Phosphorus-31 nuclear magnetic resonance assignments of biogenic phosphorus compounds in sediment of an artificial Fuyangxin River, China

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Abstract River eutrophication could drastically influence the phosphorus (P) in the water and sediment. To understand the biogenic-P species, distribution and bioconversion, five sediment samples were collected from an artificial river, and analyzed by phosphorus-31 nuclear magnetic resonance (^{31}P -NMR). The P pollution in the water and sediment were both severe. The average concentrations of total P (TP) and solution reactive phosphorus in the water were 3.0 and 2.6 mg L $^{-1}$, respectively, which surpass grade V of the national quality standard (China) and should not be used for any purpose. The river sediments accumulated significant inorganic phosphorus (Pi) and organic phosphorus (Po); in the P fractionation, the rank order of the P fractions was as follows: Ca-P > NaOH-Pi > Res-P > KCl-P > NaOH-Po, with average relative proportions of 25.1:16.8:6.6:1.7:1:0. Six P compounds were detected in the NaOH-EDTA extract by ^{31}P -NMR. Mono-P (8.96–29.58 %) was the dominant forms of biogenic-P, and other smaller fractions of biogenic-P were also observed, including pyro-P (0.22–0.86 %), DNA-P (0.75–2.03 %), phon-P (0–1.57 %), and lipids-P (0–2.66 %). The TP and biogenic-P decreased along the direction of flows, with their average relative proportions 7.97:1.20:1.49:1.00:1.00 and 40.87:2.34:3.46:1.60:1 from the upstream to downstream, respectively. The concentration and species of Po in NaOH-Po

were lower than found in ^{31}P -NMR analysis in this research. Thus, the use of 0.25 M NaOH and 50 mM EDTA extracts and solution ^{31}P -NMR analysis was a more accurate method for quantifying biogenic-P in the river sediments than P fractionation.

Keywords Phosphorus · Artificial river · Sediment · ^{31}P -NMR

Introduction

Phosphorus (P) is a primary element in freshwater ecosystems (Cade-Menun et al. 2006). P from agriculture, industrial, and municipal sources can lead to or worsen the eutrophication of freshwater, which results in water quality deterioration (Carpenter 2008). Many artificial rivers were constructed to reduce the flood hazard in the Haihe River Basin of northern China in the 1950s and 1960s of the twentieth century. Within the past 30 years, the Haihe River Basin has faced the highest rate of increase in population and economic development in China. Large amounts of wastewater from agriculture, domestic, and industrial sources are discharged into the artificial rivers, the function of which has been changed from flood control to receptacles for sewage. As a result, the nutrient (P) concentration has massively increased in the past 30 years (Pernet-Coudrier et al. 2012). The artificial rivers have smaller river curvature, a simple littoral zone and lack of change in river morphology. These rivers are located in the plain areas, which have been regions of intensified farming and dense population. Their extensive P input and peculiar river morphology make the P biogeochemical cycle in artificial rivers worthy of attention.

River sediment plays a vital role as a reservoir of external P and source of internal P (Rusu et al. 2012). The biogeochemical cycles of P in artificial river sediment are complicated,

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including physical, chemical, and biological processes (Golterman 2001; Schauser et al. 2006; Liu et al. 2009). Sediment P includes inorganic phosphorus (Pi) and organic phosphorus (Po). Several analytical methods have been developed to elucidate Po compounds, including P fractionation and study of the Po fraction using various extracts to distinguish different P-forms binding to metals (Fe, Al) or organic matter (Hieltjes and Lijklema 1980; Pierzynski 2004). Although these methods have provided useful information pertaining to P biogeochemical cycling in aquatic environments, they have not yielded sufficient insight into identification of specific P compounds (Brandes et al. 2007). Nuclear magnetic resonance (NMR) is a powerful analysis tool for environmental science, and phosphorus-31 nuclear magnetic resonance (^{31}P -NMR) spectroscopy distinguishes different P groups based on specific resonance frequencies, reflecting the chemical environment surrounding the P nuclei (Cardoza et al. 2004; Reitzel et al. 2006a, b). ^{31}P -NMR is a non-destructive, non-invasive technique for identifying chemical forms that has the advantage of enabling multiple P compounds to be characterized simultaneously with distinct binding properties (Cade-Menun 2005). Therefore, it is an ideal technique for use in analysis of Po species in marine or freshwater sediment. Many P species are detected by ^{31}P -NMR, including phosphonates, orthophosphate, orthophosphate monoesters, orthophosphate diesters (lipids-P and DNA-P), pyrophosphate, and polyphosphate (Cade-Menun et al. 2005). Therefore, it is an ideal technique for use in analysis of Po species in freshwater sediments, which not only provides sufficient insight into identification of specific P compounds, but also offers useful information pertaining to P biogeochemical cycling in the aquatic environment. ^{31}P -NMR has been used to analyze single Po or Po compounds (Bai et al. 2009; Orchard et al. 2010), the P transformation and effect factors (Ahlgren et al. 2005; Reitzel et al. 2007), P biogeochemistry (Shinohara et al. 2012), and aluminum dosing effects on eutrophication management (Reitzel et al. 2006a, b, 2009).

