figure 4-3 TP comparison of sediment collected in 2006 and 2007

highly. Vervier et al. (2009), reported that TP concentrations ranged from 269 to 465 mg kg^{-1} in hyporheic sediment and from 391 to 817 mg kg^{-1} in the finest sediments in Garonne River, France, and Owens and Walling (2002) observed that in the range of from 500 to1500 mg kg^{-1} for a fluvial sediment in a rural and industrialized river in the United Kingdom. Similarly, House and Denison (2002) found 700-3000 mg kg^{-1} of TP in river bed sediments at the Thames catchments. Also, McDaniel and David (2009) reported that TP in sediments from streams within two east-central Illinois agricultural watershed (the Embarras and the Vermilion) was up to 2,340 mg kg^{-1}, which is much higher compared to that of the Bronx River bed sediments. However, the median value of sediment TP in Illinois streams was only 360 mg kg^{-1} (McDaniel and David, 2009), which is much lower than the mean value of the Bronx River sediments (i.e., 580 mg kg^{-1} in 2006 and 793 mg kg^{-1} in 2007), indicating although TP in river sediments may vary widely, the levels of TP in the Bronx River bed sediments is in the high range, thus, could become a threat to the river ecosystems.

4.3.2 Phosphorus fractionation

4.3.2.1 Labile P pool

The labile P ($NaHCO_3$-P) values in 2006 sediments ranged from 37 to 272 mg kg^{-1} (Table 4-1) with a relative contribution of 10-25% (Fig.4-4) to TP, and had the highest value at site 4, the second highest at 14, and these two sites were not significantly different however the relative contribution at 14 of 25% was way higher than site 4 of 17%; the sites 11, 12, 3, 5 and 7 had the comparatively low mean values and those sites were not significantly different; in the Bronx Park region, site 10 at the New York Botanical

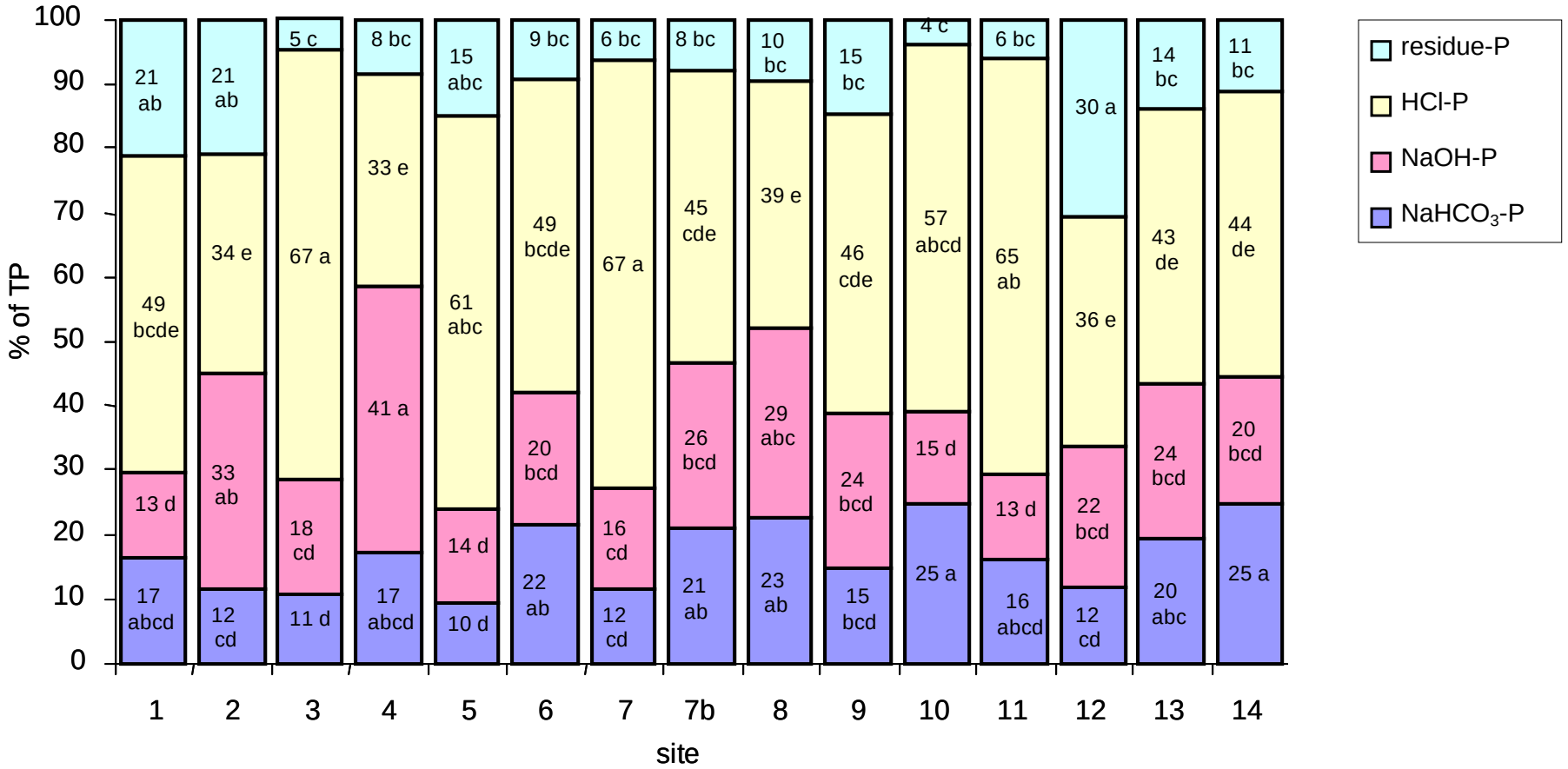


Figure 4-4 P distribution in the Bronx River bed sediments that were collected in 2006. Values in each fraction ($NaHCO_3$-P, NaOH-P, HCl-P, residue-P) with different letters are significantly different (Tukey's HSD test, $p<0.05$)

Garden (NYBG) had the highest P distribution, which was significantly higher from site 11 at the Bronx Zoo, and the estuary boundary site 12 at East Tremont Ave Bridge. The two estuary sites were significantly different from each other; site 14 at the estuary was significantly higher than site 13 at Sound View Park Station. The higher TP content sites had higher readily labile P, indicating potential higher supply of P for biological uptake.

The labile P concentrations ranged from 7 to 84 mg kg^{-1}, and the relative percentages of TP were ranged from 1 to 8% in 2007 (Fig.4-5). In sediments collected in 2007, the labile P concentrations of sites 7, 11, 12 and 14 were significantly higher than that of the other sites; sites 4, 7b, 8, 10 and 14 had higher percentage of labile P.

Generally sediments collected in 2006 had much higher labile P concentrations and represented higher percentage of TP than in sediments collected in 2007 (highest at 272 mg kg^{-1}, 25% and 84 mg kg^{-1}, 8% respectively; average at 101 mg kg^{-1}, 17% and 21 mg kg^{-1}, 4% respectively). The temporal differences in labile P in sediments collected in 2006 and 2007 could be related with the interactions between labile P and sediments during the precipitation/dissolution reactions with Fe, Al and Ca, resulting in P pool variations (Fox, 1989).

4.3.2.2 Moderately labile P pool

In sediments collected in 2006, the moderately labile P (NaOH-P) ranged from 48 to 647 mg kg^{-1} (Table 4-1) with a relative contribution of 13-41% of TP (Fig. 4-4). Site 11 had the lowest value and represented the lowest percentage of TP; site 4 had the highest

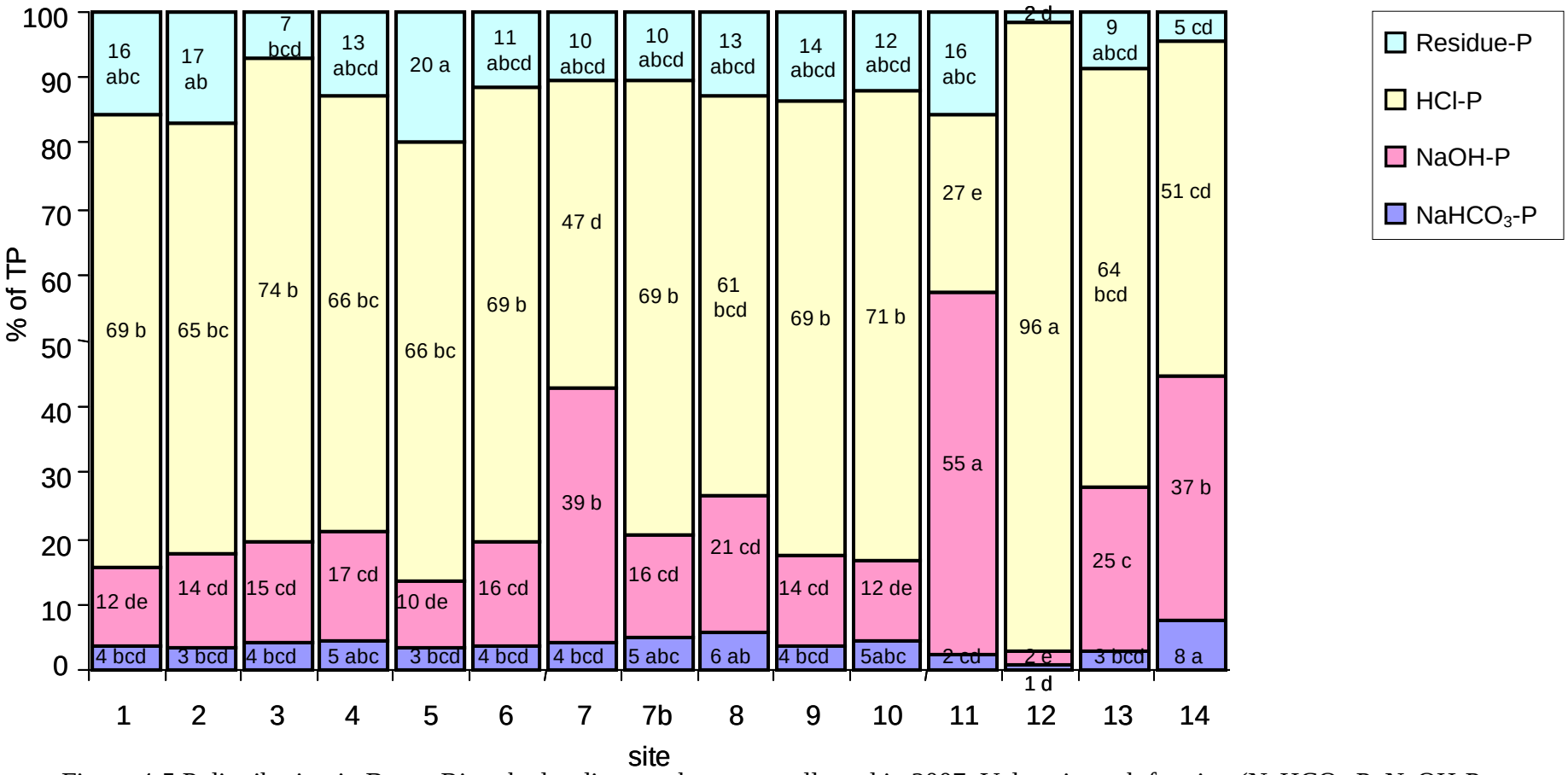


Figure 4-5 P distribution in Bronx River bed sediments that were collected in 2007. Values in each fraction ($NaHCO_3$-P, NaOH-P, HCl-P, residue-P) with different letters are significantly different (Tukey's HSD test, $p<0.05$)

NaOH-P concentrations as well as highest relative contribution to TP. The three sites 2, 4 and 14 had significantly higher moderately labile P concentrations than other sites, and sites 2 and 4 also had significantly higher NaOH-P relative contribution to TP. Meanwhile, sites 2, 4 and 14 had the highest total readily available and moderately available P values ($NaHCO_3$-P and NaOH-P). The bioavailable P, i.e., labile and moderately labile P, represented 24-59% of TP. This portion was found between 20-71% in stream sediments in Lake Okeechobee Basin (Reddy et al., 1995), between 13-53% in agricultural runoff (Sharpley et al., 1991); and around 42-44% in an agricultural creek sub-watershed (McDowell and Sharpley, 2003). The sediments particles in both sites 2 and 4 were apparently silty and clay type, very fine-grained and sticky, while sediments from site 14, an estuary site, were mixed with sandy and silty/sticky. The smaller particle size sediments tend to bound tighter with organic associated materials, such as Fe, Al oxides and hydroxides, resulting in higher NaOH-P values (Bostrom et al., 1982; Stone and Mudroch, 1989; Stone and English, 1993; Wang and Pant, 2010b). Similarly, such as microbial mineralization could also affect the OP content (Pant et al., 2002b).

In sediments collected in 2007, the moderately labile P concentrations were ranging from 22 to 576 mg kg^{-1} (Table 4-1), and the relative contribution was from 2-55% (Fig.4-5). Sites 7, 11, 13 and 14 had higher P values and represented a higher percentage of TP. Site 11 had the significantly higher moderately labile P (concentration of 576 mg kg^{-1} and P distribution of 55%) than the other sites followed by sites 7, 14, 13 with higher concentrations and relative proportion of TP than the other sites. The labile and moderately labile P ranged from 3-57% of TP. Sites 7, 11, and 14 had the highest P values and relative contribution of sum of $NaHCO_3$-P and NaOH-P. Meanwhile, the

sediments collected in sites 7, 11, and 13 were apparently composed of clay and silt, and sediments in site 14 were mixed with sandy and silty clay. As mentioned previously, the smaller particle tends to bind tighter organic matters associated with Fe, Al, Ca, and Mg (Stone and English, 1993). The P content increased as particle size decreased (Vervier et al., 2009). The sediments' texture appeared to be different in these two studied years in some of the sites, which might have been caused by the sediment transports and depositions and assimilation of temporal variations, besides the specific sampling locations could be slightly different in 2006 from 2007.

4.3.2.3 Stable (non-labile) P pool

In sediments collected in 2006, the stable P or moderately recalcitrant P (HCl-P) was ranged from 140 to 512 mg kg^{-1} (Table 4-1) with a relative contribution to TP of 33-67% (Fig.4-4). Site 4 had the highest value, followed by site 14, while the lowest value was at site 6. Site 10 and 11 had similar mean value. The estuary site 14 had significantly higher value than site 13. Sites 7 and 3 had the highest relative contribution to TP, and site 4 had the lowest relative contribution.

In 2007, the stable P concentrations were in a range from 144 to 5143 mg kg^{-1} (Table 4-1), and the contribution to TP was ranged from 27 to 96% (Fig.4-5). There were no significant differences in mean HCl-P values other than site 12 had significantly higher value than the all other sites. The HCl-P of Site 12 also represented the highest proportion of TP (96% TP), while HCl-P of Site 11 represented the lowest proportion of TP (27%). There were highest values of oxalate extracted Ox-Fe (14,274 mg kg^{-1}), HCl extracted HCl-Ca (31,464 mg kg^{-1}), HCl-Mg (5,371 mg kg^{-1}) and S_{max} (476 mg kg^{-1}) and fairly

high value of Ox-Al (416 mg kg^{-1}) in the sediments at site 12 that were collected in 2006, indicating the sediments had very high P sorption capacity (Wang and Pant, 2010b). Phosphorus precipitation as calcium phosphate is an important retention process (Reddy et al., 1999). Sediments accumulated P and Ca through precipitation of calcite and co-precipitation of P (House and Denison, 1997). The high sorption capacity sediments could adsorb more P whenever P source is available (Pant and Reddy, 2001b), and those P was not extractable by $NaHCO_3$ and NaOH but extractable with HCl. Perhaps, that is why sediments collected in 2007 from this site had extremely high HCl-P. Moreover, HCl-P fraction usually represents high percentage of TP depending on the sediment characteristics along with anthropogenic inputs. McDowell and Sharpley (2003) reported HCl-P representing 48-85% of TP in an agricultural stream environment.

4.3.2.4 Recalcitrant P pool

The recalcitrant P (residue-P) in the sediments collected in 2006 was ranged from 18 to 147 mg kg^{-1} (Table 1). Site 2 had the highest recalcitrant P, while Site 7 had the lowest. Site 12 had significantly higher value than Bronx Park sites 10 and 11. The estuary sites 13 and 14 had the close mean values without a significant difference. In sediments collected in 2006, the relative contribution of recalcitrant P to TP ranged from 4% at site 10 to 30% at site 12 (Fig.4-4). Similarly, sediments collected in 2007, sites 11 and 12 had higher concentrations of recalcitrant P, however, sites 2, 5 and 11 had higher relative proportions of TP, plus site 12 had the lowest relative proportion (Fig.4-5). The recalcitrant P in sediments collected in 2007 ranged from 27 to 167 mg kg^{-1}, and representation to TP was from 2-20%. McDowell and Sharpley (2003) also reported

somewhat similar proportion of residual P fraction of 30-36% TP in an agricultural creek environment. In general, it is indicative that relatively lesser proportion of non-bioavailable P in the river bed sediments than the potentially bioavailable P.

4.3.3 Microbial P

The 2006 sediment showed negative microbial P (MP) values (Table 4-1), ranging from –13 to -162 mg kg^{-1}. The higher negative values appeared at site 4 (-154 mg kg^{-1}) and site 14 (-162 mg kg^{-1}) that were significantly different from other sites. Sites 7 and 5 had the least negative values (-13 mg kg^{-1} and –15 mg kg^{-1}, respectively). The reason might be the microorganisms were different types, which could not be paralyzed by $CHCl_3$, thus, the resistant microorganisms continue to proliferate, in turn, take up the available P, resulting in negative values. Since the Bronx River is an urban river, it is likely to receive, occasionally, microorganisms that are resistant to cell lysis/inhibition, especially from WWTP. These appeared to be an interesting results warranting further research.

In sediments collected in 2007, the microbial P concentrations were ranged from -3 to 82 mg kg^{-1} (Table 4-1), and contributions to TP were ranged from 0-8%. The microbial P was significantly higher at the tributary of Sprain Brook (Site 7; 34 mg kg^{-1}), the site at Bronx Zoo (Site 11) had 82 mg kg^{-1}, East Tremont Ave Bridge (Site 12)-the boundary between fresh and saline water with 51 mg kg^{-1}, and the Hunts Point estuary (Site 14) of 63 mg kg^{-1}, and other than 12, all these sites had proportion of microbial P (% of TP) as well.

The regression analysis showed that sediment microbial P was correlated with sediment $NaHCO_3$-P (r=0.597), NaOH-P (r=0.872), as well as with residue-P (r=0.886).

In sediments collected in 2006, the P fractionation results showed that the largest fraction was HCl-P (33-66% of TP) and the smallest fraction was residue-P (4-30%), whereas in the sediments collected in 2007, the smallest fraction was $NaHCO_3$-P (1-8%). The average P pool rank order of the sediments collected in 2006 was HCl-P > NaOH-P > $NaHCO_3$-P > residue-P, and the relative proportions were 3.7: 2.0:1.4:1. The rank order for the sediments collected in 2007 was HCl-P > NaOH-P > residue-P > $NaHCO_3$-P, with their relative proportion of 27.8:6.2:2.7:1. Overall, the labile and moderately labile P contributed to 24-59% and 3-57% of TP in sediments collected in 2006 and 2007, respectively. The differences in anthropogenic inputs along the river may have resulted in the spatial variations (Carman et al., 2000) in the sizes of the P pools. Similarly, the changes in anthropogenic and natural sources such as land use (Owens and Walling, 2002), and local hydro-climatic conditions could result in temporal variations in the P pools (McConnell, 1980; Withers and Jarvie et al., 2008).

4.3.4 Phosphorus mineralization

The $NaHCO_3$-P was significantly decreased from 0 to 7 d incubation period that might be related with the microbial uptake of readily labile P during the initial period of incubation, and there was no significant difference among 7, 15, and 30 d incubation (Fig. 4-6). The NaOH-P was significantly increased from 0 to 7 d, but no significant differences were observed among that of 7, 15 and 30 d. The corresponding increased in moderately labile P (NaOH-P) and decreased in readily labile P ($NaHCO_3$-P), perhaps, an indicative of initial microbial proliferation during the first week of incubation. The HCl-P did not change during the entire incubation period, while residue-P was significantly

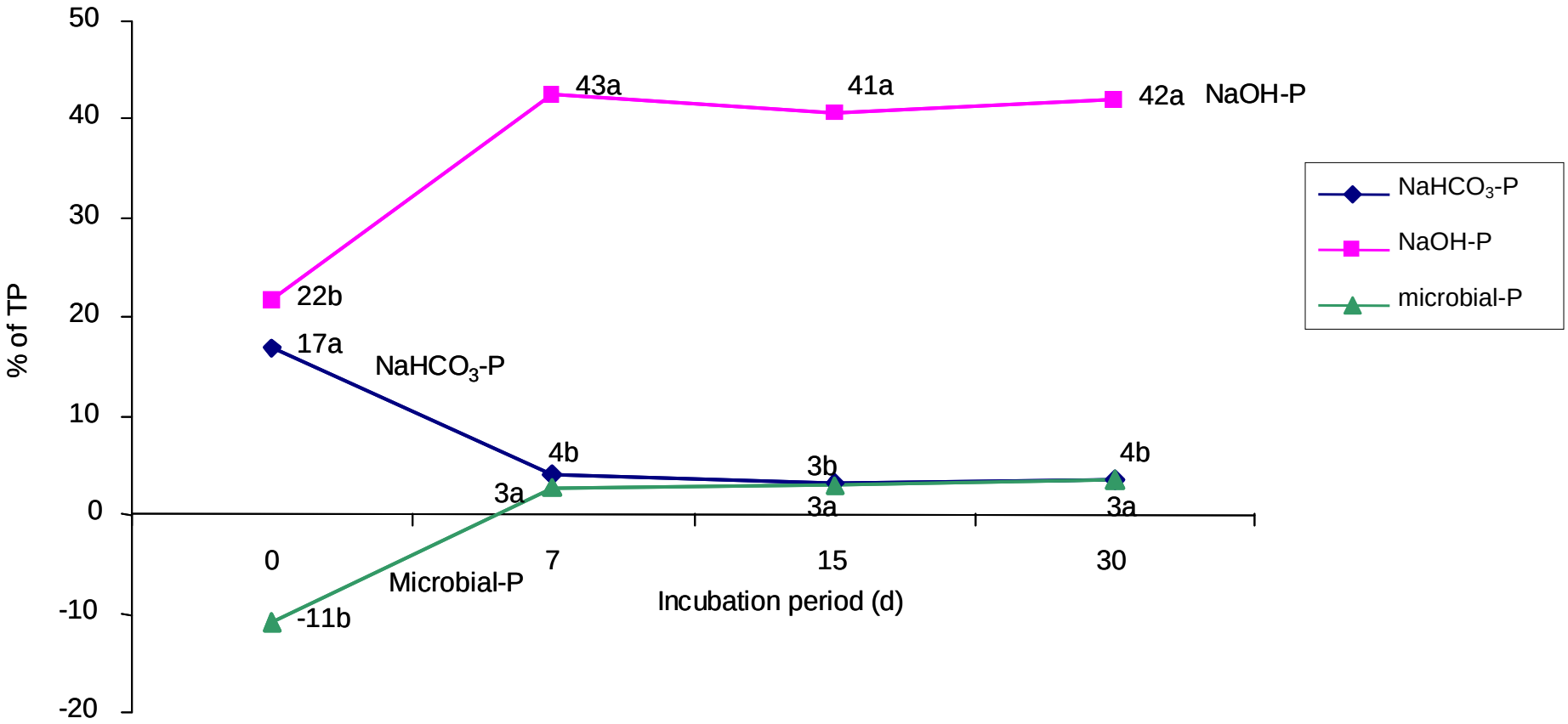


Figure 4-6 Mineralization of organic P in the river sediments at physiological temperature during various incubation periods (data for sediments collected in 2006). Values in each fraction during incubation ($NaHCO_3$-P, NaOH-P, microbial P) with different letters are significantly different (Tukey's HSD test, $p<0.05$)

decreased from 0 to 7 d incubation, and no significant differences were obtained between 7 and 15 d. Although increased in microbial P was observed during the initial phase, no significant differences were obtained at 7, 15 and 30 d incubations.

In sediments collected in 2007, microbial P increased from 0 to 7 d, indicating initial microbial proliferation, and then somewhat decreased from 7 to 15 d, and no significant differences between 15 to 30 d (Fig. 4-7). The $NaHCO_3$-P was increased from 0 to 7 d, and then decreased from 7 to 15 d remaining the same as 30 d; which was a different pattern from 2006 data, the SRP could be released from microbial activity during mineralization. Surridge et al. (2007) found the SRP increased during flooding incubation of river sediments, as well. The NaOH-P distribution increased from 0 to 15 d, and there was no significant change between 7 and 15 d, and 15 and 30 d. The HCl-P decreased from 0 to 15 d, no significant difference between 7 and 15 d, after that remained stable from 15 to 30 d. The proportion of residue-P remained the similar without significant difference. The increase of the microbial P from 0 to 7 d was accounted for the proportion increase of $NaHCO_3$-P and NaOH-P, and the proportion decrease of HCl-P. As mentioned earlier, the high HCl-P concentrations and proportions in 2007 were caused by high sorption capacity in the sediments that adsorb P whenever the P source is available (Lin et al., 2009; House and Denison, 1998). If the SRP in the water column decreased, and SRP < EPC_0 in the water column, then sediment release SRP to the water column, and the bioavailable P increased (Kunishi et al., 1972; Wang et al., 2009). The temporal differences in P pools in sediments collected during 2006 and 2007 may have caused by differential P mineralization and uptake by microorganisms (Hedley et al.,

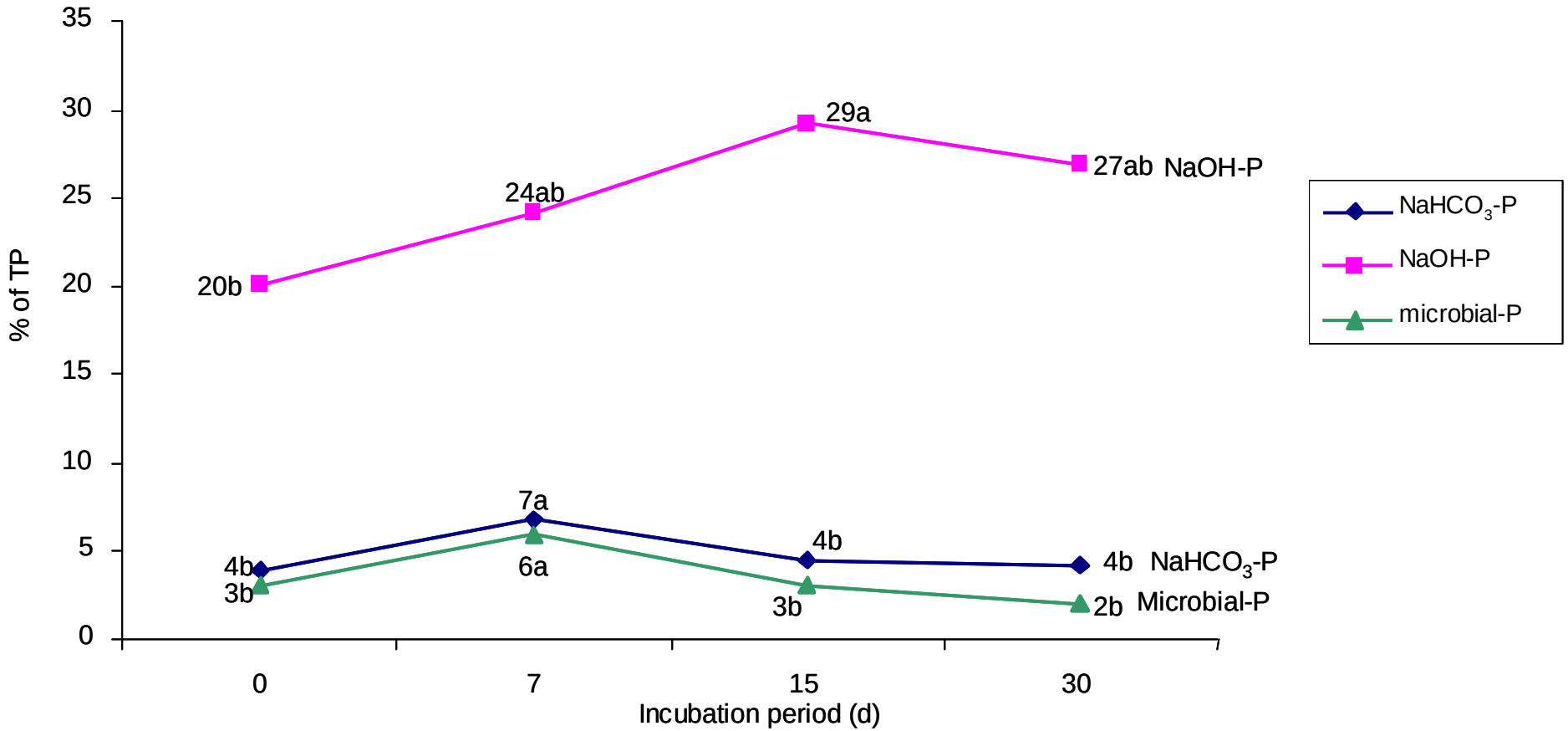


Figure 4-7 Mineralization of organic P in the river sediments at physiological temperature during various incubation periods (data for sediments collected in 2007). Values in each fraction during incubation ($NaHCO_3$-P, NaOH-P, microbial-P) with different letters are significantly different (Tukey's HSD test, $p<0.05$)

1982; Pant et al., 2002b), land use changes (Owens and Walling, 2002; Wang and Pant, 2009), and/or P associated sediments transport, deposition and assimilation (Withers and Jarvie, 2008). Similarly other factors such as anthropogenic, e.g., gas spill, raw sewer discharge (Kronvang et al., 1997; Owens and Walling, 2002; Withers and Jarvie et al., 2008), CSOs, sewer overflow from Hunts Point WWTP (House and Denison, 1997; Huaxin et al., 1997), as well as localized hydro-climatic changes could alter the relative composition of various P pools river bed sediments (Wang and Pant, 2009, 2010a, b).

4.4 Conclusion

The Bronx River sediments showed the spatial and temporal variations on P fractionation. In general, the stable P pool was the largest portion followed by moderately labile P, readily labile P and residue-P in the bed sediments during the study period. It appears that sediment transport, deposition and assimilation, different anthropogenic factors such as land use changes, localized hydro-climatic changes such as temperature, precipitation variations would account for the spatial and temporal variations in P distribution and fractionation in river sediments. The regression analysis indicated that the microbial P values were significantly correlated with $NaHCO_3$-P (r=0.597), NaOH-P (r=0.872), and residue-P (r=0.885), suggesting that a large percentage (up to 88.5%) of variability in microbial P could be explained by the sizes of the various P pools. The research is ongoing to further investigate bioavailability of various P pools in the river sediments, and estimate their potential detrimental effects in water quality, as well as in the river management.

Chapter 5

Enzymatic Hydrolysis of Organic Phosphorus in the Bronx River Bed Sediments

Abstract

Enzymatic hydrolysis of phosphorus (P) in bed sediments is an important process that maintains bioavailable P in the river systems. The P bioavailability is the criteria for assessing the eutrophication potential in rivers and streams. The objective of this research is to determine potential bioavailability of organic P (OP) in the Bronx River bed sediments using native phosphatases (NPase) and phosphodiesterase (PDEase) hydrolysis. The bed sediments collected in summer 2006 and 2007 were incubated at 37ºC for 6 h at pH 7.5 with NPase. The results showed that NPase hydrolyzed substantial amount of OP (up to 76%) under favorable temperature and pH, indicating OP could be hydrolyzed under increased temperature, in turn, increase in P availability in the river. Similarly, the sediments incubated with PDEase under 37ºC at pH 8.8, the results showed that up to 82% of OP could be hydrolyzed. Strong correlations between percentage of OP hydrolyzed by PDEase and organic matter (OM) were observed for sediments collected in 2006 ($r = 0.745$; $p \leq 0.01$) and 2007 ($r = 0.724$; $p \leq 0.01$), indicating PDEase hydrolysable P is mainly associated with OM. It is indicative that local hydro-climatic changes such as temperature increase and pH variation could hydrolyze substantial amount of OP and increase bioavailable P in water column, resulting in potential threat to the river ecosystems.

