Comparison of Phosphorus Partitioning Results in Estuarine Sediments by Sedex, Modified Sedex and Agemian Extraction Schemes

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Three sedimentary phosphorus sequential extraction schemes in sediments, namely the SEDEX scheme,[5] Modified SEDEX scheme[6] and Agemian scheme[7] were employed in the study of phosphorus partitioning in the sediment samples collected from the Mae Klong, Chao Phraya and Bang Pakong estuaries. Only a fraction of less than 125 μm of each sample was analyzed. The objective of this study is to compare phosphorus partitioning results in sediments by the three extraction schemes. In addition, the samples were analyzed for total phosphorus contents by X-ray fluorescence spectroscopy (XRF). The total phosphorus contents were used in evaluating the accuracy of the extraction schemes by comparing these values with the sum of all phosphorus fractions. All schemes show different and inconsistent results. However, in terms of accuracy, the Agemian scheme shows the most promising results. Since in comparison of the total phosphorus results with the sum of all phosphorus fractions, the Agemian scheme gives higher accuracy and precision than the SEDEX and Modified SEDEX scheme.

Key words: phosphorus partitioning, phosphorus speciation, sediment and sequential extraction.

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การเปรียบเทียบผลการสกัดรูปแบบฟอสฟอรัสในตะกอนปากแม่น้ำ จากวิธีสกัดตามลำดับขั้น SEDEX, Modified SEDEX และ Agemian

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วิธีการสกัดรูปแบบฟอสฟอรัสในตะกอนตามลำดับขั้น 3 วิธี ได้แก่ SEDEX scheme,[5] Modified SEDEX scheme[6] และ Agemian scheme[7] ถูกนำมาใช้ในการศึกษารูปแบบฟอสฟอรัสในตะกอน 3 ตัวอย่างจากปากแม่น้ำเจ้าพระยา แม่กลอง และบางปะกง โดยที่ตัวอย่างร่อนเอามาเฉพาะขนาดเล็กกว่า125 ไมครอน เพื่อเปรียบเทียบผลการศึกษารูปแบบฟอสฟอรัสที่ได้มาจากต่างวิธีสกัดในตะกอนเดียวกัน นอกจากนี้ได้ทำการวิเคราะห์ปริมาณฟอสฟอรัสรวมในตะกอนโดยใช้เทคนิคทาง X-ray fluorescence spectroscopy (XRF) เพื่อนำมาใช้ในการเปรียบเทียบความถูกต้องของวิธีสกัดรูปแบบฟอสฟอรัส โดยการนำผลรวมของรูปแบบฟอสฟอรัสมาเปรียบเทียบกับผลการวิเคราะห์ฟอสฟอรัสรวมในตะกอน ผลการศึกษาพบว่าผลการสกัดรูปแบบฟอสฟอรัสในตะกอนจากทั้ง 3 วิธีไม่สามารถนำมาเปรียบเทียบกันได้และวิธีสกัดรูปแบบฟอสฟอรัสของ Agemian (1997) เป็นวิธีสกัดที่ดีที่สุด เนื่องจากเมื่อนำผลรวมของรูปแบบฟอสฟอรัสมาเปรียบเทียบกับผลการวิเคราะห์ฟอสฟอรัสรวมในตะกอนแล้ว พบว่าวิธีนี้มีความถูกต้องและแม่นยำสูงกว่าวิธีสกัด SEDEX และ Modified SEDEX

