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ORIGINAL PAPER

Characterization of phosphorus in marshland sediments by ^{31}phosphorus nuclear magnetic resonance spectroscopy

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Abstract Phosphorus turnover is vital to understand algal blooms and nutrient export in coastal waters; however, little is known about the composition and dynamic of sediment phosphorus in marshes. In this study, the relative composition of various organic phosphorus in sodium hydroxide (NaOH) extracts of freshwater and saltwater sediments in the Pelham Bay Park, New York City, the USA, were investigated, using solution ^{31}phosphorus nuclear magnetic resonance Spectroscopy. The correlation between sodium hydroxide-extractable organic phosphorus and the organic matter content fitted to a linear model. Moreover, it is indicative that the species of organic phosphorus compounds in sediments are mainly dependent on salinity and the organic matter content in the sediments. The representative phosphorus compounds in the marshland sediments were glucose-6-phosphate nucleoside monophosphate and dihydroxyacetone phosphate. The composition of various phosphorous compounds was more complex in the saltwater sediments than in the freshwater sediments. Monoester phosphorus was the major component in most of the marshland, and diester phosphorus dominated the anoxic area.

Keywords Freshwater · Pelham Bay Park · Organic phosphorus · Phosphate compounds · Salt water

Introduction

Marshland sediments have been considered as a natural reservoir of phosphorus (P). However, the presence of P species in the sediments is dependent on the characteristics of marshland. Phosphorus is the most limiting nutrient for primary production in most water bodies (Reitze et al. 2006; Wines 2013). Therefore, effective control of P in marshland system is of prime concern in reducing the risk of eutrophication of surface water of the close bays/rivers (McCulloch et al. 2013). Phosphorus in sediments can be divided into different fractions, which include inorganic P(IP), calcium-associated P(Ca-P), Al, Fe and Mn-bound P(Fe/Al-P), organic P(OP) and total P(TP) (Zhang et al. 2008a). Among them, Fe/Al-P and OP are sorted as mobile P. Mobilization of these two fractions is the most important mechanism of P release under high pH values and aerobic conditions (Ruban et al. 2001).

Recently, accretion of organic matter has been reported as a major sink for P in wetlands (Pant et al. 2002). However, little attention has been paid to the chemical nature and dynamics of sediment organic P. It is still under investigation regarding the compositional variances in phosphorus compounds in sediments of freshwater and saltwater marshlands. In the present study, we investigated the impact factors, which affect the content and species of organic P, and determined the relative composition of functional groups of various organic P in freshwater and saltwater sediments.

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Materials and methods

Study site

Pelham Bay Park, located in the northeast corner of the Bronx (73°47′W, 40°52′N), is the largest public park in New York City and is composed of freshwater wetlands and saltwater marshes along with other structures including meadows, forests and mixed shrubs. The total area of Pelham Bay Park is 2764 acres (Fig. 1). Most of the saltwater marshlands are located in the northeastern section of the Pelham Bay Park, and freshwater marshlands are around the golf course in the southwestern section of the Park. The samples were collected from 10 sites (Fig. 1). Sites 1–6 represented freshwater marshlands, and sites 7–10 represented saltwater marshlands. Sites 1, 2, 3 and 4 are near Bartow Creek and the Lagoon; site 5 is in the Pelham Golf Course; site 6 in the Split Rock Golf Course; site 7 in the Turtle cove; site 8 near the Orchard Beach; site 9 in the Lagoon, and site 10 is in the Eastchester Bay which faces to the Landfill.

Sediment sampling and physical–chemical properties analysis

Representative sediment samples from the marshlands were collected using a hand corer below water level. Sediments samples were homogenized after removing large roots, stones and residual stuff and then kept in a cooler. The samples were thereafter brought back to the Environmental Laboratory of Lehman College of the City University of New York and stored at 4 °C.

Organic matter content was determined by Loss on Ignition (LOI). Sediment pH and electrical conductivity (EC) were measured in a 1:2.5, sediment/water mixture, by a dual pH and EC-meter (Mettler Toledo, Inc; InLab 730 Conductivity Electrode and InLab 413 pH Electrode). Unless stated otherwise, all results are means of three replicate analyses.

NaOH extraction and ^{31}P NMR spectroscopy

Sediments (50 g wet weight) were extracted with 100 mL of 0.4 M NaOH by shaking in an end-over-end shaker at 20 °C for 16 h. The suspensions were centrifuged for 30 min at $10{,}000\times g$. The decanted supernatants were concentrated around tenfold using a vacuum rotary evaporator at 35 °C. Each concentrated sample was transferred to a 5-mm NMR tube, and 10 % (by vol.) of D_2O was added to the sample to obtain a stable signal.

