CHAPTER V
DISCUSSION

This chapter mainly focuses on seven topics. The first topic is factors controlling geochemical properties of sediments. The second emphasizes on quality control in phosphate analysis. The third deals with accuracy of various total phosphorus methods. The fourth focuses on comparison of phosphorus partitioning results by three selected extraction schemes. The fifth explains about the dominant form of phosphorus in sediment. The sixth discusses about the accuracy of the selected sequential extraction schemes. Finally, the last part is about relationship between grain size and phosphorus with its implication.

5.1 Factors controlling geochemical properties of sediments

Due to the fact that environmental parameters control the geochemical properties of sediments, each sampling site was carefully chosen based on the similarity in its environmental parameters. Therefore, the analytical results of sediment samples should be comparable. Each sampling site is at the river mouths, having the same salinity (22-24 ppt). Samplings were conducted during the same tidal period (ebbing) and under the same weather condition (including season). Physico-chemical properties of bottom water and other conditions of Chao Phraya, Mae Klong and Bang Pakong estuaries while sampling are shown in Table 5.1.

Sediment preparation step is also important. To make sure that the analytical results are comparable, after freeze-drying, well mixing and subsampling by Coning and Quartering method (Walton, 1978), size of sediment to be analyzed was controlled in their uniformity and grading by dry sieving through a 125 μm sieve.

Table 5.1 Physico-chemical properties of bottom water and other environmental

conditions of sampling sites

Location	Salinity (ppt)	T (°C)	pH	DO (mg/l)	Dissolved Phosphate (μg/l)	Weather condition	Tide period
Mae Klong (M)	23.9	31.3	7.65	6.69	130.47	Sunny, calm wind	Ebbing
Chao Phraya (C)	23	28	7.52	2.62	158.33	Sunny, calm wind	Ebbing
Bang Pakong (B)	22	28.2	7.06	2.66	103.87	Sunny, calm wind	Ebbing

5.2 Quality control in phosphate analysis

To ensure that the phosphorus results are reliable, following attempts to reduce systematic errors and personal errors in extraction and colorimetry as much as possible were undertaken:

5.2.1 Labwares and reagents

- Using acid-cleaned glasswares and plasticwares to reduce the positive contamination. Acid- cleaned separatory funnels for Fe-P analysis were rinsed three times with absolute ethanol before use.

- Using double distilled-deionized water for rinsing labwares, preparing of all standards and reagents and diluting samples before phosphate analysis in order to reduce the contamination in reagent blanks, standards and samples.

- Using reagent grade chemicals that contain impurity of phosphate less than 0.0005% in extraction and phosphate analysis.

5.2.2 Spectrophotometer

- Spectrophotometer was warmed up at least 30 min before use.

- Checking the stability of spectrophotometer while measuring absorbance of samples, standards and reagent blanks against a reference cell. The reference cell is always the same one throughout each batch of measurements.

5.2.3 Extraction condition

- During extraction, sediment sample was constantly suspended in the extractant via shaking.

- Temperature of extraction was controlled at the same level as specified in the extraction schemes.

- pH of extractants were checked and adjusted before adding to sediment sample to ensure that extractant was most effective in extracting the targeted P fraction. Because some reagents such as $MgCl_2$, citrate dithionite bicarbonate (CDB), bicarbonate ditionite (BD), and sodium dodecyl sulfate (SDS), were difficult to maintain their pH at the desired level, so they had to be freshly prepared before use.

- pH of extractants were rechecked after mixing with the sediments to ensure that the extraction processes were optimized during extraction.

5.2.4 Phosphate analysis

-   Phosphate analysis was done immediately after extraction to avoid orthophosphate decomposition which affects the phosphorus results.

-   At least three replicates of samples were analyzed. Each replicate was measured three times. The absorbance values measured should not differ more than 0.001 absorbance unit.

-   Absorbance of the reagent blanks must not exceed 0.001 except $MgCl_2$+NaOAc matrix for analyzing authigenic fluorapatite (0.000-0.005) and citrate bicarbonate ditionite (CDB) matrix which employed Isobutanol method for analyzing reducible ferric bound P (0.000-0.009).

