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Organic matter and pH affect the analysis efficiency of ^{31}P -NMR

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ABSTRACT

Solution ^{31}P nuclear magnetic resonance spectroscopy (^{31}P -NMR) is a useful method to analyze organic phosphorus (Po), but a general procedure for the analysis method is lacking. The authors used solution ^{31}P -NMR, which was found to be an effective method for analysis of Po in Haihe River sediment, to analyze the Po in the surface sediment in Eastern China at the regional scale, and found that the NaOH-ethylenediaminetetraacetic acid (EDTA) extraction rate was affected by environmental factors. At the regional scale, the extraction rate showed a positive relationship with loss on ignition, when the extraction rate was lower than 60%. The extraction rate had no relationship with the loss on ignition when the extraction rate was higher than 60%. The extraction rate showed a negative relationship with pH, which means that the extraction rate was higher in acidic sediment and lower in alkaline sediment. The ratio of TC/TN (the ratio of total carbon to total nitrogen) was considered to represent the origin of organic matter in the sediment. The extraction rate was high when the TC/TN ratio was lower than 20, meanwhile the extraction rate decreased as the TC/TN ratio increased. The results show that the origin of organic matter in sediment significantly affects the NaOH-EDTA extraction rate. This study will give theoretical support for building an effective and general solution ^{31}P -NMR analysis method.

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Introduction

Phosphorus (P), which includes inorganic phosphorus (Pi) and organic phosphorus (Po), is a vital nutrient source for aquatic organisms. Po constitutes a major component in some sediment, accounting for about 10% to 90% of total phosphorus (TP) in swamp sediments, everglades histosols and lake sediments (Hesse, 1962; D'Angelo and Webster, 1991; Sommers et al., 1972). Studies show that Po can be directly used (Phon-P can be directly used by *Trichodesmium*) or mineralized into (Pi) and then used by algae (Dyhrman et al., 2006; Wang and Pant, 2010).

Although Po is an important component in the sediment, due to the complexity of Po constituents and analytical limitations, the composition and transformation of Po remain

poorly understood (Cade-Menun, 2005; Turner et al., 2005). Several analytical methods have been developed to analyze Po compounds, such as P fractionation (Hieltjes and Lijklema, 1980; Pierzynski, 2004). Although these methods have provided useful information to help understand P compounds and their cycling, they have not yielded sufficient insight into the identification of specific Po compounds (Brandes et al., 2007). Nuclear magnetic resonance (NMR) plays a key role in environmental research (Simpson et al., 2012). Phosphorus-31 nuclear magnetic resonance spectroscopy (^{31}P -NMR) 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, 2006b). Many Po species have been detected by ^{31}P -NMR, including phosphonates, orthophosphate

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monoesters, and orthophosphate diesters (Cade-Menun, 2005). Therefore, ^{31}P -NMR is an ideal technique for use in analysis of P species in marine or freshwater sediment (Cade-Menun, 2005; Simpson et al., 2011; Newman and Tate, 1980).

Po must be extracted from the sediment samples before it can be analyzed by ^{31}P -NMR. The extraction process must remove as much Po from the sediment sample as possible, and it must also keep the Po components untransformed in the extraction process. Thus the extraction processes are key factors in determining the effectiveness of the analysis. Many chemicals have been used as Po extractants, such as H_2O , NaOH, BD, NaOH and NaF, H_2SO_4 , NaHCO_3 and Chelex (Reitzel et al., 2006a; Newman and Tate, 1980; Turner and Leytem, 2004; Sumann et al., 1998; Makarov et al., 2002). Cade-Menun and Preston (1996) examined various extractants and concluded that a mixture of NaOH and ethylenediaminetetraacetic acid (EDTA) was an ideal extractant, which gave excellent and stable recovery of the various P components. The presence of the chelating agent, EDTA, along with an organometallic component, increased the extraction efficiency of the NaOH. Based on this conclusion, several P compounds have been detected using this extractant mixture (Turner et al., 2002; Sundareshwar et al., 2001; Hupfer et al., 1995).