คำสำคัญ รูปแบบฟอสฟอรัส ตะกอน วิธีสกัดตามลำดับขั้น

INTRODUCTION

Phosphorus partitioning in sediment is an important aspect in studying the biogeochemical cycle of phosphorus, because it can provide information on the origin of phosphorus in sediments, the degree of pollution from anthropogenic activities, the bioavailability of phosphorus in sediments which is responsible for eutrophication, and finally, the biological and chemical processes that occur with phosphorus in the aquatic environment. By its origin and biogeochemical processes, the phosphorus forms of interest can be classified as (1) ***Exchangeable or loosely bound P*** *or* ***labile P***, the fraction easily adsorbed and released by exchange sites. Once released, it will become available for algal growth[1-7] (2) ***Fe bound P*** *or* ***Fe and Al bound*** *P*, the fraction associated with Al, Fe and Mn oxides and hydroxides[1,2,5,7,8] (3) ***Carbonate bound P***, this fraction includes authigenic carbonate fluorapatite plus biogenic carbonate hydroxyapatit[5] (4) ***Detrital P*** *or* ***detrital apatite***, this fraction includes fluoraptite from igneous, sedimentary and metamorphic rocks[5] (5) ***Calcium bound P***, this fraction is generally referred to as apatite P [4,8] or ***Ca-bound P*** [1,7,9] (6) ***Residual P*** or ***refractory P***, this fraction includes very resistant minerals, such as monazite and xenotime[8] and those in the crystal lattices of some silicate minerals[5] (7) ***Organic P***, the nature of the organic pool is much more complex and less well described, therefore; the organic fraction is usually consider cumulatively[10] and (8) ***Polyphosphate***, this fraction can be used as the index of pollution from human activities in estuaries and coastal waters.[4,7,11]

The dominant form of phosphorus partitioning in sediment might play an important role in controlling dissolved phosphate concentration in that aquatic environment. However, the dominant form of phosphorus found was different in each estuary. This difference might be firstly caused by chemical weathering processes of parent rock materials and a wide variety of human activities along those drainage basins.[6,12-15] Secondly, it might also be due to the sequential extraction applied. Because these methods are inherently different in the classification of extracted species, type and strength of extractants, extract conditions (temperature duration of extraction, ratio of sediment to the volume of extractant and grain size of sediment), sequence of extraction schemes and the steps involved in extraction schemes. Therefore, the phosphorus partitioning results from these studies cannot be directly compared.

The prime aim of this study is to compare the same phosphorus forms extracted by various sequential extraction schemes in the same sediment. This can show which selected sequential extraction scheme is most suitable for determining phosphorus partitioning in estuarine sediments of some Thai major rivers. Furthermore, this will also be the pilot study to provide the preliminary phosphorus partitioning data of some Thai major river estuaries and the guideline for comparison of data obtained from different extraction methods.

MATERIALS AND METHODS

Selection of sequential extraction methods

In this comparison study, the SEDEX scheme[5] and the Modified SEDEX scheme[6,7] were selected as tools in investigating the phosphorus partitioning in estuarine sediments (For more details of these methods see also in Wiratchapun).[16] These extraction schemes use the same type of extractant (combination of acid-base extractant and reducing agents), but inherently differ in

classification of some extracted species, type and strength of extractants to extract the same P fraction, extract conditions (temperatures, times of extraction, ratios of the amount of sediment to volume of extractant) and sequences of extraction (as described in Table 1). The ***SEDEX scheme***[5] was developed from Chang and Jackson's,[1] Lucotte and D' Anglejan's, [17] and Aspila et al.,'s [10] extraction schemes, and is the most popular sequential extraction scheme utilized for studying sedimentary P reservoirs in estuarine and marine sediments. The ***Modified SEDEX schemes***[6] was developed from SEDEX to reduce the organic P hydrolysis that occurs in the successive steps before organic P extraction. Organic P was extracted using SDS instead of 1 M HCl before Fe-P, Authigenic and detrital apatite and residual P extraction, respectively. The other reagents are the same as in the SEDEX scheme. The ***Agemian extraction scheme*** [7] has a different origin from the other two, and was modified from Van Eck's[3] and Psenner et al.'s[4] and Jensen and Thamdrup's[18] schemes. All reagents, extract conditions and the name of phosphorus speciation differ from the SEDEX and Modified SEDEX schemes.