Solution ^{31}P NMR spectra were obtained using a Bruker 300 UltraShield ^{31}P NMR operating at 121.4948 MHz. We used a 90-pulse width and acquisition time of 5.39 s (pulse delay 1 s, acquisition time 4.39 s) at 20 °C. Chemical shift was determined according to an external standard of 85 % H_3PO_4. The identification of peaks of different P compounds in the NMR spectra was done by comparing chemical shifts of peaks in literatures (Pant et al. 1999; Carman et al. 2000; Turner et al. 2003; McDowell and Stewart, 2006). To attain the accurate interpretations of ^{31}P NMR spectra, samples were also spiked with 0.1 mL

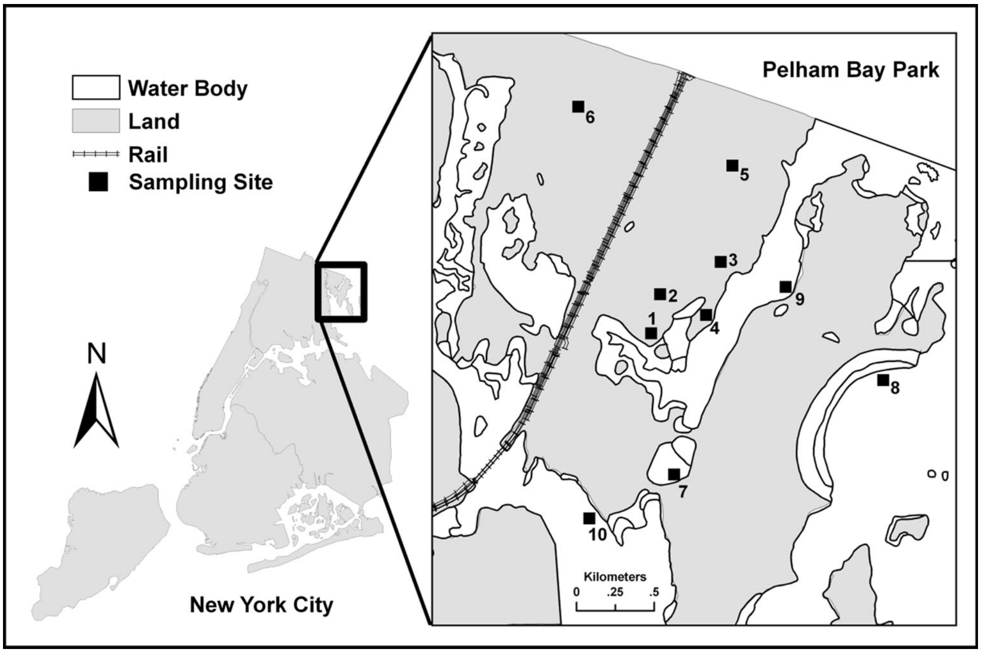

Fig. 1 Sampling sites in marshlands of Pelham Bay Park, NYC, NY

(1 mg P mL^{-1}) of potassium phosphate monobasic (KH_2PO_4) as an internal standard to ascertain the peak identifications.

Measurement of total P in sediment and NaOH extracts

Total P in sediments was measured by combustion of the sediments at 550 °C for 4 h followed by acid extraction as described by Andersen (1976). Total P and total inorganic P in NaOH extracts were also measured. The supernatants were used to measure soluble reactive P (considered as inorganic P) using ascorbic acid method as described by the United States Environmental Protection Agency (USEPA) (1993; Method 365.1). Similarly, the supernatants were digested to determine total P in NaOH extracts as described by USEPA (1993; Method 310). Organic P in the NaOH extracts was calculated as the difference between total P and inorganic P in NaOH extracts.

Results and discussion

Sediment properties

The pH of the freshwater sediments ranged from 5.4 to 6.4 and that of saltwater sediments ranged from 4.1 to 8.2 (Table 1). Water content of freshwater sediments was from 42.95 to 51.52 % and that of saltwater sediments was from 15.79 to 32.40 %. Organic matter in all sediments ranged from 0 to 128.5 g kg^{-1} sediment (mean value: 45.7 g kg^{-1}). The amount of organic matter in freshwater sediments (44.6–128.5 g kg^{-1} sediment) was higher than in saltwater sediments (0–21.24 g kg^{-1} sediment).