The authors have optimized the preparation method for Po analysis using solution ^{31}P -NMR (Zhang et al., 2013). A solution with 0.25 mol/L NaOH and 50 mmol/L EDTA was found to be the most appropriate extractant for the target sediment samples. There is significant diversity in the water ecosystems in Eastern China, as reflected in the rivers, wetlands, and lakes. Different water ecosystems have various types of sediments, which exhibit diverse P retention mechanisms. The extraction efficiency of 0.25 mol/L NaOH and 50 mmol/L EDTA for widely different types of sediments is unknown. Research on the extraction rates of the 0.25 mol/L NaOH and 50 mmol/L EDTA extractant for widely varying sediment samples is scarce, necessary and valuable. Therefore the goal of this study is to investigate the NaOH and EDTA extraction rate and the effect of environmental factors, such as pH and organic matter. The research will provide a theoretical foundation for building a ^{31}P -NMR analysis method with broad applicability.

1. Materials and methods

1.1. Site description and sampling

A total of 89 sediment samples collected from Eastern China in September 2013 were analyzed in this study (Fig. 1). The sampling stations were located in rivers, wetlands and lakes, which were arranged from the Songhuajiang River to the Zhujiang River. Three surface sediments (about 5 cm depth) were collected by a Peterson grab sampler. Samples at the same site were pooled and homogenized to obtain a representative sample. On arrival at the laboratory, samples were freeze-dried at less than -50°C (FD-1 freeze-dryer). Dried samples were ground and sieved through a 100- μm mesh sieve. A representative sample was obtained by the quartering method, and then samples were placed in a sealed plastic bag and stored at room temperature until analysis.

Fig. 1 – Location of the 89 sample sites in Eastern China. There sites were distributed in Heilongjiang Province, Liaoning Province, Hebei Province, Beijing Municipality, Tianjin Municipality, Shandong Province, Jiangsu Province, Shanghai Municipality, Anhui Province, Hubei Province, and Guangdong Province. There sites represent river, wetland and lake, which were the most common ecology systems in Eastern China. The region also has the largest population density and fastest economic development, and includes the Bohai Sea Economic Rim, Pearl River Delta Economic Rim and Yangtze River Delta Economic Rim.

1.2. Sediment analysis and ^{31}P -NMR

Organic matter (OM) in sediments was determined by loss-on-ignition (LOI) at 550°C for 4 h (Jensen et al., 1992). Total nitrogen (TN) in the sediment was analyzed by an elemental analyzer (Vario EL III, Elementar, Germany). The pH was determined by a pH electrode at a sediment:water ratio of 1:2.5 (Bai et al., 2009).

Po analysis in this study included two parts, the NaOH-EDTA extraction and the ^{31}P -NMR analysis. Sediment samples, after being ground into powder and sieved through a 100 μm -mesh, were extracted with a solution of NaOH (0.25 mol/L) and EDTA (0.05 mol/L) at room temperature at a ratio of 1:10 (sediment to extractant) (Zhang et al., 2013). An aliquot of extracts was used to analyze Pi (Pi in NaOH-EDTA) and TP (TP in NaOH-EDTA) using the molybdate colorimetry method before and after digestion by Potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$). Total Po in the NaOH-EDTA extract was determined from the difference between Pi and TP. The

Fig. 2 – Phosphorus nuclear magnetic resonance (^{31}P -NMR) spectra of NaOH-ethylenediaminetetraacetic acid (NaOH-EDTA) extracts of the surface sediments in Eastern China. Phon: phosphonate; Ortho-P: orthophosphate; Mono-P: orthophosphate monoesters; Diesters-P: orthophosphate diesters; PL-P: phospholipid; DNA-P: deoxyribonucleic acids; Pyro-P: pyrophosphates.

remaining solution was frozen and lyophilized before using for ^{31}P -NMR analysis. Freezing the extracts has been shown not to alter the P composition (Hupfer et al., 1995, 2004).