5.1 Introduction

Phosphorus (P) is a primary limiting nutrient in freshwater systems (Hecky and Kilham, 1988; Pant and Warman, 2000; Monbet, et al., 2009). Plants can uptake inorganic P (IP) directly (Monbet al., 2009), whereas OP could become bioavailable to plants only after enzymatic hydrolysis to IP (Pant et al., 1994a, b; Pant and Warman, 2000). The enzymatic hydrolysis of P-ester is an important step in P cycle, which is relevant to bioavailabe P to plants (Adams, 1992).

The total phosphorus (TP) is composed of 20-80% OP; on an average OP is around 50% in nature (Wetzel, 1999), and OP is critical on P cycling (Oehl et al., 2004; Fransson and Jones, 2007; Wang and Pant, 2009). The OP cycling relates to the mineralization to IP, and IP transport relates to uptake and release by plants and algae in water environment (Wetzel, 1999). The major OP compounds are inositol phosphates, phospholipids and nucleid acids (Anderson, 1967). Inorganic P comprises crystalline and amorphous forms of P and phosphate in adsorbed, free or complexed forms (Quiquampoix and Mousain, 2005). Plants uptake P as phosphate ions ($H_2PO_4^-$ and HPO_4^{2-}), determined by the OP mineralization or degradation (Quiquampoix and Mousain, 2005). The bioavailable OP is associated with the phosphatases, microbial capacity of uptake and assimilation and the sorption strength and amount (Berg and Joern, 2006).

Alkaline phosphatase hydrolyzes phosphomonoester (Reid and Wilson, 1971), and adenosine triphosphatase (ATPase) hydrolyzes adenosine triphosphate (ATP) (Pant et al., 2002b). Alkaline phosphatase is a primary enzyme that hydrolyzes OP in an alkaline environment (He et al., 2009). Both phosphomonoesterase (PMEase) and phospodiesterase (PDEase) hydrolyze P-esters such as nucleic acids and phospholipids

(Christmas and Whitton, 1998; Toor et al., 2003; Ellwood et al., 2008). Phosphatase enzymes regulate OP in soil and sediment, hydrolyze OP and release phosphate in a bioavailable form. Phosphodiesterase releases a phosphate monoester that could be hydrolyzed by PMEase to release free phosphate bioavailable for uptake by plants (Pant and Warman, 2000). The optimum pH for soil NPase is between pH 6 to 8 (Dick and Tabatabai 1984). Acid and alkaline phosphatases have their optimum activities in acidic and alkaline pH, respectively (Tabatatai, 1982). In most soils, the optimum pH for phosphodiesterase is pH=8 (Bowman and Tabatabai, 1978). Phosphatases, however, could be inhibited by different factors such as fluoride; metal ions including silver, zinc, mercury, copper, iron and manganese; polyvalent anions including phosphate, molybdate and arsenate (Quiquampoix and Mousain, 2005).

It is known that enzymes participate in nutrient cycling as catalysts to facilitate and increase the reaction rates but not undergo the chemical reactions, and enzymes behave differently depending on different pH, temperature and ionic strength (Tabatabai, 1982). Phosphatases catalyze hydrolytic reaction of the OP esters releasing IP (Jasson et al., 1988; Crouse et al., 2002; Monbet et al., 2007 and 2009). Organic P is mineralized by various extracellular phosphatases including alkaline phosphatase (APase) and phosphodiesterase (PDEase) that originate from plant roots, fungi, or bacteria, and those phosphatases exist in waters, sediments or soils either soluble or insoluble forms (Pant and Warman, 2000; Pant et al, 2002b). It is, therefore, enzymatic hydrolysis using phosphatases (alkaline phosphatase, phosphodiesterase, and phytase) is an important tool on characterization of dissolved organic phosphorus (DOP) pool (Pant et al., 2002a). Moreover, phosphatases are also used for sewage treatment to hydrolyze the

enzymatically available fraction of DOP to IP before chemical precipitation following the aluminum sulfate addition (Monbet et al., 2007), and P bioavailability is the criteria for assessing the eutrophication potential in rivers and streams (Maynard et al., 1999). Thus, determining potential bioavailability of P compounds using different phosphatase-induced hydrolyses is crucial. The objectives of the study were to assess the capacity of the NPase to hydrolyze organic P in the Bronx River sediments, as well as to estimate the potential hydrolysis of organic P using commercial PDEase.

5.2 Materials and Methods

5.2.1 Sediment physico-chemical analysis

A Mettler Toledo InLab 730 conductivity electrode was used to determine the electrical conductivity (EC) and a Mettler Toledo InLab413 pH electrode was used to determine the pH of the sediments.

5.2.2 Sediment extraction and phosphorus analysis

2 g wet sediment was extracted with 30 ml 0.5 M NaOH for 16 h by shaking in an end-over-end shaker at 25$\pm$2ºC, 230 l/min. The suspensions were centrifuged at 21$\pm$2ºC, 10,000 X *g* for 10 min after each extraction, then supernatants were decanted into 25 ml vials. The TP in the NaOH sediment extract (NaOH-TP) was determined by persulfate digestion method using digestion block (Method 365.4, USEPA, 2003). After the digestion process, NaOH-TP and the NaOH sediment extracts SRP were determined using automated ascorbic acid method (ESS Method 310.1, USEPA, 1992). The NaOH-OP referring to unreactive P was calculated by subtracted SRP from TP.

5.2.3 Enzymatic hydrolysis

5.2.3.1 Native phosphatases incubation

Took 0.1 ml NaOH sediment extracts, added 3.1 ml Tris(Tris(hydroxymethy-aminomethane)-HCl buffer solution (pH 7.5), plus 1 ml DDI (de-ionized distilled water) and 0.02 ml toluene were added as well. The vials were then incubated at 37ºC for 6 h. After that, the SRP in the solutions was measured by the automated ascorbic acid method as described previously. Toluene had been widely used to inhibit microbial activity, and it is necessary for enzyme incubation process (Tabatatai, 1982). The amount of NPase hydrolyzed OP was calculated by subtracting the SRP measured in the sample without incubation from that of the sample incubated with the NPase. The percentage of OP hydrolyzed by NPase was calculated to estimate the potential capability of NPase to hydrolyze OP at favorable/increased temperature.

5.2.3.2 Phosphodiesterase incubation

Phosphodiesterase l, Type IV from Crotalus atrox, (one unit hydrolyzing 1.0 μmol of bis-ρ-nitrophenyl phosphate per min at pH 8.8 and at 37ºC), was used for the study. The pH 8.8 buffer solution was prepared with tris-HCl. The PDEase was prepared by 25 mg of PDEase dissolved in 60 ml of pH 8.8 buffer (0.02 units of activity per mg solid of PDEase). In 0.1 ml NaOH sediment extracted sample, 1 ml PDEase (0.04 unit per sample) in pH 8.8 buffered solution, and 3.1 ml pH 8.7 buffered solution, plus 0.02 ml toluene were added, incubated at 37ºC for 6 h. The amount of PDEase hydrolyzed OP during the 6 h incubation was calculated by subtracting the SRP measured in the sample without incubation from the sample incubated with enzymes.

5.2.4 Statistical analyses

The data was analyzed by SAS JMP Version 7.0 (SAS Inc., 2008) statistical software for one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test at $p<0.05$ level were used. All the analyses were carried out in triplicate, and data were normally distributed ($p<0.01$) unless otherwise stated. Pearson correlation coefficients (r values) at $p\leq0.01$ and $p\leq0.05$ were used to determine whether there was significant correlations between NPase and PDEase hydrolyzed P, and OM, OP, and Ox-Al using SPSS v.15 for Windows (SPSS Inc., 2006).

5.3 Results and discussion

5.3.1 P hydrolysis by phosphodiesterase and native phosphatases in sediments collected in 2006

The 2006 data showed that PDEase hydrolyzed greater percentage of OP than NPase in most sites (Fig. 5-1). However, three sites 11, 13 and 14 had higher NPase% (% of NPase hydrolyzed OP) than PDEase% (% of PDEase hydrolyzed OP), and this is different from the general pattern that PDEase% was higher than NPase%. The NPase in the sediments might be partially different from PDEase, e.g., the sediments may have PDEase as well as PMEase, which could hydrolyze P-monoesters, including the byproducts of PDEases (Pant and Warman, 2000), resulting in higher percentage of OP hydrolyzed by NPase than that by PDEase (Wang and Pant, 2010a).

The amount of PDEase hydrolysis on 2006 bed sediments (Fig.5-1) ranged from 7 to 82% of OP, varied considerably between different sites along the river. The highest PDEase hydrolyzed P percentage in 2006 sediment NaOH extracts showed the highest

Table 5-1 Selected physico-chemical characteristics of the bed sediments collected in 2006 and 2007 (Enzyme)

Site	PDEase-P		Npase-P		OP		TP		OP/TP		OM		pH	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
	mg kg^{-1}								%					
1	42 cde	14 d	41 bc	83 b	182 def	472 bc	315 g	621 cd	58 abc	70 ab	0.7	1.0	7.1	7.2
2	68 bcde	73 abcd	0 c	21 b	401 b	168 bc	909 b	309 cd	44 bcd	45 ab	3.3	0.5	6.8	7.5
3	13 e	33 cd	0 c	39 b	183 def	85 c	430 ef	266 d	43 bcd	32 ab	1.9	1.2	6.6	7.4
4	352 a	76 abcd	170 a	67 b	1377 a	193 bc	2730 a	325 cd	51 abcd	54 ab	10.7	1.1	6.5	7.3
5	25 de	13 d	21 bc	24 b	108 gh	173 bc	254 h	315 cd	43 bcd	54 ab	0.1	0.3	7.6	7.9
6	54 cde	26 d	44 abc	41 b	195 def	893 abc	392 f	1075 bc	50 abcd	78 a	0.1	0.8	7.3	7.7
7	70 bcde	237 a	56 abc	95 b	86 h	1667 a	138 I	2469 a	62 a	67 ab	0.3	3.5	6.8	7.1
7b	137 b	53 bcd	84 abc	37 b	397 b	288 bc	678 c	478 cd	58 ab	56 ab	1.7	1.0	6.6	7.4
8	101 bcd	45 bcd	49 bc	34 b	224 cde	237 bc	479 e	409 cd	47 abcd	54 ab	1.0	1.1	7.1	7.8
9	40 cde	83 abcd	34 bc	30 b	169 ef	350 bc	314 g	448 cd	54 abcd	72 a	1.7	0.6	6.8	7.7
10	118 bc	89 abcd	91 abc	32 b	238 cd	121 bc	552 d	212 d	43 bcd	55 ab	3.7	0.3	7.0	8.2
11	33 de	216 abc	44 bc	495 a	94 h	644 bc	243 h	3080 a	39 d	21 b	3.2	6.7	6.8	7.1
12	26 de	15 d	13 bc	13 b	92 h	337 bc	251 h	648 cd	37 d	51 ab	3.8	1.1	7.9	7.1
13	35 de	226 ab	110 abc	46 b	159 fg	943 ab	394 f	1637 b	40 cd	57 ab	4.9	16.7	7.8	8.2
14	91 bcde	63 abcd	122 ab	32 b	274 c	808 bc	659 c	1595 b	41 bcd	48 ab	3.6	3.4	7.8	8.3

Values in the same column with different letters are significantly different (Tukey's HSD test, $p \leq 0.05$)

PDEase-P: PDEase hydrolyzed OP, mg kg^{-1}

Npase-P: Native phosphatases hydrolyzed OP, mg kg^{-1}

OP: organic P, mg kg^{-1}

TP: total P, mg kg^{-1}

OP %: OP/TP%

OM: organic matter, %

value at site 7, followed by sites 10, 8 and 7b; site 3 is the lowest one. The enzymatic hydrolysis of OP by PDEase in natural rivers contributes to eutrophication of freshwater systems (Monbet et al., 2007; Wang and Pant, 2010a).

The 2006 data indicated that NPase hydrolyzed percentage of OP ranged from 0 to 68% (Fig. 5-1); site 13 in the estuary had the highest NPase%, followed by sites 7, 11, 14 and 10. There was no NPase hydrolyzed OP in sediments from sites 2 and 3. The OP constitutes 37 to 62% of TP (Table 5-1), and significant native/inherent phosphatase activities hydrolyzed OP into IP when bed sediments were incubated at 37ºC (without any addition of phosphatases), showing that OP could be hydrolyzed when temperature increased by hydro-climatic changes, in turn, the increased bioavailable P transport in the water column and resulting in potential threat to the river ecosystems (Pant et al., 1994 a, b; Toor et al., 2003; Wang and Pant, 2010a).

Site 7, a tributary site at Sprain Brook, had relative higher values of both PDEase% and NPase %. Sites 10, 11 and 14 were all had considerably higher values for PDEase% and NPase%. The results indicated that the tributary, the zoo, the garden and the estuary area all had the potential to release P under favorable temperature and pH conditions (Wang and Pant, 2010a). Sites 2 and 3 had the lowest PDEase% and NPase%, indicating that the substantial amount of OP left not being hydrolyzed by NPase or PDEase, suggesting a substantial portion of the P pool was inaccessible to phosphatases/enzymes to be hydrolyzed at these sites (Wang and Pant, 2010a).

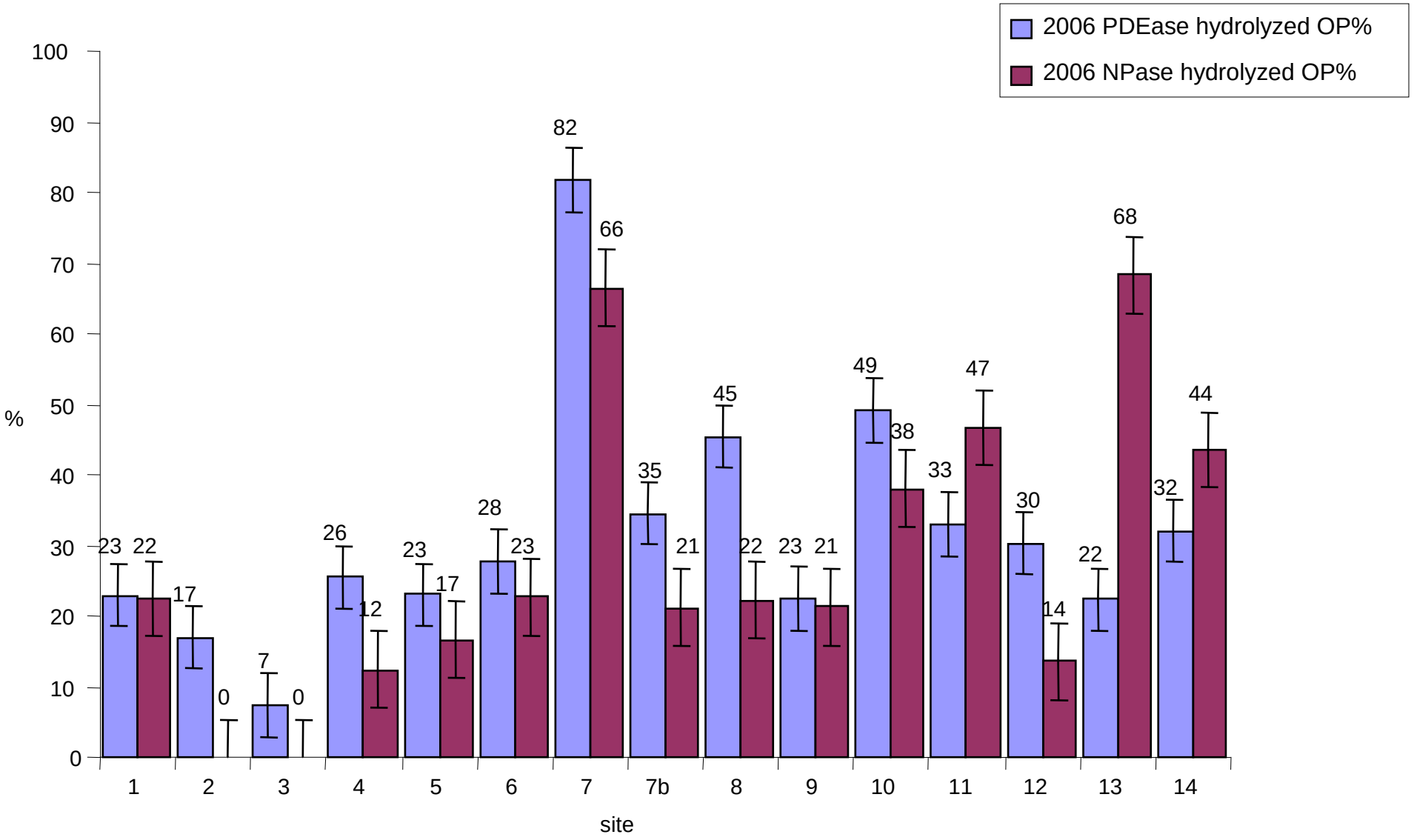


Figure 5-1 PDEase hydrolyzed OP vs. NPase hydrolyzed OP in sediments collected in 2006 (%)

5.3.2 P hydrolysis by phosphodiesterase and native phosphatases in sediments collected in 2007 and the temporal variations from 2006 data

Sediments collected in 2007, similar as in the sediments collected in 2006, there were10 sites had the pattern of PDEase% > NPase%, one site (Site 6) PDEase%=NPase% and 4 sites (sites 1,3,5,11) PDEase%<NPase% (Fig. 5-2). The percentage of OP in 2007 ranged from 21 to 78% of TP. The PDEase% of 2007 ranged from 3-81% (Fig. 5-2). Site 10 had the highest at 81%, significantly higher than all other 14 sites; followed by sites 4, 9, 3, and 8. Sites 5, 14, 12, 6 and 1 had the PDEase% significantly lower.

Compared with the PDEase% in 2006, there was a different pattern in 2007. The maximum PDEase% of 82% of Site 7 in 2006, and 81% of site 10 in 2007 were observed (Fig. 5-3). Those values significantly higher in 2006 than in 2007 were: sites 1, 5, 6, 7, 7b, 12 and 14 (Fig. 5-3); PDEase% significantly lower in 2006 than 2007 were: sites 2, 3, 4, 9 and 10; no significant differences in PDEase% were observed at sites 8, 11 and 13. The temporal variations could be caused by P transport along the river (Wang and Pant, 2009, 2010b). The average PDEase% in 2006 was 32%, while that was 27% in 2007; both were higher than the relative PDEase activity of 23% observed by Gibson and Mitchell (2005) in extracellular fungi culture after incubation at 20°C for 14 d. In general, our study indicated that the PDEase hydrolysable OP in the Bronx River constitutes a substantial component of the bioavailable P pool (Wang and Pant, 2010a).

The NPase% of 2007 ranged from 3 to 76% (Fig. 5-2). Site 11 had significantly high value of 76%, followed by sites 4, 3, 8 and 10; sites 13, 6, 14 and 12 had the lowest values. The highest NPase% in 2006 was at sites 13 and 7. The NPase% had significantly higher values in 2006 than 2007 at sites 6, 7, 10, 12, 13 and 14; NPase% were lower in

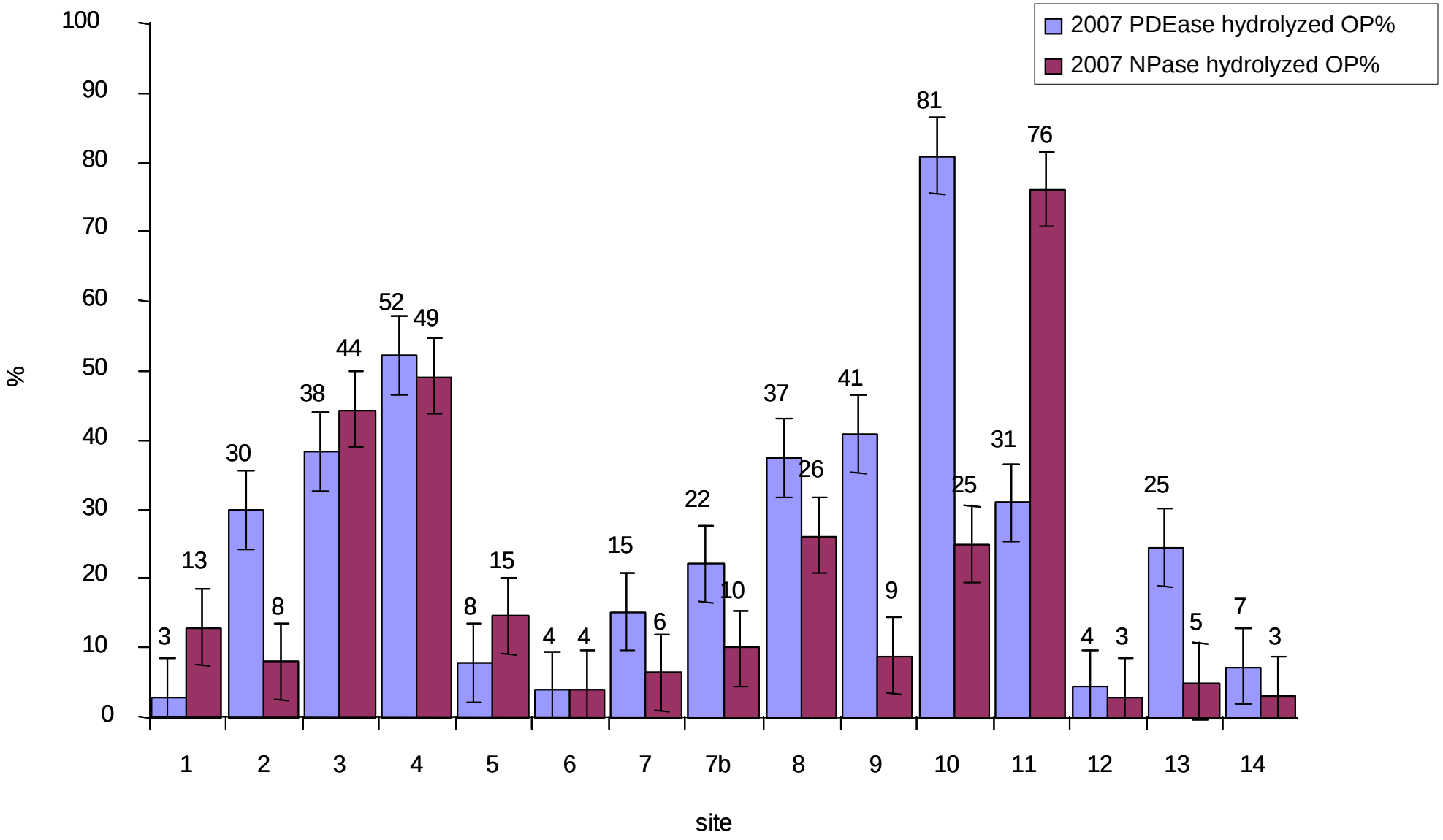


Figure 5-2 PDEase hydrolyzed OP vs. NPase hydrolyzed OP in sediments collected in 2007 (%)

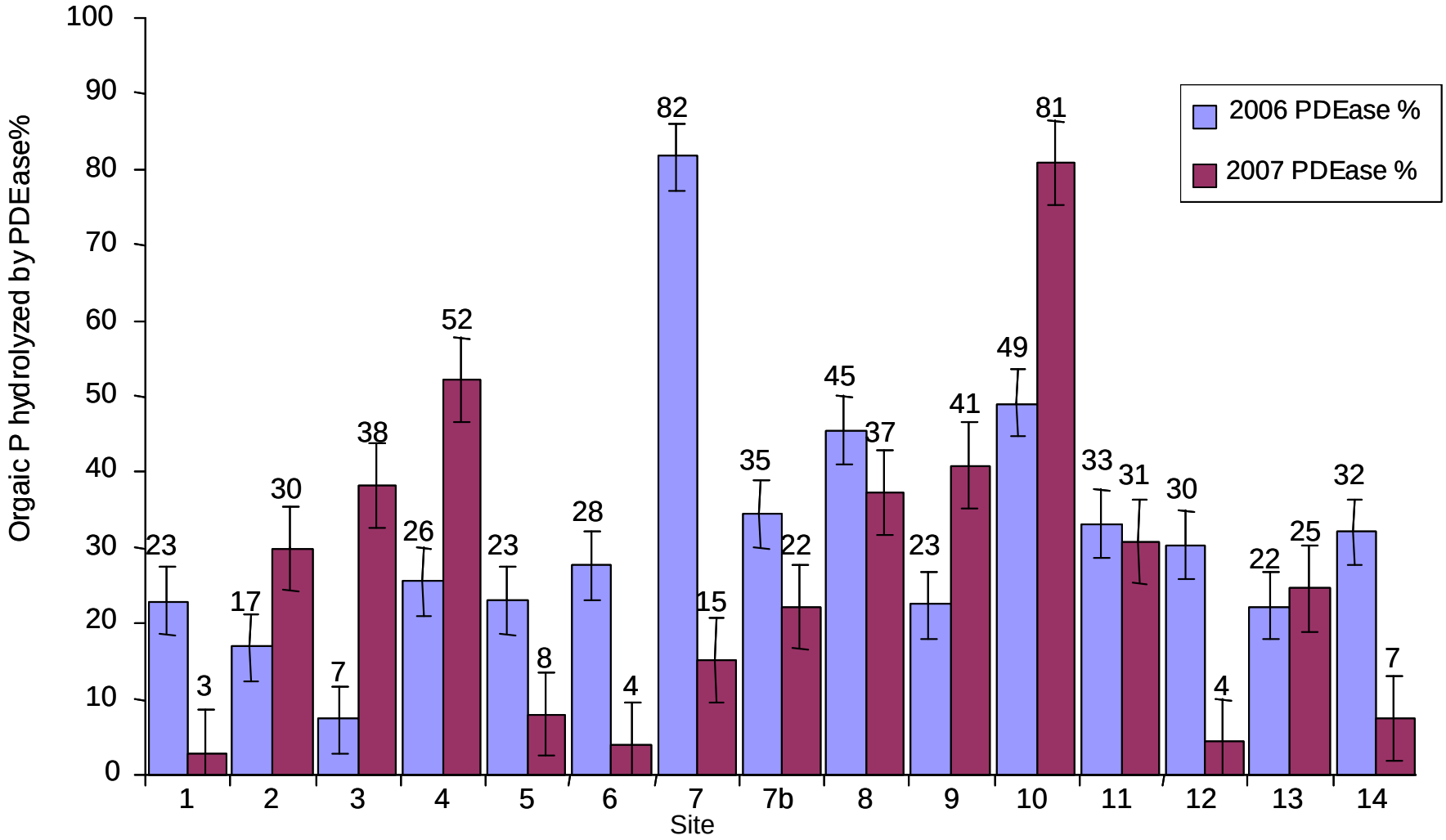


Figure 5-3 Percentage of PDEase hydrolyzed OP (PDEase%) in sediments collected in 2006 and 2007

2006 at sites 2, 3, 4 and 11, but no significant difference were observed among sites 1, 5 and 8 (Fig. 5-4).

The average NPase% for sediments collected in 2006 was 28%, which was slightly higher than 20% that of sediments collected in 2007. The spatial variations during these two years were very different, most of the high values in 2007 appeared upstream at Troublesome Brook tributary and the Bronx Zoo instead of the Sprain Brook tributary and the estuary sites in 2006, which was possibly associated with P transport by water, suspended sediment and microorganisms and their subsequent re-deposition in the bed sediments (Wang and Pant, 2010a). The NPase% values in the Bronx River sediments were in substantial amounts while comparing with the native/inherent phosphatases activities in leachate at 37°C (Toor et al. 2003). They stated that 10-21% hydrolysis of the total unreactive P, and OP was 85-88% of TP in the leachate, which is larger than maximum OP values obtained for bed sediments collected in the Bronx River in both years. It is thus, indicative that OP in river bed sediments presents a greater threat to freshwater ecosystems, especially due to P transports (Wang and Pant, 2010a).

In sediments collected in 2006, the PDEase hydrolyzed P (Table 5-1; values expressed in PDEase-P, mg kg^{-1}) showed a strong correlation ($p \leq 0.01$) with OP ($r=0.946$), TP ($r=0.931$) and OM ($r=0.745$). The NPase hydrolyzed P (i.e., NPase expressed in mg kg^{-1}) was also correlated with OP ($r=0.651$, $p=0.009$), TP ($r=0.647$, $p=0.009$) and OM ($r=0.683$, $p=0.005$), and PDEase ($r=0.763$, $p=0.001$). Both PDEase and NPase hydrolyzed P were correlated with OP and OM, suggesting OM associated P may have high potential for bioavailability (Wang and Pant, 2010a).

Also, in sediments collected in2007, the PDEase hydrolyzed P (Table 5-1; values

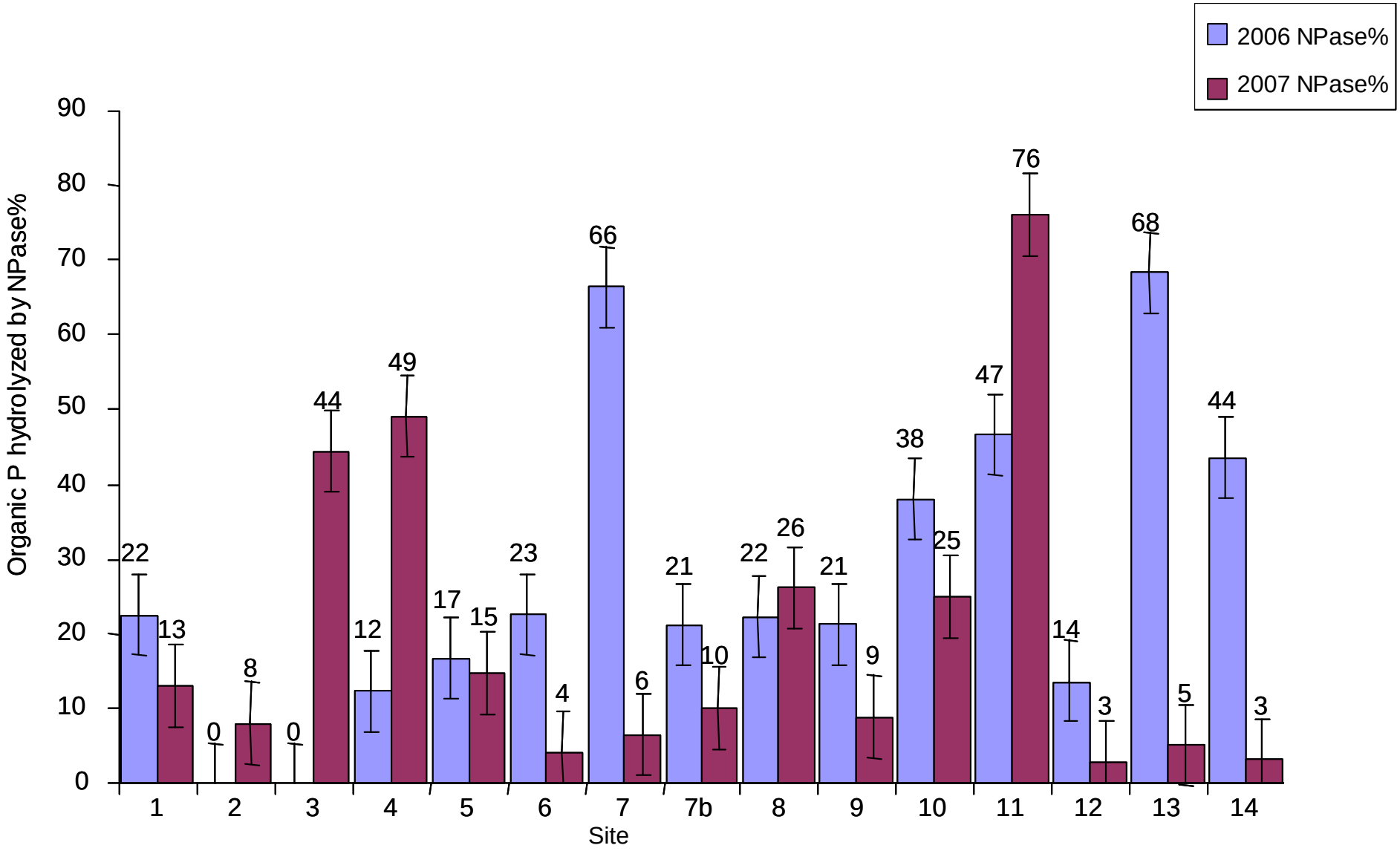


Fig. 5-4 Percentage of native phosphatases hydrolyzed OP (NPase%) in sediments collected in 2006 and 2007

expressed in mg kg^{-1}) was significantly correlated with OP (mg kg^{-1}) (r=0.658, p=0.008), TP (mg kg^{-1}; r=0.788, p=0.000) and OM (r=0.724, p=0.002) in sediments. The TP and OP content variations in the river sediments are related to the land use (Owens and Walling, 2002) and other characteristics along the Bronx River, and they may affect enzymatic hydrolysis process (Wang and Pant, 2010a). The tributaries and the estuaries had higher P content and NPase hydrolysis for both years. The NPase was also strongly correlated with TP (r=0.734, p<0.002). Moreover, the data indicated that P hydrolyzed by PDEase and NPase in sediments collected in both years were correlated to each other (r=0.537, p<0.05) indicating the NPase include PDEase, as well, in turn, suggesting possible use of NPase hydrolyzed P to predict the potential bioavailability of overall P in the sediments under changing sediment/water chemistry in rivers (Wang and Pant, 2010a).