Table 1 Selected properties of the marshland sediments from Pelham Bay Park

Sediment series	pH (1:2.5)	EC (μs cm^{-1})	Moisture content (%)	Organic matter (g kg^{-1} sediment)
1. Freshwater wetland 1	5.6	185.3	49.4	56.9
2. Freshwater wetland 2	5.5	87.9	51.5	74.3
3. Freshwater wetland 3	5.4	106.4	46.7	59.1
4. Freshwater wetland 4	6.4	213	48.1	128.5
5. Pelham Golf Course	5.4	97.1	42.9	44.6
6. Split Rock Golf Course	5.5	540	44.5	46.5
7. Turtle cove	4.1	5120	32.4	21.2
8. Orchard Beach	8.2	2150	15.8	0
9. Lagoon	7.4	2720	23.4	12.0
10. Eastchester Bay (near Landfill)	7.2	2990	24.85	13.76

Total sediment P and NaOH-extractable P

Total sediment P ranged from 4.57 to 130.8 mg P kg^{-1} sediment (Table 2). The corresponding recovery of total P extracted with NaOH alone ranged from 61.05 to 87.47 % (mean value: 77.36 %). Besides NaOH-EDTA solution (Zhang et al. 2008b), NaOH solution has also been used to extract P selectively from soils and sediments (Condron et al. 1990; Carman et al. 2000), and thus, NaOH-extract was used as a pretreatment extractant of Solution ^{31}P NMR in this study.

Organic P concentration in the NaOH extracts was from 0.36 to 95.84 mg P kg^{-1} sediment, representing that the NaOH-extractable P was from 11.43 to 97.47 %, respectively. A positive significant correlation between the organic matter content and NaOH-extractable organic P was observed (Fig. 2). The liner model ($R^2 = 1$, $P < 0.001$):

$$\mathrm{OP} = 0.565 + 0.739\mathrm{OM}$$

OP: NaOH-extractable organic P (mg P kg^{-1} sediment); OM: organic matter (g P kg^{-1} sediment).

It could be deduced that the composition of sediment organic P in marshlands was dependent on the organic matter content in sediments. In this case, the content of organic matter in freshwater sediments was higher than in

Table 2 Total sediment P and NaOH-extractable P fraction in the marshland sediments from Pelham Bay Park

Site	Total sediment P (mg P kg^{-1} sediment)	NaOH-extractable P (mg P kg^{-1} sediment)		
		Total P[a]	Inorganic P[b]	Organic P[b]
1.	130.78	114.39 (87.47)	70.97 (62.04)	43.42 (37.96)
2.	128.13	105.94 (82.68)	51.55 (48.66)	54.39 (51.34)
3.	131.62	110.87 (84.23)	66.78 (60.23)	44.09 (39.77)
4.	118.28	98.33 (83.13)	2.49 (2.53)	95.84 (97.47)
5.	127.54	94.21 (73.87)	60.94 (64.68)	33.27 (35.31)
6.	128.76	108.46 (84.23)	73.79 (68.03)	34.67 (31.97)
7.	75.25	49.52 (65.81)	32.68 (65.99)	16.84 (34.01)
8.	4.57	3.79 (61.05)	2.79 (88.57)	0.36 (11.43)
9.	70.9	46.82 (68.92)	36.88 (78.77)	9.94 (21.23)
10.	43.59	33.65 (77.20)	23.39 (69.51)	10.26 (30.49)

[a] Values in parentheses are the proportion (%) of the total sediment P

[b] Values in parentheses represents the percentage of NaOH-extractable P

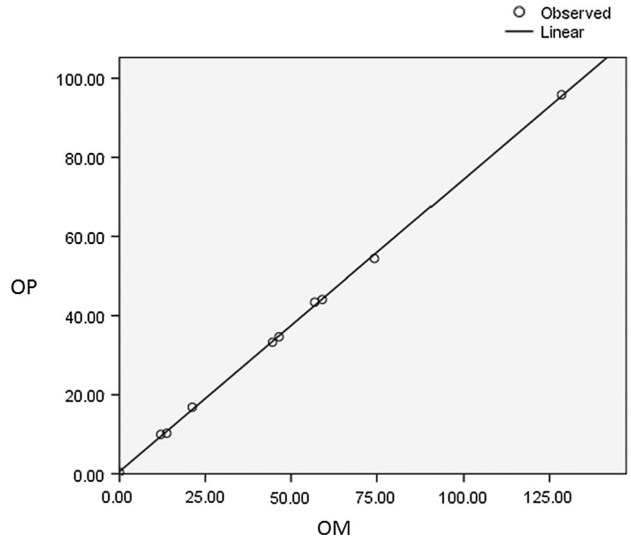

Fig. 2 Relationship between organic matter (OM; g kg^{-1}) in sediments and NaOH-extractable organic P (OP; mg kg^{-1}) in sediments

saltwater sediments. Thus, concentration of organic P in freshwater sediments was relatively greater than in saltwater sediments.