Po might be lost through the process of freeze-drying and re-dissolution because of incomplete dissolution. However, lyophilized samples in this research were completely re-dissolved. So 0.3 g of the lyophilized NaOH-EDTA extracts were re-dissolved with a mixed solution consisting of 0.6 mL D_2O and 0.1 mL 10 mol/L NaOH. The Po solution was ultrasonicated for 30 min, and then equilibrated for 5 min. To reduce interference from paramagnetic ions, such as Fe^{3+} and Mn^{2+} , 2% (V/V) of BD (0.11 mol/L NaHCO_3 and 0.11 mol/L $\text{Na}_2\text{S}_2\text{O}_4$) was added to the extracts. The supernatants were centrifuged for 15 min at 14,000 r/min (rpm) (Eppendorf: Centrifuge 5418, Germany) 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 degree observation pulse, relaxation delay 3 sec and acquisition time 0.6 sec. Spectra were collected after between 3000 and 20,000 scans, depending on the concentration of Po (Beijing NMR center). Although the T_1 relaxation times were not explicitly analyzed in this study, a T_1 relaxation time of 3 sec is thought to be adequate to obtain quantitative spectra for a variety of P forms from most samples (70%) analyzed for Mn and Fe. Chemical shifts were recorded relative to 85% H_3PO_4 standard ($\delta = 0$ ppm). Signals were assigned to P species based on data in the literature (Cade-Menun, 2005; Turner et al., 2003b). Peak assignments were made using ^{31}P -NMR chemical shifts of phosphonate (phon-P: 12 to 23 ppm), orthophosphate (ortho-P: 6 to 7 ppm), orthophosphate monoesters (mono-P: 4 to 6 ppm), phospholipids (lipids-P: 1 to 3 ppm), deoxyribonucleic acid phosphorus (DNA-P: 0 ppm), and pyrophosphate (pyro-P: -3.5 to -4.5 ppm) (Fig. 2). The peak areas were calculated by visual inspection and an

automated peak analysis tool. From the areas for the different P species, the contribution of each P compound group was calculated relative to TP in the NaOH-EDTA extract, determined by the molybdenum blue method (Cade-Menun, 2005; Turner et al., 2003b).

2. Results and discussion

2.1. Characteristics of sediment in eastern China

The characteristics of sediments are as following TP 87.3–6124.7 mg/kg with mean 549.54 mg/kg, Po 6.1–319.8 mg/kg with mean 116.51 mg/kg, loss-on-ignition 0.5%–16.7% with mean 5.70%, TC/TN 6.1–107.8 with mean 16.16, and pH 4.6–8.9 with mean 7.78. The highest concentration of TP and Po was in the Haihe River System. The main reason for the high P concentration in the Haihe River System was that sewage was the predominant P source in this watershed. The pH also varied in different watersheds in Eastern China. The NaOH-EDTA extraction rates ranged from 2% to 102%, and the average extraction rate was 46% for different freshwater systems in Eastern China.

Four Po compositions were detected by solution ^{31}P -NMR in the surface sediment in Eastern China, including phon-P, mono-P and diesters-P (PL-P and DNA-P). Mono-P is an important group of Po compounds in sediment that includes inositol P, sugar phosphates and mononucleotides. Inositol phosphates are considered to be the most abundant Po in aquatic sediment, which can be mineralized under anaerobic or aerobic conditions (Turner et al., 2002). In this study, the mean concentration of mono-P was 50.42 mg/kg, making it the major Po in sediment. Diesters-P is a mixture of numerous compounds with varying lability (Turner et al., 2005). In this study, DNA-P and lipids-P were detected in the surface sediment. The concentrations of DNA-P and PL-P were 8.93 and 4.54 mg/kg, respectively. The phon-P, which was only detected in some surface sediment, is considered a chemically stable compound because of the direct C–P bond (Turner et al., 2005); the concentration of phon-P was 2.90 mg/kg.

2.2. EDTA-NaOH extraction rate and characteristics of sediment

According to comprehensive analysis, the characteristics of LOI, pH and the ratio of TC to TN affect the NaOH-EDTA extraction rate (ER). The relationship of LOI and ER is shown in Fig. 3a. The ER were positively related with LOI for ER under 60% ($R^2 = 0.10$, $p < 0.05$). That indicated the ER of Po increased with increasing LOI, and that there was no relationship between LOI and the ER of Po when the ER was above 60%. Studies have shown that Po is positively correlated with LOI in the sediments. Therefore the ER of NaOH-EDTA increased with increasing LOI. The main reason for this phenomenon is that the increase of Po ER affects the total ER in eastern region sediments. For some sediments, the ER had no relationship with LOI when the ER was above 60%. The reason for this phenomenon was that most of Po was extracted when the ER was above 60%, and the remaining P was the ortho-P and acid extract-P (Cade-Menun, 2005; Turner et al., 2005; Cade-Menun and Liu, 2013).