5.4 Conclusions

The results showed that OP in bed sediments comprised up to 62% of TP in 2006, and up to 78% of TP in 2007. The PDEase and NPase hydrolysable P constitutes a substantial component of the P pools in the Bronx River bed sediments. The PDEase hydrolyzed OP was up to 82% in 2006 and 81% in 2007. The extent of NPase hydrolyzable P in the sediments suggested a strong inherent presence of NPase in the river sediments. This means that OP could be hydrolyzed to IP and released to the water columns, and transported in the river under favorable conditions such as temperature increase caused by local hydro-climatic changes. It is indicative that P hydrolyzed by PDEase and NPase in sediments collected in both years were correlated to each other (r=0.537, p<0.05) suggesting that NPase include PDEase, as well, in turn, indicating possible use of NPase

hydrolyzed P to predict the potential bioavailability of overall P in the river bed sediments. There were spatial and temporal variations of P hydrolysis by PDEase and NPase along the river. Thus, future research on effects of land use on P transport and hydrolysis may help to formulate environmental policies and P regulation strategies in WC and the Bronx.

Chapter 6

Enzymatic Hydrolysis of P by Native Phosphatases in the Bronx River Water Samples

Abstract

Phosphorus (P) is a primary limiting nutrient in rivers and streams, and excessive P results in eutrophication of freshwater systems, in turn, excessive algal growth/toxic algal blooms and oxygen depletion, i.e., water quality degradation. This study analyzed P pool, and hydrolysis of organic P (OP) by native phosphatases (NPase) in the water samples collected in the Bronx River. The soluble reactive P (SRP) of most of the sites' water collected in 2006 and 2007 were higher (average 67 μg L^{-1} and 68 μg L^{-1}, respectively) than the US Environmental Protection Agency's (EPA) standard of 15 μg L^{-1}. The SRP% (SRP/TP%) average was 27% in 2006, much lower than in 2007 of SRP% average 83%. The OP% (OP/TP%) average was 73% in 2006, which was much higher than that of in 2007 (which was only 17%). The SRP concentrations and distributions (%), and the total P (TP) concentrations were in substantial amounts compared with other rivers. The NPase hydrolyzed OP % was up to 100% in 2006 and 2007 water sample. The average of NPase% was 59% in 2006 and 73% in 2007. The NPase average concentrations were 348 μg L^{-1} in 2006, and 175 μg L^{-1} in 2007. The NPase hydrolyzed up to 100% of OP% in the Bronx River water samples at 37ºC, indicating a potential threat of eutrophication of freshwater systems as the global rise in temperature may continue to occur.

6.1 Introduction

Phosphorus is commonly limiting nutrient in rivers and streams (Neal et al., 2002); however, excess P results in eutrophication and water quality degradation in rural and urban freshwater systems (Edwards and Withers, 1998; Correll, 1999). Nutrient enrichment results in excessive algal growth and toxic algal blooms, resulting in dissolved oxygen level decrease and water quality degradation (Neal et al., 2000a, b). The sewage effluent is a major P source (Neal et al., 2002). P concentrations in the water column varied by the bed sediments released P, the anthropogenic discharge, and particulate P in suspended sediments (House et al., 1998). Various P compounds assimilated or deposited in sediments and biota could be chemically or enzymatically hydrolyzed to orthophosphate releasing to water column (Correll, 1999). Orthophosphate is the only P form that autotrophs could assimilate. Extracellular enzymes hydrolyze OP to phosphate. Phosphatases hydrolyze phosphorus esters to orthophosphate P in a bioavailable form (Ellwood et al., 2008). P cycling and transport in the river were controlled by physico-chemical factors and biological factors (Withers and Jarvie, 2008). Anthropogenic nutrient enrichment of natural water can lead to water quality declines, low dissolved oxygen concentrations, toxic algal blooms and fish-kills (Duda, 1993; Carpenter et al., 1998; Bowes et al., 2003). Anthropogenic factors often result in large amount of P from point source (e.g. wastewater treatment plant) or non point source (e.g. fertilizer application to lawn, garden) discharged into freshwater systems (Jarvie et al., 1997; Correll, 1999; Wang and Pant, 2009).

The P bioavailability is the criteria for assessing the eutrophication potential in rivers and streams (Maynard et al., 2009, Wang and Pant 2009, 2010a,b). This research is ongoing

to analyze the physcio-chemical characteristics, the P pool and OP hydrolysis by NPase in the water samples collected in the Bronx River, providing reference data on P bioavability and estimation of the potential threat on water quality caused by NPase hydrolysis of OP under increased temperature.

6.2 Methodology

6.2.1 Water sample collection and physico-chemical analysis

Water samples were collected from 14 sites along the river (Fig 1), and were transported to Environmental Laboratory of Department of Environmental, Geographic and Geological Sciences at Lehman College of The City University of New York at the end of each sampling day, and stored at 4ºC in a Fisher Scientific Isotemp Laboratory Refrigerator until further experimentation. Electrical Conductivity (EC) and pH combination electrodes were used to determine the EC and pH of the water samples. Put 25 ml water sample from each site in a 50 ml beaker, and then the pH and EC were measured. The water samples were analyzed for SRP by UV-2501PC UV-VIS Recording Spectrophotometer (Shimadzu Corporation) using ascorbic method at wavelength 880 nm (ESS Method 310.1; USEPA, 1992). The TP of water samples were determined by persulfate digestion block method (Method 365.4; USEPA, 2003). An automatic ascorbic acid method (ESS Method 310.1; USEPA, 1992) is used to determine the TP in the extracts. The difference between TP and SRP is considered as OP.

6.2.2 P hydrolysis by native phosphatases

1ml water sample and 3.2 ml Tris(Tris(hydroxymethy)- aminomethane)-HCl buffered pH=7 solution, plus 1 drop of toluene were incubated at 37ºC for 6 h, and then measured

the SRP in the solution. The NPase hydrolyzed P was measured by difference of the SRP after the incubation and the SRP without incubation; the percentage of OP hydrolyzed by NPase was calculated, in order to estimate the potential capability of NPase to hydrolyze OP under increased temperature in the river.

6.3 Results and discussion

6.3.1 Selected physico-chemical characteristics of water samples

There was no significant difference on pH along the river that ranged from 7.3 to 8.0 in 2006 and from 7.4 to 8.0 in 2007, so was EC (ranged from 530 to 353,000 μs cm^{-1} in 2006 and 540 to 378,000 μs cm^{-1} in 2007) other than the two estuary sites 13 and 14 had significantly higher EC values (Table 6-1). The SRP in the Bronx River ranged from 2 to 221μg L^{-1} in 2006 and from 27 to 162 μg L^{-1} in 2007 (Fig. 6-1); the SRP average was 67 μg L^{-1} in 2006 and 68 μg L^{-1} in 2007, both were way higher than the EPA standard of water quality value for P is less than of 15 μg L^{-1} for all source water reservoirs within New York City Watershed (Spitzer, 2006). The SRP concentrations in sites 11, 12, 13 and 14 in both years were considerably higher than other sites, and the phosphate levels varied with salinity meanwhile affected by dilution factors in estuary (Kunish and Glotfelty, 1985). The TP ranged from 30 to 1,113 μg L^{-1} in 2006 (the Sprain Brook tributary sites 7 and 8, the city line site 9, the estuary sites 13 and 14 had comparatively higher TP concentrations), and from 30 to 197 μg L^{-1} in 2007 (the Sprain Brook tributary site 6, Bronx Zoo site 11, estuary sites 13 and 14 had higher TP concentrations) (Fig. 6-

Table 6-1 Selected physico-chemical characteristics of the water samples collected in 2006 and 2007

Site	Npase-P		OP		SRP %		OP %		Npase-P %		pH		EC	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
	μg L^{-1}				%								μs cm^{-1}	
1	28	10	53	56	3	33	97	67	52	18	7.9	7.9	530	540
2	1818	79	82	0	6	100	94	0	100	100	7.8	7.8	396	621
3	154	135	11	0	62	100	38	0	100	100	7.9	7.9	651	739
4	286	17	5	0	82	100	18	0	100	100	8.0	7.9	674	786
5	428	22	49	2	21	94	79	6	100	100	7.9	7.7	1257	1415
6	80	0	333	115	9	35	91	65	24	0	7.9	7.9	853	770
7	208	1387	1030	0	4	99	96	1	20	100	7.9	7.9	846	777
8	55	61	964	63	5	30	95	70	6	97	7.9	8.0	806	701
9	5	0	714	0	9	100	91	0	1	0	8.0	7.9	766	678
10	42	507	51	0	64	100	36	0	82	100	8.0	7.9	820	727
11	1165	40	193	0	53	100	47	0	100	100	8.0	7.9	685	569
12	404	47	360	0	22	100	78	0	100	100	7.9	7.9	786	569
13	88	145	979	35	12	82	88	18	9	100	7.4	7.5	34500	25400
14	109	0	379	22	24	87	76	13	29	0	7.3	7.5	35300	37800
Ave	348	175	372	21	27	83	73	17	59	73	7.9	7.8		

Npase-P: Native phosphatases hydrolyzed OP, mg kg^{-1}

OP: organic phosphorus

SRP%: SRP/TP%

OP%: OP/TP%, percentage of OP

Npase-P%: Npase-P/OP%, percentage of native enzyme hydrolyzed OP

2). The OP ranged from 5 to 1,030 µg L^{-1} in 2006, and from 0 to 115 µg L^{-1} in 2007 (Table 1); the tributary and estuary sites were having higher OP values. There is only SRP (OP=0 µg L^{-1}) in seven sites of 2007 data. The average TP and OP levels in 2006 (438 µg L^{-1}, 372 µg L^{-1}) were much higher than in 2007 (89 µg L^{-1}, 21 µg L^{-1}). The SRP% (SRP/TP%) was from 3 to 82%, average 27% in 2006, which was much lower than in 2007 of SRP% average 83% that ranged from 33 to 100% (Table 6-1). The OP% (OP/TP%) average in 2006 was 73%, ranged from 18 to 97% (Table 6-1), which was much higher than in 2007 of OP% average only 17% ranging from 0-70%.

6.3.2 SRP and TP comparison with other rivers

The SRP concentrations were ranged from 70-80 µg L^{-1} in interstitial water (IW) and 70-120 µg L^{-1}in surface water (SW) and the average SRP was 47% of TP in Garonne River, southern France (Vervier et al., 2009). The maximum SRP concentrations in Bronx river water sample in both years were higher than Garonne River, and the average SRP% in 2006 was lower and in 2007 was higher than that of Garonne River. The TP concentrations ranged from 160-180µg L^{-1} in IW and 160-220µg L^{-1} in SW, which was a smaller range than Bronx River water sample, the maximum values were lower than those in 2006 of Bronx River sample. The SRP ranged from 2-50 µg L^{-1} in Wye River located in Queenstown, MD (Kunish and Glotfelty, 1985) at spring season and it was in the range of SRP in Bronx River water sample, in summer the SRP reached the peak of 400 µg L^{-1} that was much higher than that of the Bronx River. The SRP and TP in streams within two east-central Illinois agricultural watershed (the Embarras and the

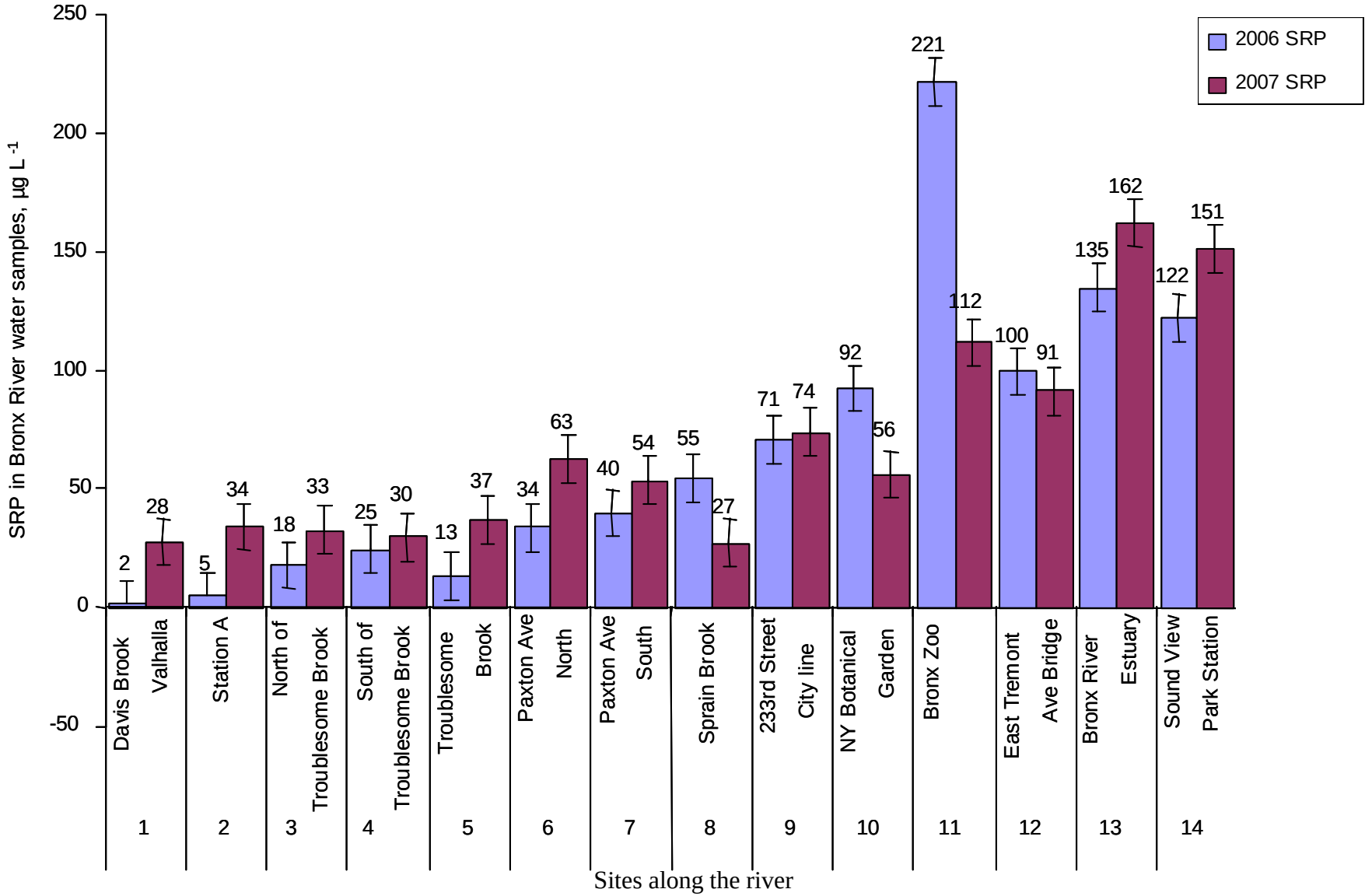


Figure 6-1 SRP in water samples collected in 2006 and 2007

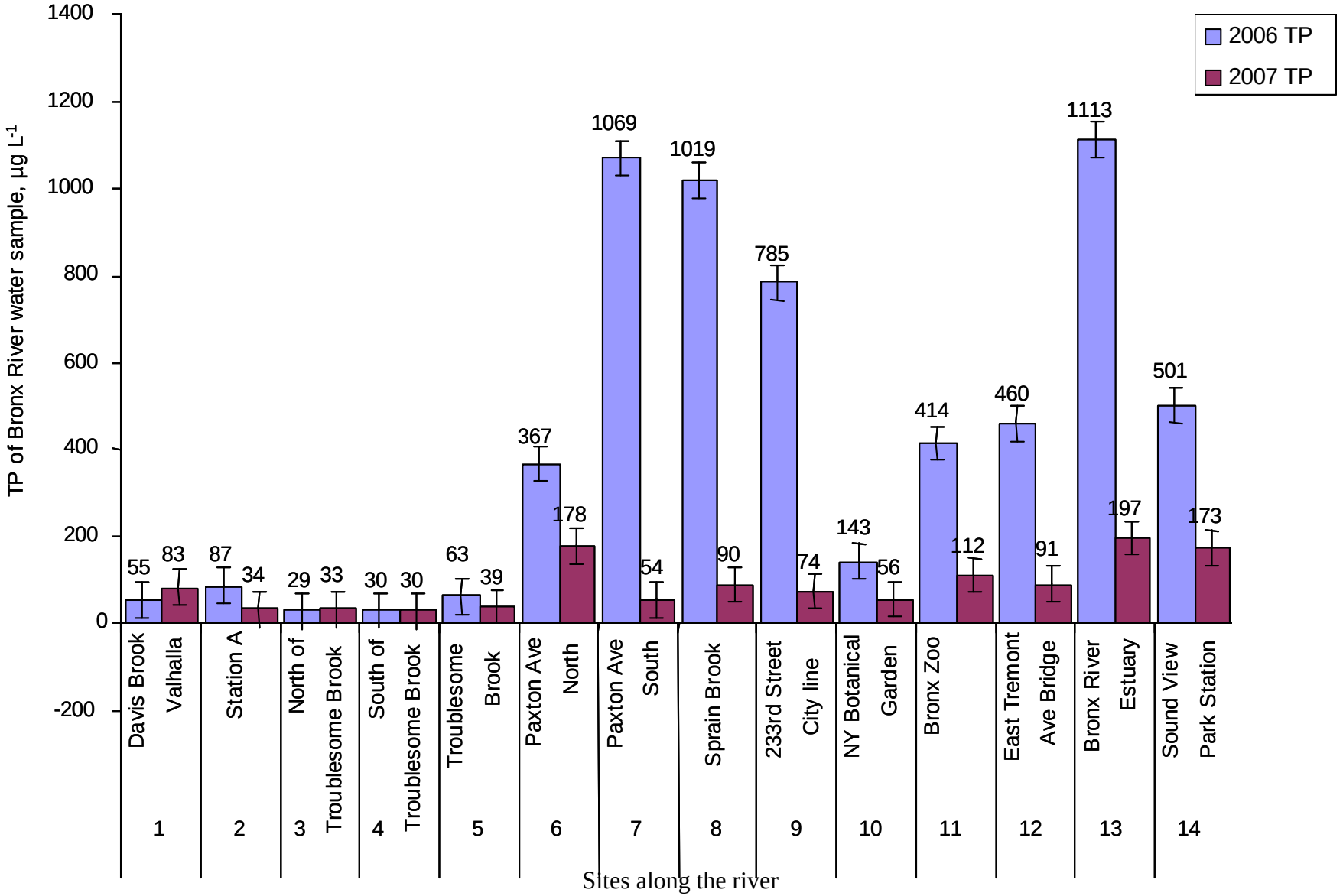


Figure 6-2 TP in water samples collected in 2006 and 2007

Vermilion) was up to the same maximum concentrations of 2,800 µg L^{-1} (McDaniel and David, 2009), which was much higher than SRP and TP maximum values in Bronx River water samples. The median SRP was 81 µg L^{-1} in Illinois streams, which shared similarity of SRP average values (67 µg L^{-1} in 2006 and 68µg L^{-1} in 2007) in the Bronx River, and the median TP was 168 µg L^{-1} (McDaniel and David, 2009), which was much lower than the average in 2006 (438 µg L^{-1}) and higher than in 2007 (89 µg L^{-1}). The peak SRP concentration in River Lambourn during storm was 218 µg L^{-1}, and 267 µg L^{-1}in River Enborne (Evans and Johns, 2004), which were close to the Bronx River maximum SRP concentration in 2006.

The mean TP concentrations of the upper Thames is 6,600 µg L^{-1}, and SRP form was 91%; There were six small sewage treatment plants in this agricultural sub-catchment Rivers (Withers and Jarvie, 2008). There is only Hunts Point WWTP in the estuary of the Bronx River compared with more WWTPs in the agricultural sub-catchment of Kennet and Dun Rivers, resulting in the TP concentration and SRP portion in upper Thames were way higher than those in the Bronx River for both years (Withers and Jarvie, 2008). The TP in 2006 was much higher than the TP in Chesapeake Bay in 1970's varied from 150 to 200µg L^{-1}, and the SRP for both years were also much higher than the dissolved orthophospate of 5-8 µg L^{-1} in Chesapeake Bay in 1970's (Correll, 1981). The TP ranged from 19.6 to 679.6 µg L^{-1}, and the average ranged from 145.3 to 296.6 µg L^{-1} in four upland streams in northern England in year 1 (Ellwood et al., 2008); the TP concentrations and the average TP values were lower than the Bronx River water sample TP in 2006 (average TP=438µg L^{-1}) and greater than water sample TP in 2007 (average TP=89µg L^{-1}). In year 2, the TP ranged from 10-316.2µg L^{-1}, average TP from 174.2 to

316.2 μg L^{-1} in the four stream in England; which were much lower than year 1 and still higher than Bronx River year 2-2007 data. Total P in streamwater samples in the Elk Creek, Canada showed that the TP_{max} increased from upper Elk of 15μg L^{-1} to middle Elk of 20 μg L^{-1} and mouth of Elk of 60μg L^{-1} (Schendel et al., 2004), which were way lower than TP average concentrations in the Bronx River. The spatial pattern of TP increase from upper to lower river in Elk was not found in the Bronx River, instead the tributary and the estuary tended to have higher TP. The hydrochemistry spatial and temporal variations lead to P concentration and proportion and their bioavailability variations (Wade et al., 1999; Turner et al., 2003). The phosphate needed to maintain equilibrium algal growth varied from 0.003 to 0.8 μg L^{-1} (Grover, 1989). There was a great potential for algal growth in the Bronx River. The released P from sediments and hydrolyzed P from microorganisms were potentially bioavailable for algal growth, endangered dissolved oxygen level and water quality.

6.3.3 The Npase hydrolysis of OP in water samples and comparison with other studies

The NPase hydrolyzed OP % (NPase%) ranged from 1 to 100% for Bronx River water sample collected in 2006 and from 0 to 100% in 2007 water sample (Table 6-1). The average of NPase% was 59% in 2006 and 73% in 2007. The NPase concentrations ranged from 5 to 1,818 μg L^{-1} with a mean of 348 μg L^{-1} in 2006, and 0-1,387 μg L^{-1} with a mean of 175μg L^{-1} in 2007 The results showed that fair amount of OP had been hydrolyzed to SRP that available to plants in the river without any addition of enzyme at

37°C. It is a potential threat on water quality when the temperature increases (Shand and Smith, 1997; Pant and Warman, 2000; Toor et al., 2003; Wang and Pant, 2010a).

The total enzymatically hydrolysable phosphorus (EHP) pool ranged from 1.1 to 15µg L^{-1}, and EHP fraction of DOP was 23-100%, with a mean of 68% in the waters of the Tamar estuary in SW England (Monbet et al., 2009). The EHP concentration range was much narrower than that of Bronx River water, and the maximum value was only around 1% of that of Bronx River water. The average hydrolyzed P% was in between that in 2006 and 2007. The treated of inflow and outflow agricultural drainage water hydrolyzed by phosphodiesterases was 54 µg L^{-1} that was 71% of OP (Pant et al., 2002b). The native enzymatically hydrolysable P in the Bronx River water was in a substantial amount compared with other studies.

6.4 Conclusion

The TP and OP average values in 2006 were much higher than in 2007, however, SRP was similar on average in both years. The SRP concentrations were much higher than EPA standard. Compared with other rivers, the P concentrations (SRP, OP, and TP) in the Bronx River were in substantial amounts and had a great potential for bioavailability. The average SRP% in 2007 was almost three times of 2006, and average OP% in 2006 was more than 4 times of 2007. The NPase hydrolyzed up to 100% of OP% in the Bronx River water samples at 37ºC, indicating a potential threat of eutrophication of freshwater systems as the global rise in temperature may continue to occur.

Concluding Remarks

This dissertation research was focused on P bioavailability predication, P exchange between sediment and water column, OP hydrolysis, and spatial and temporal variations in P transport. The results were in accordance with the hypothesis, experiments were conducted to fulfill the objectives, and the dissertation had answered the research questions: (1) P compound identification and bioavailability, (2) P pool and hydrolysis of OP effect on spatial and temporal variations of P bioavailability and P transport, (3) P sorption between sediment and water column effect on maintaining P pool.

The water samples from the Bronx River were mostly around neutral (pH 7), and the EC values were extremely high in the two estuary sites. The SRP was much higher than the EPA standard of 15 μg L^{-1}. The SRP concentrations and its distributions (%) (SRP_{ave}=67 μg L^{-1}, 27% in 2006; SRP_{ave}=68 μg L^{-1}, 83%), and the TP concentrations (TP_{ave}=438 μg L^{-1} in 2006; TP_{ave}=89 μg L^{-1} in 2007) were in a substantial amounts compared with other rivers (Garonne River in southern France, Wye River in Queenstown, MD). The native phosphatases (NP) hydrolyzed up to 100% of OP in Bronx River water samples collected in 2006 and 2007at temperature 37^{o}C, indicating a potential threat on water quality as the temperature may substantially increase in the future.

Sediment either release P to the water column or sorbed P from the water column. Phosphorus sorption maxima (S_{max}) were significantly correlated with oxalate-extractable iron (Ox-Fe) and aluminum (Ox-Al), and acid-extractable calcium (HCl-Ca) and magnesium (HCl-Mg) along with total organic matter (OM), suggesting that not only metal ions affected P sorption characteristics, but organic matter also influenced the P sorption processes. The sorption experiments also showed that originally sorbed P (S_0)

was significantly correlated with Ox-Fe, Ox-Al, HCl-Mg, and OM. The extremely high values of the percentage of sorbed P retained in sediments (>98%for all sites except the two estuary sites- site 13 of 88% and site 14 of 92%) suggest that a large flux of P to the water column from the sediments could potentially occur under changing hydro-climatic conditions such as the changes in pH, ionic strength and redox conditions, in turn, creation of eutrophic conditions, and subsequent algal blooms.

Phosphorus fractionation and mineralization showed the temporal and spatial variations on P pool. The results show that the average P pool rank order was: HCl-P > NaOH-P > $NaHCO_3$-P > residueP, and the relative proportion of 3.7 : 2.0 : 1.4 : 1 in 2006 sediment; HCl-P > NaOH-P > residue-P > $NaHCO_3$-P, with their relative proportion of 27.8 : 6.2 : 2.7 : 1 in 2007 sediment. The P pool variations indicated that the microbial activity changes over incubation time. The strong correlation between microbial P and NaOH-P distributions indicated that OP was mineralized by microorganisms to become bioavailable. Similarly, P compound identification showed spatial differences along the river, meanwhile the two years data indicated temporal changes, as well. The ^{31}P-NMR spectra showed that the dominant P species in Bronx River bed sediments were orthophosphate monoester, and lesser phosphate diesters and pyrophosphates (pyro-P). The P compounds were mostly glycerophosphate (GlyP), nucleoside monophosphates (NMP), and polynucleotides (PolyN). A few sites showed a small amount of dihydroxyacetone phosphate (DHAP), inosine monophosphate (IMP), and pyrophosphates (pyro-P).

Moreover, the results showed that native enzymes hydrolyzed substantial amount of OP (up to 76%) under favorable temperature and pH, indicating OP could be hydrolyzed

under increased temperature, in turn, increase in P availability in the river. The sediment incubated with PDEase under 37ºC for 6 h at pH=8.8, the results showed up to 82% of OP had been hydrolyzed. The enzymatic hydrolysis of OP to inorganic P (IP) by NPase in natural water could contribute to eutrophication in a river. This study indicates that the total enzyme hydrolysable P in the Bronx River sediments constitutes a substantial portion of the total P.

The sediment transport, deposition, assimilation, the exchange of P between sediment and water column, mineralization and enzymatic hydrolysis, the land use changes, as well as raw sewer discharge, runoffs from Bronx River Parkway, sewer overflow from Hunts Point WWTP, P fertilizer applications in lawns, golf courses and garden, manure from the zoo, and other anthropogenic factors, plus the hydro-climatic changes could result in the spatial and temporal variations in P distributions and transports along the Bronx River. Utilizing data from P sorption, P pools, ^{31}P-NMR, PDEase and NPase hydrolysis that were obtained from this study will help determine the spatial and temporal variations of P transport/distribution, and estimate impacts of anthropogenic P inputs on water quality of the Bronx River, in turn, help regulate P management along the river.

Appendix

Appendix 1

^{31}P-NMR spectra of NaOH-extracts of the sediments

Table 1. 2006 sediment P compound identification by ^{31}P-NMR (Table updated on 2/27/09 Fri)

Site#	Location	Peak (ppm)	P compound	Relative composition (%)
1	Davis Brook, Valhalla	5.47	GlyP	100
2	Station A	5.34	GlyP	95
		4.49	NMP	1.8 (trace)
		4.08	PolyN	1.7 (trace)
		-1.00	IMP	1.5 (trace)
3	North of Tb Brook	4.97	GlyP	98
		3.61	PolyN	1 (trace)
		-5.38	PyroP	1 (trace)
4	South of Tb Brook repeat	6.40	DHAP	95
		5.50	GlyP	2
		5.08	NMP	2
		-0.01	IMP	1 (trace)
5	Troublesome Brook	5.39	GlyP	100
6	Paxton Ave North	5.34	GlyP	96
		4.03	PolyN	3
		-1.00	IMP	1 (trace)
7	Paxton Ave South	5.50	GlyP	100
7B	Paxton Ave Southwest	4.70	NMP	96
		3.03	PolyN	2 (trace)
		-2.25	IMP	1 (trace)
		-5.15	PyroP	1 (trace)
8	Sprain Brook	5.39	GlyP	100
9	233rd St City Line	5.36	GlyP	100
10	NY Botanical Garden	5.35	GlyP	97
		3.93	PolyN	1 (trace)
		-1.25	IMP	1 (trace)
		-5.00	PyroP	1 (trace)
11	Bronx Zoo	5.57	GlyP	100
12	East Tremont Ave Bridge	5.35	GlyP	96
		4.01	PolyN	2
		-1.04	IMP	2
13	Bronx River estuary	5.60	GlyP	99
		4.01	PolyN	1 (trace)
14	Sound View Park Station	5.47	GlyP	96
		4.94	NMP	4

GlyP=glycerophophate, NMP=nucleoside monophosphates, PolyN=polynucleotides DHAP=dihydroxyacetone phosphate, G6P=gluecose-6-phosphate, IMP=inosine monophosphate, PyroP=pyrophosphates.
Trace: =<1.5% of relative composition

Table 2. 2007 sediment P compound identification by ^{31}P-NMR (updated on 2/27/09 Fri)

Site#	Location	Peak (ppm)	P compound	Relative composition (%)
1	Davis Brook, Valhalla	5.28	GlyP	100
2	Station A	5.34	GlyP	100
3	North of Tb Brook	5.45 4.06 -4.79	GlyP PolyN PyroP	98 1 (trace) 1 (trace)
4	South of Tb Brook	5.43 4.07	GlyP PolyN	98 2
5	Troublesome Brook	5.61 -4.41	GlyP Pyrop	99 1 (trace)
6	Paxton Ave North	5.55	GlyP	100
7	Paxton Ave South	5.53 4.09 -1.10	GlyP PolyN IMP	98 1.5 (trace) 0.5 (trace)
7B	Paxton Ave Southwest	5.55 -4.98	GlyP Pyrop	99 1 (trace)
8	Sprain Brook	5.60 4.08	GlyP PolyN	99 1 (trace)
9	233rd St City Line	4.91 -5.03	GlyP Pyrop	98 2
10	NY Botanical Garden	5.48	GlyP	100
11	Bronx Zoo	5.85 5.44	DHAP GlyP	10 90
12	East Tremont Ave Bridge	5.55	GlyP	100
13	Bronx River estuary	4.88 4.52 -5.75	GlyP NMP PyroP	98 1 (trace) 1 (trace)
14	Sound View Park Station	5.65	GlyP	100

GlyP=glycerophophate, NMP=nucleoside monophosphates, PolyN=polynucleotides
DHAP=dihydroxyacetone phosphate, G6P=gluecose-6-phosphate,
PyroP=pyrophosphates.