-   Turbidity blanks were measured to correct the absorbance of unfiltered sediment extracts.

-   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 error of phosphate analysis. Extracted solution that has pH before dilution less than 1 need to be neutralized before adding mixed reagent because strong acid can inhibit the complex formation.

-   Dilution of extracted solution has to be done prior to phosphate analysis to make the intensity of phosphomolybdate complex suitable for the standard calibration curve. In addition, dilution can help reducing

interference of silicate, arsenate and fluoride in solution (Blomqvist et al., 1993). Furthermore, dilution can also help reducing effect of the extractant so that the optimum pH of phosphomolybdate blue complex formation is easily controlled. However, too much dilution causes the intensity of phosphomolybdate blue to be below the detection limit. Too less dilution causes phosphomolybdate blue complex to be outside the reliable range (linear) of standard calibration curve. Finally, dilution can also affect on slope of standard calibration curves for phosphorus. In other words, slope of the calibration curve varies with degree of dilution.

- Neutralization needs to be done in order to reduce the matrix effect of some extractants, especially for strong acids or solutions that have pH less than 1, due to the fact that strong acid inhibits the formation of phosphomolybdate blue complex. However, it was found that using too weak base to neutralize strong acid, such as using 0.5 M $NaHCO_3$ to neutralize 6 M HCl extract as recommended by Vink et al. (1997), can cause too much dilution effect as described above. Therefore, to reduce the effect of too much dilution, it is recommended that a few droplets of strong base like 1 M NaOH be used first to overcome the acidity and then follows by 0.5 M $NaHCO_3$ until the extracted solution is neutralized.

5.2.5 Standard calibration curve and calculation

- 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 matching each others. In every step of sequential extraction schemes including total phosphorus analytical methods, 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 (as shown in Tables 5.2.1 and 5.2.2) range from 0.0183 to 0.0209 for Strickland and Parsons (1972)'s method and range from 0.0208 to 0.0280 for Koroleff (1976)'s method. The slope deviations are due to the matrix of extractants. More details of matrix effect of extractants on standard calibration curves are shown in Appendix F.

Table 5.2.1 Matrix effect of extractants used in preparing standard calibration curve for phosphate analysis – Strickland and Parsons (1972)'s method

Matrix	Dilution	Slope	Deviation	R^2	$A_{reagent\ blank}$
Deionized water	1	0.0200–0.0209	0.0000	1.0000	0.000
1 M MgCl$_2$	5	0.0200-0.0217	0.0000 -0.0008	0.9986 -1.0000	0.000
	10	0.0200	0.0000	1.0000	0.000
0.5 M NaCl	10	0.0200	0.0000	1.0000	0.000
1 M NaOAc	20	0.0204-0.0210	-0.0002-0.0006	0.9999	0.000
1 M NaOAc+1 M MgCl$_2$	1	0.0183-0.0192	-0.0006-0.0000	0.9991-1.0000	0.000
0.5 M HCl	20	0.0200	0.0000	1.0000	0.000
1 M HCl	10	0.0200	0.0000	1.0000	0.000
	100	0.0200-0.0209	-0.0006-0.0000	0.9996-1.0000	0.000
6 M HCl	30	0.0204-0.0205	-0.0002	0.9996-0.9999	0.000
10% SDS+ 6M HCl	30	0.0205	-0.0002	0.9996	0.000
0.1 M NaOH	2	0.0209	-0.0002	0.9999	0.000
	5	0.0208	0.0002	0.9999	0.000
	10	0.0209	-0.0002	0.9999	0.000
1 M NaOH	5	0.0209	0.0008	0.9999	0.001

Table 5.2.2 Matrix effect of extractants used in preparing standard calibration curve

for phosphate analysis – Koroleff (1976)'s method

Matrix	Dilution	Slope	Deviation	R^2	$A_{reagent\ blank}$
Deionized water	1	0.0230	0.0000	1.0000	0.000
1 M MgCl$_2$	5	0.0230-0.0250	-0.0008-0.0002	0.9995-1.0000	0.000
1 M NaOAc	20	0.0250-0.0253	-0.0002-0.0005	0.9997-0.9999	0.000
1 M NaOAc+1 M MgCl$_2$	1	0.0208-0.0280	-0.0009-0.0008	0.9888-1.0000	0.000-0.005
1 M HCl	10	0.0215-0.0228	-0.0002-0.0004	0.9997-1.0000	0.000
	20	0.0218-0.0229	-0.0002-0.0006	0.9999-1.0000	0.000
	25	0.0227-0.0229	0.0003-0.0007	0.9998-1.0000	0.000

