Information on the chemical composition of sediment phosphorus (P) is fundamental to understanding P dynamics and eutrophication in lake ecosystems. In this study, the surface (10 cm) sediments were collected from seven lakes representing two contrasting ecological areas in China: the middle and lower reaches of Yangtze River region and the Southwestern China Plateau. Phosphorus in these sediments was extracted by NaOH-EDTA and characterized by solution 31P nuclear magnetic resonance spectroscopy. Results show that P in the extracts was dominated by inorganic orthophosphate (76.7–97.4% of the extracted P) and orthophosphate monoesters (1.8–14.3%), with smaller amounts of orthophosphate diesters (0.4–8.9%), pyrophosphate (0.1–0.7%), and phosphonates (0.1–0.2%). The relative abundance of orthophosphate was higher in hypertrophic and shallow lake sediments than in eutrophic and mesotrophic and deep lake sediments, whereas the relative abundance of orthophosphate monoesters was the opposite. These observations suggested that the relative abundance of the two types of P forms in sediments might be related to the degree of lake eutrophication.
logical areas (Zhang et al., 2008). Most lakes in these two areas were under mesotrophic or eutrophic conditions due to the rapidly expanded township enterprises, large population densities, the generally agrochemical and chemical fertilizer over-use, discharge of the municipal sewage, and the scale of cultivation (Wu et al., 2001; Qin, 2002). Phosphorus has been recognized as a major factor responsible for lake eutrophication (Jin et al., 1990), and much attention has been paid to inorganic P fractions and their bioavailability in the lacustrine environment (Zhou et al., 2001). However, little is known about the composition of organic P and its role in lake eutrophication even though it has been reported to be an important component in many Chinese lake sediments (Jin et al., 1990; Wang, 2001).

To increase our knowledge of the role of organic P in lake eutrophication, a sequential fractionation scheme has been applied to characterize the distributions of inorganic and organic P in lake sediments from these two areas (Zhang et al., 2008). This work found the presence of a greater organic P fraction in heavily polluted sediments than in moderately polluted and nonpolluted sediments. Although the chemical fractionation provides valuable information on P lability, it does not provide information on the P identities in these sediments. Therefore, the purpose of this study was to (i) use ³¹P NMR spectroscopy to investigate the form and distribution of P, particularly the organic P, in seven lake sediments from these two areas and (ii) discuss the differences in the occurrence of P forms affected by lake environmental conditions.

**Materials and Methods**

**Sediment Collection and Analysis**

Sediments from seven lakes were collected. In addition to the six lakes listed in Zhang et al. (2008), Lake East Taihu located in the middle and lower reaches of the Yangtze River region was included in this study. These lakes represented different trophic levels and other characteristics of lakes in China (Table 1).

Sediments from the center of each lake were collected using a core sampler (30 cm length, 5 cm diameter) Plexiglas cylinder tube in May 2005. The sediment cores were sliced, and the top 10 cm of the samples were transported to the laboratory in air-sealed plastic bags that were stored in ice. Total P (TP), inorganic P (Pi), and organic P (Po) in sediments from Lake East Taihu were determined in the same manner as described previously (Zhang et al., 2008), according to the procedure of Aspila et al. (1976). Inorganic P was analyzed by direct extraction with 1 mol L⁻¹ HCl (16 h), and TP was measured by treating at 500°C (2 h), followed by HCl extraction. The concentration of P, in sediment was calculated as the difference between TP and P, i.e., the organic matter content was quantified as the loss on ignition based on the weight loss after drying and combustion of the sediments at 500°C (Jensen et al., 1992).