Table 3. 2006 sediment site 4 mineralization effect on 0, 7, 15 & 30days

Site#	Location	Peak (ppm)	P compound	Relative composition (%)
4 0day	South of Tb Brook	6.40 5.50 5.08 -0.01	GlyP NMP PolyN IMP	95 2.1 2.5 0.4 (trace)
4 7day	South of Tb Brook	5.01 -5.88	DHAP Pyrop	95 5
4 15day	South of Tb Brook	5.33 -5.20	GlyP PyroP	95 5
4 30day		5.27 -5.42	G6P PyroP	95 5

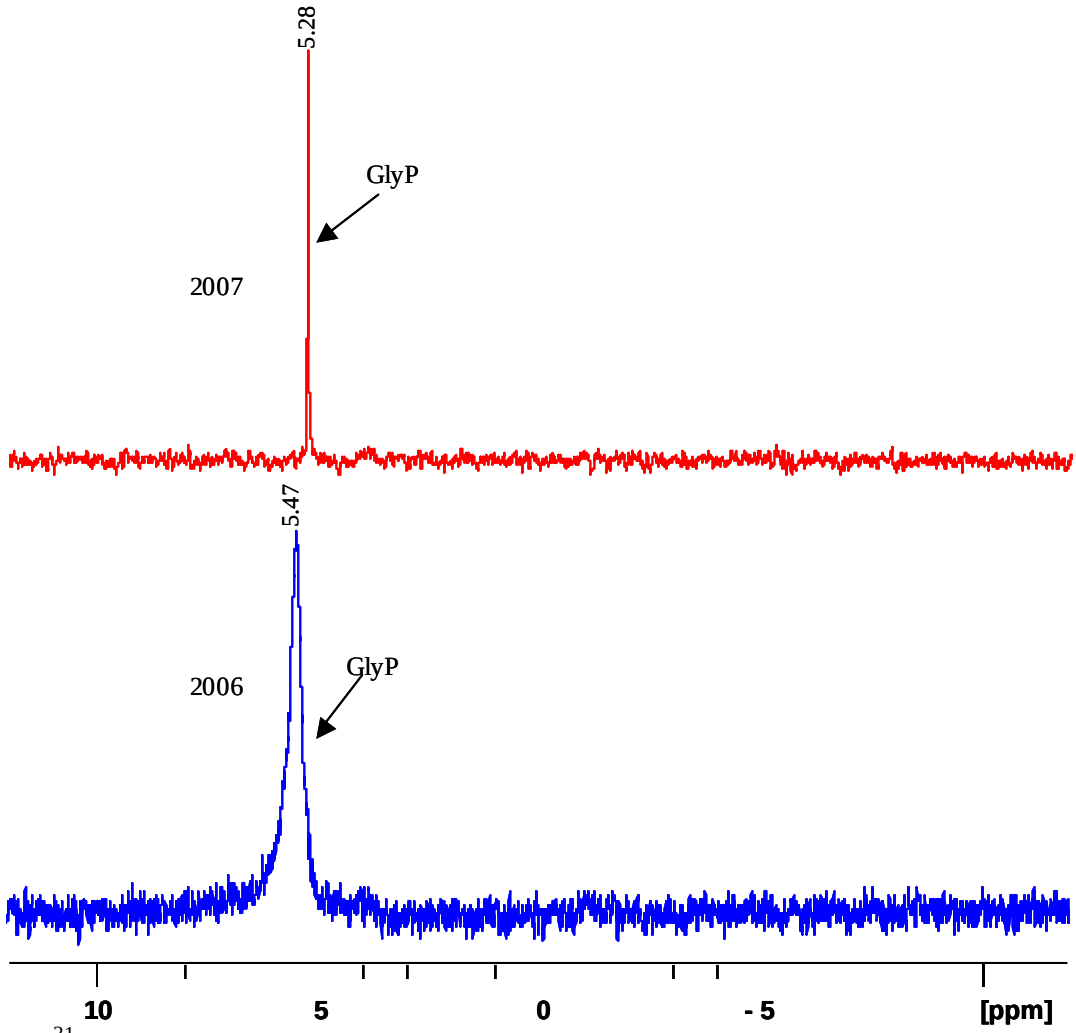


Fig. 1. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 1, Davis Brook, Valhalla, collected in 2006 and 2007.

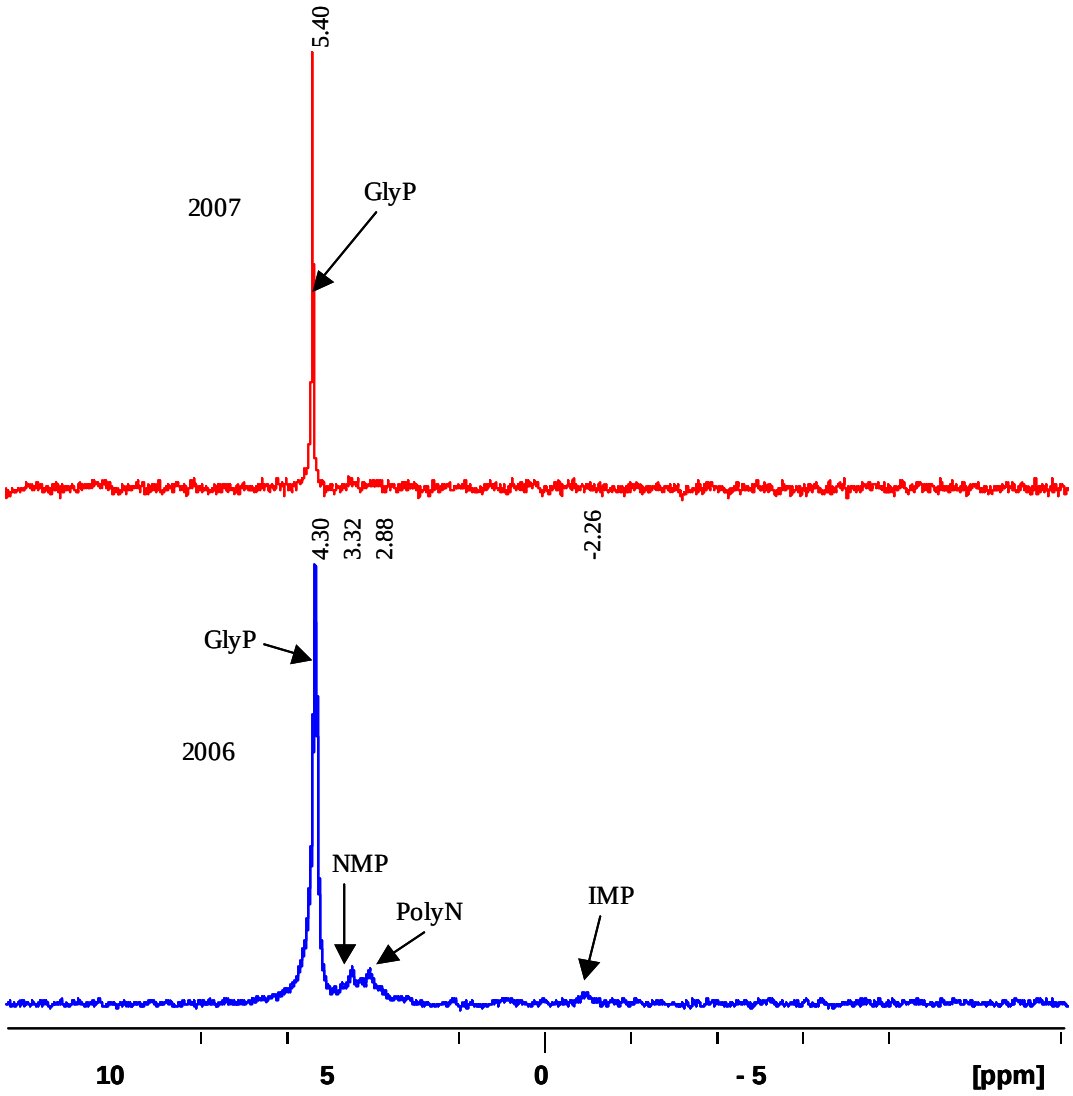


Fig. 2. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 2 Station A, collected in 2006 and 2007.

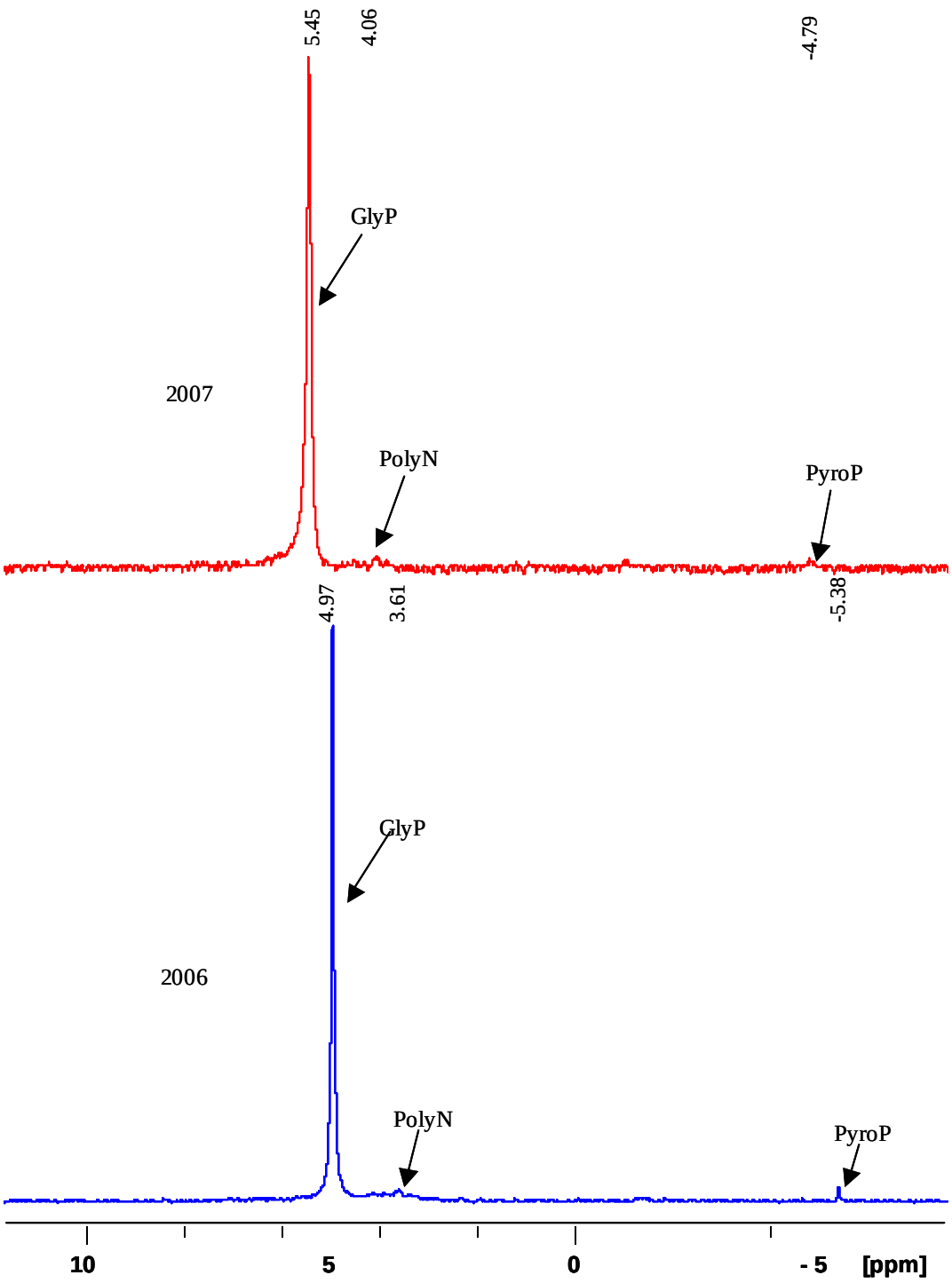


Fig. 3. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 3, North of Troublesome Brook, collected in 2006 and 2007.

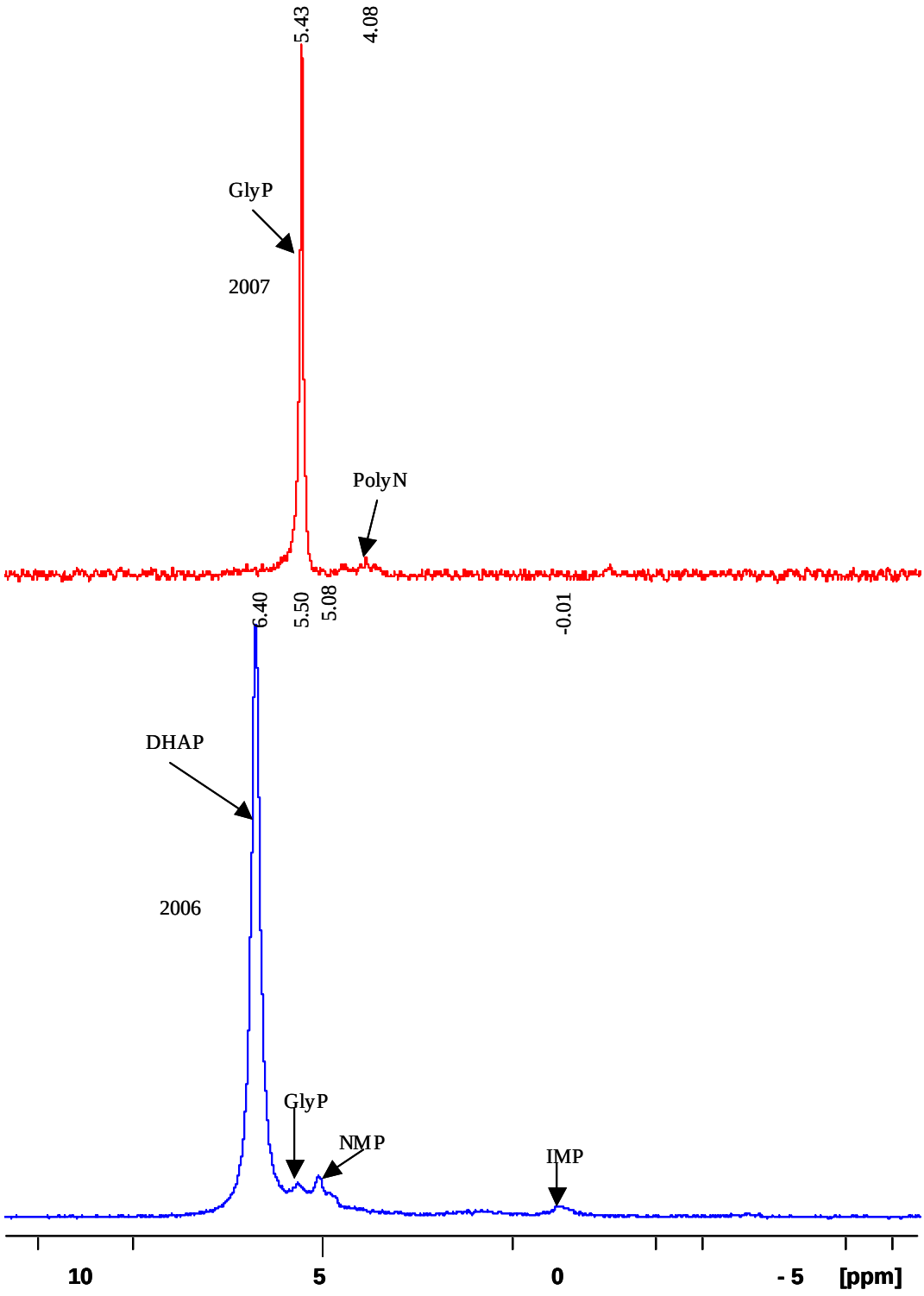


Fig. 4. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 4, South of Troublesome Brook, collected in 2006 and 2007.

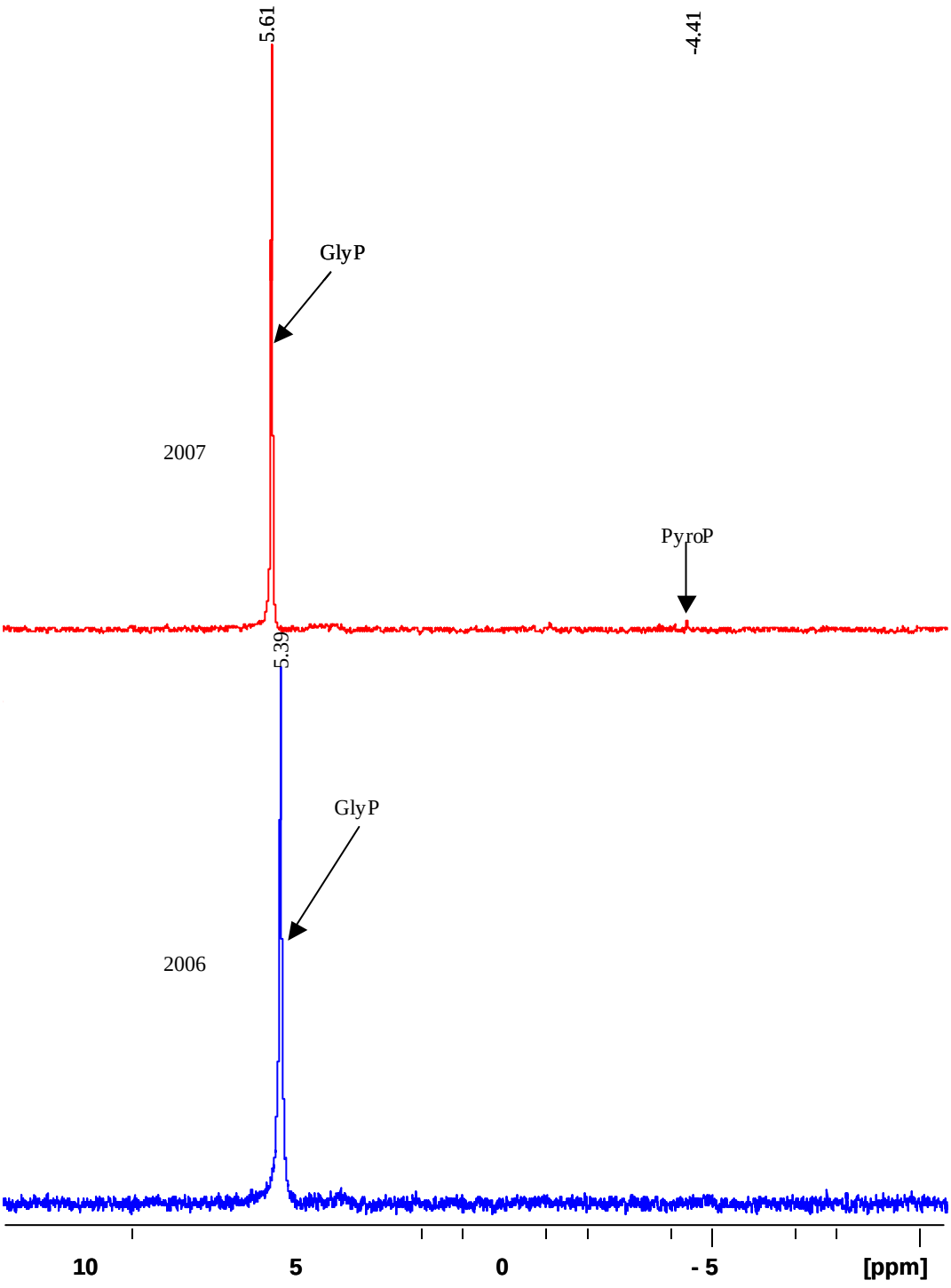


Fig. 5. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 5, Troublesome Brook, collected in 2006 and 2007.

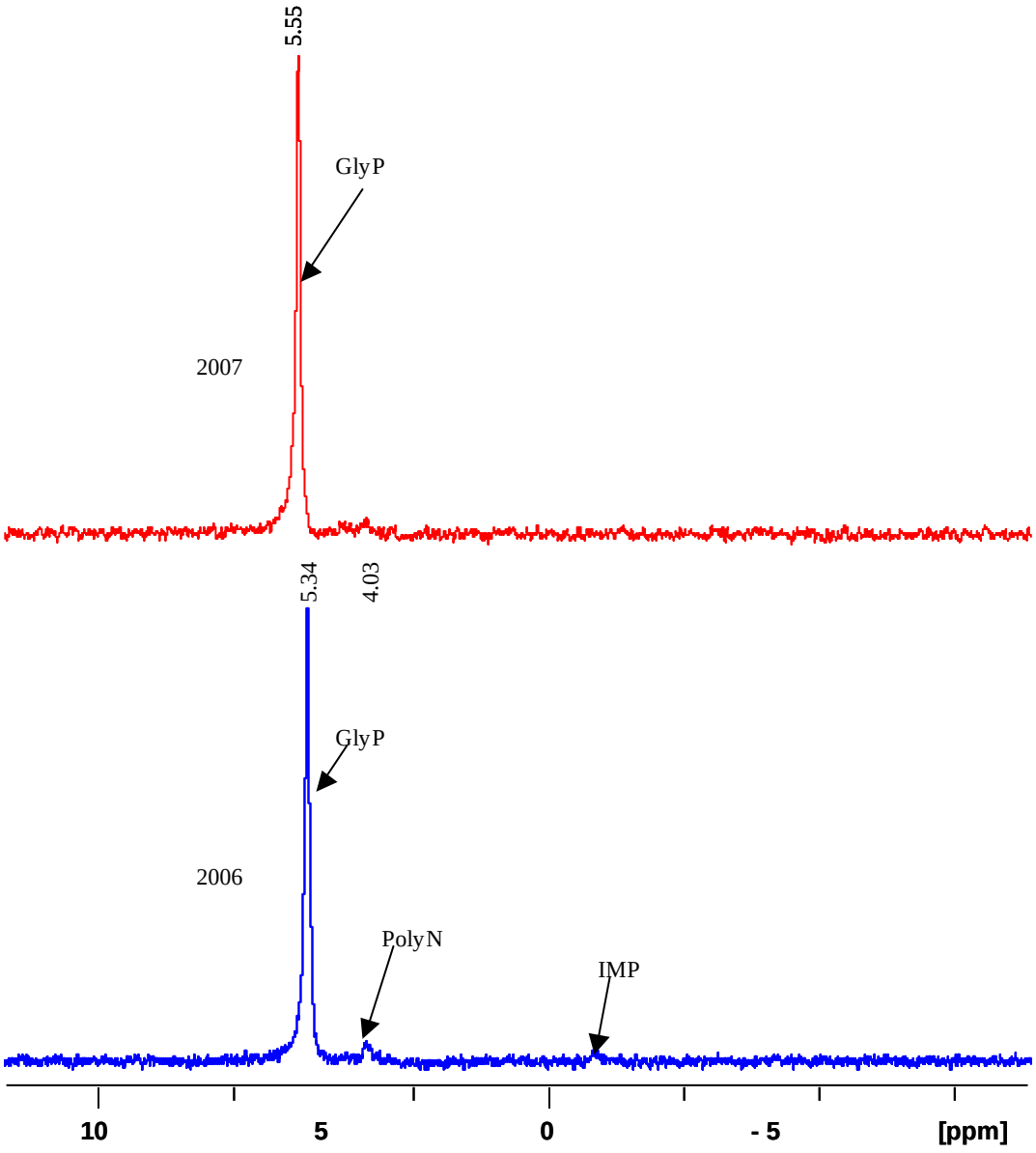


Fig. 6. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 6, Paxton Ave North, collected in 2006 and 2007.

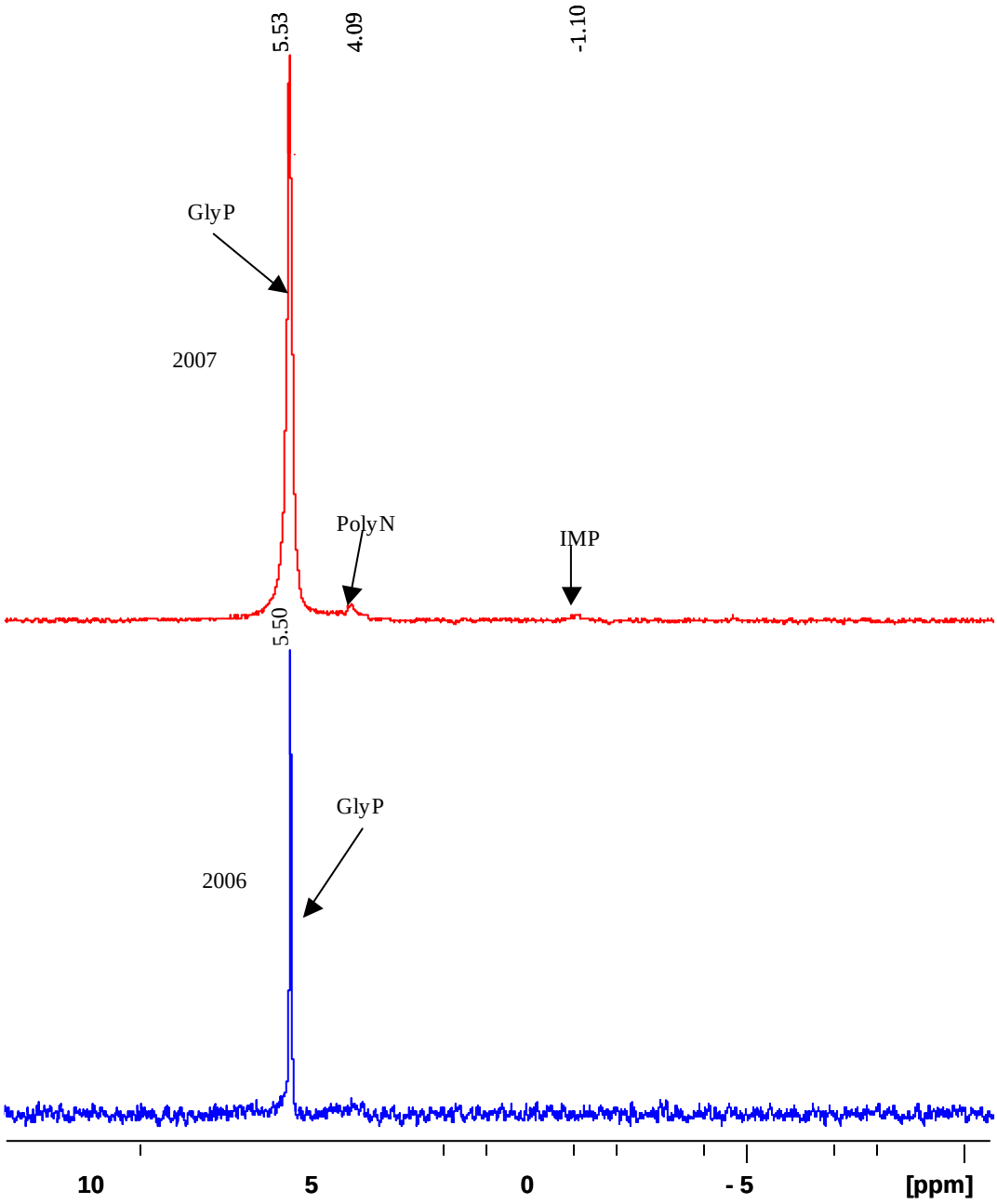


Fig. 7. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 7, Paxton Ave South, collected in 2006 and 2007.

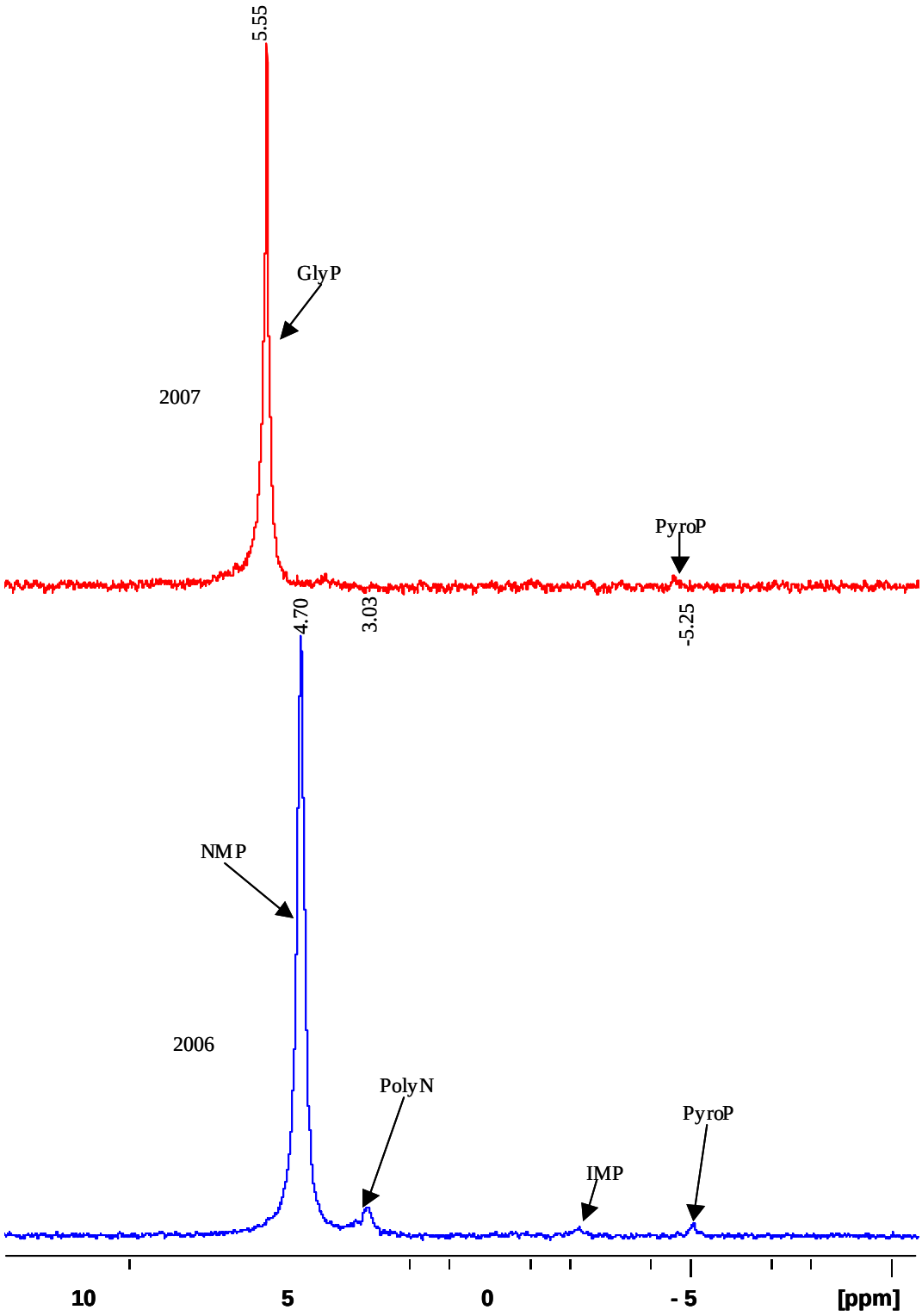


Fig. 8. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 7b, Paxton Ave Southwest, collected in 2006 and 2007.