5.3 Accuracy of various total phosphorus methods

In the beginning, the author believed that Aspila method (Aspila et al., 1976) is the most reliable (as mentioned in the first chapter) because it was adopted in many researches. However, after applying many total phosphorus analytical methods to analyze the same samples, it was found that total phosphorus results from XRF is the best. This is due to the fact that XRF apply series of certified reference materials to calibrate its accuracy. In addition, amount of sediment sample used per analysis in XRF is at least 100 percent more than other techniques (as shown in Table 5.3.1). The average values from larger amount of sample per analysis can make the accuracy of XRF better than other techniques. Furthermore, its preparation technique (fused cast bead method) has less risk of sediment lost than that of other methods. Boiling hot acid in total digestion can cause spillage. Loss of samples during transferring into acid digestion container and/or other vessels is unavoidable.

Table 5.3.1 Amount of sediment (g) used in various methods for total P analysis

XRF Fused cast bead	Ignition+1 M HCl Aspila et al.(1976)	Ignition+6M HCl Vink et al.(1997)	Ignition+1 M HCl Agemian(1997)	Total digestion HF-HClO$_4$-HNO$_3$
1.00	0.25	0.25	0.50	0.25

Accordingly, total phosphorus calculated from XRF is chosen as reference for comparing with total phosphorus from other analytical methods (as shown in Tables 5.3.2, 5.3.3 and 5.3.4) in order to find the best digestion method for total phosphorus analysis in this study. It should be noted that both solubilization method and phosphate analysis are evaluated separately.

Mae Klong sediment

It was found that accuracy of total digestion methods (HF-$HClO_4$-HNO_3) is either higher or comparable to the ignition methods. The total digestion method that employed soluble phosphate analysis of Koroleff (1976)'s is the most accurate.

Among three ignition methods, it was recognized that the procedure of Vink et al. (1997) is relatively better than the procedures of Agemian (1997) and Aspila et al. (1976), respectively.

Table 5.3.2 Accuracy of total phosphorus results in Mae Klong sediment relative to the XRF result

Methodology		Total P	%Difference
Digestion method	Phosphate analysis	(μg/g sediment)	From XRF
HF-$HClO_4$-HNO_3 (Windom et al., 1984)	Koroleff (1976)	394.25	9.78
Ignition-6 M HCl 16 h (Vink et al., 1997)	Strickland&Parsons (1972)	359.14	17.82
HF-$HClO_4$-HNO_3 (Windom et al., 1984)	Strickland&Parsons (1972)	354.83	18.80
Ignition-1 M HCl 16 h (Agemian, 1997)	Strickland&Parsons (1972)	309.46	29.19
Ignition-1 M HCl 16 h (Aspila et al., 1976)	Koroleff (1976)	287.24	34.27

Note: average total phosphorus by XRFS is 437.00 μg/g sediment.

Chao Phraya sediment

Here, it was observed that accuracy of total digestion methods ($HF-HClO_4-HNO_3$) is again higher or comparable to ignition methods. The total digestion method that employed soluble phosphate analysis of Strickland and Parsons (1972) is relatively better than the procedure of Koroleff (1976). Among three ignition methods, it was discovered that the procedure of Vink et al. (1997) is relatively better than the procedure of Agemian (1997) and Aspila et al. (1976), respectively.

Table 5.3.3 Accuracy of total phosphorus results in Chao Phraya sediment relative to the XRF result.