^{31}P NMR spectroscopy

Eleven ^{31}P NMR peaks were observed in the NaOH extracts in total, indicating that 11 different groups of P compounds were identified in the study area (Table 3; Fig. 3). Our measurements indicate that signals from orthophosphate monoesters in alkaline extracts occur at ppm values between 3 and 6, which is consistent with the findings of previous studies (Hawkes et al. 1984; Condron and Goh 1985; Ingall et al. 1990). These peaks represent a wide range of organic P compounds including nucleotides, sugar phosphates and phosphatidic acid, which constitute the major portion of the total NaOH-extractable P. A dominant proportion of the peak area (>40 %) is situated between 4 and 5.5 ppm. Organic phosphate groups between 4 and 5.5 ppm are assigned to, for instance, polynucleotides, nucleoside monophosphates, sugar phosphates and choline phosphate (Newman and Tate 1980; Condron and Goh 1985; Adams and Byrne 1989; Pant et al. 1999).

Table 3 Chemical shift assignments for various P compounds/functional groups present in the marshland sediments from Pelham Bay Park

Site	Chemical shift (ppm)	P compound	Functional group	Relative composition (%)
1.	4.7	Nucleoside monophosphates	Monoester	100
2.	5.5	Glucose-6-phosphate	Monoester	100
	−1.1	Inosine monophosphate	Diester	Trace
3.	5.5	Glucose-6-phosphate	Monoester	100
4.	5.5	Glucose-6-phosphate	Monoester	89.8
	5.1	Phosphatidic acid	Monoester	5.1
	4.8	ß-Glycerophosphate		5.1
5.	5.5	Glucose-6-phosphate	Monoester	100
6.	4.7	Nucleoside monophosphate	Monoester	100
7.	4.7	Nucleoside monophosphate	Monoester	95.5
	3.5	Polynucleotides	Diester	Trace
	3.3	Glucose-1-phosphate	Monoester	Trace
	3.0	Inorganic phosphate	monoester	4.5
8.	4.7	Nucleoside monophosphate	Monoester	100
9.	5.6	Dihydroxyacetone phosphate	Monoester	96.0
	4.7	Nucleoside monophosphate	Monoester	Trace
	4.1	Choline phosphate	Diester	Trace
	−4.6	Pyrophosphate	Monoester	3.0
10.	5.6	Dihydroxyacetone phosphate	Monoester	83.6
	4.1	Choline phosphate	Diester	11.5
	-4.6	Pyrophosphate	Monoester	4.9

Trace: Means <2 % relative composition

The concentration of monoesters P was likely overestimated in studies involving alkaline extraction, due to the degradation of some orthophosphate diesters, specifically RNA and phosphatidyl choline, during extraction and analysis (Makarov et al. 2002). This degradation appears unavoidable, considering the requirement for alkaline extraction and lengthy machine time unless NaOH is removed prior to sample concentration/preparation for NMR analysis (Pant et al. 1999, 2002). Solid-state ^{31}P NMR spectroscopy offers a promising alternative for the analysis of sediment P composition, which avoids the extraction and potential degradation. However, the current technique lacks adequate sensitivity required for the analyses of soils and sediments.

Unlike NaOH-EDTA extracts, no polyphosphate was detected in the NaOH extracts from any of the ten marshland sediment samples in this study, which may have been due to the lack of interaction between NaOH and sediment minerals. Complex reactions may occur between