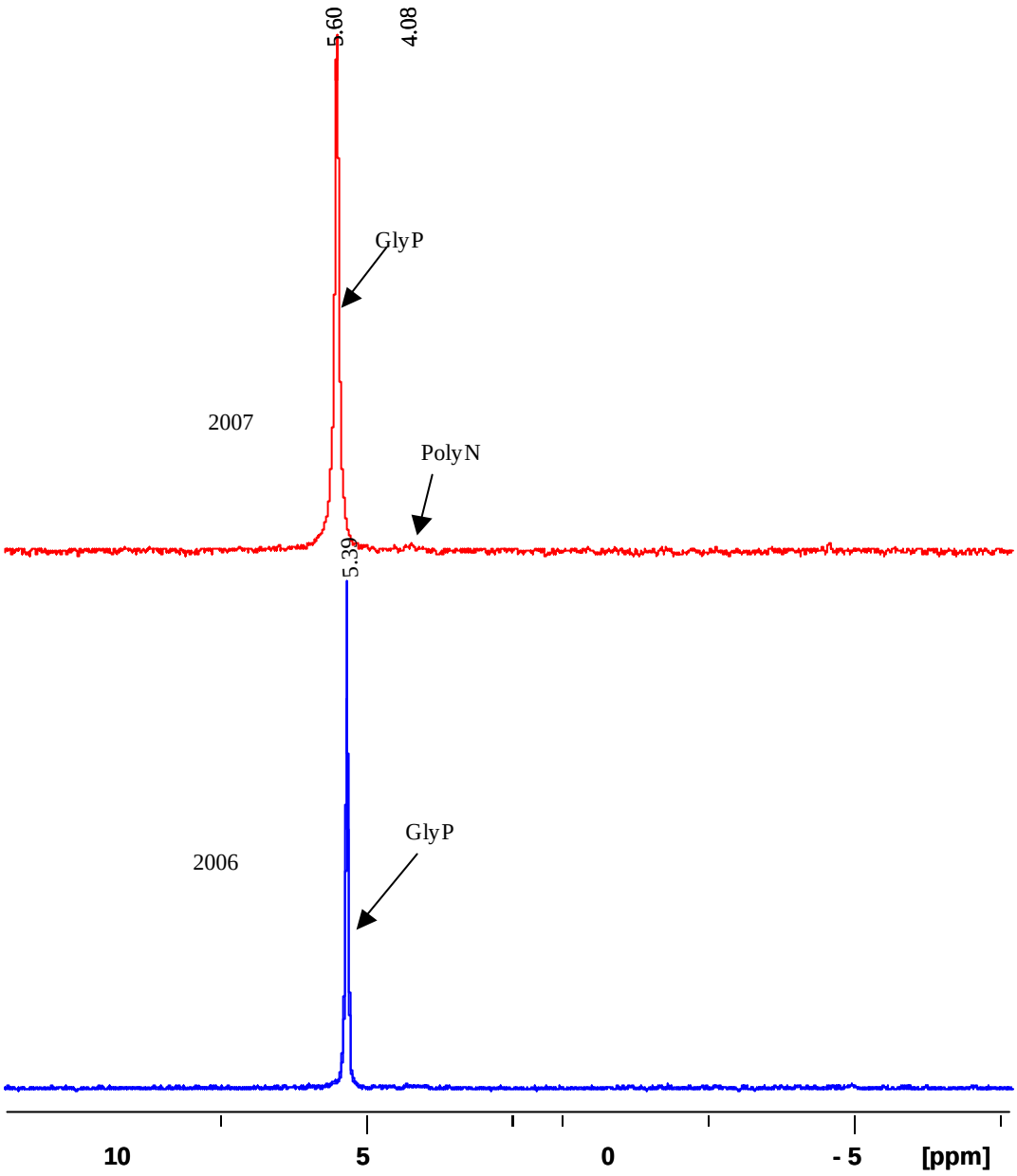


Fig. 9. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 8, Sprain Brook, collected in 2006 and 2007.

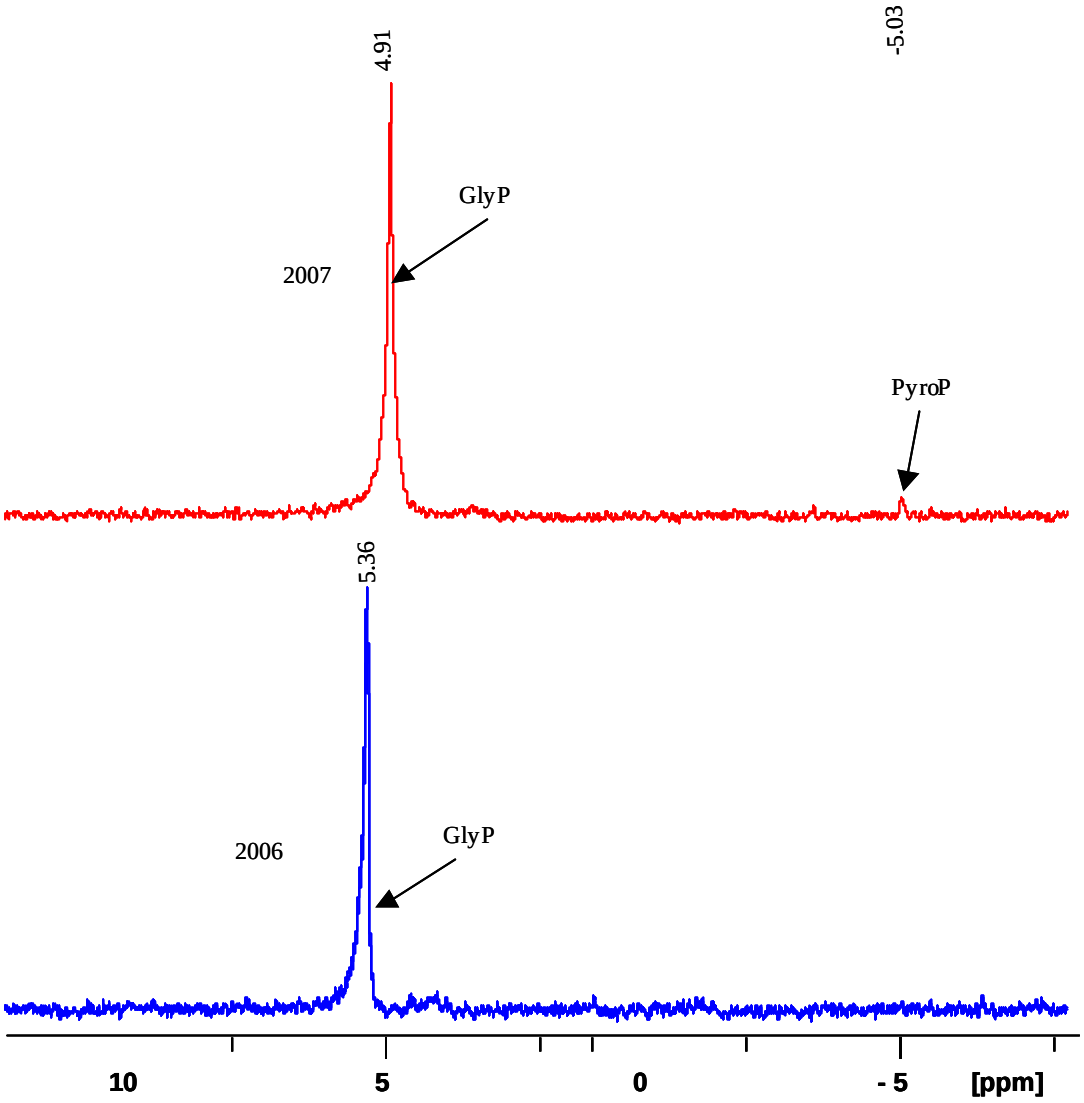


Fig. 10. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 9, 233rd St City Line, collected in 2006 and 2007.

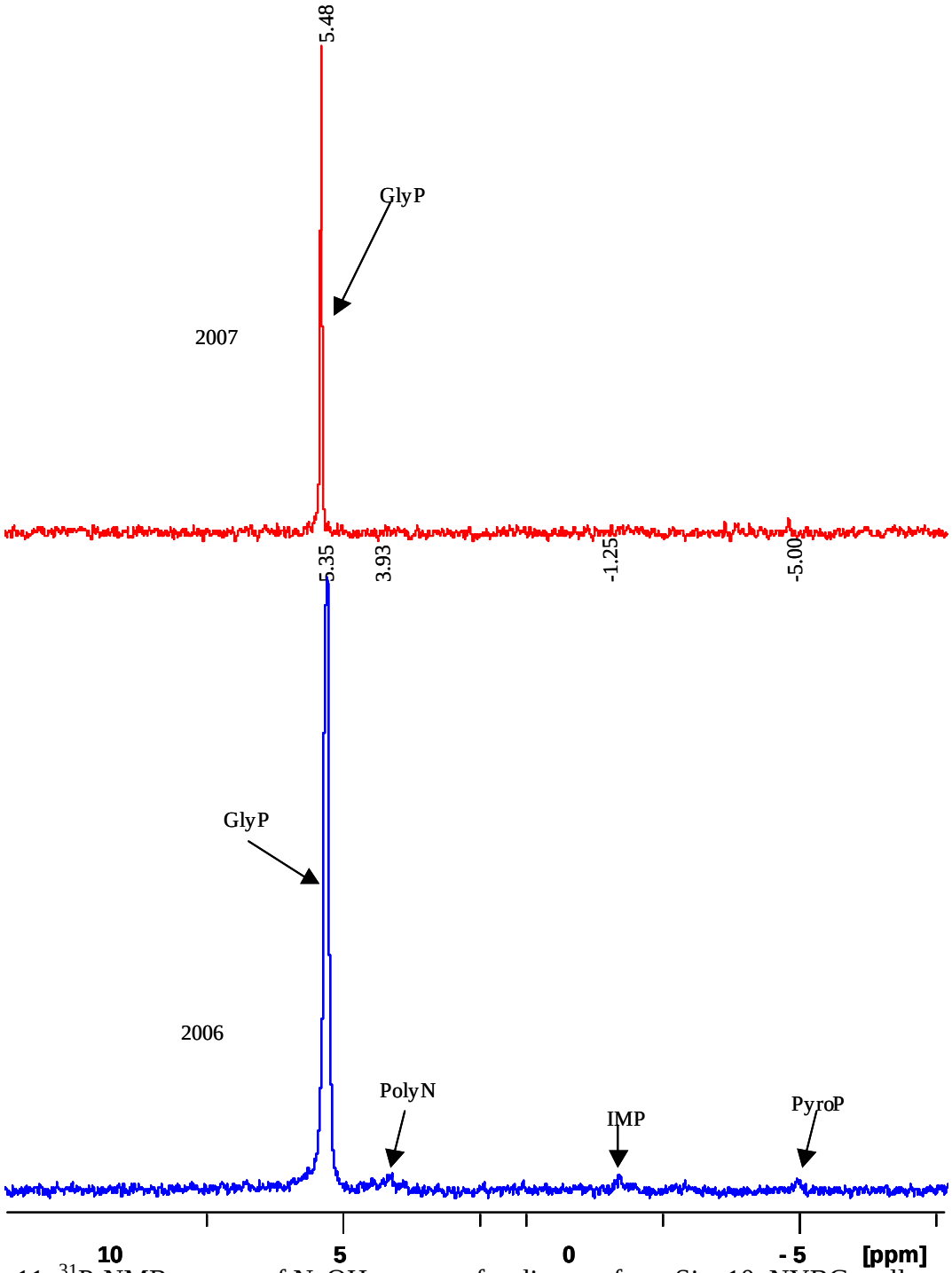


Fig. 11. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 10, NYBG, collected in 2006 and 2007.

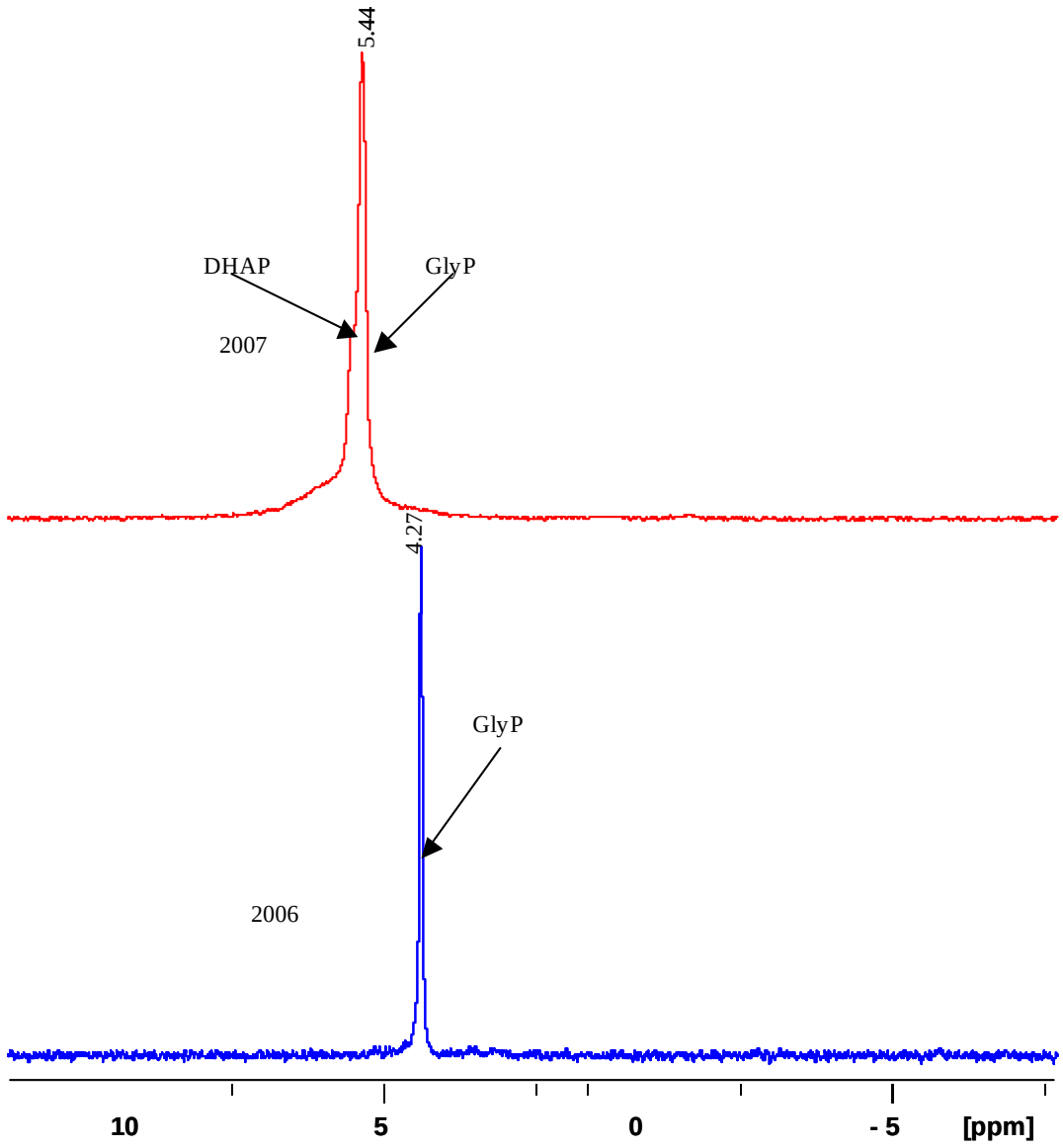


Fig. 12. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 11, Bronx Zoo, collected in 2006 and 2007.

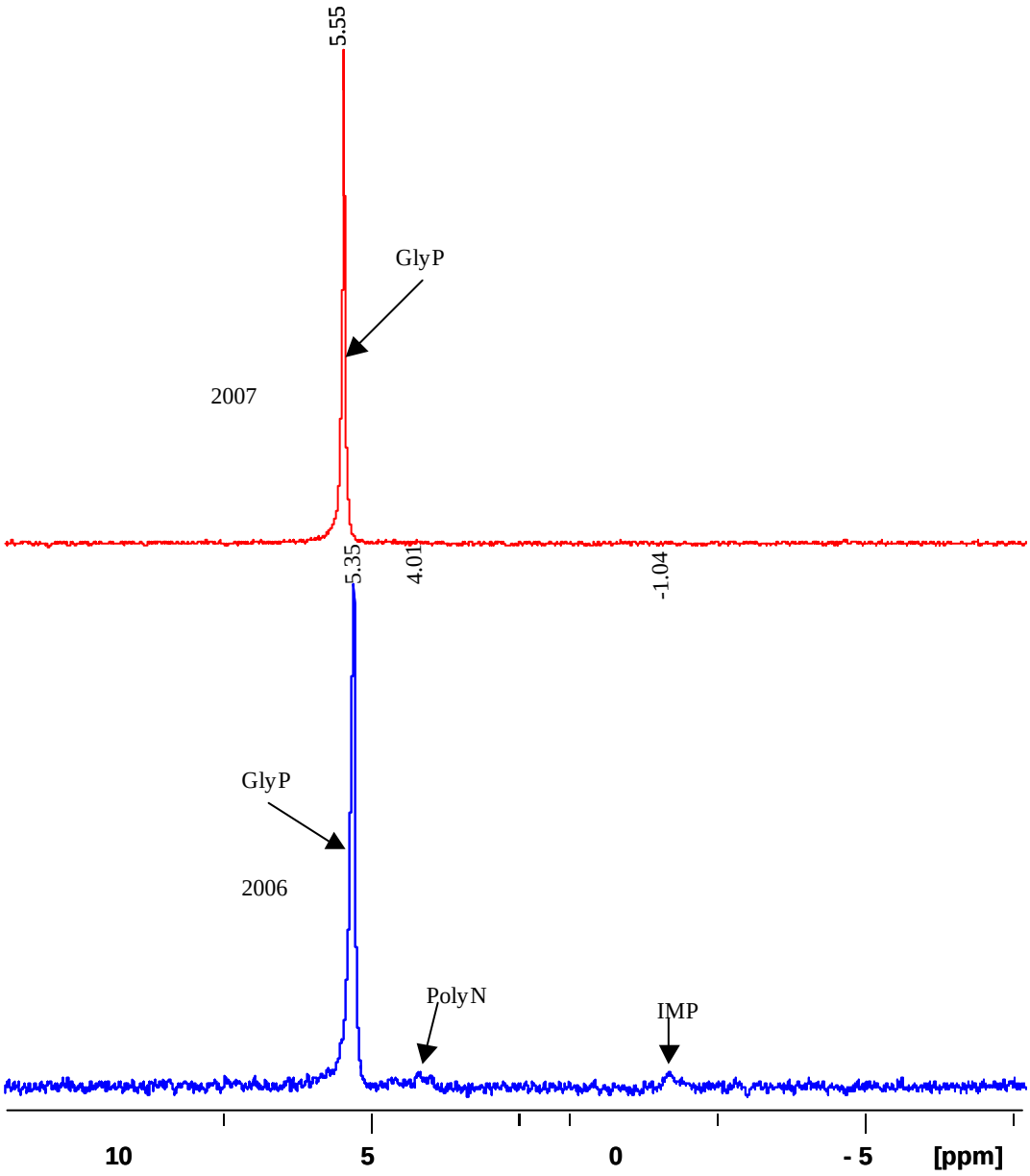


Fig. 13. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 12, East Tremont Ave. Bridge, collected in 2006 and 2007.

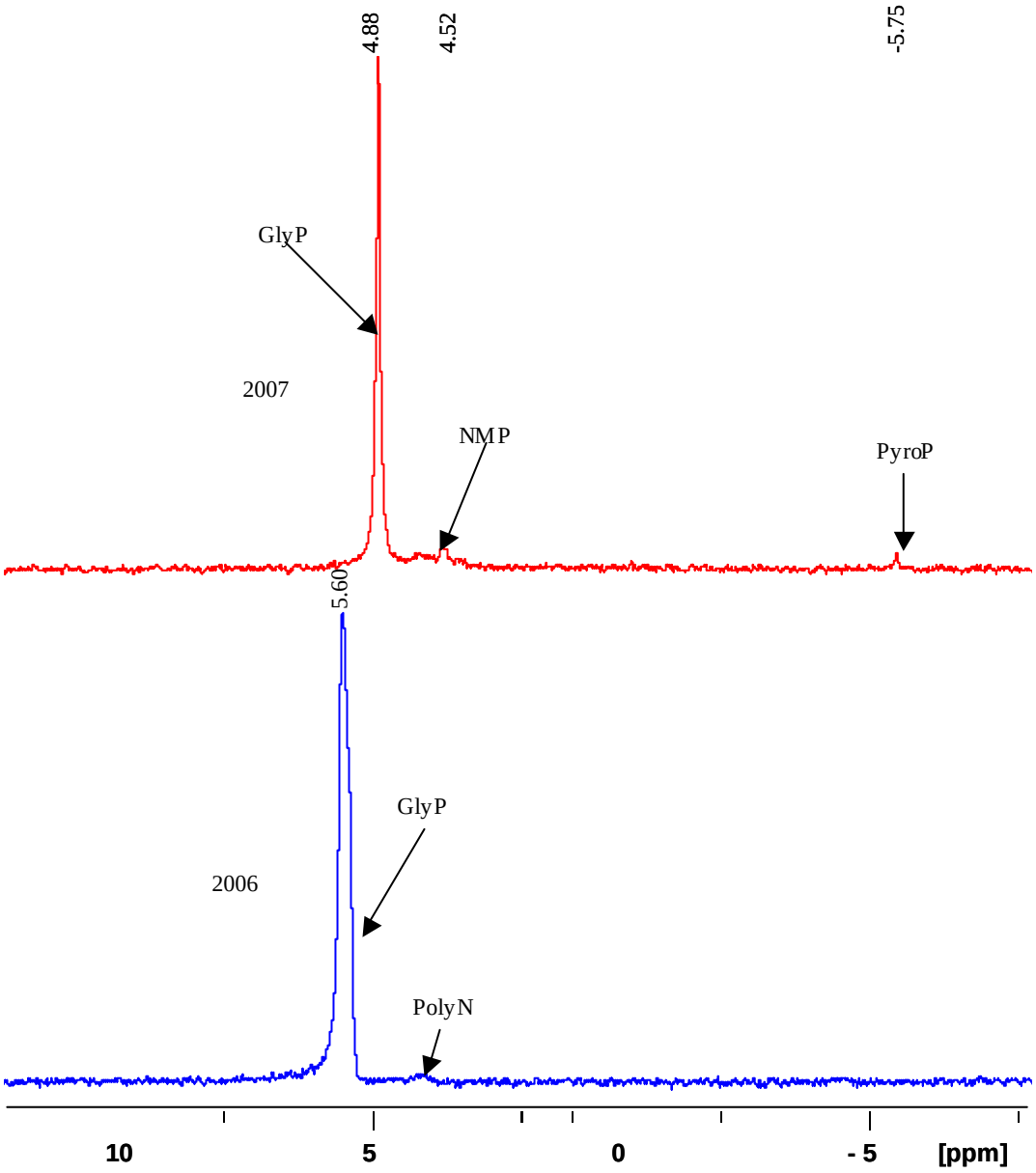


Fig. 14. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 13, Bronx River estuary, collected in 2006 and 2007.

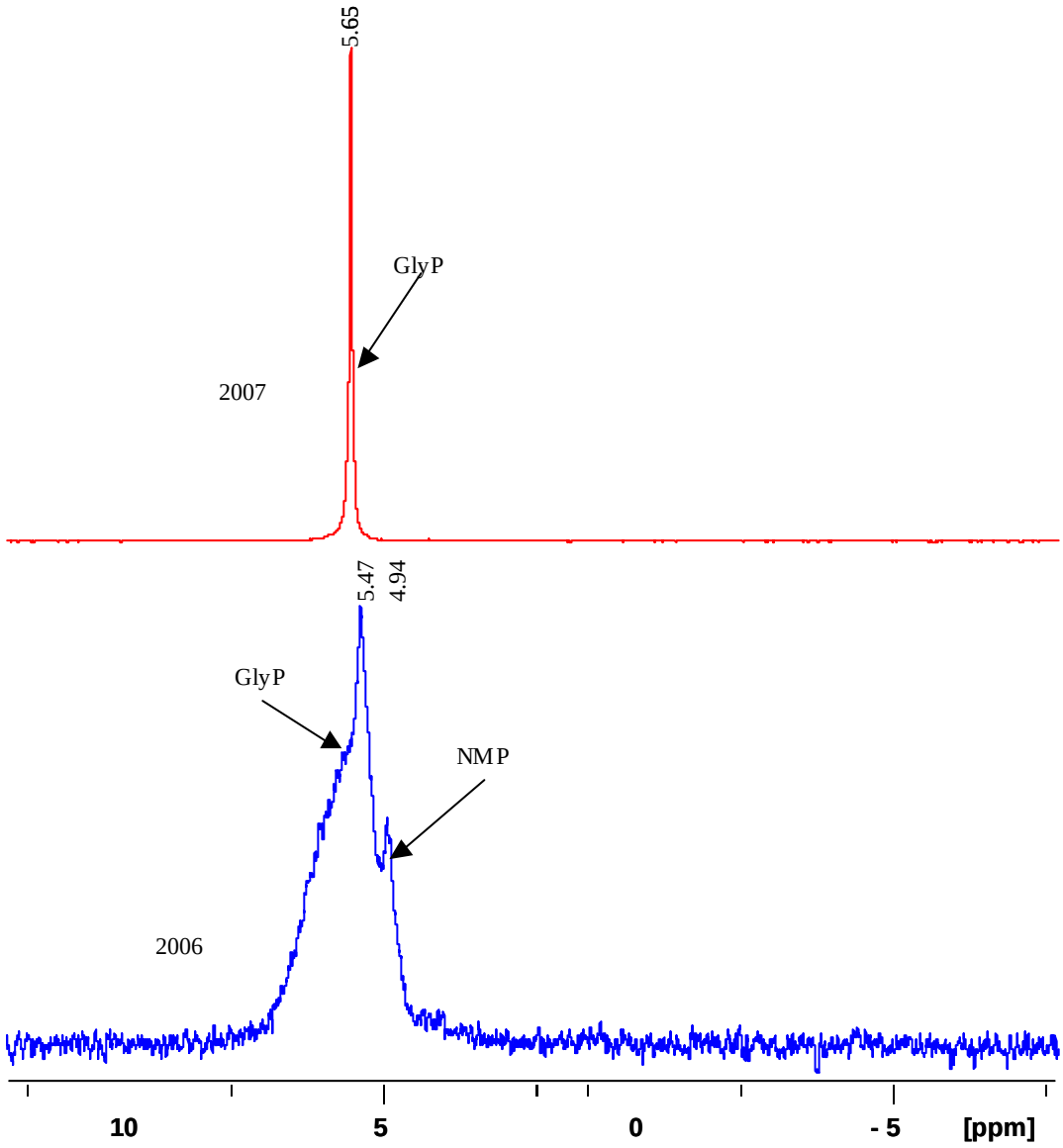


Fig. 15. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 14, Sound View Park Station, collected in 2006 and 2007.

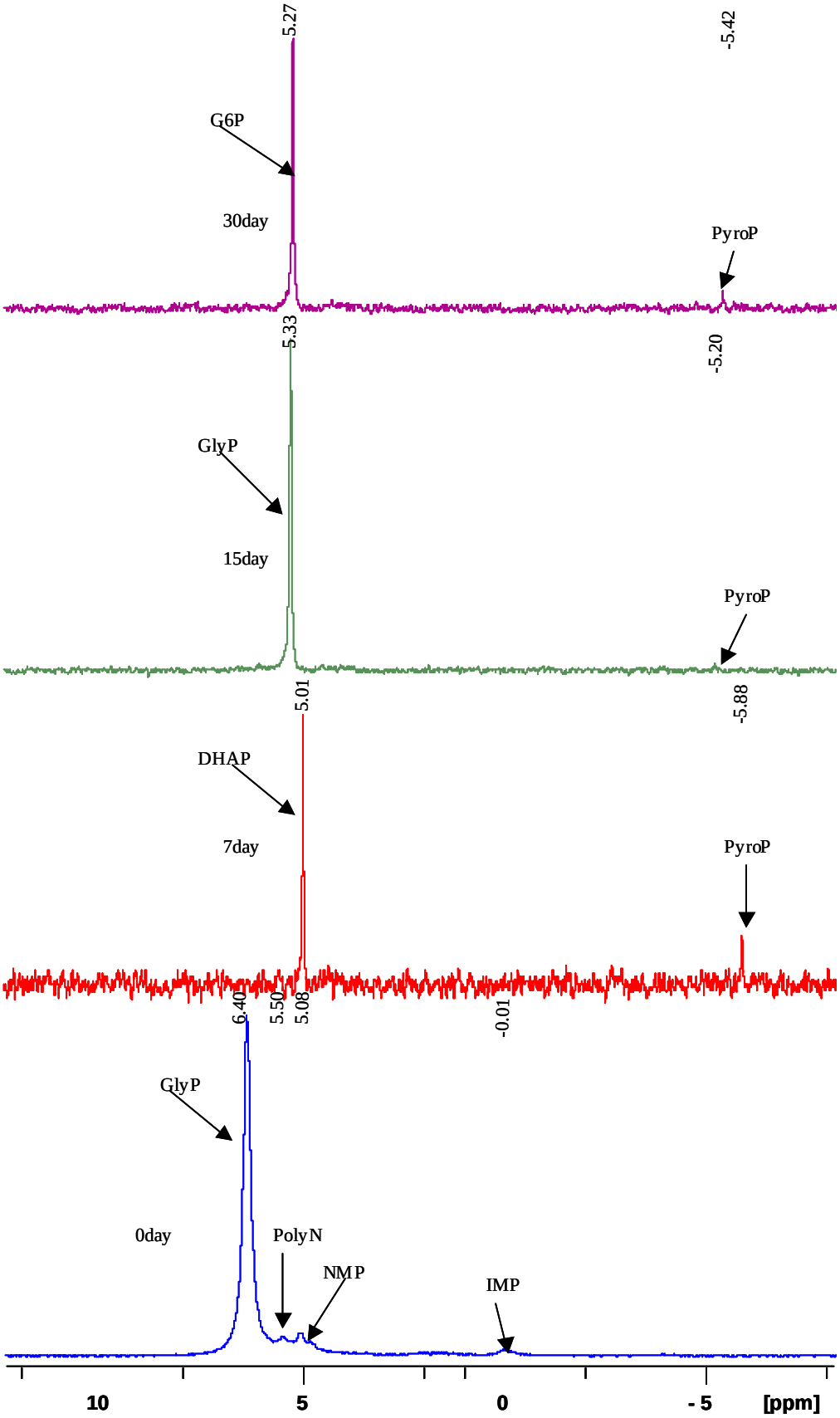


Fig. 16. ^{31}P-NMR spectra of NaOH-extract of sediments from Site 4, South of Troublesome Brook, collected in 2006 under flooding incubation for 0, 7, 15, and 30 d.