Methodology		Total P	%Difference
Digestion method	Phosphate analysis	(μg/g sediment)	From XRF
$HF-HClO_4-HNO_3$ (Windom et al., 1984)	Strickland&Parsons (1972)	480.40	0.08
Ignition-6 M HCl 16 h (Vink et al., 1997)	Strickland&Parsons (1972)	491.27	2.35
$HF-HClO_4-HNO_3$ (Windom et al., 1984)	Koroleff (1976)	505.85	5.39
Ignition-1 M HCl 16 h (Agemian, 1997)	Strickland&Parsons (1972)	418.47	12.82
Ignition-1 M HCl 16 h (Aspila et al., 1976)	Koroleff (1976)	398.2	17.04

Note: average total phosphorus by XRFS is 480.00 μg/g sediment.

Bang Pakong sediment

The same trend as in the Chao Phraya sediment is found in this sample. However, among three ignition methods, it was found that the procedure of Agemian (1997) is relatively better than the procedure of Vink et al. (1997) and Aspila et al. (1976), respectively.

Table 5.3.4 Accuracy of total phosphorus results in Bang Pakong sediment relative to the XRF result.

Methodology		Total P	%Difference
Digestion method	Phosphate analysis	(μg/g sediment)	From XRF
HF-HClO$_4$-HNO$_3$ (Windom et al., 1984)	Strickland&Parsons (1972)	634.54	3.85
Ignition-1 M HCl 16 h (Agemian, 1997)	Strickland&Parsons (1972)	552.41	9.59
HF-HClO$_4$-HNO$_3$ (Windom et al., 1984)	Koroleff (1976)	674.41	10.38
Ignition-6 M HCl 16 h (Vink et al., 1997)	Strickland&Parsons (1972)	687.62	12.54
Ignition-1 M HCl 16 h (Aspila et al. 1976)	Koroleff (1976)	531.90	12.95

Note: average total phosphorus by XRFS is 611.00 μg/g sediment.

In general, all total digestion methods for determining total phosphorus in sediment is relatively better than ignition methods. Between the two total digestion methods with different methods of soluble phosphate analysis, total digestion method with Strickland and Parsons (1972)'s method is relatively more promising for total P analysis than the other because it provide more accurate results in two (the Chao Phraya and Bang Pakong sediments) out of three. At this stage, total digestion and soluble phosphate analysis by Strickland and Parsons's is recommended for analyzing total P in sediment. However, further investigation should be able to confirm these findings.

5.4 Comparison of phosphorus partitioning results by three extraction schemes

The similar fractions of phosphorus partitioning results in the same sediment analyzed by SEDEX scheme (Ruttenberg, 1992) and Modified SEDEX scheme (Vink et al., 1997) and Agemian scheme (Agemian, 1997), as shown in Figures 5.4.1, 5.4.2 and 5.4.3, are quantitatively incomparable because they are highly dependent on the extraction scheme utilized.
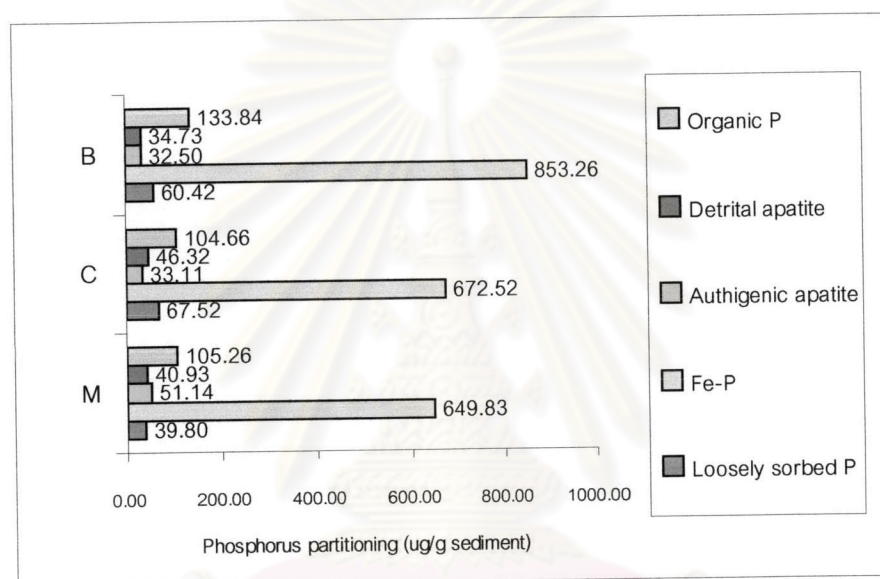