Fuyangxin River was in the Hebei province, which mainly accepts sewage from Shijiazhuang, Handan, and other cities. As the economic development and land use change, more and more sewage water flow to this river. For example, the area of city increased 179.05 and 72.00 km² from 1980 to 2005 in Shijiazhuang and Handan, respectively, and the sewage water were also increased 85,290 and 37,020 kt from 1980 to 2005 in Shijiazhuang and Handan, respectively. Although some environmental regulations and future environmental policy perspective was drafted, such as the twelfth five-year guideline of the ecological protection in Hebei, Hebei water pollution control ordinance, and Shijiazhuang city drinking water source protection areas groundwater pollution prevention and control regulations, abundant of P flow to the river as sewage. Because of the different morphological characteristics, the P species and cycle might be different to natural

ivers. In this study, surface sediment and water quality were researched in Fuyangxin River. P was analyzed by P fractionation and solution ^{31}P -NMR. The extract of NaOH–total phosphorous (TP) in P fractionation was also analyzed by ^{31}P -NMR. Through this research, we will identify the P compounds in the surface sediment of the Fuyangxin River and the relationship between P compounds and water quality and other factors of the river, and the variation of P compounds in artificial river systems. This study may provide deep insight into P biogeochemical cycling in artificial river systems.

Materials and methods

Site description

The Haihe River Basin, with area of about 300,000 km² and population of 145 million, is located in northern China. Beijing, Tianjin, and many other cities are included in this river basin, and its urbanization level is over 45 %. The Fuyangxin River (N 37°30′–38°11′, E 115°03′–116°04′) is an important component of the Haihe river system. The length of the main channel is 133 km and width is 1,600 to 1,700 m, crossing the cities of Hengshui and Xingtai. The Fuyangxin River was built during 1967 to 1968 for improve the Fuyang River flood discharge capacity. This river mainly accepts the floods from the Beili River, Xiao River, and Fuyang River. With its high growth in economic development and population, the region faces increasing water scarcity and water pollution. The Fuyangxin River has become a river that mainly accepts sewage from Shijiazhuang, the capital of Hebei province, Xingtai, Handan, and other cities. The high pollution discharge results in the deterioration of water quality, and P is the primary pollutant in the Fuyangxin River. A total of five sampling sites were set up over the whole river. Site 1 was set up near Aixinzhuang Dam. Site 2 was located upstream of Hengshui city, and sites 3, 4, and 5 were set up downstream of Hengshui city successively (Fig. 1).

Sample collection and analysis

Sediment sample collection was conducted in July 2012. Three surface (about 5 cm) sediment samples of each site were collected by Peterson grab sampler (the distance between the three parallel samples was not less than 500 m). Samples at the same site were pooled and homogenized to obtain a representative sample. All samples were frozen and stored at –18 °C immediately. Water samples were collected at the surface and water quality was monitored from 2010 to 2012.

Total P in sediment was measured by treating at 500 °C (2 h), followed by 1 mol L^{–1} HCl extraction (16 h). Pi was determined by direct extraction with 1 mol L^{–1} HCl (16 h). Po concentrations in the sediments were calculated as the

Fig. 1 Location of sampling sites in Fuyangxin River

difference between TP and Pi (Aspila et al. 1976). Total Fe, Al, Mg, Mn, and Ca were measured using ICP-OES/MS after digesting sediments with HNO_3 -HCl-HF (MARSSXpress, CEM, USA). Sample pH was determined in a sediment suspension in deionized water with a sediment/water ratio of 1:2.5. Organic matter (OM) in sediments was analyzed as the loss on ignition at 550 °C for 4 h (Jensen et al. 1992). TP in the water was measured using the molybdenum blue method. An annual average for TP in the water column was calculated for each site by using the monitoring data from 2010 to 2012. Three replicates were performed for each index, and the mean value was calculated (Wang and Pant 2012).