METHODOLOGY

Three sediment samples were collected at river mouths (salinity 22-24 ppt) from the Mae Klong, Chao Phraya, and Bang Pakong Estuaries by a Petersen Grab sampler in September 1999. After collection, sediments were stored frozen in plastic bags until freeze-dried. After freeze-drying, sediments were gently crushed in an agate mortar, well mixed and subsampled by the Conning and Quartering method[19] before analysis. Then subsampled sediments were dry-sieved through a 125 μm stainless steel test sieve (Encotte®). At least three replicates of <125 μm dried sediments were conducted for Total P by a Siemens SRS 3000 XRF spectrophotometer and for phosphorus partitioning by the selected sequential extraction methods (SEDEX, Modified SEDEX and Agemian extraction schemes (as described in Wiratchapun).[16] Milton Roy® Spectronic 401 (spectrophotometer), Centurion Scientific® 1040 series (centrifuge), Accumet® BASIC AB15 (pH meter), waterbath-shaker and muffle furnace (Thermolyne, Sybron Corporation) were used in this study. Soluble phosphate in all sediment extracts, except CDB- and BD-extracts, were colorimetrically analyzed by either the phosphomolybdate blue complex method of Koroleff according to SEDEX scheme or Strickland and Parsons according to Modified SEDEX and Agemian schemes. CDB–and BD-extracts were analyzed for soluble phosphate by the isobutanol method according to Watanabe and Oilsen.[20]

Table 1. The selected sequential extraction schemes applied in this study.

Sequential extraction scheme	Extractant	Proposed fraction
SEDEX scheme (Ruttenberg, 1992)	1 M $MgCl_2$ pH 8.0	Exchangeble or loosely sorbed P
	CDB[1], pH 7.6	Easily reducible or reactive Fe bound P Carbonate fluorapatite, biogenic
	1 M Na-acetate in acetic acid, pH 4.0	hydroxyapatite, $CaCO_3$ bound P
	1 M HCl	Detrital fluorapatite-bound P
	1 M HCl after ignition at 550 °C	Organic P
Modified SEDEX (Vink et al., 1997)	1 M $MgCl_2$ pH 8.0	Exchangeble or loosely sorbed P
	10% SDS, HCO_3^- buffer, pH 8.57, 80 °C	Organic P
	CDB[1], pH 7.6	Easily reducible or reactive Fe bound P Carbonate fluorapatite, biogenic
	1 M Na-acetate in acetic acid, pH 4.0	hydroxyapatite, $CaCO_3$ bound P
	1 M HCl	Detrital fluorapatite-bound P
	6 M HCl after ignition at 550 °C	Residual P
Agemian scheme (Agemian, 1997)	0.5 M NaCl	Loosely bound P
	Bicarbonate ditionite (BD), pH 7.0	Fe and Al bound P
	0.1 M NaOH	Polyphosphates
	0.5 M HCl	Calcium bound P
	1 M NaOH, 85 °C	Refractory P

1 CDB = citrate dithionite bicarbonate

Quality control in phosphate extraction and analysis

To reduce contamination in phosphate analysis, all glassware and plasticware were soaked in 10% (v/v) HCl overnight, rinsed three times with double distilled- deionized water, dried and kept in clean plastic bags until use.[21] Double distilled-deionized water and analytical grade chemicals (containing less than 0.0005% P) were used throughout this work. To control the quality of sequential extraction, extract conditions (esp. sediment suspension, temperature and pH) were controlled to be the same as described in the SEDEX, Modified SEDEX and Agemian schemes. At least three replicates of samples were analysed. To control the quality of measurement, soluble phosphate analysis was done immediately after extraction (or after dilution or neutralization). The absorbance values did not differ more than 0.001 absorbance unit. Absorbance of the reagent blanks did not exceed 0.001 except $MgCl_2$ +NaOAc matrix for analyzing authigenic fluorapatite (0.000-0.005) and citrate bicarbonate dithionite (CDB) matrix which employed the isobutanol method for analyzing reducible ferric bound P (0.000-0.009). Turbidity blanks were measured to correct the absorbance of unfiltered

sediment extracts. Subsequently, pH of diluted extracted solution after adding mixed reagents was checked and controlled at 0.7 to 1.1 in order to eliminate interference of silicate in all sediment extracts. This would let the absorbance of phosphomolybdate complex to be constant once the optimum time is reached. If the phosphomolybdate complex occurs with interference of silicate, the absorbance will increase with time and this can cause errors in phosphate analysis. Extracted solutions that have a pH before dilution less than 1 need to be neutralized before addition of mixed reagent because strong acid can inhibit the complex formation. Standard calibration curves of phosphate were prepared by using standards and reagent blanks with extractants at the same degree of dilution as sediment extracts to make their solution matrix match. In every step of sequential extraction schemes, the calibration curve was specifically and separately prepared for that step and/or determination. Linear regression analysis was employed in calculating the relationship between absorbance and concentration of standards. Most of the relative coefficients (r^2) of these linear relationships are not less than 0.9995 but their slopes range from 0.0183 to 0.0209 for the Strickland and Parsons method[25] and range from 0.0208 to 0.0280 for the Koroleff method.[24] The slope deviations are due to the matrix of extractants. More details of matrix effect of extractants on standard calibration curves are shown in Wiratchapun.[16]