Figure 5.4.1 Phosphorus partitioning in the Mae Klong (M), Chao Phraya (C) and Bang Pakong (B) estuarine sediments from SEDEX scheme (Ruttenberg, 1992)
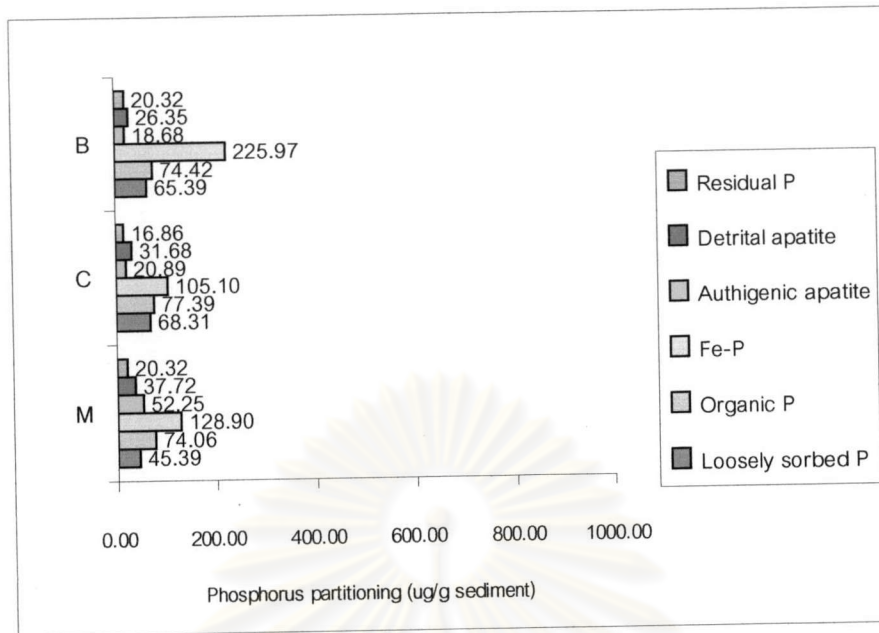
Figure 5.4.2 Phosphorus partitioning in the Mae Klong (M), Chao Phraya (C) and
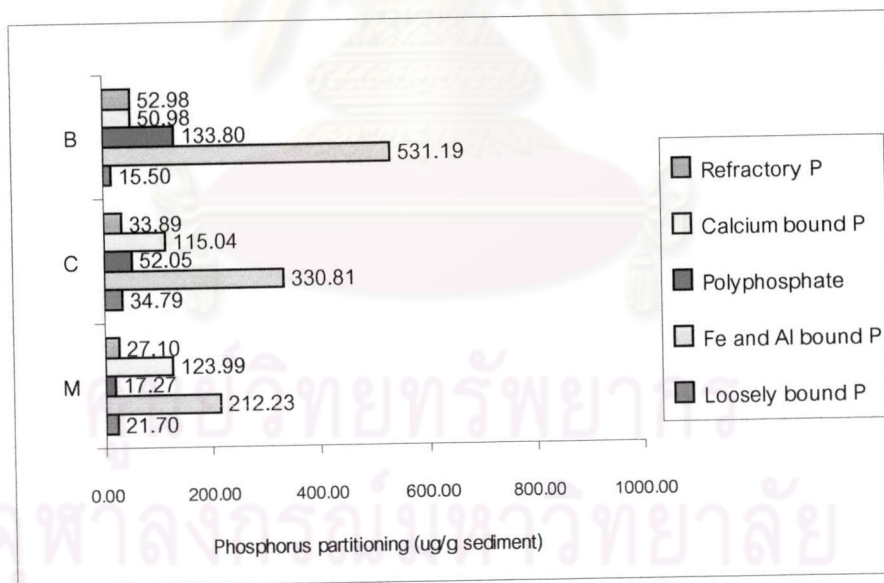Bang Pakong (B) estuarine sediments from Vink et al. (1997) 's scheme

Figure 5.4.3 Phosphorus partitioning in the Mae Klong (M), Chao Phraya (C)
and Bang Pakong (B) estuarine sediments from Agemian(1997)' s scheme