Fig. 3 – EDTA-NaOH extraction rate plotted against the characteristics of the sediment. (a) extraction rate (ER) and the Loss on ignition (LOI) of the sediment. Positive relationship was found between ER and LOI under the condition of ER under 60% ($R^2 = 0.10$, $p < 0.05$). The ER and the LOI show no correlation when the ER exceeds 60%. (b) ER and pH of the sediment. Negative relationship was found between EDTA-NaOH extraction rate and the pH of the sediments ($R^2 = 0.38$, $p < 0.01$), which means the ER was increased with the decrease of the pH of the sediment.

The ER had a negative relationship with pH, meaning that the ER decreased as the pH increased ($R^2 = 0.38$, $p < 0.01$) (Fig. 3b). For all sediments, the pH ranged from 6.5 to 9.0, and the ER ranged from 2% to 102%. The lowest ER was found for the Yellow River sediment sample. The Yellow River sediment samples were composed of fine sand, which had low Po. The same results were also discovered in soil samples, but not in sediments at the regional scale (McDoell and Stewart, 2006; Dou et al., 2009). Turner et al. (2003a) suggested that higher adsorptive surface area and lower microbial activity are beneficial for the existence of Po. On the contrary, alkaline soil, which is mainly characterized by CaCO_3 , has a lower ability for holding organic carbon and Po, so the NaOH-EDTA had a weak ER in extracting Po from calciculous soil (Turner et al., 2003a). In this study, the reason that ER and pH show a positive relationship is that acidic sediment, which has a higher ability to hold organic matter than alkaline sediment, was distributed in southern China. So the ER was higher in acidic sediment than that in alkaline sediment. However, the mechanism for the difference in ER in different types of sediments needs to be studied in more depth in terms of sediment physicochemical structure and composition and NaOH-EDTA extraction mechanism.

The ratio of TC to TN is another factor affecting the NaOH-EDTA extraction ratio (Fig. 4). For freshwater sediment, the ratio of TC/TN was an indicator for the source of the organic matter. Low aquatic plants are enriched in protein, giving a TC/TN ratio under 7. However, the ratio of TC/TN in terrestrial vascular plants is usually greater than 20. Therefore, the organic matter most likely originates from the decomposition of aquatic plants when the TC/TN ratio is under 7. When the ratio of TC/TN is greater than 20, terrestrial input is the source of the organic matter in the freshwater sediment (Chen and Wan, 2000; Reitzel et al., 2007).

The ratio of TC/TN (organic matter origin) showed a positive relationship with the ER ($R^2 = 0.65$, $p < 0.01$). The ER decreased as the TC/TN ratio increased, which means the NaOH-EDTA ER for the terrestrial input in sediment was

lower, and the ER for the sediment originating from aquatic plants was higher. Large amounts of wastewater input lead to the accumulation of P in the sediment, because of the rapid socio-economic development in Eastern China. The complex composition of man-made pollutant input will influence the composition of Po in the sediment, and affect the NaOH-EDTA extraction rate. For the aquatic plant enriched sediment, most of the Po originated from the decomposition of aquatic plants, such as algae, and the composition of Po was relatively simple. Thus, the NaOH-EDTA extraction rate was high. The present work is a simple study on the effect of sediment type on NaOH-EDTA extraction rate, and much more in-depth study needs to be carried out in the future to more fully elucidate the NaOH-EDTA extraction mechanism.

Solution ^{31}P -NMR is a useful method for Po analysis. However, the NaOH-EDTA extraction rate was found to be affected by the sediment physico-chemical properties at the large regional scale. At the regional scale, the ER showed a positive relationship with LOI, when the ER was lower than 60%. The ER had no relationship with LOI when the ER was higher than 60%. ER showed a negative

Fig. 4 – The ratio of total carbon to total nitrogen (TC/TN) ratio and extraction rate (ER) show an exponential relationship ($R^2 = 0.6466$).

relationship with pH, meaning that the ER was higher in acidic sediments, while the opposite was the case in alkaline sediments. The ratio of TC/TN was considered to represent the origin of organic matter in the sediment. The ER was high when the TC/TN ratio was lower than 20, but the ER decreased as the ratio of TC/TN increased. The results show that the origin of organic matter in sediment can significantly affect the NaOH-EDTA extraction rate.