The P fractionation procedure was primarily based on the Hietjes-Lijklema scheme (Hietjes and Lijklema 1980). P in the sediment was classified into KCl-P, NaOH-Pi, NaOH-Po, Ca-P, and Res-P. KCl-P was extracted with 1 M KCl for 1 h with shaking at room temperature (about 25 °C). Then the sediment was extracted with 0.1 M NaOH for 17 h with shaking at room temperature. NaOH-Pi and NaOH-TP were analyzed using the molybdenum blue method before and after digestion by potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$). NaOH-Po was calculated as their difference. Ca-P was extracted with 0.25 M H_2SO_4 for 1 h with shaking at 25 °C. The Res-P was the difference of TP in sediment and KCl-P, NaOH-TP, and Ca-P. The P concentration in all extracts was estimated colorimetrically according to the method by Murphy and Riley (1962). In order to detect the P compounds in the NaOH-TP, the NaOH-

TP extract was freeze-dried and then dissolved with 0.6 ml D_2O and 0.1 ml 10 mol L^{-1} NaOH, ultrasonicated for 30 min and then equilibrated for 5 min. Two percent (v/v) of bicarbonate-buffered dithionite (0.11 M NaHCO_3 +0.11 M $\text{Na}_2\text{S}_2\text{O}_4$) was added to extracts to reduce interference from paramagnetic ions, such as Fe and Mn. The supernatants were centrifuged for 15 min at 14,000 rpm and transferred to 5-mm NMR tubes for ^{31}P -NMR analysis.

^{31}P -NMR analysis

Three grams of sediment samples were pre-extracted with 30 ml of a solution composed of 0.05 mol L^{-1} EDTA+0.25 mol L^{-1} NaOH for 16 h at room temperature (Turner et al. 2003a). An aliquot of the NaOH-EDTA extracts was used to analyze Pi (Pi in NaOH-EDTA) and TP (TP in NaOH-EDTA) using the molybdenum blue method before and after digestion by potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$). The Po in extractions was calculated as their difference. The remaining solution was frozen and lyophilized and used for ^{31}P -NMR analysis. Some studies have shown that freezing the extracts does not alter the P composition (Hupfer et al. 1995, 2004).

Of the lyophilized extracts, 0.3 mg was re-dissolved in 0.6 ml D_2O and 0.1 ml 10 mol L^{-1} NaOH, ultrasonicated for 30 min and then equilibrated for 5 min. Two percent (v/v) of bicarbonate-buffered dithionite (0.11 M NaHCO_3 +0.11 M $\text{Na}_2\text{S}_2\text{O}_4$) was added to extracts to reduce interference of

paramagnetic ions, such as Fe and Mn. The supernatants were centrifuged for 15 min at 14,000 rpm and transferred to 5-mm NMR tubes. Solution ^{31}P -NMR spectra were obtained using a Bruker 400 MHz spectrometer (Bruker, Billerica, MA, USA) operating at 129.53 MHz at 25 °C. We used a 90 °C observation pulse, relaxation delay 2 s, and acquisition time 0.6 s. Spectra were collected with around 20,000 scans and the spectra were taken at around 14 h (Beijing NMR center, Beijing). Chemical shifts were recorded relative to an 85 % H_3PO_4 standard ($\delta=0$ ppm). Signals were assigned to P species based on data in the literature reports (Turner et al. 2003b; Cade-Menun 2005). The peak area was calculated by visual inspection and an automated peak analysis tool. From the different P species areas, the contribution of each of the P compound groups (ortho-P, phon-P, pyro-P, mono-P, and diesters-P (DNA-P and lipid-P)) was calculated relative to the TP in the NaOH-EDTA extraction determined by the molybdenum blue method. The author verified the chemical shifts of P compounds by carrying out spike experiments using known compounds to assign peaks (such as $\text{Na}_4\text{P}_2\text{O}_7 \cdot 10\text{H}_2\text{O}$) (Wang and Pant 2010c).