5.5 Dominant form of phosphorus in sediments

Eventhough the phosphorus partitioning results by three selected extraction schemes are quantitatively incomparable, all three schemes can qualitatively provide trends of phosphorus partitioning in sediments and the dominant form of phosphorus that plays an important role in controlling soluble phosphate in the Mae Klong, Chao Phraya and Bang Pakong Estuaries. From Figures 5.4.1, 5.4.2 and 5.4.3, phosphorus partitioning results from the selected extraction schemes were ranked for the dominant form of phosphorus species as shown in the box below:

Mae Klong sediment

Ruttenberg's Fe-P > Organic P> Authigenic apatite> Detrital apatite> Loosely sorbed P

Vink's Fe-P > Organic P > Authigenic apatite> Detrital apatite> Loosely sorbed P>Residual P

Agemian's Fe and Al bound P> Calcium bound P> Refractory P >Loosely sorbed P> Polyphosphate

Chao Phraya Sediment

Ruttenberg's Fe-P > Organic P> Loosely sorbed P > Detrital apatite> Authigenic apatite

Vink's Fe-P > Organic P > Loosely sorbed P > Detrital apatite> Authigenic apatite>Residual P

Agemian's Fe and Al bound P> Calcium bound P> Polyphosphate >Loosely bound P> Refractory P

Bang Pakong Sediment

Ruttenberg's Fe-P > Organic P> Loosely sorbed P > Detrital apatite> Authigenic apatite

Vink's Fe-P > Organic P > Loosely sorbed P > Detrital apatite> Authigenic apatite>Residual P

Agemian's Fe and Al bound P> Polyphosphate > Refractory P>Calcium bound P >Loosely bound P

From the ranking above, all three extraction schemes show the same trends of having Fe-P or Fe and Al bound P as the most dominant fractions in all sediment samples from the Mae Klong, Chao Phraya and Bang Pakong Estuaries. The results of this study is similar to those from the Amazon and the Mississippi delta (cited in Berner et al., 1993), the Cochin Estuary (Balchand and Nair, 1994), the Tamagawa Estuary (Suzumura and Koike, 1995), the Potomac and Anacostia Estuaries (Huanxin et al.,

1997). The finding of this study supports the concept that Fe–P plays an important role in the cycling of phosphorus in estuarine environment (as mentioned in chapter 2). Other fractions show different relative dominant in different samples, which may have caused by the extraction method employed, the geological conditions and weathering processes in watersheds and human activities.

5.6 Accuracy of the selected sequential extraction schemes

Since the certified reference material of phosphorus for controlling quality of the selected sequential extraction methods is not available, the accuracy of these extraction schemes cannot be directly evaluated. However, the accuracy of these schemes can be indirectly determined by comparing sum of all P fractions with total P from XRF (the best total P analysis discussed in section 5.3) as shown in Table 5.6.1. The accuracy of each selected sequential extraction methods is shown in Table 5.6.2.

Table 5.6.1 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	Sum of phosphorus speciation		
	from XRF	Ruttenberg (1992)	Vink et al. (1997)	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 5.6.2 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		
	Ruttenberg (1992)	Vink et al. (1997)	Agemian (1997)
Mae Klong	+95	-18	- 8
Chao Phraya	+93	-33	+18
Bang Pakong	+82	-29	+22

5.6.1 Ruttenberg (1992)'s extraction scheme

Ruttenberg (1992)'s extraction scheme or SEDEX scheme provides relatively less accurate results than other schemes because sum of phosphorus speciation in three sediment samples are more than 80% overestimated when compared with total phosphorus from XRF (Table 5.6.2). Overestimated results are mainly caused by the poor precision Fe-P results [in term of coefficient of variation (%)] from isobutanol extraction method after extraction with CDB reagent (as shown in Table 5.6.3). Ruttenberg (1992) found the similar problem that could not be resolved during the development of the SEDEX method. Eijsink et al. (1997) and Vink et al. (1997) who followed and modified SEDEX method, respectively, also discovered the same problems. Recently, Ruban et al. (1999) also claimed that reliable Fe-P extraction with CDB reagent following SEDEX scheme was difficult to achieve due to the isobutanol extraction method before the molybdate blue determination. Precision of the isobutanol method according to CDB extraction from the previous researches is shown in Table 5.6.4. Excess Fe in $FeCl_3$ adding to eliminate excess citrate in CDB extract might affect on phosphomolybdate complex formation like silicate, arsenate and fluoride. In addition, dilution of CDB extract in isobutanol method might also alter the CDB matrix that causes the poor precision of Fe-P results.