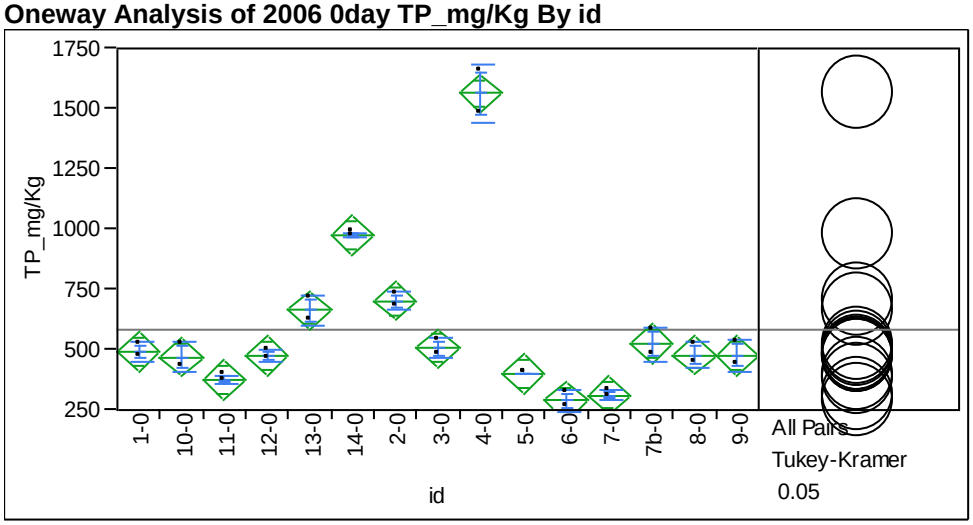
Appendix 2 Statistical analysis of phosphorus pools

2.1 2006 P pool

2.2 2007 P pool

Appendix 2.1 2006 P pool

Oneway Analysis of 2006 0day TP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.984983
Adj Rsquare	0.970966
Root Mean Square Error	54.02322
Mean of Response	579.7427
Observations (or Sum Wgts)	30

Analysis of Variance

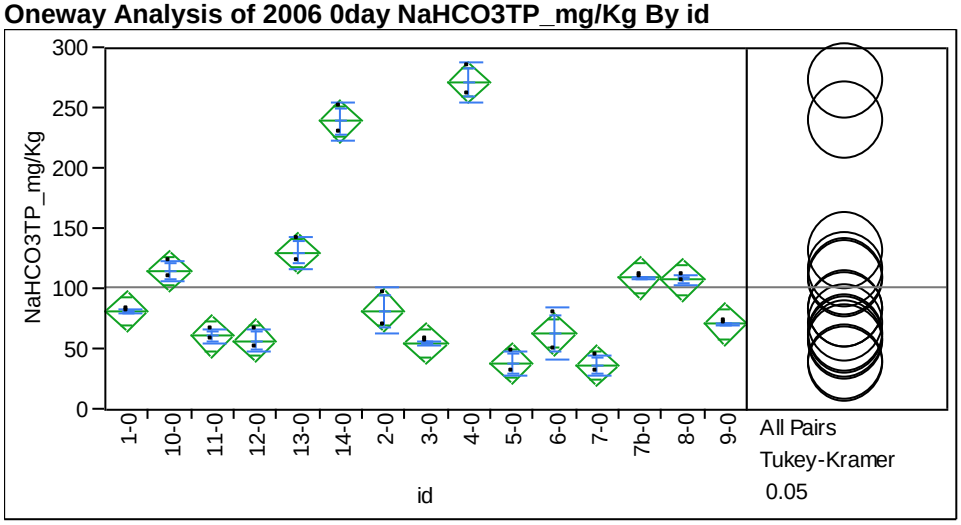
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	2871332.9	205095	70.2740	<.0001
Error	15	43777.6	2919		
C. Total	29	2915110.5			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
4-0	A					1562.8250
14-0		B				973.8450
2-0			C			702.6150
13-0			C	D		663.3200
7b-0			C	D	E	523.1500
3-0			C	D	E	505.8200
1-0			C	D	E	F 491.6600
8-0				D	E	F 478.5650
9-0				D	E	F 477.0600
12-0				D	E	F 473.0050
10-0				D	E	F 469.6250
5-0					E	F 398.3750
11-0					E	F 376.3300
7-0					E	F 311.7450
6-0						F 288.2000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day NaHCO3TP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.985459
Adj Rsquare	0.971888
Root Mean Square Error	11.40961
Mean of Response	101.322
Observations (or Sum Wgts)	30

Analysis of Variance

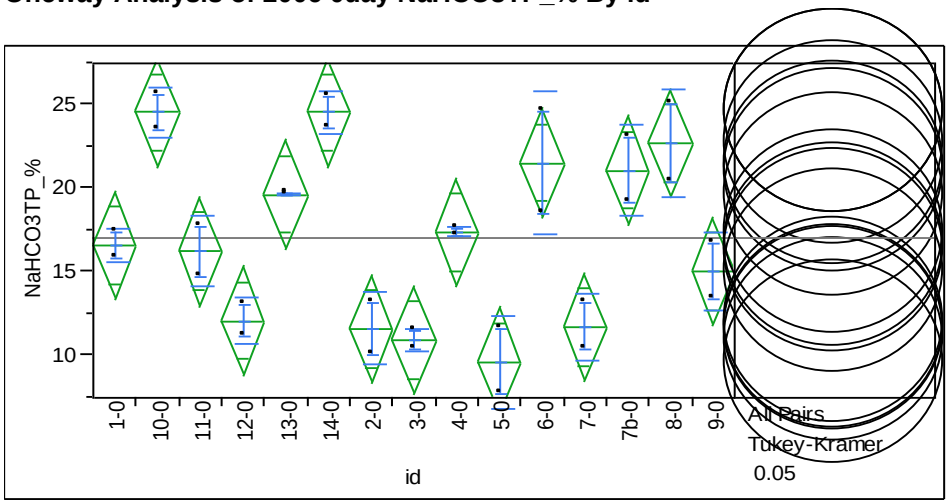
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	132337.05	9452.65	72.6126	<.0001
Error	15	1952.69	130.18		
C. Total	29	134289.73			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
4-0	A				271.73500
14-0	A				239.29500
13-0		B			130.30000
10-0		B	C		114.89000
7b-0		B	C		109.35500
8-0		B	C	D	107.91500
2-0			C	D	E	82.08000
1-0			C	D	E	81.44500
9-0			C	D	E	71.02500
6-0				D	E	63.01000
11-0					E	60.97000
12-0					E	57.45500
3-0					E	55.22000
5-0					E	38.35500
7-0					E	36.78000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day NaHCO3TP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.912803
Adj Rsquare	0.831419
Root Mean Square Error	2.164184
Mean of Response	17.03333
Observations (or Sum Wgts)	30

Analysis of Variance

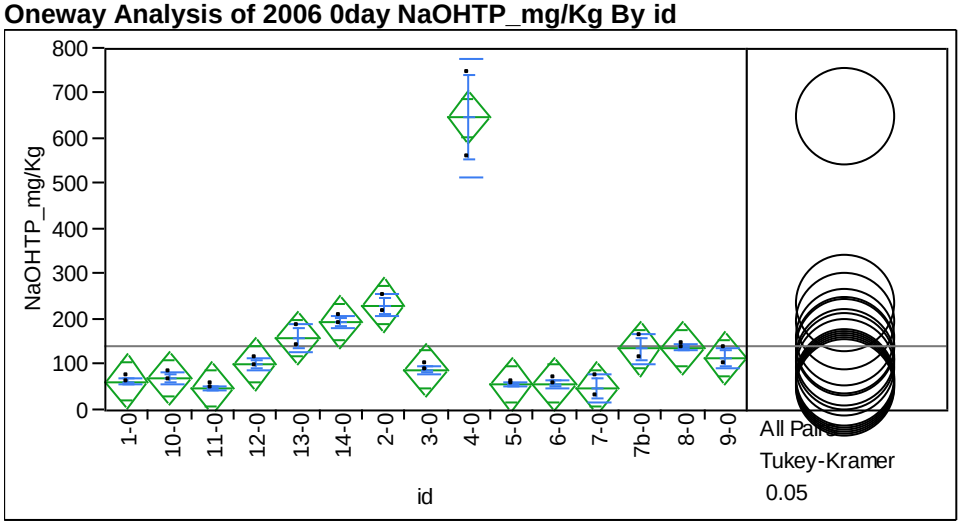
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	735.45287	52.5323	11.2160	<.0001
Error	15	70.25540	4.6837		
C. Total	29	805.70827			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
10-0	A				24.570000
14-0	A				24.565000
8-0	A	B			22.725000
6-0	A	B			21.545000
7b-0	A	B			21.090000
13-0	A	B	C		19.645000
4-0	A	B	C	D	17.400000
1-0	A	B	C	D	16.610000
11-0	A	B	C	D	16.250000
9-0		B	C	D	15.045000
12-0			C	D	12.110000
7-0			C	D	11.735000
2-0			C	D	11.625000
3-0				D	10.945000
5-0				D	9.640000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day NaOHTP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.965724
Adj Rsquare	0.933733
Root Mean Square Error	38.56116
Mean of Response	144.295
Observations (or Sum Wgts)	30

Analysis of Variance

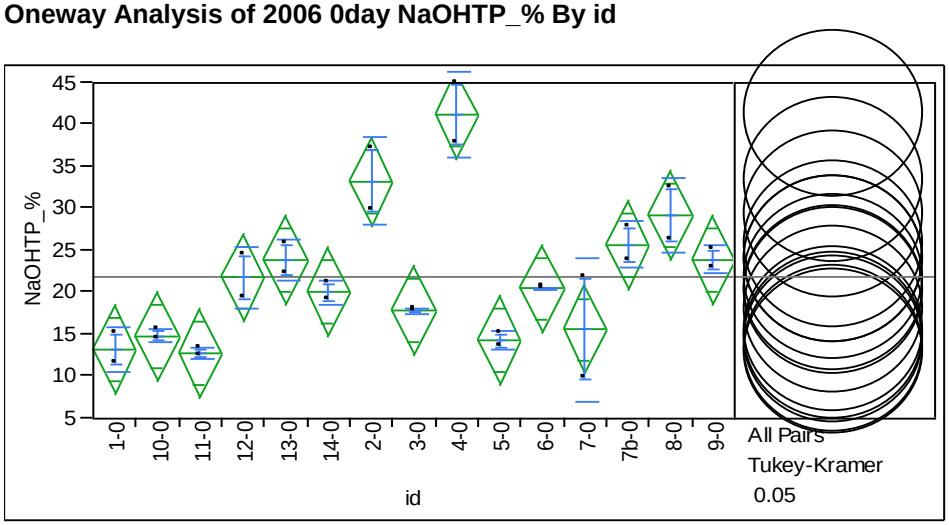
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	628422.00	44887.3	30.1872	<.0001
Error	15	22304.45	1487.0		
C. Total	29	650726.45			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level				Mean
4-0	A			647.14500
2-0		B		233.13000
14-0		B	C	195.21500
13-0		B	C	158.96500
8-0		B	C	138.57500
7b-0		B	C	135.71000
9-0		B	C	114.80000
12-0		B	C	102.58000
3-0		B	C	90.12000
10-0			C	70.04500
1-0			C	64.42000
6-0			C	58.94000
5-0			C	56.88000
7-0			C	49.42000
11-0			C	48.48000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day NaOHTP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.90635
Adj Rsquare	0.818944
Root Mean Square Error	3.533153
Mean of Response	21.876
Observations (or Sum Wgts)	30

Analysis of Variance

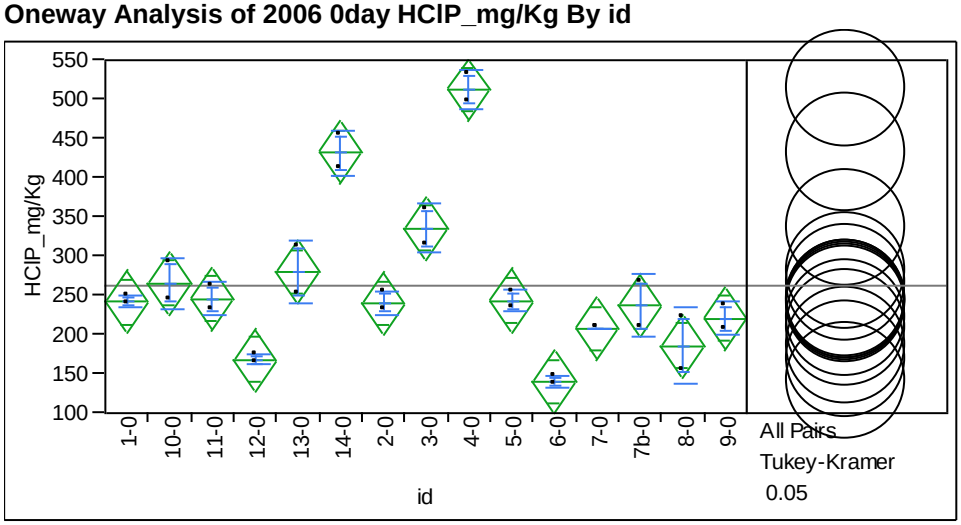
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	1812.2019	129.443	10.3694	<.0001
Error	15	187.2476	12.483		
C. Total	29	1999.4495			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
4-0	A				41.205000
2-0	A	B			33.315000
8-0	A	B	C		29.195000
7b-0		B	C	D	25.750000
9-0		B	C	D	23.955000
13-0		B	C	D	23.845000
12-0		B	C	D	21.780000
6-0		B	C	D	20.445000
14-0		B	C	D	20.055000
3-0			C	D	17.810000
7-0			C	D	15.565000
10-0				D	14.860000
5-0				D	14.280000
1-0				D	13.210000
11-0				D	12.870000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day HClP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.961486
Adj Rsquare	0.925539
Root Mean Square Error	26.68745
Mean of Response	263.6823
Observations (or Sum Wgts)	30

Analysis of Variance

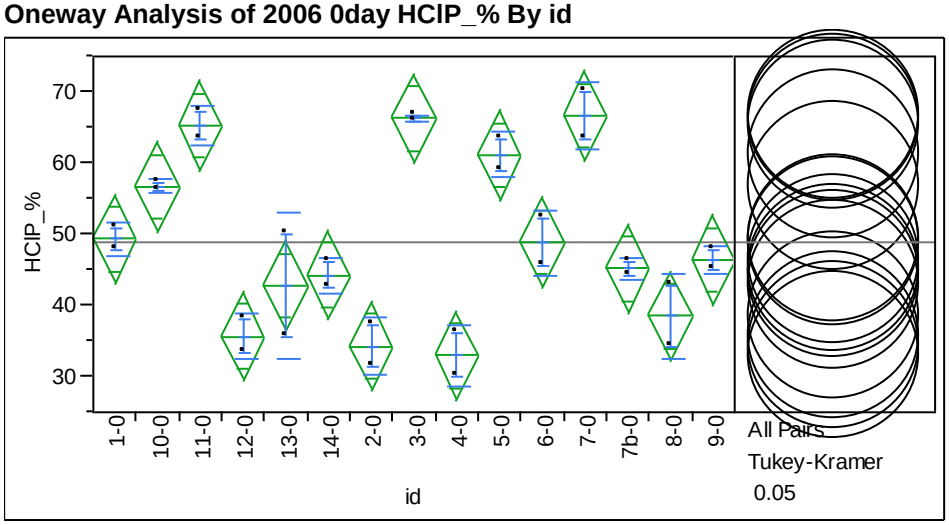
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	266700.71	19050.1	26.7474	<.0001
Error	15	10683.30	712.2		
C. Total	29	277384.01			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
4-0	A					512.28500
14-0	A	B				431.48000
3-0		B	C			335.34000
13-0			C	D		280.04500
10-0			C	D	E	265.85000
11-0			C	D	E	F 245.70000
5-0			C	D	E	F 243.63500
1-0			C	D	E	F 242.06500
2-0			C	D	E	F 240.10500
7b-0			C	D	E	F 236.69000
9-0				D	E	F 220.77500
7-0				D	E	F 207.20000
8-0				D	E	F 185.82500
12-0					E	F 168.36500
6-0						F 139.87500

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day HClP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.935201
Adj Rsquare	0.874722
Root Mean Square Error	4.216453
Mean of Response	48.94
Observations (or Sum Wgts)	30

Analysis of Variance

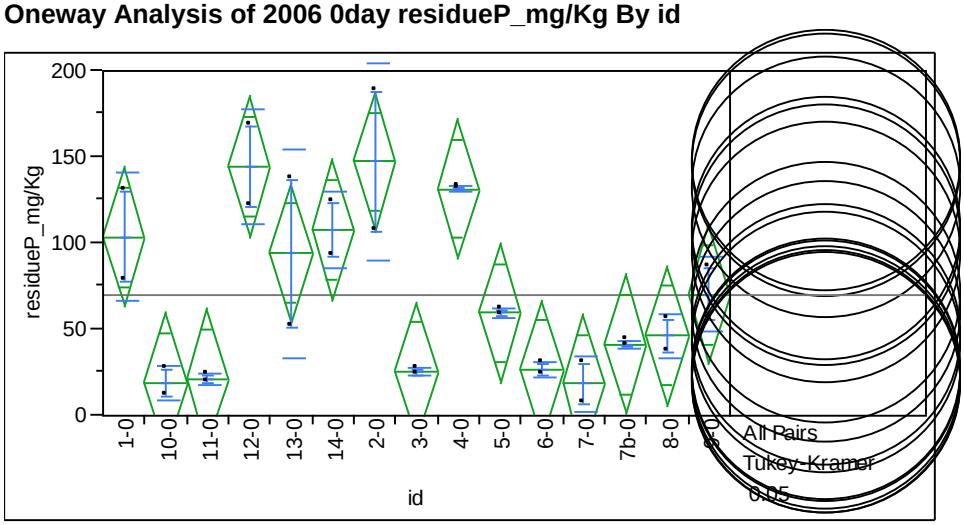
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	3848.7699	274.912	15.4632	<.0001
Error	15	266.6771	17.778		
C. Total	29	4115.4470			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
7-0	A					66.625000
3-0	A					66.275000
11-0	A	B				65.230000
5-0	A	B	C			61.145000
10-0	A	B	C	D		56.675000
1-0		B	C	D	E	49.320000
6-0		B	C	D	E	48.875000
9-0			C	D	E	46.400000
7b-0			C	D	E	45.145000
14-0				D	E	44.290000
13-0				D	E	42.715000
8-0					E	38.505000
12-0					E	35.675000
2-0					E	34.275000
4-0					E	32.950000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day residueP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.852021
Adj Rsquare	0.713906
Root Mean Square Error	27.03657
Mean of Response	70.44267
Observations (or Sum Wgts)	30

Analysis of Variance

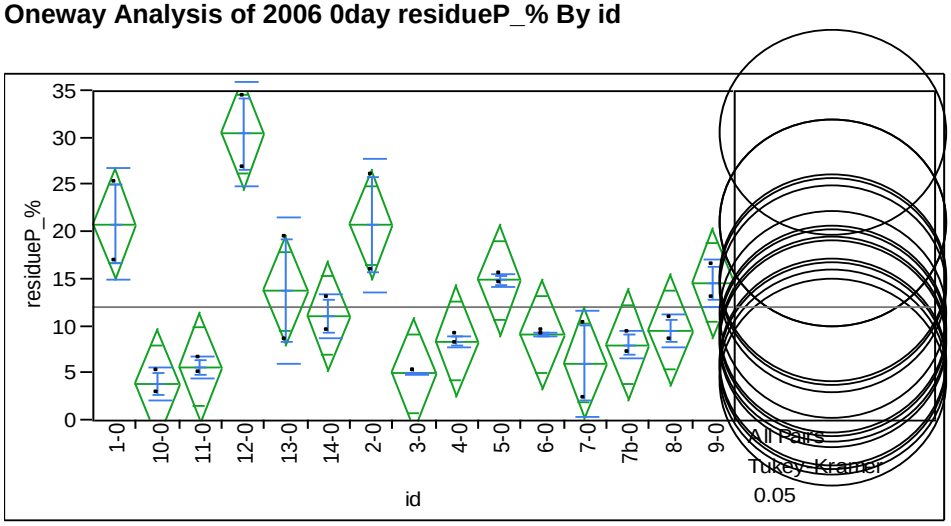
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	63131.087	4509.36	6.1690	0.0006
Error	15	10964.643	730.98		
C. Total	29	74095.730			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level				Mean
2-0	A			147.29500
12-0	A			144.60500
4-0	A	B		131.66000
14-0	A	B	C	107.85000
1-0	A	B	C	103.74000
13-0	A	B	C	94.01000
9-0	A	B	C	70.46000
5-0	A	B	C	59.51000
8-0	A	B	C	46.25000
7b-0	A	B	C	41.39500
6-0		B	C	26.37500
3-0		B	C	25.14000
11-0			C	21.17500
10-0			C	18.83500
7-0			C	18.34000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day residueP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.866628
Adj Rsquare	0.742147
Root Mean Square Error	3.916801
Mean of Response	12.15033
Observations (or Sum Wgts)	30

Analysis of Variance

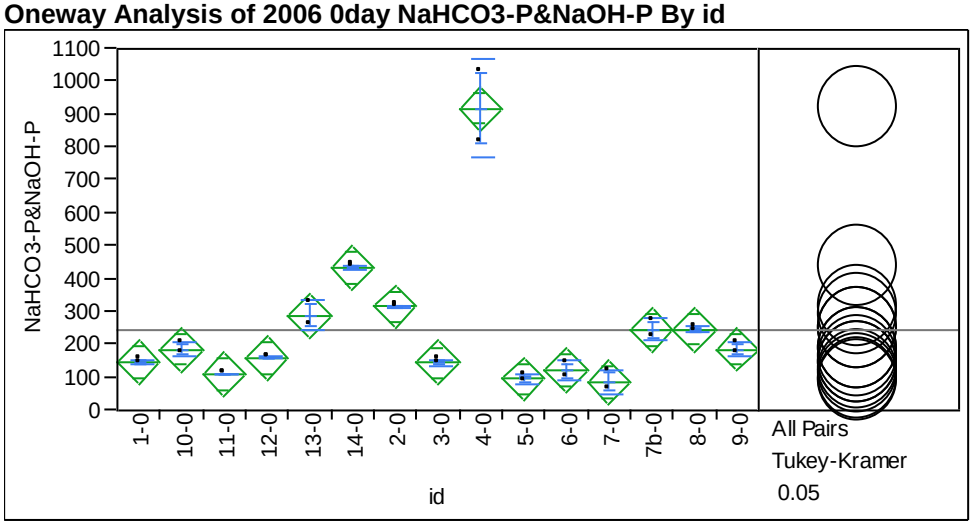
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	1495.2763	106.805	6.9619	0.0003
Error	15	230.1199	15.341		
C. Total	29	1725.3963			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level				Mean
12-0	A			30.435000
1-0	A	B		20.860000
2-0	A	B		20.780000
5-0	A	B	C	14.940000
9-0		B	C	14.595000
13-0		B	C	13.795000
14-0		B	C	11.090000
8-0		B	C	9.570000
6-0		B	C	9.135000
4-0		B	C	8.445000
7b-0		B	C	8.015000
7-0		B	C	6.080000
11-0		B	C	5.650000
3-0			C	4.970000
10-0			C	3.895000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day NaHCO3-P&NaOH-P By id



Oneway Anova
Summary of Fit

Rsquare	0.976667
Adj Rsquare	0.954889
Root Mean Square Error	44.15866
Mean of Response	245.6173
Observations (or Sum Wgts)	30

Analysis of Variance

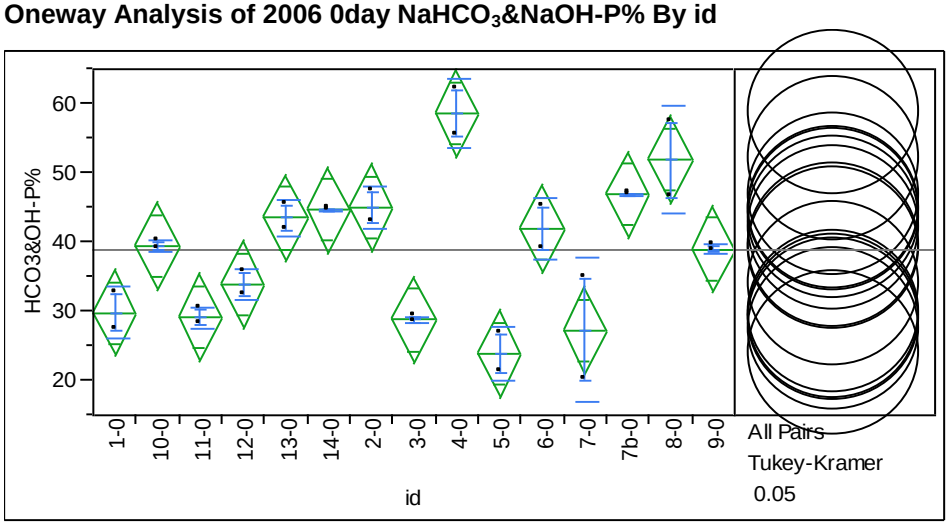
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	1224322.6	87451.6	44.8473	<.0001
Error	15	29249.8	1950.0		
C. Total	29	1253572.5			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
4-0	A				918.88000
14-0		B			434.51000
2-0		B	C		315.21000
13-0		B	C	D	289.26500
8-0			C	D	E	246.48500
7b-0			C	D	E	245.06500
9-0			C	D	E	185.82500
10-0			C	D	E	184.93500
12-0			C	D	E	160.04000
1-0			C	D	E	145.86000
3-0			C	D	E	145.34000
6-0				D	E	121.95000
11-0					E	109.45500
5-0					E	95.23500
7-0					E	86.20500

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day $NaHCO_3$&NaOH-P% By id



Oneway Anova
Summary of Fit

Rsquare	0.912977
Adj Rsquare	0.831756
Root Mean Square Error	4.197416
Mean of Response	38.908
Observations (or Sum Wgts)	30

Analysis of Variance

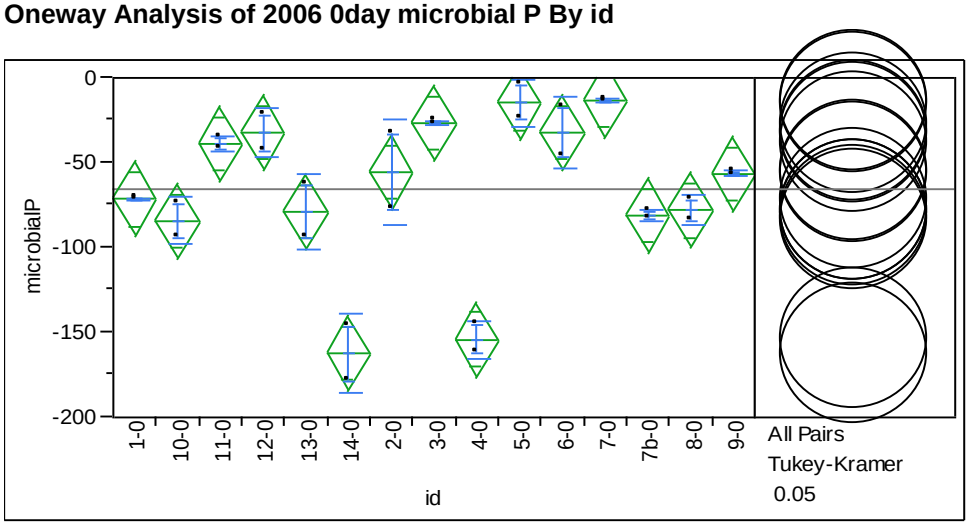
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	2772.5772	198.041	11.2407	<.0001
Error	15	264.2745	17.618		
C. Total	29	3036.8517			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
4-0	A					58.605000
8-0	A	B				51.920000
7b-0	A	B	C			46.840000
2-0	A	B	C	D		44.945000
14-0	A	B	C	D		44.615000
13-0	A	B	C	D	E	43.485000
6-0	A	B	C	D	E	41.990000
10-0		B	C	D	E	F 39.430000
9-0		B	C	D	E	F 39.000000
12-0			C	D	E	F 33.890000
1-0				D	E	F 29.810000
11-0				D	E	F 29.120000
3-0				D	E	F 28.755000
7-0					E	F 27.295000
5-0						F 23.920000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day microbial P By id



Oneway Anova
Summary of Fit

Rsquare	0.945025
Adj Rsquare	0.893716
Root Mean Square Error	14.74398
Mean of Response	-65.3997
Observations (or Sum Wgts)	30

Analysis of Variance

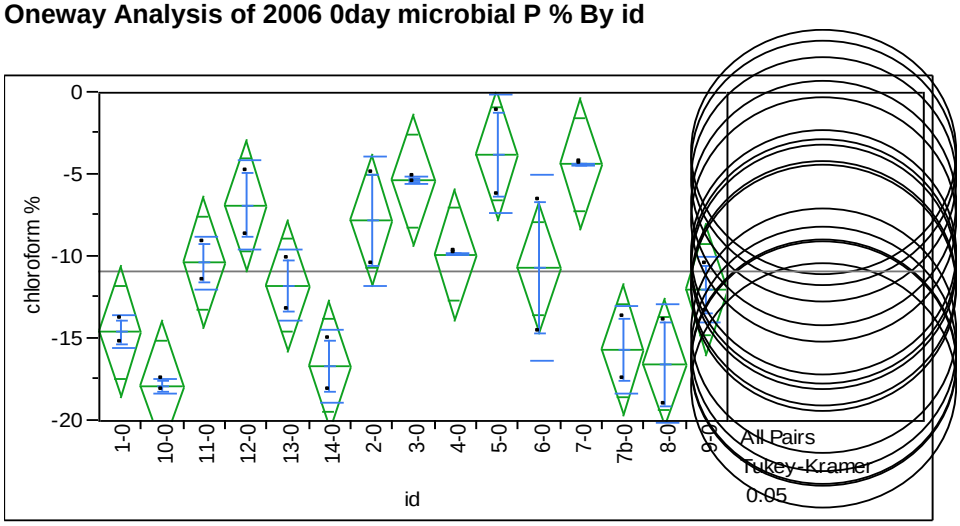
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	56053.504	4003.82	18.4181	<.0001
Error	15	3260.775	217.39		
C. Total	29	59314.279			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level				Mean
7-0	A			-13.4400
5-0	A			-14.8700
3-0	A	B		-26.9700
6-0	A	B		-32.1550
12-0	A	B		-32.5150
11-0	A	B		-38.7900
2-0	A	B		-55.5250
9-0	A	B		-56.4000
1-0	A	B		-71.6150
8-0		B		-78.2000
13-0		B		-78.8150
7b-0		B		-81.2300
10-0		B		-84.1800
4-0			C	-153.9900
14-0			C	-162.3000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 0day microbial P % By id



Oneway Anova
Summary of Fit

Rsquare	0.852029
Adj Rsquare	0.713922
Root Mean Square Error	2.657129
Mean of Response	-10.9433
Observations (or Sum Wgts)	30

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	609.80867	43.5578	6.1694	0.0006
Error	15	105.90500	7.0603		
C. Total	29	715.71367			

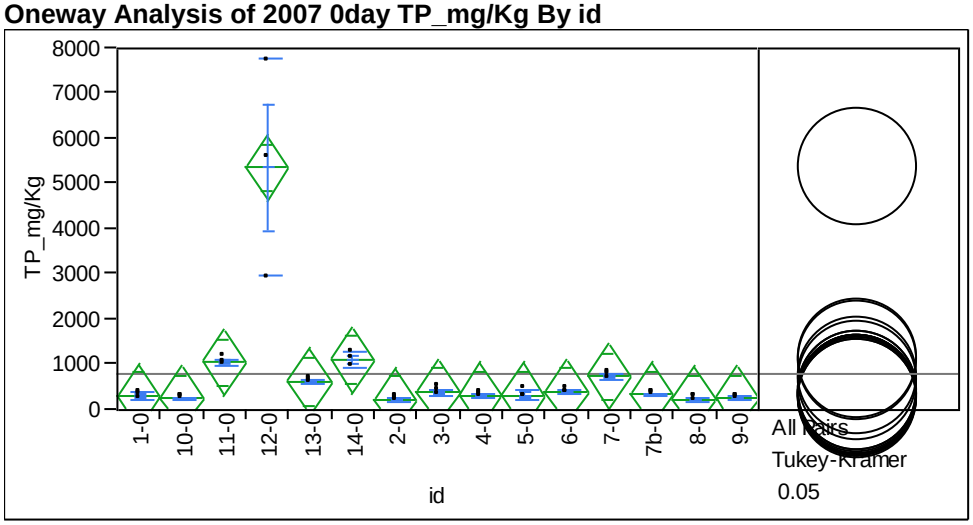
Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
5-0	A					-3.75000
7-0	A	B				-4.35000
3-0	A	B	C			-5.35000
12-0	A	B	C	D		-6.85000
2-0	A	B	C	D	E	-7.80000
4-0	A	B	C	D	E	-9.85000
11-0	A	B	C	D	E	-10.35000
6-0	A	B	C	D	E	-10.70000
13-0	A	B	C	D	E	-11.75000
9-0	A	B	C	D	E	-12.00000
1-0		B	C	D	E	-14.60000
7b-0			C	D	E	-15.70000
8-0				D	E	-16.55000
14-0				D	E	-16.65000
10-0					E	-17.90000

Levels not connected by same letter are significantly different.