Results

Sediment and water characteristics

TP in the water and sediment characteristics are shown in Table 1. For the five sediments, the concentration of TP varied greatly, ranging from 852.38 to 6,804.10 mg kg^{-1} . The highest concentration appeared at point 1, which was near Aixinzhuang Dam, and was nearly 7,000 mg kg^{-1} . The average of TP was 2,160.29 mg kg^{-1} and the Pi was the main P fraction. The concentration of Po, which accounted for 15.6 to 42.8 %, ranged from 132.93 to 2,909.98 mg kg^{-1} . The concentration of total Al was from 18.42 to 31.71 g kg^{-1} , which was the same as that in an artificial lake (21.82 g kg^{-1} , Liu et al. 2009), lower than Taihu Lake (61.72 g kg^{-1} , Bai et al. 2009) and higher than the Bronx River (0.34 g kg^{-1} , 0.34 g kg^{-1} (oxalate-extractable), Wang and Pant 2010b). The concentration of total Ca ranged from 12.17 to 17.19 g kg^{-1} , which was higher than the Bronx River (7.72 g kg^{-1} (HCl-extractable), Wang and Pant 2010b) and Taihu Lake (7.91 g kg^{-1} , Bai et al. 2009) and lower than an artificial lake (44.72 g kg^{-1} , Liu et al. 2009). The concentration of total Fe ranged from 34.38 to 43.63 g kg^{-1} , which was higher than that in an artificial lake (27.16 g kg^{-1} , Liu et al. 2009), Taihu Lake (32.42 g kg^{-1} , Bai et al. 2009), and the Bronx River (5.20 g kg^{-1} (oxalate-extractable), Wang and Pant 2010b). The concentration of total Mg ranged from 3.47 to 4.10 g kg^{-1} , which was higher than that in the Bronx River (2.29 g kg^{-1} (HCl-extractable), Wang and Pant 2010b). The concentration of total Mn ranged from 0.43 to

0.66 g kg^{-1} , which was the same as that in an artificial lake (0.58 g kg^{-1} , Liu et al. 2009). The pH ranged from 6.69 to 8.53, and the average of pH was 7.59. The concentrations of OM varied along with those of TP in the sediments, which ranged from 4.15 to 18.45 %, and the average of OM was 7.62 %. TP and solution reactive phosphorus (SRP) in water also varied greatly, ranging from 2.23 to 3.48 mg kg^{-1} and 1.52 to 3.58 mg kg^{-1} , respectively. The EC of sediments were 2,224.0, 422.2, 1,636.0, 459.9, and 1,518.0 $\mu\text{S cm}^{-1}$, and the EC of the water were 5,786.6, 1,879.5, 3,177.0, 1,789.0, and 2,855.7 $\mu\text{S cm}^{-1}$ as the river flow from site 1 to site 5.

Phosphorus fractionation in sediments

The results from P fractionation in the sediment are shown in Fig. 2. The rank order of the P fractions was as follows: Ca-P > NaOH-Pi > Res-P > KCl-P > NaOH-Po, with their average relative proportion 25.1:16.8:6.6:1.7:1:0 (the proportion was based on the average proportion of the NaOH-Po). Ca-P and NaOH-Pi were the dominant fractions of sediment P in the sediment, and ranged from 503.93 to 2,179.42 mg kg^{-1} and 181.50 to 3,897.70 mg kg^{-1} with relative contributions of 32.0 to 59.3 % and 21.3 to 57.3 %, respectively. Res-P ranged from 123.68 to 498.16 mg kg^{-1} with relative contribution of 7.3 to 18.4 %. The concentrations of KCl-P and NaOH-Po were lower than the others, and ranged from 5.62 to 13.48 mg kg^{-1} and 8.57 to 215.58 mg kg^{-1} with relative contributions of 0.3 to 5.7 % and 0.7 to 3.2 %, respectively. The concentrations of KCl-P, Ca-P, and Res-P decreased along the river flow direction (site 1 to site 5). The concentration of NaOH-Pi decreased with the river flow except for site 3. The concentration of NaOH-Pi was 567.55 mg kg^{-1} in site 3, which was higher than that in sites 2, 4, and 5. The concentrations of sites 2, 4, and 5 were 228.54, 181.50, and 194.54 mg kg^{-1} , respectively.

Phosphorus-31 nuclear magnetic resonance assignments

^{31}P -NMR spectra of NaOH-EDTA extracts of sediments The NMR spectra for the river sediments are shown in Fig. 3. Six ^{31}P -NMR peaks from the NaOH-EDTA extracts were observed. All NMR-spectra showed peaks in the area of phon-P (12 to 23 ppm), ortho-P (6 to 7 ppm), mono-P (4 to 6 ppm), lipids-P (1 to 3 ppm), DNA-P (0 ppm), and pyro-P (−3.5 to −4.5 ppm) (Turner et al. 2003a; Cade-Menun 2005; Cade-Menun et al. 2006; Liu et al. 2009). Phon-P and lipids-P were not detected in sites 2 and 4 and sites 4 and 5, respectively.