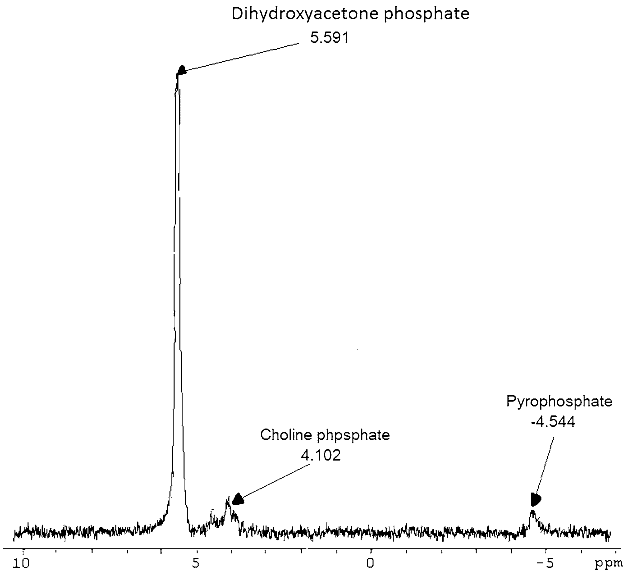

Fig. 3 P peaks in saltwater sediments as determined by ^{31}P-NMR Spectroscopy (e.g., Site 10)

EDTA and sediments minerals, thus appearance of polyphosphates in NaOH-EDTA extracts. Although arguably, the occurrence of poly-P in sediments has been considered as an indicator of biological/microbial activity. Bacteria and several other organisms (such as fungi, algae and protozoa) accumulate long-chained poly-P during aerobic conditions and subsequent hydrolysis during anoxic conditions. The lack of poly-P in marshland sediments may also result from, at least periodically, the lack of oxygen in the whole sediment profile, which might have prevented the development of significant amount of poly-P. Moreover, it has been observed that various metal ions catalyze the degradation of poly-P to tri-meta-P (Hupfer et al. 1995a, b). Therefore, the higher concentration of metal ions in sediments might be an additional factor for the lack of poly-P in all of our marshland sediments.

A small pyrophosphate peak was visible in the ^{31}P NMR spectra of sites 8 and 9, which represent saltwater marshlands. Carman et al. (2000) reported peaks for orthophosphate and polyphosphate from sediments of seas and lakes. Usually, peaks corresponding to orthophosphate in all alkaline extracts are present around 6-7 ppm, and it may contribute from a hydrolysis of some ester components (Newman and Tate 1980; Ingall et al. 1990). The orthophosphate may also, to some extent, originate from its association with metals that are tightly bound in humic matrixes.

Species of organic P compounds

The number of organic P compound species in saltwater sediments (mean value: 3) was more than in freshwater sediments (mean value: 1.5). The representative P compounds in freshwater sediments were glucose-6-phosphate and nucleoside monophosphates, while dihydroxyacetone phosphate dominated in the saltwater sediments. Monoester P dominated most of the marshland, while diester P dominated the remaining anoxic area.

The saltwater sediments exhibited relatively higher EC and concentrations of metal ions compared with the freshwater sediments, which may lead to more species of organic P compounds in saltwater sediments than in freshwater sediments. One exception was site 8 where only one species of organic P compounds existed. There was no appreciable amount of organic matter in this saltwater sediment site. Therefore, the species of organic P compounds in sediment may mainly depend on salinity and potentially on organic matter content.

It has been reported that organic P is mainly composed of monoester and diester P compounds in lake sediments (Zhang et al. 2008a). In the present study, we found the somewhat similar composition of organic P in marshland sediments, too. Monoester P compounds dominated seven of the ten sites in the study area. The other three sites were dominated by diester P where anoxia was common (Site 7, a lagoon), which indicates that the breakdowns of organic P diesters are less effective during anoxic conditions. Although a justified calculation of the relative proportion of peak areas from the sediments was difficult to perform, it could be evaluated by visual inspection that there was relatively higher amount of diester P in the sediment samples where anoxia prevails.

Conclusion

In the present study, eleven different groups of P compounds were identified in study areas by Solution ^{31}P NMR. Solution ^{31}P NMR, for NaOH extracts, was a competent tool for proper direct characterization of the distribution of different P compounds, as well as the identification of functional P groups within various sediment environments. However, for accurate determination of unique P compound, other supplemental techniques should be used such as HPLC/MS and HPLC/NMR. Also, the use of different chromatographic methods is sometimes limited. Therefore, the application of ^{31}P NMR is indispensable in studying possible diagenetic reaction and potential pathways of particular P in all aquatic environments.

In summary, the contents of sediment organic P in marshlands mainly depended on the content of organic matter in the sediments, and the species of organic P compounds in sediments mainly depended on salinity and potentially on the organic matter content. The linear mode of organic matter content and NaOH-extractable organic P has a practical significance in assessment of organic P content in marshland sediments. The composition of various P compounds was more complex in the saltwater sediments than in the freshwater sediments. Monoester P compounds dominated seven of the ten sites in the study area, while the other three sites were dominated by diester P where anoxia was common.

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