Appendix 2.2: 2007 P pool

Oneway Analysis of 2007 0day TP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.85788
Adj Rsquare	0.791557
Root Mean Square Error	623.8097
Mean of Response	793.4109
Observations (or Sum Wgts)	45

Analysis of Variance

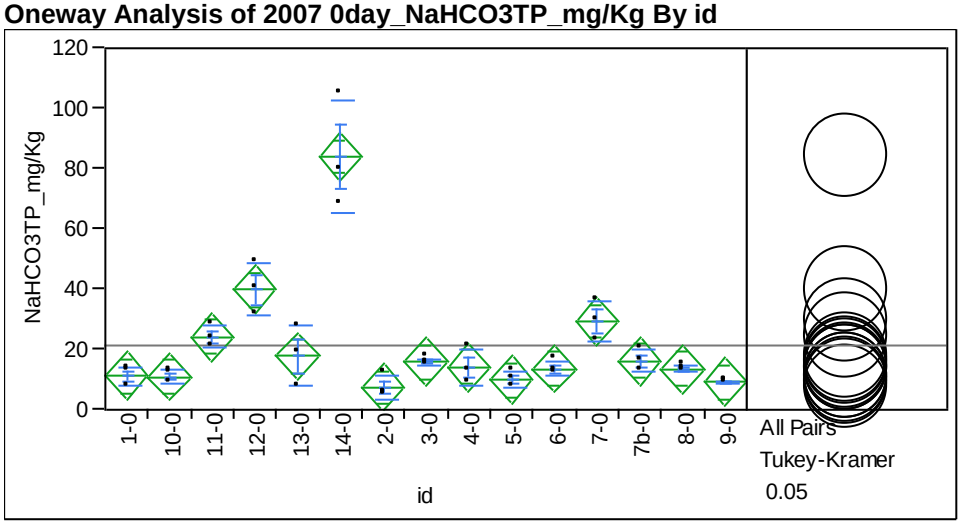
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	70468705	5033479	12.9349	<.0001
Error	30	11674155	389138		
C. Total	44	82142859			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level		Mean
12-0	A	5359.7533
14-0	B	1106.1733
11-0	B	1051.2600
7-0	B	735.1367
13-0	B	620.8533
3-0	B	396.2467
6-0	B	392.6067
7b-0	B	334.6800
5-0	B	318.0000
4-0	B	310.7033
1-0	B	306.0200
9-0	B	257.0367
10-0	B	254.7067
8-0	B	238.5600
2-0	B	219.4267

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day_NaHCO3TP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.9215
Adj Rsquare	0.884866
Root Mean Square Error	6.703735
Mean of Response	21.12178
Observations (or Sum Wgts)	45

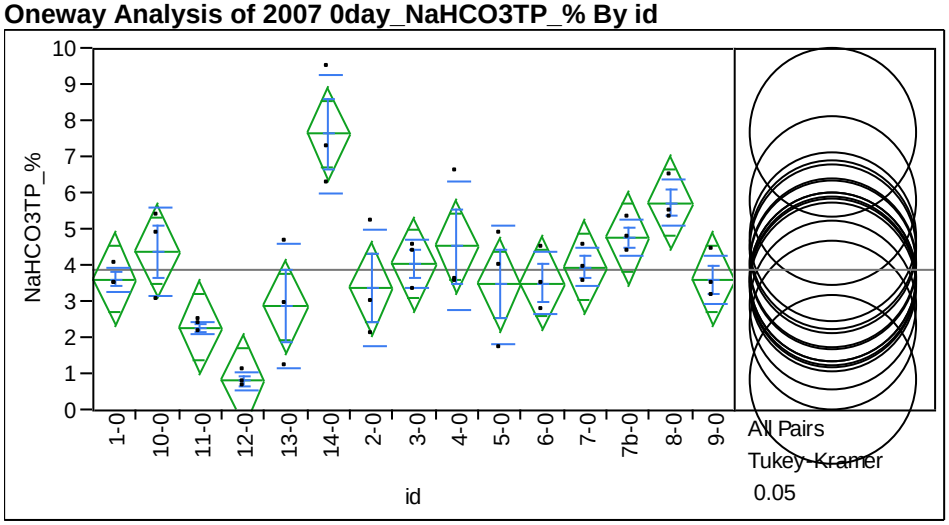
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	15826.319	1130.45	25.1546	<.0001
Error	30	1348.202	44.94		
C. Total	44	17174.521			

Level				Mean
14-0	A			84.036667
12-0		B		39.896667
7-0		B	C	29.323333
11-0		B	C D	24.133333
13-0			C D	17.733333
7b-0			C D	16.103333
3-0			C D	15.770000
4-0			C D	14.106667
6-0			C D	13.616667
8-0			C D	13.536667
1-0			C D	11.126667
10-0			C D	10.983333
5-0			C D	9.876667
9-0			C D	9.180000
2-0			D	7.403333

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day_NaHCO3TP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.727152
Adj Rsquare	0.599823
Root Mean Square Error	1.108309
Mean of Response	3.915778
Observations (or Sum Wgts)	45

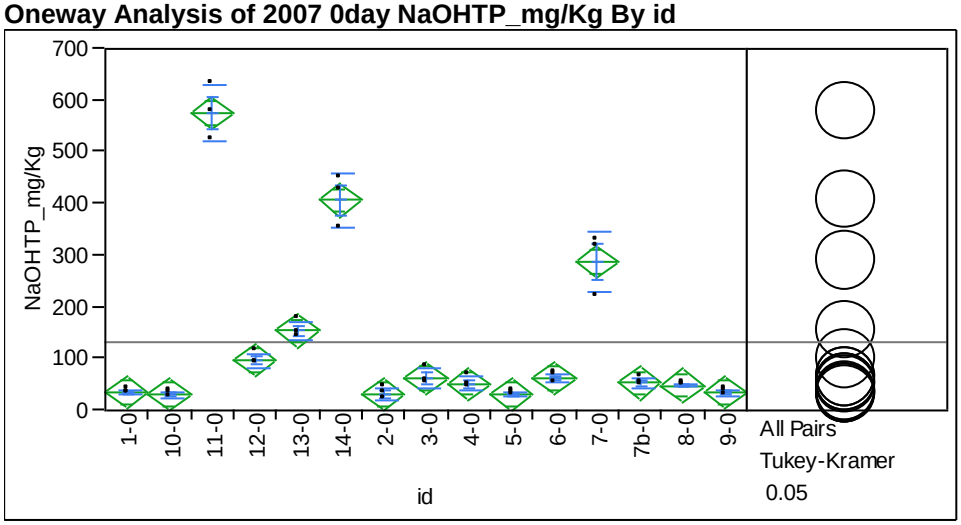
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	98.20823	7.01487	5.7108	<.0001
Error	30	36.85047	1.22835		
C. Total	44	135.05870			

Level					Mean
14-0	A				7.6433333
8-0	A	B			5.7433333
7b-0	A	B	C		4.7733333
4-0	A	B	C		4.5366667
10-0	A	B	C		4.3966667
3-0		B	C	D	4.0500000
7-0		B	C	D	3.9600000
9-0		B	C	D	3.6333333
1-0		B	C	D	3.6266667
6-0		B	C	D	3.5266667
5-0		B	C	D	3.4800000
2-0		B	C	D	3.3833333
13-0		B	C	D	2.8800000
11-0			C	D	2.2900000
12-0				D	0.8133333

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day NaOHTP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.981337
Adj Rsquare	0.972628
Root Mean Square Error	26.82191
Mean of Response	130.65
Observations (or Sum Wgts)	45

Analysis of Variance

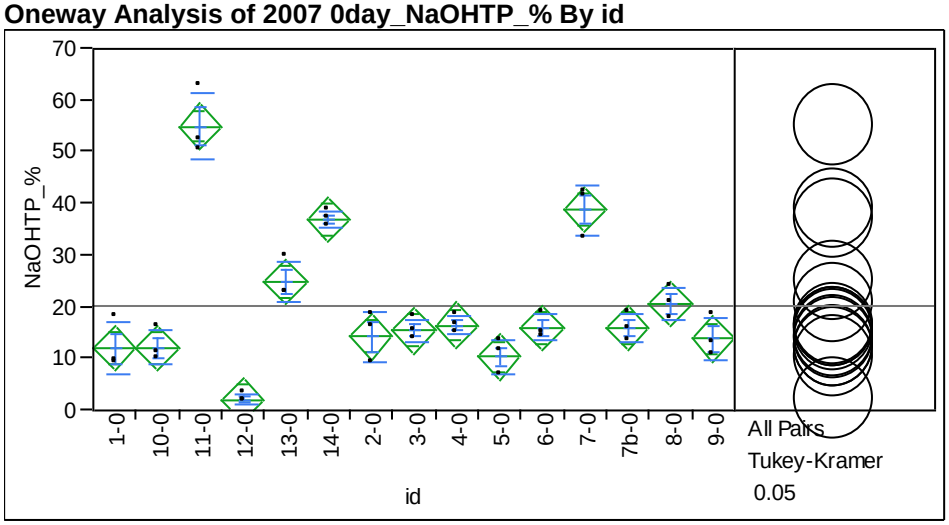
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	1134849.6	81060.7	112.6758	<.0001
Error	30	21582.5	719.4		
C. Total	44	1156432.1			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
11-0	A				576.47000
14-0		B			406.82000
7-0			C		287.72000
13-0				D	153.78333
12-0				D	E 96.60667
6-0					E 62.35667
3-0					E 61.87667
7b-0					E 52.98333
4-0					E 51.66333
8-0					E 48.33000
9-0					E 34.67333
1-0					E 34.22000
2-0					E 31.31667
5-0					E 30.51667
10-0					E 30.41333

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day_NaOHTP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.949324
Adj Rsquare	0.925675
Root Mean Square Error	3.702014
Mean of Response	20.31289
Observations (or Sum Wgts)	45

Analysis of Variance

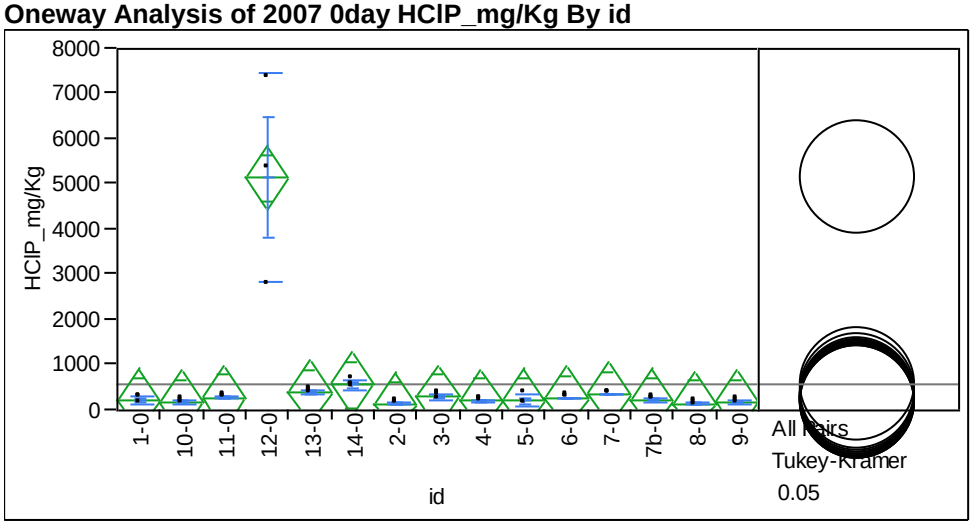
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	7702.0945	550.150	40.1425	<.0001
Error	30	411.1472	13.705		
C. Total	44	8113.2417			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
11-0	A				55.026667
7-0		B			38.853333
14-0		B			36.850000
13-0			C		24.886667
8-0			C	D	20.620000
4-0			C	D	16.513333
6-0			C	D	15.970000
7b-0			C	D	15.906667
3-0			C	D	15.446667
2-0			C	D	14.273333
9-0			C	D	13.830000
10-0				D	E	12.153333
1-0				D	E	11.993333
5-0				D	E	10.320000
12-0					E	2.050000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day HClP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.862386
Adj Rsquare	0.798167
Root Mean Square Error	597.8023
Mean of Response	587.6484
Observations (or Sum Wgts)	45

Analysis of Variance

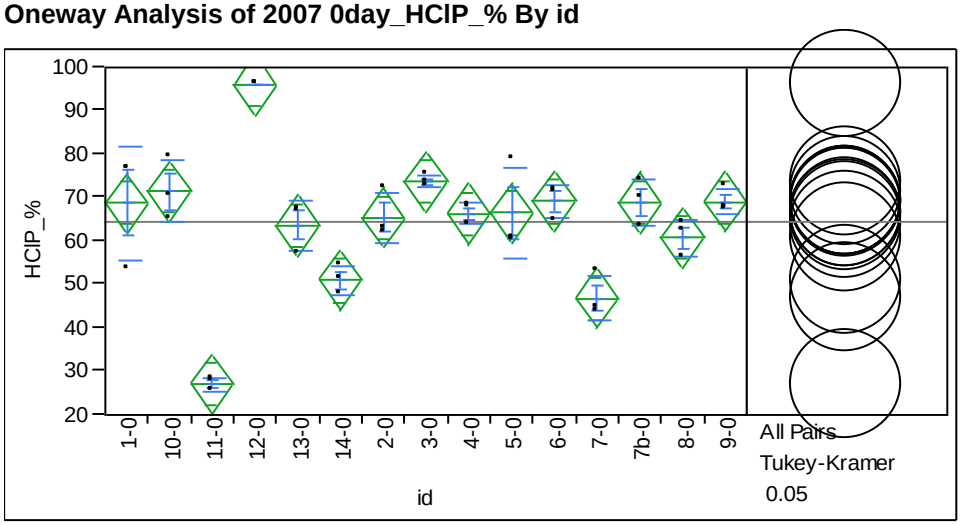
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	67185651	4798975	13.4287	<.0001
Error	30	10721029	357368		
C. Total	44	77906680			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level		Mean
12-0	A	5142.5500
14-0	B	564.8167
13-0	B	396.0333
7-0	B	342.0300
3-0	B	291.4267
11-0	B	283.9967
6-0	B	270.0633
7b-0	B	231.2700
5-0	B	219.3867
1-0	B	216.7767
4-0	B	205.1533
10-0	B	183.1933
9-0	B	177.9033
8-0	B	146.0333
2-0	B	144.0933

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day_HClP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.899447
Adj Rsquare	0.852523
Root Mean Square Error	5.90981
Mean of Response	64.27178
Observations (or Sum Wgts)	45

Analysis of Variance

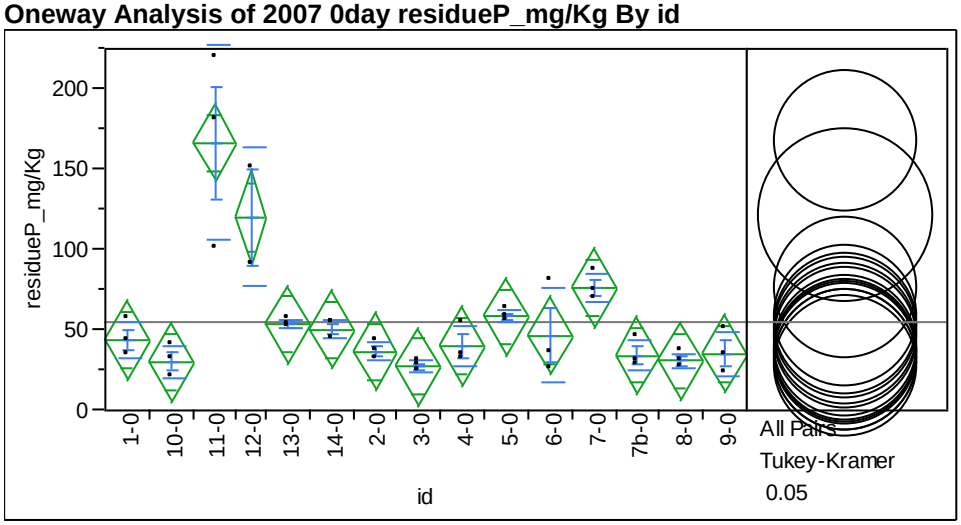
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	9372.392	669.457	19.1679	<.0001
Error	30	1047.775	34.926		
C. Total	44	10420.167			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
12-0	A				95.923333
3-0		B			73.606667
10-0		B			71.396667
6-0		B			69.116667
9-0		B			69.013333
7b-0		B			68.956667
1-0		B			68.730000
5-0		B	C		66.470000
4-0		B	C		66.273333
2-0		B	C		65.496667
13-0		B	C	D	63.626667
8-0		B	C	D	60.730000
14-0			C	D	50.920000
7-0				D	46.846667
11-0					E 26.970000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day residueP_mg/Kg By id



Oneway Anova
Summary of Fit

Rsquare	0.821671
Adj Rsquare	0.735581
Root Mean Square Error	20.84584
Mean of Response	55.18205
Observations (or Sum Wgts)	44

Analysis of Variance

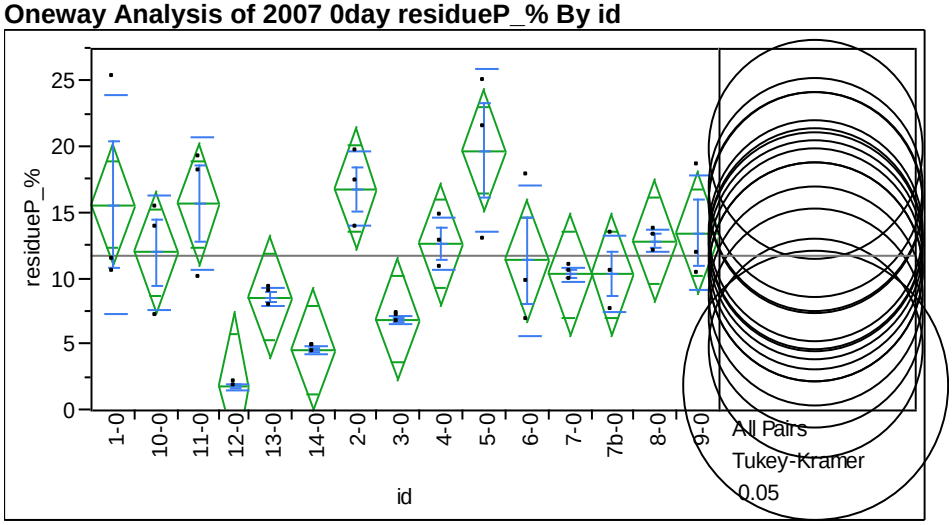
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	58064.635	4147.47	9.5443	<.0001
Error	29	12601.928	434.55		
C. Total	43	70666.562			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level				Mean
11-0	A			166.66000
12-0	A	B		120.30500
7-0		B	C	76.06333
5-0		B	C	58.21667
13-0		B	C	53.30333
14-0		B	C	50.49333
6-0			C	46.56667
1-0			C	43.89333
4-0			C	39.78333
2-0			C	36.61000
9-0			C	35.27667
7b-0			C	34.32333
8-0			C	30.66333
10-0			C	30.11000
3-0			C	27.17000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day residueP_% By id



Oneway Anova
Summary of Fit

Rsquare	0.650799
Adj Rsquare	0.482219
Root Mean Square Error	3.949222
Mean of Response	11.75886
Observations (or Sum Wgts)	44

Analysis of Variance

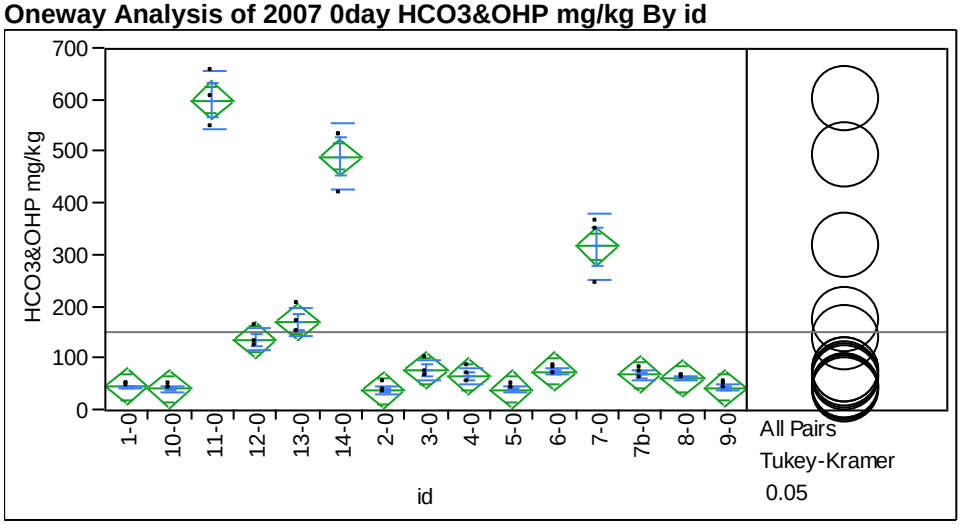
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	842.9317	60.2094	3.8605	0.0010
Error	29	452.2943	15.5964		
C. Total	43	1295.2260			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
5-0	A				19.730000
2-0	A	B			16.846667
11-0	A	B	C		15.716667
1-0	A	B	C		15.653333
9-0	A	B	C	D	13.520000
8-0	A	B	C	D	12.900000
4-0	A	B	C	D	12.673333
10-0	A	B	C	D	12.053333
6-0	A	B	C	D	11.386667
7b-0	A	B	C	D	10.366667
7-0	A	B	C	D	10.340000
13-0	A	B	C	D	8.603333
3-0		B	C	D	6.893333
14-0			C	D	4.583333
12-0				D	1.795000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day HCO3&OHP mg/kg By id



Oneway Anova
Summary of Fit

Rsquare	0.97964
Adj Rsquare	0.970139
Root Mean Square Error	30.18646
Mean of Response	151.7556
Observations (or Sum Wgts)	45

Analysis of Variance

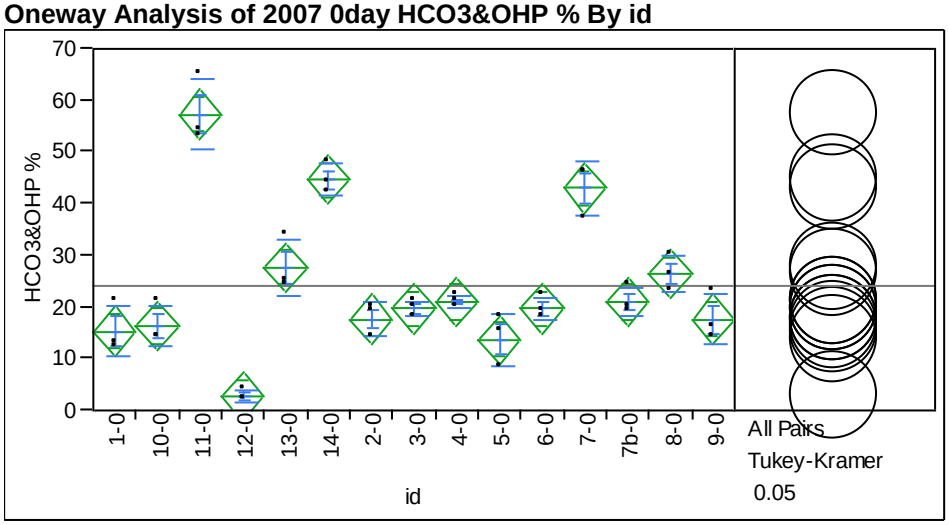
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	1315329.6	93952.1	103.1056	<.0001
Error	30	27336.7	911.2		
C. Total	44	1342666.3			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
11-0	A					600.66667
14-0		B				491.00000
7-0			C			317.00000
13-0				D		171.33333
12-0				D	E	136.66667
3-0					E	F 77.33333
6-0					E	F 75.66667
7b-0					E	F 69.00000
4-0					E	F 66.00000
8-0					E	F 62.00000
1-0						F 45.33333
9-0						F 43.66667
10-0						F 41.33333
5-0						F 40.66667
2-0						F 38.66667

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day HCO3&OHP % By id



Oneway Anova
Summary of Fit

Rsquare	0.945211
Adj Rsquare	0.919642
Root Mean Square Error	3.991658
Mean of Response	24.24444
Observations (or Sum Wgts)	45

Analysis of Variance

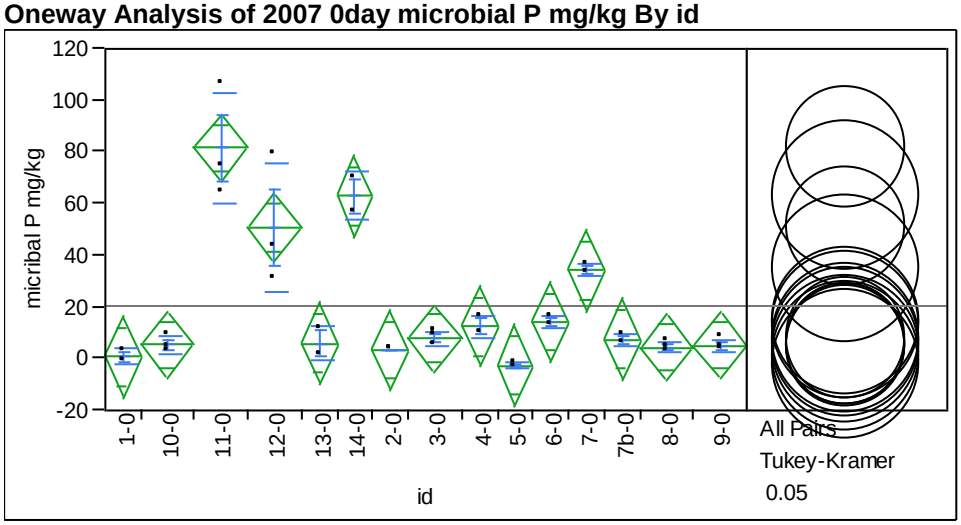
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	8246.3111	589.022	36.9679	<.0001
Error	30	478.0000	15.933		
C. Total	44	8724.3111			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level						Mean
11-0	A					57.333333
14-0		B				44.666667
7-0		B				43.000000
13-0			C			27.666667
8-0			C	D		26.333333
7b-0			C	D	E	21.000000
4-0			C	D	E	21.000000
3-0			C	D	E	19.666667
6-0			C	D	E	19.666667
9-0			C	D	E	17.666667
2-0			C	D	E	17.666667
10-0			C	D	E	16.333333
1-0				D	E	15.333333
5-0					E	F	13.666667
12-0						F	2.666667

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day microbial P mg/kg By id



Oneway Anova
Summary of Fit

Rsquare	0.908396
Adj Rsquare	0.847326
Root Mean Square Error	10.73314
Mean of Response	20.63583
Observations (or Sum Wgts)	36

Analysis of Variance

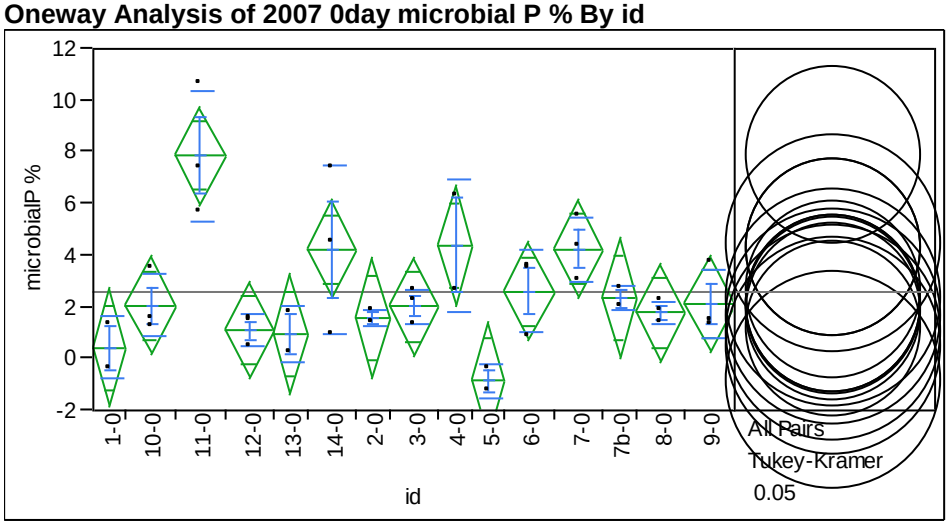
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	23990.108	1713.58	14.8748	<.0001
Error	21	2419.208	115.20		
C. Total	35	26409.316			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level					Mean
11-0	A				81.55333
14-0	A	B			62.99500
12-0	A	B	C		50.78333
7-0		B	C	D	34.25500
6-0			C	D	14.26000
4-0				D	12.52500
3-0				D	7.89667
7b-0				D	7.44500
13-0				D	6.00500
10-0				D	5.30333
9-0				D	5.11000
8-0				D	4.37000
2-0				D	3.34000
1-0				D	0.67500
5-0				D	-2.58000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day microbial P % By id



Oneway Anova
Summary of Fit

Rsquare	0.722481
Adj Rsquare	0.560595
Root Mean Square Error	1.590051
Mean of Response	2.61
Observations (or Sum Wgts)	39

Analysis of Variance

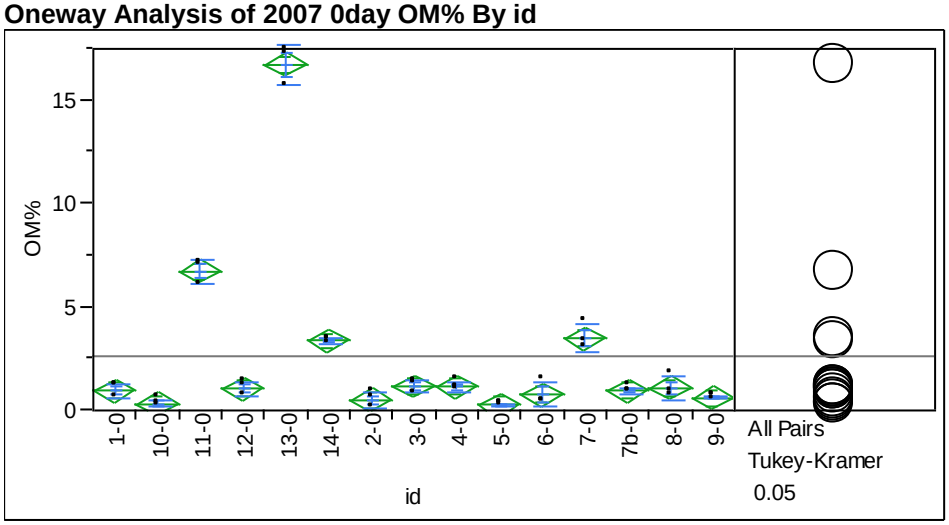
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	157.96748	11.2834	4.4629	0.0007
Error	24	60.67832	2.5283		
C. Total	38	218.64580			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

Level			Mean
11-0	A		7.863333
4-0	A	B	4.390000
7-0	A	B	4.246667
14-0	A	B	4.236667
6-0		B	2.610000
7b-0		B	2.340000
9-0		B	2.113333
10-0		B	2.060000
3-0		B	2.013333
8-0		B	1.783333
2-0		B	1.580000
12-0		B	1.106667
13-0		B	0.965000
1-0		B	0.440000
5-0		B	-0.870000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 0day OM% By id



Oneway Anova
Summary of Fit

Rsquare	0.992079
Adj Rsquare	0.988383
Root Mean Square Error	0.451171
Mean of Response	2.610222
Observations (or Sum Wgts)	45

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
id	14	764.87323	54.6338	268.3975	<.0001
Error	30	6.10667	0.2036		
C. Total	44	770.97990			

Means Comparisons
Comparisons for all pairs using Tukey-Kramer HSD

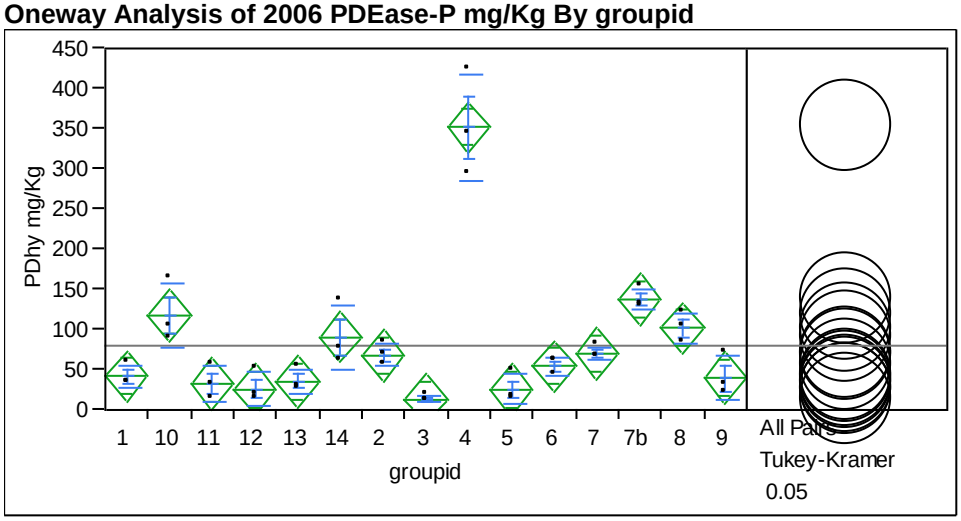
Level				Mean
13-0	A			16.743333
11-0		B		6.720000
7-0			C	3.493333
14-0			C	3.360000
3-0			D	1.166667
4-0			D	1.146667
8-0			D	1.076667
12-0			D	1.056667
1-0			D	0.953333
7b-0			D	0.950000
6-0			D	0.766667
9-0			D	0.616667
2-0			D	0.500000
10-0			D	0.340000
5-0			D	0.263333

Levels not connected by same letter are significantly different.