3. Conclusions

This research study has used NaOH-EDTA as extractant to analyze the P composition in the surface sediment in Eastern China by solution ^{31}P -NMR. The results show that the extraction rate was affected by environmental factors. The main conclusions are as follows: At the regional scale, the ER showed a positive relationship with LOI, when the ER was lower than 60%. The ER had no relationship with LOI when the ER was higher than 60%.

ER showed a negative relationship with pH, meaning that the ER was higher in acidic sediments, and the opposite was the case in alkaline sediments.

The TC/TN ratio was considered to represent the origin of organic matter in the sediment. The ER was high when the TC/TN ratio was lower than 20, but the ER decreased as the ratio of TC/TN increased.

The results show that the origin of organic matter in sediment significantly affects the ER of NaOH-EDTA at the regional scale.

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REFERENCES

- Bai, X.L., Ding, S.M., Fan, C.X., Liu, T., Shi, D., Zhang, L., 2009. Organic phosphorus species in surface sediments of a large shallow, eutrophic lake, Lake Taihu, China. *Environ. Pollut.* 157 (8–9), 2507–2513.
- Brandes, J.A., Ingall, E.D., Paterson, D., 2007. Characterization of minerals and organic phosphorus species in marine sediments using soft X-ray fluorescence spectromicroscopy. *Mar. Chem.* 103 (3–4), 250–265.
- Cade-Menun, B.J., 2005. Characterizing phosphorus in environmental and agricultural samples by ^{31}P nuclear magnetic resonance spectroscopy. *Talanta* 66 (2), 359–371.
- Cade-Menun, B.J., Liu, C.W., 2013. Solution phosphorus-31 nuclear magnetic resonance spectroscopy of soils from 2005 to 2013: a review of sample preparation and experimental parameters. *Soil Sci. Soc. Am. J.* 78 (1), 19–37.
- Cade-Menun, B.J., Preston, C.M., 1996. A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. *Soil Sci.* 161 (11), 770–785.
- Cardoza, L.A., Korir, A.K., Otto, W.H., Wurrey, C.J., Larive, C.K., 2004. Applications of NMR spectroscopy in environmental science. *Prog. Nucl. Magn. Reson. Spectrosc.* 45 (3), 209–238.
- Chen, J.A., Wan, G.J., 2000. Environmental records in recent sediments of lake Chenhai, Yunnan province. *Acta Mineral. Sin.* 20 (2), 112–116.
- D'Angelo, D.J., Webster, J.R., 1991. Phosphorus retention in stream draining pine and hardwood catchments in the southern Appalachian Mountains. *Freshw. Biol.* 26 (3), 335–345.
- Dou, Z.X., Ramberg, C.F., Toth, J.D., Wang, Y., Sharpley, A.N., Boyd, S.E., et al., 2009. Phosphorus speciation and sorption-desorption characteristics in heavily manured soils. *Soil Sci. Soc. Am. J.* 73 (1), 93–101.
- Dyhrman, S.T., Chappell, P.D., Haley, S.T., Moffett, J.W., Orchard, E.D., Waterbury, J.B., et al., 2006. Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. *Nature* 439 (7072), 68–71.
- Hesse, P.R., 1962. Phosphorus fixation in mangrove swamp muds. *Nature* 193, 295–296.
- Hieltjes, A.H.M., Lijklema, L., 1980. Fractionation of inorganic phosphorus in calcareous sediments. *J. Environ. Qual.* 9 (3), 405–407.
- Hupfer, M., Gächter, R., Ruegger, H., 1995. Polyphosphate in lake sediments: ^{31}P NMR spectroscopy as a tool for its identification. *Limnol. Oceanogr.* 40 (3), 610–617.
- Hupfer, M., Rube, B., Schmieder, P., 2004. Origin and diagenesis of polyphosphate in lake sediments: a ^{31}P -NMR study. *Limnol. Oceanogr.* 49 (1), 1–10.
- Jensen, H.S., Kristensen, P., Jeppesen, E., Skytthe, A., 1992. Iron: phosphorus ratio in surface sediment as an indicator of phosphate release from aerobic sediments in shallow lakes. *Hydrobiologia* 235/236 (1), 731–743.
- Makarov, M.I., Haumaier, L., Zech, W., 2002. The nature and origins of diester phosphates in soils: a ^{31}P -NMR study. *Biol. Fertil. Soils* 35 (2), 136–146.
- McDoell, R.W., Stewart, I., 2006. The phosphorus composition of contrasting soils in pastoral native and forest management in Otago, New Zealand: sequential extraction and ^{31}P NMR. *Geoderma* 130 (1–2), 176–189.
- Newman, R.H., Tate, K.R., 1980. Soil phosphorus characterization by ^{31}P nuclear magnetic resonance. *Commun. Soil Sci. Plant* 11 (9), 835–842.
- Pierzynski, G.M., 2004. Methods of phosphorus analysis for soils, sediments, residuals, and waters. *Southern Cooperative Series Bulletin No.396*. North Carolina State University Press, Raleigh.
- Reitzel, K., Ahlgren, J., Gogoll, A., Jensen, H.S., Rydin, E., 2006a. Characterization of phosphorus in sequential extracts from lake sediments using ^{31}P nuclear magnetic resonance spectroscopy. *Can. J. Fish. Aquat. Sci.* 63 (8), 1686–1699.
- Reitzel, K., Ahlgren, J., Gogoll, A., Rydin, E., 2006b. Effects of aluminum treatment on phosphorus, carbon, and nitrogen distribution in lake sediment: a ^{31}P NMR study. *Water Res.* 40 (4), 647–654.
- Reitzel, K., Ahlgren, J., DeBrabandere, H., Waldebäck, M., Gogoll, A., Tranvik, L., et al., 2007. Degradation rates of organic phosphorus in lake sediment. *Biogeochemistry* 82 (1), 15–28.
- Simpson, A.J., McNally, D.J., Simpson, M.J., 2011. NMR spectroscopy in environmental research: from molecular interactions to global processes. *Prog. Nucl. Magn. Reson. Spectrosc.* 58, 97–175.
- Simpson, A.J., Simpson, M.J., Soong, R., 2012. Nuclear magnetic resonance spectroscopy and its key role in environmental research. *Environ. Sci. Technol.* 46 (21), 11488–11496.
- Sommers, L.E., Harris, R.F., Williams, J.D.H., Armstrong, D.E., Syers, J.K., 1972. Fractionation of organic phosphorus in lakes sediments. *Soil Sci. Soc. Am. J.* 36, 51–54.
- Sumann, M., Amelung, W., Haumaier, L., Zech, W., 1998. Climatic effects on soil organic phosphorus in the north American great