**NaOH-EDTA Extraction and Solution Phosphorus-³¹N Nuclear Magnetic Resonance Spectroscopy**

Sediment samples (10 g) for the ³¹P NMR analysis were extracted in 100 mL of a solution containing 0.25 mol L⁻¹ NaOH and 0.05 mol L⁻¹ EDTA for 16 h at room temperature (Cade-Menun et al., 2002). After centrifugation of the extracts at 10,000 x g for 30 min, the supernatants were filtered through 0.2-μm polysulfone membranes. Subsamples were collected for analyzing the concentrations of TP and P, before 5 times pre-concentration by rotary evaporation at 30°C. Inorganic P was determined in diluted extracts (1:100) by molybdate colorimetry (Murphy and Riley, 1962). The P, fraction is mainly ortho-P but can include acid-labile organic and condensed P compounds (Dick and Tabatabai, 1977). Total P was measured by a similar procedure after acid-persulphate digestion of the samples (Rowland and Haygarth, 1997). Organic P was calculated as the difference between TP and P,. This fraction also includes poly-P (Shand et al., 2000). However, we use the terms P, and P, for clarity as in Turner et al. (2003a). The concentrations of P determined by molybdate colorimetry were used as the basis to calculate the concentrations of various P compounds measured with ³¹P NMR analyses, which can only give the relative abundance or percentage. Experiments were performed in triplicates, and results were reported as the average with standard errors (not shown) less than ±5% of the mean value.

To reduce interference from paramagnetic ions such as Fe and Mn in ³¹P NMR measurement, 5% (v/v) of bicarbonate buffered dithionite solution (0.11 mol L⁻¹ NaHCO₃ + 0.11 mol L⁻¹ Na₂S₂O₄) was added to the concentrated extracts (Reitzel et al., 2006b; Ahlgren et al., 2007). The extracts were then frozen at −18°C and lyophilized over several days. Freezing the extracts has been shown not to alter the phosphorus composition (Hupfer et al., 1995, 2004). Freeze-dried extracts (approximately 300 mg) were re-dissolved in 0.5 mL of D₂O (for signal lock) and 0.2 mL of 10 mol L⁻¹ NaOH and were ultrasonicated for 30 min and then equilibrated for 5 min. The supernatants were centrifuged for 10 min at 4000 x g and transferred to 5-mm NMR tubes. Solution ³¹P NMR spectra were obtained using a JEOL ECX 500 MHz spectrometer (Tokyo, Japan) operating at 202.5 MHz at 25°C. We used a 90° observe pulse, a total acquisition time of 5.6 s (acquisition time 1.6 s, pulse delay 4 s), and broadband proton decoupling. Spectra were collected with 10,000 to 40,000 scans.

Chemical shifts were recorded relative to an external 85% H₃PO₄ standard (δ = 0 ppm). Peaks were assigned based on data in the literature (Carman et al., 2000; Cardoso et al., 2003; Turner et al., 2003b; Cade-Menun, 2005). The spectra were processed using visual inspection and automated peak analysis tools for peak-picking and spectra integration. From these peak areas, the contribution of the individual P compound groups (ortho-P, pyro-P, poly-P, monoester-P, diester-P, and phosphonates) was calculated relative to the TP in the extracts determined as described previously.

**Results and Discussion**

**Phosphorus Recovery by NaOH-EDTA Extraction**

The chemical component concentrations in the lake sediments are presented in Table 2. The concentrations of organic matter in the sediments from Lake Meiliang, Yuantouzhu,
The percentage of total P and organic P extracted with NaOH-EDTA.

§ The concentrations of various P forms in the NaOH-EDTA extracts.

‡ Various P forms in sequentially extracted fractions. Data are from Zhang et al. (2008) except for those of Lake East Taihu.