Appendix 3 Statistical analysis of enzymatic hydrolysis

Appendix 3.1 2006 Enzyme hydrolysis

Oneway Analysis of 2006 PDEase-P mg/Kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.930938
Adj Rsquare	0.898709
Root Mean Square Error	26.9058
Mean of Response	80.28222
Observations (or Sum Wgts)	45

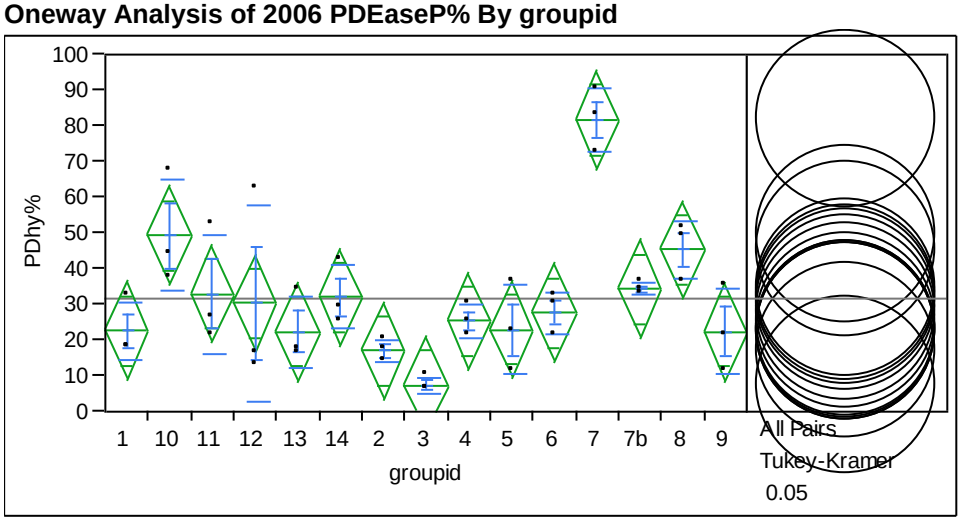
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	292747.03	20910.5	28.8850	<.0001
Error	30	21717.66	723.9		
C. Total	44	314464.69			

Level						Mean
4	A					351.56667
7b		B				137.13333
10		B	C			117.60000
8		B	C	D		101.46667
14		B	C	D	E	90.60000
7		B	C	D	E	69.70000
2		B	C	D	E	67.86667
6			C	D	E	54.16667
1			C	D	E	41.60000
9			C	D	E	39.56667
13				D	E	35.36667
11				D	E	32.86667
12				D	E	26.10000
5				D	E	25.23333
3					E	13.40000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 PDEaseP% By groupid



Oneway Anova
Summary of Fit

Rsquare	0.754934
Adj Rsquare	0.64057
Root Mean Square Error	11.68094
Mean of Response	31.57778
Observations (or Sum Wgts)	45

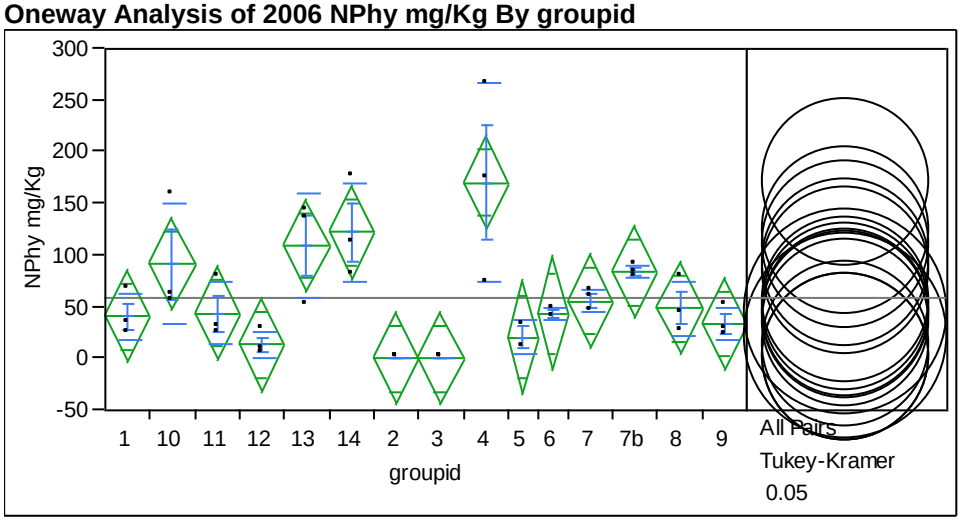
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	12609.644	900.689	6.6011	<.0001
Error	30	4093.333	136.444		
C. Total	44	16702.978			

Level				Mean
7	A			81.666667
10	A	B		49.333333
8		B		45.333333
7b		B	C	34.333333
11		B	C	33.000000
14		B	C	32.000000
12		B	C	30.333333
6		B	C	27.666667
4		B	C	25.333333
5		B	C	23.000000
1		B	C	22.666667
9		B	C	22.333333
13		B	C	22.333333
2		B	C	17.000000
3			C	7.333333

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 NPhy mg/Kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.702876
Adj Rsquare	0.554315
Root Mean Square Error	38.13405
Mean of Response	59.7907
Observations (or Sum Wgts)	43

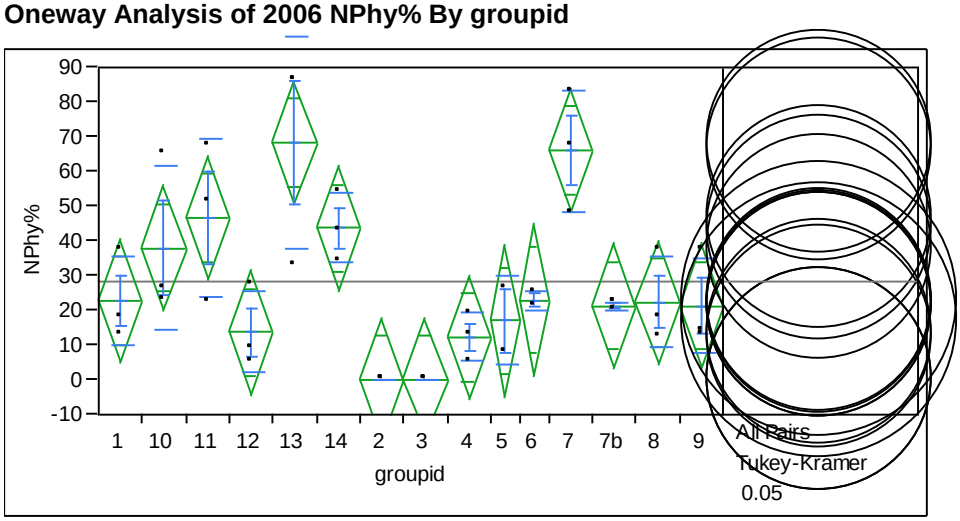
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	96322.08	6880.15	4.7312	0.0002
Error	28	40717.76	1454.21		
C. Total	42	137039.84			

Level				Mean
4	A			170.40000
14	A	B		122.26667
13	A	B	C	109.53333
10	A	B	C	91.40000
7b	A	B	C	83.73333
7	A	B	C	55.96667
8		B	C	48.90000
11		B	C	43.96667
6	A	B	C	43.70000
1		B	C	40.83333
9		B	C	33.73333
5		B	C	20.85000
12		B	C	13.23333
2			C	0.00000
3			C	0.00000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 NPhy% By groupid



Oneway Anova
Summary of Fit

Rsquare	0.738613
Adj Rsquare	0.607919
Root Mean Square Error	15.12094
Mean of Response	28.11628
Observations (or Sum Wgts)	43

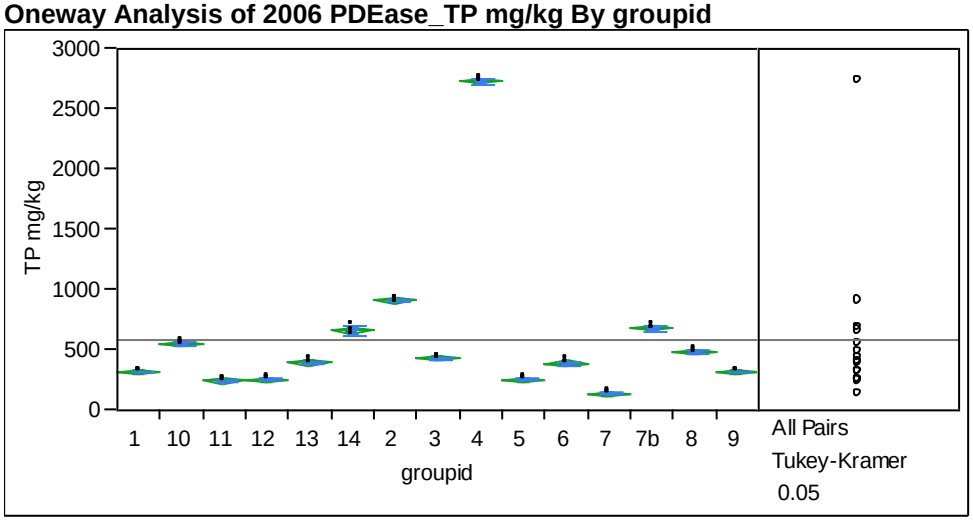
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	18090.419	1292.17	5.6515	<.0001
Error	28	6402.000	228.64		
C. Total	42	24492.419			

Level					Mean
13	A				68.333333
7	A	B			66.000000
11	A	B	C		46.666667
14	A	B	C	D	43.666667
10	A	B	C	D	38.000000
6	A	B	C	D	23.000000
1	A	B	C	D	22.666667
8		B	C	D	22.333333
9		B	C	D	21.333333
7b		B	C	D	21.333333
5		B	C	D	17.000000
12			C	D	13.666667
4			C	D	12.333333
2				D	0.000000
3				D	0.000000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006 PDEase_TP mg/kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.999491
Adj Rsquare	0.999254
Root Mean Square Error	16.74788
Mean of Response	582.5289
Observations (or Sum Wgts)	45

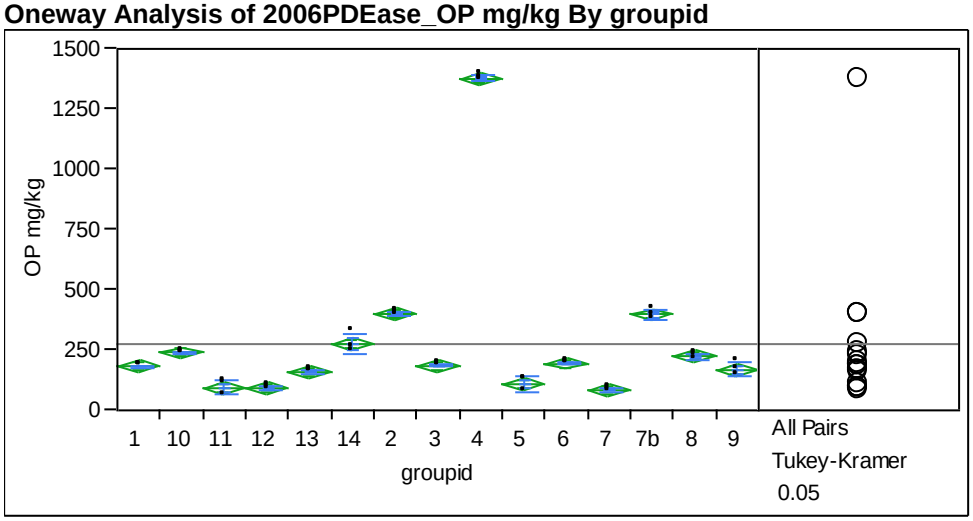
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	16537024	1181216	4211.239	<.0001
Error	30	8415	280		
C. Total	44	16545439			

Level	Mean
4 A	2729.6000
2 B	908.6333
7b C	678.4667
14 C	659.1667
10 D	551.6000
8 E	479.3333
3 E F	430.3000
13 F	394.4333
6 F	391.5667
1 G	314.7333
9 G	313.6667
5 H	253.6333
12 H	251.4667
11 H	243.0667
7 I	138.2667

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006PDEase_OP mg/kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.997229
Adj Rsquare	0.995936
Root Mean Square Error	19.9165
Mean of Response	278.5889
Observations (or Sum Wgts)	45

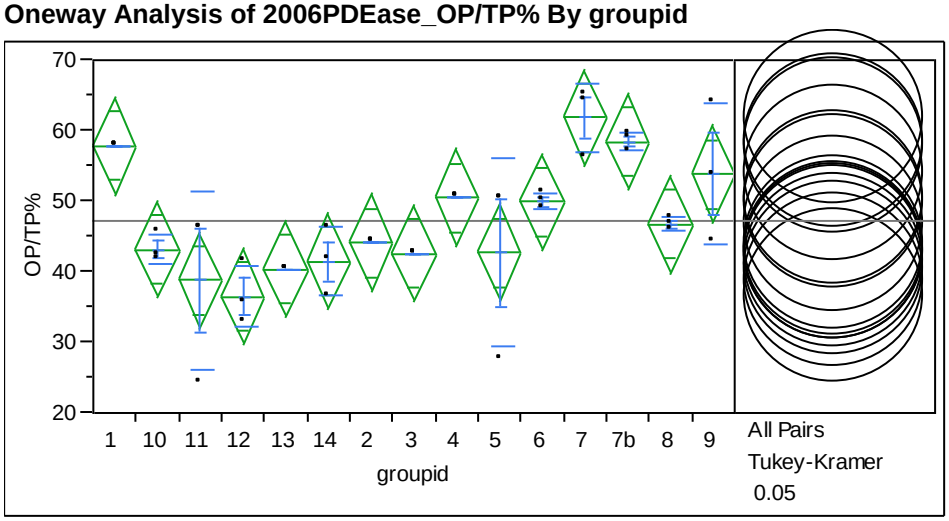
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	4282230.5	305874	771.1091	<.0001
Error	30	11900.0	397		
C. Total	44	4294130.5			

Level								Mean
4	A							1377.2333
2		B						400.5667
7b		B						396.7667
14			C					274.2667
10			C	D				237.9667
8			C	D	E			223.8000
6				D	E	F		195.1333
3				D	E	F		183.4000
1				D	E	F		181.9000
9					E	F		168.8667
13						F	G	159.3333
5							G H	108.1333
11							H	94.2333
12							H	91.7000
7							H	85.5333

Levels not connected by same letter are significantly different.

Oneway Analysis of 2006PDEase_OP/TP% By groupid



Oneway Anova
Summary of Fit

Rsquare	0.714057
Adj Rsquare	0.580617
Root Mean Square Error	5.830514
Mean of Response	47.25556
Observations (or Sum Wgts)	45

Analysis of Variance

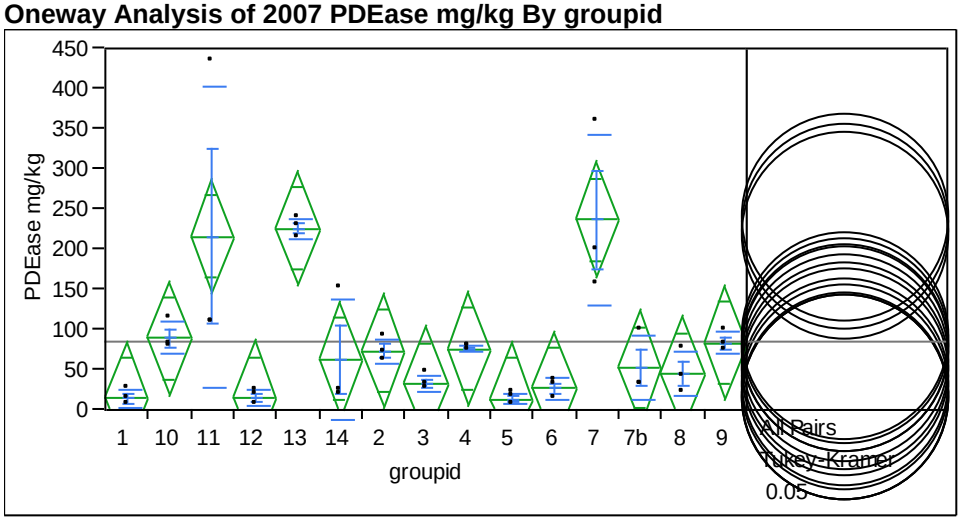
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	2546.7644	181.912	5.3511	<.0001
Error	30	1019.8467	33.995		
C. Total	44	3566.6111			

Level					Mean
7	A				61.866667
7b	A	B			58.466667
1	A	B	C		57.800000
9	A	B	C	D	53.866667
4	A	B	C	D	50.500000
6	A	B	C	D	49.900000
8	A	B	C	D	46.700000
2		B	C	D	44.100000
10		B	C	D	43.133333
5		B	C	D	42.766667
3		B	C	D	42.600000
14		B	C	D	41.466667
13			C	D	40.400000
11				D	38.766667
12				D	36.500000

Levels not connected by same letter are significantly different.

Appendix 3.2 2007 Enzyme hydrolysis

Oneway Analysis of 2007 PDEase mg/kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.69541
Adj Rsquare	0.553269
Root Mean Square Error	60.89189
Mean of Response	84.2
Observations (or Sum Wgts)	45

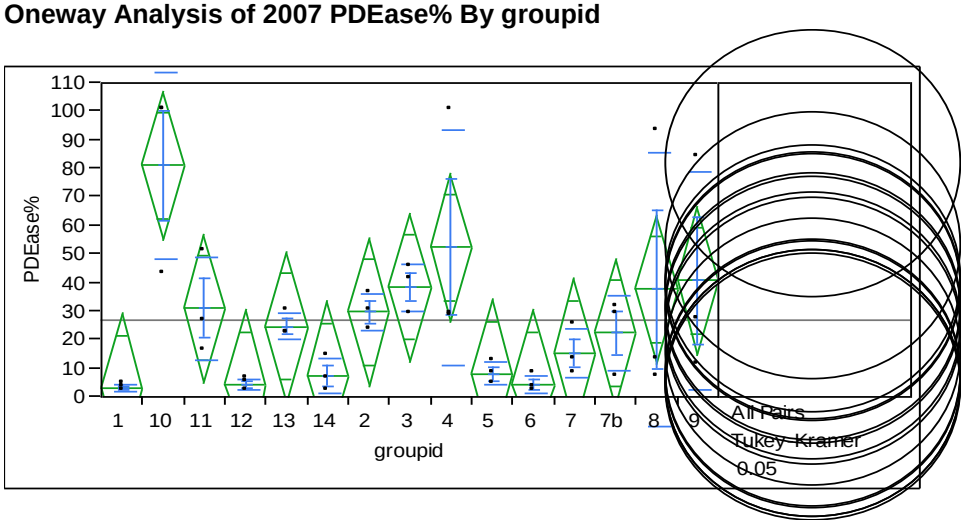
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	253960.53	18140.0	4.8924	0.0001
Error	30	111234.67	3707.8		
C. Total	44	365195.20			

Level					Mean
7	A				237.00000
13	A	B			226.00000
11	A	B	C		215.66667
10	A	B	C	D	89.33333
9	A	B	C	D	83.66667
4	A	B	C	D	75.66667
2	A	B	C	D	73.33333
14	A	B	C	D	63.00000
7b		B	C	D	52.66667
8		B	C	D	45.33333
3			C	D	32.66667
6				D	26.33333
12				D	15.00000
1				D	14.00000
5				D	13.33333

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 PDEase% By groupid



Oneway Anova
Summary of Fit

Rsquare	0.571181
Adj Rsquare	0.371066
Root Mean Square Error	22.12942
Mean of Response	26.66667
Observations (or Sum Wgts)	45

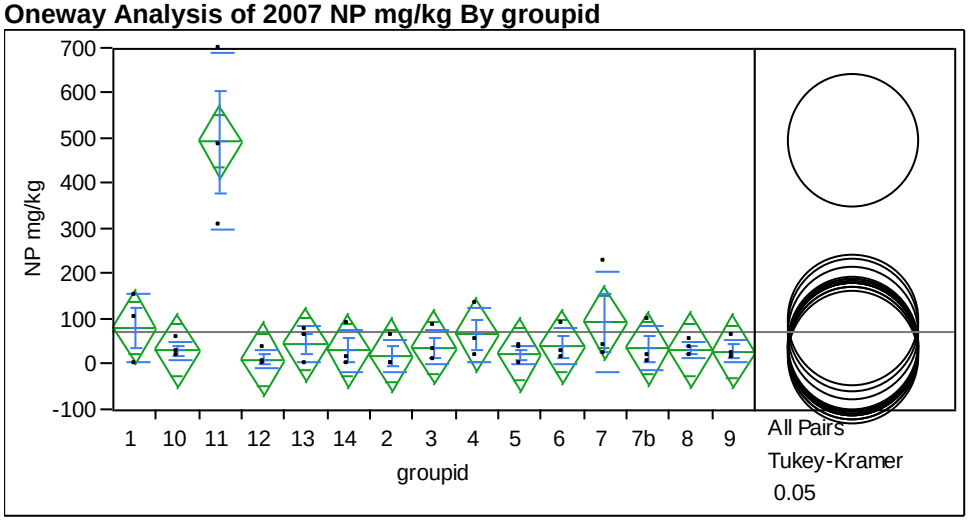
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	19568.667	1397.76	2.8543	0.0078
Error	30	14691.333	489.71		
C. Total	44	34260.000			

Level			Mean
10	A		81.000000
4	A	B	52.333333
9	A	B	40.666667
3	A	B	38.333333
8	A	B	37.666667
11	A	B	31.000000
2	A	B	29.666667
13	A	B	24.666667
7b	A	B	22.333333
7	A	B	15.333333
5		B	8.000000
14		B	7.333333
12		B	4.333333
6		B	4.333333
1		B	3.000000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 NP mg/kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.803089
Adj Rsquare	0.711197
Root Mean Square Error	69.75672
Mean of Response	72.55556
Observations (or Sum Wgts)	45

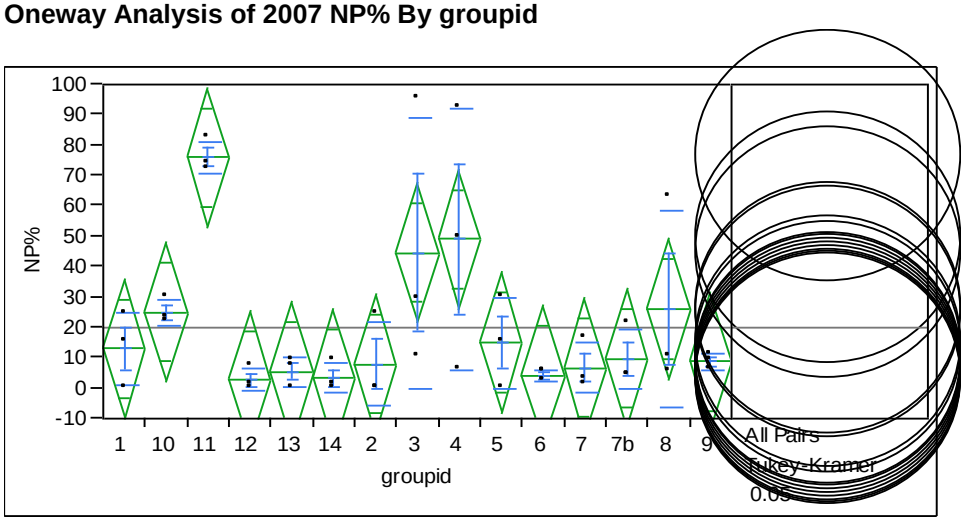
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	595369.11	42526.4	8.7395	<.0001
Error	30	145980.00	4866.0		
C. Total	44	741349.11			

Level		Mean
11	A	495.00000
7	B	95.33333
1	B	83.00000
4	B	67.00000
13	B	45.66667
6	B	41.00000
3	B	39.00000
7b	B	37.33333
8	B	33.33333
14	B	32.33333
10	B	32.00000
9	B	30.33333
5	B	23.66667
2	B	20.66667
12	B	12.66667

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 NP% By groupid



Oneway Anova
Summary of Fit

Rsquare	0.624064
Adj Rsquare	0.448627
Root Mean Square Error	19.5215
Mean of Response	19.8
Observations (or Sum Wgts)	45

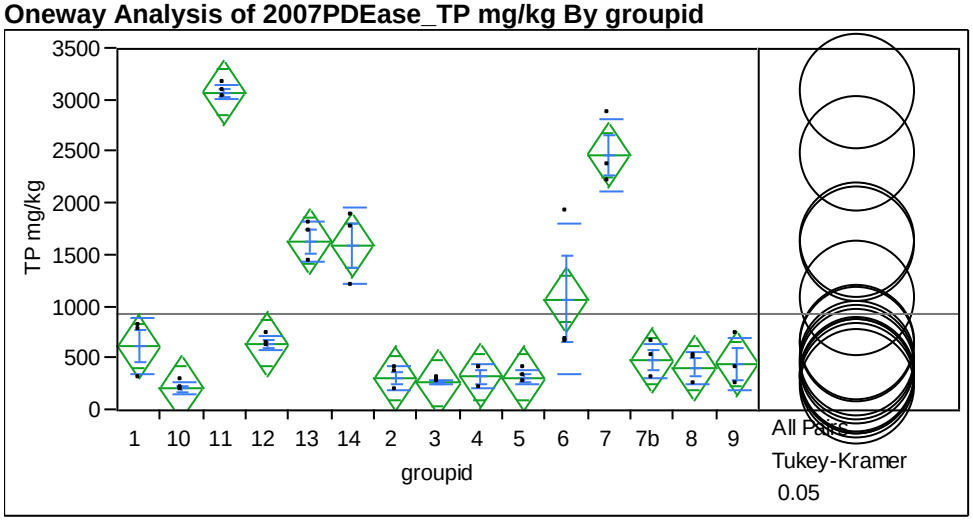
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	18978.533	1355.61	3.5572	0.0017
Error	30	11432.667	381.09		
C. Total	44	30411.200			

Level			Mean
11	A		76.000000
4	A	B	49.000000
3	A	B	44.666667
8	A	B	26.000000
10	A	B	25.000000
5		B	15.000000
1		B	13.000000
7b		B	9.666667
9		B	8.666667
2		B	8.000000
7		B	6.666667
13		B	5.333333
6		B	4.000000
14		B	3.333333
12		B	2.666667

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007PDEase_TP mg/kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.939425
Adj Rsquare	0.911157
Root Mean Square Error	265.3891
Mean of Response	925.8889
Observations (or Sum Wgts)	45

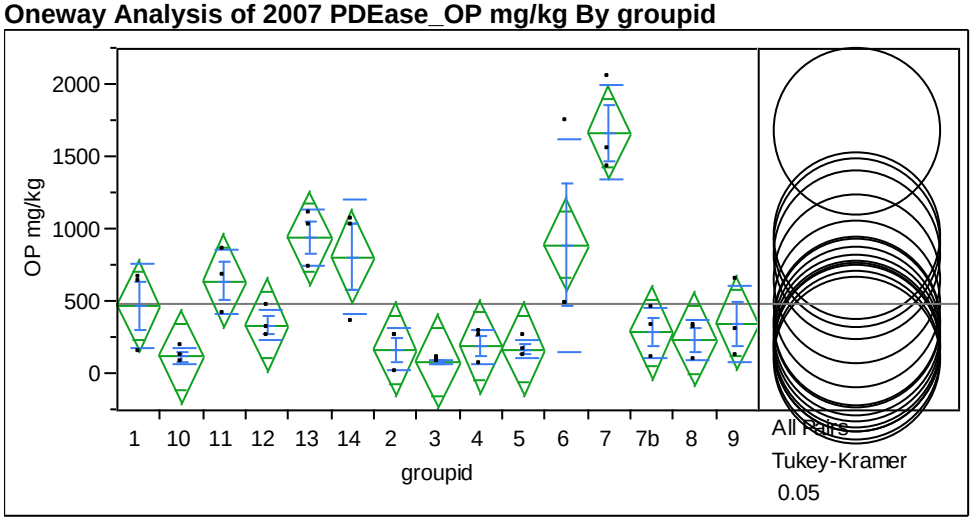
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	32768464	2340605	33.2324	<.0001
Error	30	2112941	70431		
C. Total	44	34881404			

Level				Mean
11	A			3079.6667
7	A			2469.6667
13		B		1637.0000
14		B		1595.0000
6		B	C	1074.6667
12			C	D	648.3333
1			C	D	621.3333
7b			C	D	478.3333
9			C	D	448.0000
8			C	D	409.6667
4			C	D	324.6667
5			C	D	315.3333
2			C	D	309.0000
3				D	265.6667
10				D	212.0000

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 PDEase_OP mg/kg By groupid



Oneway Anova
Summary of Fit

Rsquare	0.774124
Adj Rsquare	0.668715
Root Mean Square Error	276.4768
Mean of Response	492
Observations (or Sum Wgts)	45

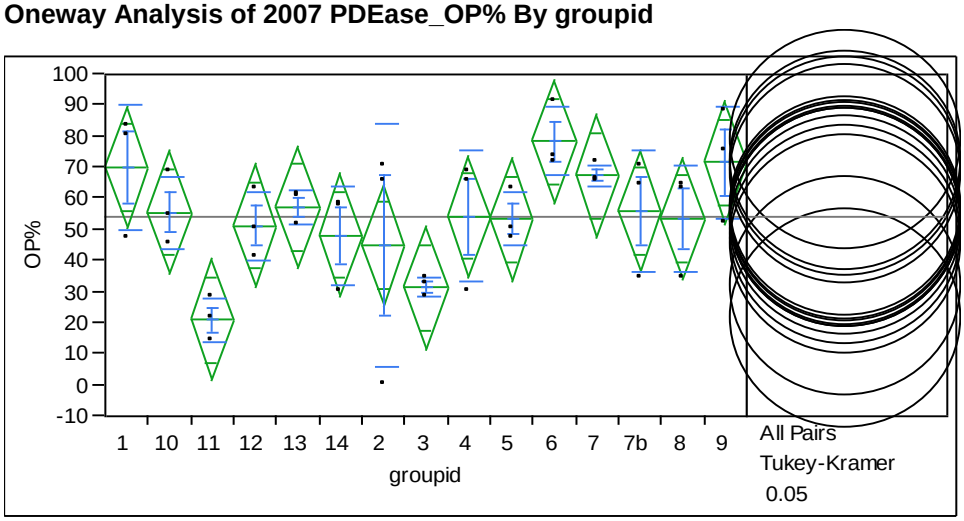
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	7859216	561373	7.3440	<.0001
Error	30	2293182	76439		
C. Total	44	10152398			

Level				Mean
7	A			1667.3333
13	A	B		943.3333
6	A	B	C	893.0000
14		B	C	808.0000
11		B	C	644.3333
1		B	C	472.0000
9		B	C	350.0000
12		B	C	337.3333
7b		B	C	288.0000
8		B	C	236.6667
4		B	C	193.3333
5		B	C	173.0000
2		B	C	167.6667
10		B	C	121.3333
3			C	84.6667

Levels not connected by same letter are significantly different.

Oneway Analysis of 2007 PDEase_OP% By groupid



Oneway Anova
Summary of Fit

Rsquare	0.525643
Adj Rsquare	0.304277
Root Mean Square Error	16.66667
Mean of Response	54.31111
Observations (or Sum Wgts)	45

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
groupid	14	9234.311	659.594	2.3745	0.0230
Error	30	8333.333	277.778		
C. Total	44	17567.644			

Level			Mean
6	A		78.333333
9	A		71.666667
1	A	B	70.000000
7	A	B	67.333333
13	A	B	57.333333
7b	A	B	56.000000
10	A	B	55.666667
4	A	B	54.333333
8	A	B	53.666667
5	A	B	53.333333
12	A	B	51.333333
14	A	B	48.333333
2	A	B	45.000000
3	A	B	31.333333
11		B	21.000000

Levels not connected by same letter are significantly different.

Appendix 4 Multiple Regression

S_{max} vs. Ox-Fe, Ox-Al Multiple Regression

Variables Entered/Removed(b)

Model	Variables Entered	Variables Removed	Method
1	OXFE, OXAL(a)	.	Enter

a All requested variables entered.
b Dependent Variable: SMAX

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.748(a)	.560	.480	84.026

a Predictors: (Constant), OXFE, OXAL

ANOVA(b)

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	98717.148	2	49358.574	6.991	.011(a)
	Residual	77664.895	11	7060.445		
	Total	176382.043	13			

a Predictors: (Constant), OXFE, OXAL
b Dependent Variable: SMAX

Coefficients(a)

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	91.976	53.671		1.714	.115
	OXAL	.309	.189	.388	1.632	.131
	OXFE	.014	.007	.463	1.947	.078

a Dependent Variable: SMAX

Smax=91.976+0.309Ox-Al+0.014Ox-Fe (R^2=0.56, sig=0.011)
Multiple regression model indicates that 56% of Smax could be explained or predicted by Ox-Al, Ox-Fe.

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