The concentrations and relative distribution of various P compound groups in river sediments are presented in Table 2. Po in the NaOH-EDTA fraction ranged from 85.59 to 1,671.42 mg kg^{-1} , with the recovery rate ranging from 56.15 to 74.69 %. The ortho-P was the dominant form of P in the NaOH-EDTA extracts of the river sediment, and ranged from

Table 1 Properties of the sediments used in the experiments

Site	pH	OM (%)	Sediment								Water	
			TP mg kg ⁻¹	Po (%)	Al g kg ⁻¹	Ca	Fe	Mg	Mn	EC μs cm ⁻¹	TP mg L ⁻¹	EC μs cm ⁻¹
1	7.15	18.45	6,804.10	2,909.98 (42.8) ^a	18.42	14.38	43.63	3.47	0.66	2,224.0	2.23±0.71	5,786.6
2	7.91	5.45	1,022.58	204.84 (20.0)	18.17	17.19	35.74	4.10	0.57	422.2	3.48±0.43	1,879.5
3	7.13	5.77	1,269.11	245.32 (19.3)	31.71	12.87	36.44	4.00	0.43	1,636.0	3.31±0.27	3,177.9
4	8.02	4.15	852.38	168.88 (19.8)	29.71	13.23	37.55	3.73	0.50	459.9	2.97±0.20	1,789.0
5	7.75	4.30	853.29	132.93 (15.6)	20.07	14.72	34.38	4.06	0.53	1,518.0	3.00±0.10	2,855.7

^a The proportion of Po to TP in sediments (%)

303.50 to 2,820.70 mg kg⁻¹, accounting for 63.30 to 89.37 % of TP in the NaOH-EDTA extracts. As another Pi component, the concentration of pyro-P was much less than for ortho-P, and ranged from 0.81 to 38.16 mg kg⁻¹, accounting for 0.2 to 0.8 % of TP in the sediment extracts. Mono-P was the dominant Po in the NaOH-EDTA fraction of river sediments, and ranged from 33.71 to 1,318.04 mg kg⁻¹, accounting for 9.0 to 29.6 % of TP in the sediment extracts. DNA-P ranged from 5.09 to 90.37 mg kg⁻¹, with relative contribution of 0.7 to 2.0 % of the extracted P in river sediment. Lipids-P and phon-P ranged from 2.55 to 118.70 mg kg⁻¹ and 0.40 to 69.80 mg kg⁻¹, with their relative contributions 0.2 to 2.7 % and 0.1 to 1.6 % of the extracted P in river sediments, respectively.

³¹P-NMR spectra of NaOH-TP in P fractionation The ³¹P-NMR peaks of NaOH-TP were different from the NaOH-EDTA extracts. For the five sample sites, the peaks show six P compounds in the NaOH-TP of P fraction in the sediments, including phon-P (12 to 23 ppm), ortho-P (6 to 7 ppm), mono-P (4 to 6 ppm), lipids-P (1 to 3 ppm), DNA-P (0 ppm), and pyro-P (−3.5 to −4.5 ppm) (Turner et al. 2003a; Cade-Menun 2005; Cade-Menun et al. 2006; Liu et al. 2009). Phon-P and

lipids-P were only detected in site 1. Pyro-P was not detected in sites 4 and 5.

The concentrations and relative distribution of various P compound groups in NaOH-TP extracts are presented in Table 2. Po in the NaOH-TP extract ranged from 8.57 to 215.58 mg kg⁻¹, with the percent of TP in NaOH ranging from 1.56 to 11.48 %. The ortho-P was the dominant P in the NaOH-TP extracts, and ranged from 181.13 to 3,835.24 mg kg⁻¹, accounting for 83.68 to 93.24 % of TP in the NaOH-TP extracts. Mono-P was the dominant Po in the NaOH-TP extracts of river sediments, and ranged from 15.24 to 220.29 mg kg⁻¹, accounting for 5.36 to 13.16 % of TP in the NaOH-TP extracts. DNA-P ranged from 3.56 to 23.57 mg kg⁻¹, with a relative contribution of 0.57 to 2.53 % of the TP in the NaOH-TP extracts. As another Pi, the concentration of pyro-P was much less than for ortho-P, ranging from 0 to 6.55 mg kg⁻¹, accounting for 0 to 0.15 % of TP in the NaOH-TP extracts. Lipids-P and phon-P ranged from 0 to 16.48 mg kg⁻¹ and 0 to 11.15 mg kg⁻¹, with their relative contribution being 0 to 0.40 % and 0 to 0.27 % of the extracted P in NaOH-TP extracts, respectively.