Zhang et al.: A 31P NMR Study of P Composition in Lake Sediments

Sediment samples. Similarly, He et al. (2007) reported that NaOH-EDTA was not capable of extracting all P in the seven lake sediments, TP varied greatly, ranging from 440 to 1691 mg kg$^{-1}$. Inorganic P$_i$ ranged from 296 to 1496 mg kg$^{-1}$, and P$_o$ ranged from 97 to 423 mg kg$^{-1}$. Relative distributions of P$_i$ and P$_o$ to TP ranged from 56 to 81%, although recovery rates by NaOH-EDTA might have reflected the high Ca content in poultry manure. An additional step of HCl extraction recovered the remaining 33% of P in poultry manure. Because the total P was determined with both HCl and NaOH extraction (Zhang et al., 2008), the different recovery rates by NaOH-EDTA might have reflected the fact that there were different amounts of Ca-relevant P species among the seven sediment samples.

### Phosphorus Forms Identified by Phosphorus-31 Nuclear Magnetic Resonance Spectroscopy

The NMR spectra for the lake sediments are shown in Fig. 2. Five 31P NMR peaks were observed in the NaOH-EDTA extracts of lake sediments. In all of these spectra, signals were interpreted as ortho-P at δ = 6 to 8 ppm, pyro-P at δ = −2.5 to −5 ppm, and poly-P at δ = −20 ppm; as monoester-P at δ = 2.5 to −1 ppm, and phosphonates at δ = 17.5 to 21 ppm.

Concentrations and relative distributions of various P compound groups in these lake sediments are presented in Table 3. In all sediment extracts, ortho-P was the dominant P form, followed by monoester-P, diester-P, pyro-P, and phosphonates. Trace amounts of phosphonates were present in two sediments only from Lake Meiliang and Hongfeng. Similar trends have
been reported in most lacustrine sediments (Hupfer et al., 2004; Reitzel et al., 2006b, 2007), with the exception of the absence of poly-P in this study. Ortho-P, monoester-P, diester-P, pyro-P, and phosphonates were in the range of 146.3 to 1326.2 mg kg$^{-1}$, 21.4 to 82.7 mg kg$^{-1}$, 5.4 to 21.1 mg kg$^{-1}$, 0.5 to 5.8 mg kg$^{-1}$, and 0.5 to 2.7 mg kg$^{-1}$, respectively, with their relative contribution of 76.7 to 97.4%, 1.8 to 14.3%, 0.4 to 8.9%, 0.1 to 0.7%, and 0.1 to 0.2% of the extracted P in those sediments, respectively.

Pyro-P may partially originate from esters or poly-P that can be hydrolyzed during alkaline extraction (Hupfer et al., 1995). Ahlgren et al. (2005) demonstrated that pyro-P was present in the upper 5 cm of sediment and rapidly declined with increasing sediment depth. We detected this P species in our seven top (0–10 cm) sediment samples. However, the relative abundances of pyro-P were different among these sediments, suggesting that the abundance of this P group in the sediment was influenced by different biogeochemical processes.

The absence of peaks in the poly-P region might not indicate that there was no poly-P in the sediments. Poly-P was considered as one of the most labile P forms—its lability was further supported by the finding of an extremely short half-life time (0.8–2.0 yr) (Reitzel et al., 2006b, 2007)—and can be transformed to pyro-P or tri-metal-phosphate with catalyzing by various metal ions (Hupfer et al., 1995, 2004). It has been reported that the large amount of metal ions that occur in those sediments (Wang, 2001; Wang et al., 2002) may affect the fate of poly-P (Carman et al., 2000). Moreover, the insufficiency of the bicarbonate-buffered dithionite solution pre-extraction

![Fig. 1. Relationship between total phosphorus, determined using the procedure of Aspila et al. (1976), and NaOH-EDTA extracted P from seven lake sediments (0–10 cm) in the middle and lower reaches of Yangtze River region and the Southwestern China Plateau.](image)