RESULTS AND DISCUSSIONS

Comparison of phosphorus partitioning results

Similar fractions of phosphorus partitioning results from the same sediment analyzed by SEDEX schemes[5] and Modified SEDEX schemes[6,7] as shown in Figures 1, are quantitatively incomparable because they are highly dependent on the extraction scheme utilized.

Accuracy of the Results Obtained Through the Selected Sequential Extraction Schemes

Since the certified reference material for phosphorus for control of the quality of the selected sequential extraction methods was not available before the year 2001, the accuracy of these extraction schemes cannot be directly evaluated. However, the accuracy of these schemes can be indirectly determined by comparing the sum of all P fractions with total P determined by XRF as shown in Table 2. The accuracy of each selected sequential extraction methods as shown in Table 3 were calculated from the difference between the sum of all P fractions and total P from XRF. The deviation values are reported from the total P from XRF. From Table 2 and 3, it can be concluded that the Agemian scheme[7] provides the most promising results with higher accuracy and precision than the SEDEX[5] and modified SEDEX schemes.[6]

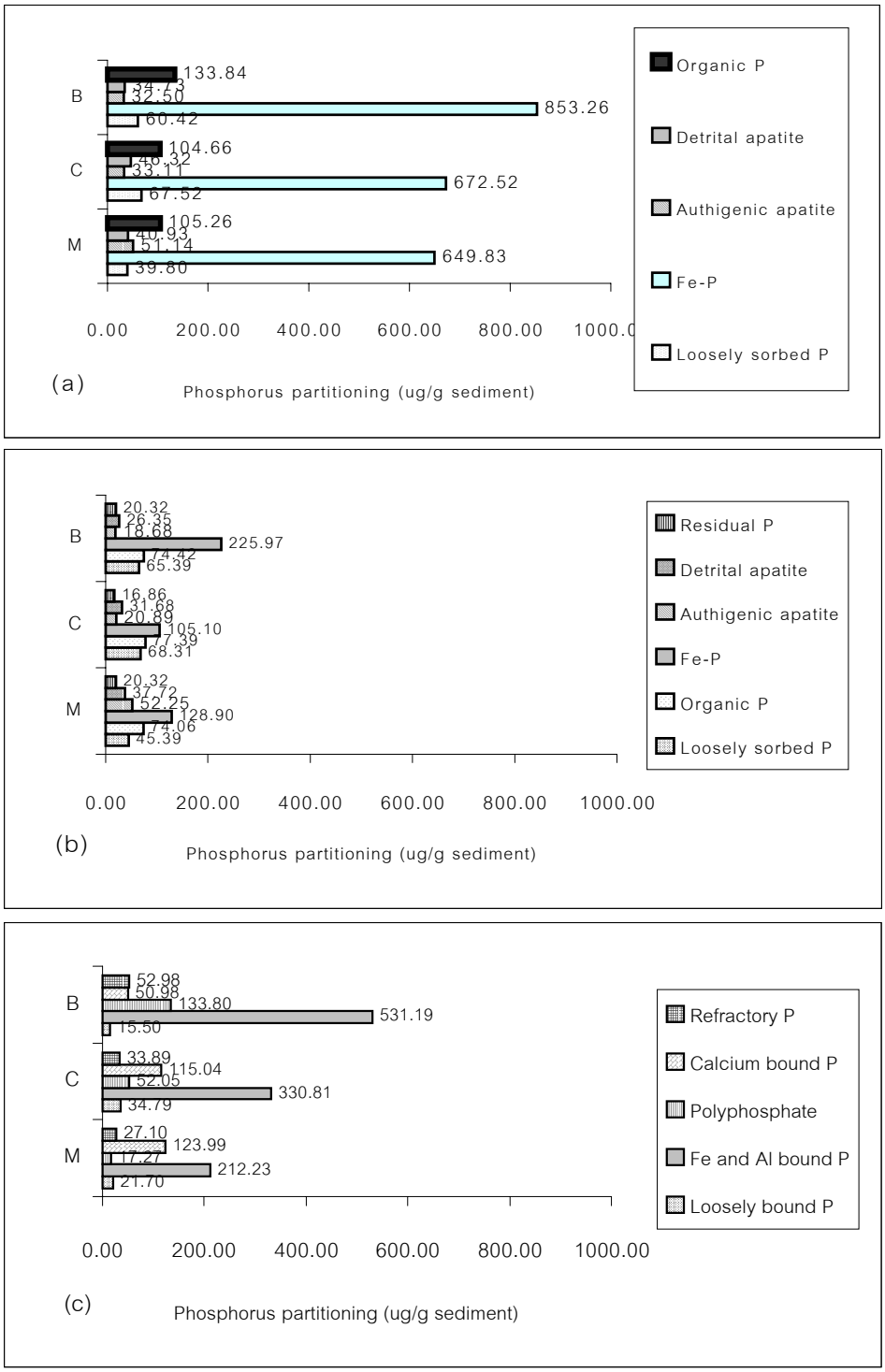

Figure 1. Phosphorus partitioning in the Mae Klong (M), Chao Phraya (C) and Bang Pakong (B) estuarine sediments from (a) SEDEX schemes,[5] (b) Modified SEDEX schemes,[6] and (c) Agemian schemes.[7]

Table 2. Comparison between total P by XRF and sum of all phosphorus fractions (mean±SD μg/g sediment) from the selected sequential extraction methods.

Sediment	Total P from XRF	Sum of phosphorus speciation		
		SEDEX (Ruttenberg, 1992)	Modified SEDEX (Vink et al., 1997)	Agemian (Agemian, 1997)
Mae Klong	437±6.56	851.14±223.99	358.64±68.19	402.79±6.85
Chao Phraya	480±7.20	924.13±102.11	320.23±15.93	566.58±17.28
Bang Pakong	611±9.17	1114.75±141.22	431.13±131.94	748.45 ±24.44

Table 3. Accuracy of various phosphorus extraction schemes reported in % difference between total phosphorus from XRF and sum of all phosphorus fractions from various extraction schemes.