Fig. 2 Concentrations (left) and percentages (right) of P fractionations in the sediments

Fig. 3 ^{31}P -NMR spectra of NaOH-EDTA extracts (*left*) and NaOH-TP extracts in P fraction (*right*) of the sediments in Fuyangxin river (A phon-P, B ortho-P, C mono-P, D diesters-P; E lipids-P, F DNA-P, G pyro-P)

Discussion

Sediment P pollution in the artificial river

The P pollution in the Fuyangxin River system was serious. The average concentrations of TP and SRP in the water were 3.0 and 2.6 mg L⁻¹, respectively, which means that the water

pollution status surpasses grade V of the national quality standard, and that the water should not be used for any purpose. The concentration of TP and SRP were higher than Taihu Lake (TP, 0.12 mg L⁻¹) and the Bronx River (TP, 0.438 and 0.089 mg L⁻¹) (Bai et al. 2009; Wang and Pant 2011, 2012). The artificial river sediment P had accumulated significantly, such that the concentration of TP in the sediment was

Table 2 Concentrations of P compounds in NaOH-EDTA extracts of the sediments determined by solution ^{31}P -NMR

Site	TP mg kg ⁻¹	Po (%)	Pi		Po			
			Ortho-P	Pyro-P	Mono-P	Lipids-P	DNA-P	Phon-P
1	4,455.77	1,671.42 (57.4) ^a	2,820.70 (63.30) ^b	38.16 (0.86)	1,318.04 (29.58)	118.70 (2.66)	90.37 (2.03)	69.80 (1.57)
2	469.88	115.01 (56.1)	376.21 (80.06)	2.08 (0.44)	80.22 (17.07)	3.08 (0.65)	8.30 (1.77)	n.d.
3	898.51	183.22 (74.7)	760.00 (84.58)	2.76 (0.30)	124.60 (13.87)	2.55 (0.28)	6.78 (0.75)	1.80 (0.20)
4	367.57	105.65 (62.6)	303.50 (82.56)	1.01 (0.27)	58.04 (15.79)	n.d.	5.03 (1.37)	n.d.
5	376.27	85.59 (64.4)	336.26 (89.37)	0.81 (0.22)	33.71 (8.96)	n.d.	5.09 (1.35)	0.40 (0.11)
NaOH-TP extracts in P fractionation analyzed by ^{31}P -NMR								
1	4,113.28	215.58 (5.24) ^c	3,835.24 (93.24) ^b	6.55 (0.16)	220.29 (5.36)	16.48 (0.40)	23.57 (0.57)	11.15 (0.27)
2	237.11	8.57 (3.61)	198.41 (83.68)	0.98 (0.41)	31.21 (13.16)	n.d.	6.50 (2.74)	n.d.
3	576.53	8.98 (1.56)	524.13 (90.91)	1.24 (0.22)	46.92 (8.14)	n.d.	4.22 (0.73)	n.d.
4	200.78	19.28 (9.60)	181.13 (90.21)	n.d.	15.24 (7.59)	n.d.	4.41 (2.20)	n.d.
5	219.77	25.23 (11.48)	197.92 (90.06)	n.d.	18.29 (8.32)	n.d.	3.56 (1.62)	n.d.

n.d. not detected

^a The proportion of Po in NaOH-EDTA extraction to total Po in sediments (%)

^b The proportion of individual P fraction to total P in NaOH-EDTA extraction

^c The proportion of Po to total P in NaOH-TP of P fraction in river sediments

2,160.29 mg kg⁻¹. The concentration of P in the sediment was higher than that in soils, lake sediments, and marine sediment, and even compared with that found in manures (Table 3). Although the Fuyangxin River was constructed for flood control originally, the function of accepting sewage became more prominent with socio-economic development. Large cities and farmland are distributed on the banks of the river, so that the wastewater from the domestic and farm sources carries a large amount of P into the river, with the result that the concentration of P in the river sediment is high.