![Fig. 2. Solution $^{31}$P nuclear magnetic resonance spectra of NaOH-EDTA extracts of the lake sediments (0–10 cm) from the middle and lower reaches of Yangtze River region and the Southwestern China Plateau. The sharp peak of orthophosphate was truncated for the clarity of other peaks.](image)

| Table 3. Concentrations and percentage of extracted P compounds in NaOH-EDTA extracts of the sediments determined by solution $^{31}$P nuclear magnetic resonance spectroscopy. |
|---|---|---|---|---|
| Sediment | Ortho-P | Pyro-P | Poly-P | Monoester-P | Diester-P | Phosphonate | Monoester to diester ratio |
| Lake Meiliang | 1326.2 (97.4)$^+$ | 2.7 (0.2) | ND$^+$ | 24.5 (1.8) | 5.4 (0.4) | 2.7 (0.2) | 5.0 |
| Lake Yuantouzhu | 529.3 (92.7) | 2.9 (0.5) | ND | 29.1 (5.1) | 9.7 (1.7) | ND | 3.0 |
| Lake Gonghu | 146.3 (76.7) | 0.4 (0.2) | ND | 27.1 (14.2) | 17.0 (8.9) | ND | 1.6 |
| Lake East Taihu | 208.9 (88.0) | 1.4 (0.6) | ND | 21.4 (9.0) | 5.7 (2.4) | ND | 3.8 |
| Lake Hongfeng | 397.3 (82.4) | 0.5 (0.1) | ND | 68.9 (14.3) | 14.9 (3.1) | 0.5 (0.1) | 4.7 |
| Lake Baihua | 851.5 (88.6) | 5.8 (0.6) | ND | 82.7 (8.6) | 21.1 (2.2) | ND | 4.0 |
| Lake Erhai | 399.2 (84.3) | 3.3 (0.7) | ND | 56.3 (11.9) | 14.7 (3.1) | ND | 3.8 |

$^+$ Numbers in parentheses are percentages.
$^+$ ND, not detected.
step with respect to poly-P might have been an additional reason for the lack of poly-P in the event of the presence of high concentrations of metal ions, despite this procedure being recommended by Reitzel et al. (2007).

Monoester-P represents a wide range of important P compounds (e.g., sugar phosphates, mononucleotides, phospholipids, and inositol phosphate [inositol P] of varying phosphorylation degrees) (Reitzel et al., 2007). Distinct signals at 4 to 5.5 ppm in all extracts may indicate large concentrations of inositol P (Carman et al., 2000; Turner et al., 2003b). Inositol P is considered to be the most abundant organic component in sediments and possibly a major constituent of the humic matrix (Suzumura and Kamatani, 1999) because of its resistance during acid and base hydrolysis (De Groot and Golterman, 1993). Zhang et al. (2008) suggested that residual P (mainly phytate) was a major P fraction and accounted for 4.1 to 23.5% of TP in these sediments. However, the other monoester-P cannot be identified as specific compounds due to the overlapping of peaks in our spectra (Fig. 2).

Diester-P is a mixture of numerous compounds with varying lability (e.g., DNA-P phospholipids and teichoic-P) (Turner et al., 2003b, Cade-Menun, 2005). A dominant proportion of the peak area in this region is situated between 0 and –1 ppm (>80%). Organic P compounds between 0 and –1 ppm were generally assigned to DNA-P (Turner et al., 2003b). DNA-P in sediment originated from bacterial DNA and a certain addition for example from decomposing phytoplankton and may thus be considered as an indicator of the bacterial abundance in the soil or sediments. The highest content of DNA-P present in the Lake Gonghu sediment could be attributed to the rich submerged macrophyte there (Fan et al., 1997), although the amount of microbe in the sediment was lower than other sediments from the Yangtze River region (Geng et al., 2006). Furthermore, higher concentrations of DNA-P were detected in the sediments from Lake Hongfeng and Erhai, supporting the findings by Wei et al. (2005), who reported that DNA and microbe activities prevailed in these surface sediments.

The monoester to diester ratio ranged between 1.6 and 5.0. This value was comparable to the result of Makarov et al. (2005) (2.0–4.0) and was lower than values in other soil P studies: 8.3 to 33.3 (Cardoso et al., 2003) and 5.1 to 25.9 (Turner et al., 2003b). Apparently, this ratio was not a reliable indicator of the origins of P in soil and the sediments, as indicated by Makarov et al. (2002, 2005).