Sediment	Sequential extraction schemes		
	SEDEX (Ruttenberg, 1992)	Modified SEDEX (Vink et al., 1997)	Agemian (Agemian, 1997)
Mae Klong	+95	-18	- 8
Chao Phraya	+93	-33	+18
Bang Pakong	+82	-29	+22

The More than 80% overestimation results of the SEDEX scheme[5] in this study was mainly caused by the poor precision Fe-P results in the isobutanol extraction method (as shown in terms of coefficient of variation (%) in Table 4). This problem was also found by Ruttenberg[5], Eijsink et al.,[14], Vink et al.,[6] and Ruban et al.,[21] as described in Table 5). Ruttenberg[5] found this problem and could not resolve it during the development of the SEDEX method. Eijsink et al.[14] and Vink et al.[6] who followed and modified the SEDEX method, respectively, also encountered the same problem. Recently, Ruban et al.[21] claimed that reliable Fe-P extraction with CDB reagent following the SEDEX scheme was difficult to achieve due to the isobutanol extraction method before the molybdate blue determination. Excess Fe in $FeCl_3$ addition to eliminate excess citrate in the CDB extract might affect the phosphomolybdate complex formation like silicate, arsenate and fluoride. In addition, dilution of the CDB extract in the isobutanol method might also alter the CDB matrix and cause the poor precision of Fe-P results. Furthermore, in this study the precision of the standard calibration curve for phosphorus prepared from CDB with the same matrix of sediment is varied. The slope values shifted daily from 0.006 to 0.008 even though the relative coefficient (r^2) of each standard calibration curve was not less than 0.9995 and the absorbance of the reagent blank was 0.000. Thus, the average slope and deviation were calculated and applied for calculating Fe-P in sediments. Moreover, according to Wiratchapun[16] organic P in sediment samples from SEDEX method is nearly twice as high as that from the ignition method of Aspila et al.[10] This is opposite to the findings of Ruttenberg[5] and Berner et al.[22] The different nature of sediment in this study might be the cause of the opposite finding.