- plains identified by phosphorus-31 nuclear magnetic resonance. *Soil Sci. Soc. Am. J.* 62 (6), 1580–1586.
- Sundareshwar, P.V., Morris, J.T., Pellechia, P.J., Cohen, H.J., Porter, D.E., Jones, B.C., 2001. Occurrence and ecological implications of pyrophosphate in estuaries. *Limnol. Oceanogr.* 46 (6), 1570–1577.
- Turner, B.L., Leytem, A.B., 2004. Phosphorus compounds in sequential extracts of animal manures: chemical speciation and a novel fractionation procedure. *Environ. Sci. Technol.* 38 (22), 6101–6108.
- Turner, B.L., Papházy, M.J., Haygarth, P.M., McKelvie, I.D., 2002. Inositol phosphates in the environment. *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.* 357 (1420), 449–469.
- Turner, B.L., Cade-Menun, B.J., Westermann, D.T., 2003a. Organic phosphorus composition and potential bioavailability in semi-arid arable soils of the western United States. *Soil Sci. Soc. Am. J.* 67 (4), 1168–1179.
- Turner, B.L., Mahieu, N., Condron, L.M., 2003b. The phosphorus composition of temperate pasture soils determined by NaOH-EDTA extraction and solution ^{31}P NMR spectroscopy. *Org. Geochem.* 34 (8), 1199–1210.
- Turner, B.L., Cade-Menun, B.J., Condron, L.M., Newman, S., 2005. Extraction of soil organic phosphorus. *Talanta* 66 (2), 294–306.
- Wang, J.Y., Pant, H.K., 2010. Identification of organic phosphorus compounds in the Bronx River bed sediments by phosphorus-31 nuclear magnetic resonance spectroscopy. *Environ. Monit. Assess.* 171 (1–4), 309–319.
- Zhang, W.Q., Shan, B.Q., Zhang, H., Tang, W.Z., 2013. Assessment of preparation methods for organic phosphorus analysis in phosphorus-polluted Fe/Al-rich Haihe River sediments using solution ^{31}P -NMR. *PLoS One* 8, e76525.