Phosphonates are the chemically stable compounds due to their direct C-P bond (Kittredge and Roberts, 1969). The pH may be important to the abundance of phosphonates because the occurrence of bacteria that possess phosphonatase enzyme seems to be governed by high pH (Wetzel, 2001), and the pH in these lacustrine environments often varied greatly (Table 1).

**Relationships between Sediment Phosphorus Composition and Lake Environmental Conditions**

For the hypertrophic sediment, the relative distribution of ortho-P was higher than that for eutrophic and mesotrophic sediment, whereas the relative distributions of monoester-P and diester-P were lower, and that of pyro-P and phosphonates was almost the same (Fig. 3). The different P distributions in these trophic sediments may indicate differences in lability. For example, clustered monoesters-P, such as phytate and humic-bound P, due to the higher charge density, could form stronger complexes with metal cations than orthophosphate or other simple P species (He et al., 2006a, 2006b). Thus, these clustered monoester-P species are less labile to decomposition (He et al., 2006b, Reitzel et al., 2006a). Furthermore, Reitzel et al. (2007) reported the half-life times of common P species in lake sediments in the order of ortho-P (1 yr)<poly-P (2 yr)<DNA-P (22 yr)<monoester-P (29 yr). Thus, the lower lability of the complicated cluster monoester-P species could partly explain the different P composition in our lake sediments. Moreover, the proportion of monoester-P in shallow lake sediments was lower than that in the deep lakes, the proportions of ortho-P and diester-P were higher, and other P compounds were almost the same (Fig. 4). The P composition in the sediments changed further as a result of bio-physical-chemical processes that occur during the settling of particles and subsequent changes during burial (Gonsiorczyk et al., 1998). In deep lakes, autochthonous and allochthonous particulate matter undergoes a series of diagenetic reactions, and only a small, recalcitrant fraction (e.g., phosphonates, inositol P) reaches the surface sediments during sedimentation (De Groot and Golterman, 1993; Ergin et al., 1996). Those processes are likely to explain the differ-
ent proportions of various P compound groups in these sediments from the two studied areas (Fig. 4). Further studies of fine-cut sediment cores, such as those sliced with 1-cm intervals (Reitzel et al., 2007), are needed for a better understanding of the relationship between the early diageneis and the P composition in these lake sediments.

Conclusions

The impact of P on eutrophication of freshwater lakes is a pressing global environmental concern. Information on P composition in the lacustrine environment is helpful to understand its biogeochemical cycles. In this study, P compounds in the sediments of seven lakes representing two contrasting ecological and serious eutrophication areas in China were investigated using 31P NMR study. Phosphorus in these sediments was extracted by NaOH-EDTA and analyzed by solution 31P NMR spectroscopy. The results show that the extracts were dominated by ortho-P (76.7–97.4% of the extracted P) and monoester-P (1.8–14.3%), with smaller amounts of diester-P (0.4–8.9%), pyro-P (0.1–0.7%), and phosphonates (0.1–0.2%). Analysis of the relationships between P composition and lake environmental conditions indicated that various P compounds were present with different extents because the relative abundance of ortho-P was greater in hypertrophic and shallow lake sediments than in eutrophic and mesotrophic or deep lake sediments. This observation warrants further investigation of the contribution of P dynamics to eutrophication in these aquatic ecosystems.

Acknowledgments

This research was jointly supported by Chinese Academy of Sciences (kzcx2-yw-102), National Natural Science Foundation of China (40525011, 40632011) and International cooperation project of CAS. We acknowledge the assistance from the colleagues of the Chinese Research Academy of Environment Sciences. Two anonymous reviewers are thanked for their constructive comments, which greatly improved the manuscript.

References