Table 4. Coefficient of variation (%) of Fe-P analysis from various extraction schemes (in this study).

Sediment	Sequential extraction scheme		
	SEDEX Ruttenberg (1992)	Modified SEDEX Vink et al. (1997)	Agemian Agemian (1997)
Mae Klong	33	38	2
Chao Phraya	14	1	2
Bang Pakong	16	56	3

Note: Step II–CDB (Ruttenberg, 1992), Step III-CDB (Vink et al., 1997) and Step II-BD (Agemian, 1997)

Table 5. Precision of the isobutanol method (%) according to CDB extraction.

Ruttenberg (1992)	Eijsink et al. (1997)	Vink et al. (1997)	Ruban et al. (1999)
10-20 up to 50	35	60	>150

The Modified SEDEX scheme provides relatively more accurate results than the SEDEX scheme even though Fe-P analysis uses the same CDB reagent and isobutanol method. This might be caused by the higher CDB blank values (0.009) of the Modified SEDEX scheme (utilizing 2.5 ml more of CDB than SEDEX scheme). Therefore, the calculated Fe-P concentration is not as high as that of the SEDEX scheme. However, the precision of the standard calibration curve prepared by CDB in the same matrix of samples has the same problem of Fe-P analysis as described in the SEDEX scheme.

In addition, the extraction steps of Fe-P of the SEDEX scheme and the Modified SEDEX schemes are different (Step II- SEDEX scheme and Step III- Modified SEDEX scheme). It is possibly that SDS (step II of Modified SEDEX scheme) may have either removed the iron oxides phase or altered the nature of the phase so that it was no longer soluble in the CDB reagent. Therefore, sequence of extraction step is important in Fe-P analysis.

Moreover, organic P in sediment extracted by SDS possibly causes the underestimated sum of P speciation because organic P in sediment in this study is possibly more refractory or less soluble in SDS extraction. The presence of refractory organic P in sediment was found from observing that the quantity of organic P obtained from the difference between total P and inorganic P extracted by the stronger extractant (6M HCl) is relatively higher than SDS.[16] Therefore, it indicates that SDS is not suitable for extracting organic P in sediment in this study.

The Sum of P speciation obtained by the Agemian scheme is closer to the total P from XRF than other schemes. The Bicarbonate-ditwionite (BD-reagent) of the Agemian scheme was proved to provide more precise results in all sediment samples than the CDB reagent of Modified SEDEX and SEDEX schemes (as shown in table 4). The absence of citrate in the extractant might be the main reason that the BD-reagent can provide higher precision. The use of $FeCl_3$ to eliminate interference by citrate might cause problems related to phosphomolybdate complex formation in the isobutanol method. However, the accuracy of the BD-reagent in comparison with the CDB reagent for measuring Fe-P is still debated because there has been no certified reference material available to check the accuracy of both reagents until 2001 (J.F. Lopez

Sanchez, personal communication). Therefore, further study for improving the isobutanol extraction method and checking the accuracy of Fe-P analysis, by CDB or BD reagent, with certified reference material should be conducted.

At the beginning of this study (in 1999), there was no certified reference material (CRM) for phosphorus partitioning analysis available. It has just been made commercially available at the beginning of the year 2001 after the European Commission, with the Standards, Measurements and Testing Programmer (formerly Community Bureau of Reference, BCR) started a project to harmonise the methodology and to produce CRMs in 1996. After a thorough discussion following several interlaboratory tests among 20 experts[21] a modified Williams scheme was agreed to be used in the certification of material. However, only a CRM of lake sediment is now available[23] Therefore, the accuracy of the selected extraction schemes in this study can be further investigated directly by utilizing the CRM of lake sediment accompanied by the modified Williams extraction scheme. This could be the best answer to the question that which selected sequential extraction method is the best for analyzing phosphorus partitioning in sediment.

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