Pernet-Coudrier et al. (2012) reported that 4.4 t P day⁻¹ was discharged into the Haihe river system with the wastewater from Beijing and its suburbs (Pernet-Coudrier et al. 2012). We could deduce that the wastewater from the cities of Shijiazhuang, Xingtai, and Hengshui introduced a large amount of P into the Fuyangxin River. Qu and Kroeze's (2010) study showed that the main sources of DIN and DIP were agriculture and sewage, respectively, between 1970 and 2000. They also predicted that these sources will be dominant in 2030 and 2050 (Qu and Kroeze 2010). Our study showed that the source of P was complicated and the emissions have continued increasing, which means that the source of P was not only agriculture but also sewage (unpublished data). And the P pollution will be more serious in the future if it is not controlled.

³¹P-NMR spectroscopy and P fractionation

Our study showed that the 0.25 M NaOH+50 mM EDTA fraction was an ideal extract for the study of Haihe River Basin sediments (unpublished data). Therefore, we chose to study the 0.25 M NaOH+50 mM EDTA extract for Fuyangxin river sediments. The NaOH assured the high recovery of Po and EDTA can prevent the poly-P hydrolysis during extraction and reduce the concentration of paramagnetic ions, such as Fe and Mn (Hupfer et al. 1995; Cade-Menun et al. 2002; Turner et al. 2005). The recovery of Po in NaOH-EDTA to total Po in sediments was 56.15 to 74.69 %, which was an ideal recovery level for Po analysis (Zhang et al. 2009; Shinohara et al. 2012). The quality of ³¹P-NMR spectra in NaOH-TP extracts was poorer than for NaOH-EDTA; the main reasons may be that there was no EDTA in the NaOH-TP extracts and much higher levels of paramagnetic ions (such as Fe and Mn) existed in the extracts. Turner et al. (2006) reported that the Po in wetland soils was overestimated by alkaline extraction and molybdate colorimetry (Turner et al. 2006). The concentration of total Po derived from ³¹P-NMR analysis of NaOH-TP extracts was higher than that by P fractionation (excluding site 5). The main reason for this result was that some stable Po was not extracted by 0.1 M NaOH, which was considered the stable Po (such as some inositol phosphates) belonging to Res-P in the P fraction system. The concentration of Po varied under different pH, and some

researcher suggested the better way to get more accurate results of sediment/soil samples is to remove NaOH before analysis with ³¹P-NMR. But in this research (Wang and Pant 2010c), the result showed that use of the 0.25 M NaOH+50 mM EDTA extracts and solution ³¹P-NMR analysis was a more accurate method for quantifying Po in the river sediments than P fractionation.

Biogenic P species

In the Fuyangxin River sediment, biogenic P included phon-P, mono-P, lipid-P, DNA-P, and pyro-P. The concentration of biogenic-P ranged from 40.00 to 1,635.07 mg kg⁻¹, accounting for 10.63 to 36.70 %. Poly-P was not detected in the NaOH-EDTA extracts. Poly-P was considered to be a more labile form of P in the sediment that mineralizes more quickly (Hupfer et al. 2004; Reitzel et al. 2006a, b). This might be the reason there were no poly-P peaks observed in this research. Mono-P was the dominant biogenic-P in the NaOH-EDTA extracts of the river sediments. The inositol phosphates, from manures, plants, zooplankton, and algae, were the main compounds of mono-P, which were considered to be bio-unavailable, immobile, and refractory previously (Turner et al. 2002; McKelvie 2007). Some studies, however, showed that the inositol phosphates were a possible source of phosphate release under anaerobic and aerobic conditions (Livingstone et al. 1982; Golterman et al. 1998). The concentration of DO in the surface water was 0.87 mg L⁻¹, and the DO concentration at the sediment–water interface might be hypoxic. The main reason for the hypoxia-DO depletion was the high concentration of NH₄⁺-N and COD_{Cr} in the surface water. The concentration of NH₄⁺-N and COD_{Cr} were from 1.44 to 14.38 mg L⁻¹ and 76.5 to 145.5 mg L⁻¹ (based on the investigation from 2010 to 2012). Lots of the oxygen-consumption substances will deplete the DO in the river water. The mono-P would be mineralized under hypoxic conditions. Diesters-P was labile P in the sediment and its half-life was 21 years, and was a mixture compounds, such as DNA-P, lipids-P, and teichoic-P (Turner et al. 2003a; Ahlgren et al. 2005; Cade-Menun 2005). Pyro-P partially originated from esters or poly-P that was hydrolyzed during alkaline extraction (Hupfer et al. 1995). The diesters-P and pyro-P might the main P source in the river sediments.

P variation and the river systems

The P compounds showed different characteristics of change with the river flow. There were many dams in the river system in the Haihe River Basin for irrigation dating from 1960s, which were similar to the Bronx River (Kensico Dam) (Wang and Pant 2011, 2012). With the water scarcity, the operation of river dams caused intermittent fluctuations in flows (Pernet-Coudrier et al. 2012). Intermittent flows in a river system lead

Table 3 Literature data concerning extracted TP from environmental samples

Samples	TP (mg kg ⁻¹)	Literature
River sediment (Bronx River, USA)	138–2,730	Wang and Pant (2010a)
Soil (Everglades, USA)	190–1,570	Dierberg et al. (2011)
Lake sediments (Taihu, China)	399–1,830	Bai et al. (2009)
Manure (cattle, Deer and Sheep)	5,584–7,919	McDowell et al. (2006)
Artificial lake sediments (China)	888–1,984	Liu et al. (2009)
Marine sediment (Baltic Sea)	1,371	Ahlgren et al. (2006)
River sediment (North Canal River)	654.9–1,285.4	Li et al. (2010)
River sediment (Haihe River Mainstream)	928–2,528	An and Huang(2007)

to zero flow in some river segments, and also cause sediment accumulation at the front or behind dams. In this research, site 1 was located behind the Aixinzhuang Dam, which is a large dam for regulating the water for the Fuyang River and Fuyangxin River. The accumulation of sediment led to the TP and biogenic-P in site 1 being higher than other sites, at 6,804.10 and 1,635.07 mg kg⁻¹, respectively. TP and biogenic-P in the sediment was attenuated along the direction of flows because of the character of the artificial river, with their average relative proportion being 7.97:1.20:1.49:1.00:1.00 and 40.87:2.34:3.46:1.60:1 from upstream to downstream, respectively. The concentrations of TP and biogenic-P decreased 87 and 98 % from site 1 to site 5. The concentrations of TP and biogenic-P decreased to 853.29 and 40.00 mg kg⁻¹ (Fig. 4). The P compounds showed different characteristics of change. The concentration of ortho-P, pyro-P, mono-P, and DNA-P were reduced along the river flow direction. Lipid-P was not detected at sites 4 and 5. Phon-P was found at sites 1, 3, and 5. The main reason for the P variation might be changes of the physical–chemical characteristics of the water and sediment along the flow direction (Wang and Pant 2010a). Site 1 was located behind of the Aixinzhuang Dam, which was a large dam for regulation the water for Fuyang River and Fuyangxin River. The accumulation of sediment lead to the

TP and biogenic-P in site 1 was higher than other sites. From the upstream to the downstream, the sediment accepted the P from the surface water was decrease, and also part of biogenic-P was mineralized. This phenomenon was not detected in the North Canal River (Li et al. 2010) and Bronx River (Wang and Pant 2010c).

Conclusions

This research revealed the P pollution status in terms of aspects of P species, distribution, and bioconversion in sediments of an artificial river affected by water scarcity and heavy pollution, the Fuyangxin River, by P fractionation and ³¹P-NMR. We also evaluated the P fraction and ³¹P-NMR for biogenic-P analysis. The average concentrations of TP and SRP in the water were 3.0 and 2.6 mg L⁻¹, respectively, which means that the water pollution status surpasses grade V of the national quality standard and the water should not be used for any purpose. The river sediments accumulated significant Pi and Po. The rank order of the P fractions was as follows: Ca-P>NaOH-Pi>Res-P>KCl-P>NaOH-Po, with their average relative proportion 25.1:16.8:6.6:1.7:1.0. Six P compounds were detected in NaOH-EDTA extracts by ³¹P-NMR. Mono-P (8.96–29.58 %) was the dominant forms of biogenic-P, and other smaller components of biogenic-P were also observed, including pyro-P (0.22–0.86 %), DNA-P (0.75–2.03 %), phon-P (0–1.57 %), and lipids-P (0–2.66 %). Along the direction of flows, the TP and biogenic-P decreased, with their average relative proportion 7.97:1.20:1.49:1.00:1.00 and 40.87:2.34:3.46:1.60:1 from upstream to downstream, respectively. The concentration and species of Po in the NaOH-Po fraction were lower than was found by ³¹P-NMR analysis in this research. Thus the use of 0.25 M NaOH+50 mM EDTA extracts and solution ³¹P-NMR analysis was a more accurate method for quantifying biogenic-P in the river sediments than P fractionation.

Fig. 4 The concentrations of TP and biogenic-P as the distance from Aixinzhuang dam (km)

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