Table 5.6.3 Coefficient of variation (%) of Fe-P analysis from various extraction schemes

Sediment	Sequential extraction scheme		
	Ruttenberg (1992)	Vink et al. (1997)	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 5.6.4 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

Furthermore, the precision of standard calibration curve for phosphorus prepared from CDB in the same matrix of sediment is varied. The slope values shifted daily from 0.006 to 0.008 even though relative coefficient (r^2) of each standard calibration curve was not less than 0.9995 and absorbance of reagent blank was 0.000. Thus, the average slope and deviation were calculated and applied for calculating Fe-P in sediments.

Moreover, organic phosphorus in sediments can also be responsible for the overestimation in sum of P speciation by SEDEX method. According to table 4.8 of the previous chapter, it was found that organic P in sediment samples from SEDEX method is nearly twice higher than that from ignition method of Aspila et al. (1976). This is opposite to what Ruttenberg (1992) and Berner et al. (1993) found. The different nature of sediment in this study might be the cause of the opposite finding.

5.6.2 Vink et al. (1997)'s extraction scheme

Vink et al. (1997)'s extraction scheme provides relatively more accurate results than SEDEX scheme even though Fe-P analysis also applied the same CDB-reagent and isobutanol method (Table 5.6.2). This might be caused by the higher CDB blank values (0.009) of Vink's extraction scheme (utilizing more 2.5 ml of CDB than SEDEX scheme). Therefore, calculated Fe-P concentration is not as high as that of SEDEX scheme. However, the precision of standard calibration curve prepared by CDB in the same matrix of samples has the same problem of Fe-P analysis as described in Ruttenberg's scheme.

In addition, the extraction steps of Fe-P of SEDEX (Ruttenberg, 1992) and Vink's extraction schemes are different (Step II-Ruttenberg (1992) and Step III-Vink et al. (1997)). It is possibly that SDS (step II of Vink's extraction scheme) may have either removed the iron oxides phase or altered the nature of the phase so that it was no

longer soluble in 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 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 (according to table 4.8). Therefore, it indicates that SDS is not suitable for extracting organic P in sediment of this study.

5.6.3 Agemian (1997)'s extraction scheme

Sum of P speciation obtained by Agemian (1997) is relatively closer to total P from XRFS than other schemes. Bicarbonate-ditionite (BD-reagent) of Agemian (1997)'s extraction scheme was proved to provide relatively more precise results in all sediment sample than CDB-reagent of Vink et al. (1997)'s and Ruttenberg (1992)'s schemes (as shown in table 5.6.3). The absence of citrate in extractant might be the main reason that BD reagent can provide relatively higher precision. The use of $FeCl_3$ to eliminate interference of citrate might cause problems related to phosphomolybdate complex formation in isobutanol method. However, the accuracy of BD-reagent comparing with CDB-reagent for measuring Fe-P is still debated because there has been no certified reference material available to check accuracy of both reagents until 2001 (J.F. Lopez Sanchez, personal communication). Therefore, the 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.

From the discussion above, it can be concluded that Agemian scheme (Agemian, 1997) provides the most promising results with higher accuracy and precision than SEDEX scheme (Ruttenberg 1992) and modified SEDEX scheme (Vink et al., 1997).

At the beginning of this study (in 1999), there was no certified reference material for phosphorus partitioning analysis available. It has just been commercially available at the beginning of the year 2001 after the European Commission, within the Standards, Measurements and Testing Programme (formerly Community Bureau of Reference, BCR) started a project to harmonise the methodology and to produce certified reference materials (CRMs) in 1996. After a thorough discussion following several interlaboratory tests among 20 experts (see Ruban et al., 1998), a modified Williams scheme was agreed to be used in the certification of material. However, only a CRM of lake sediment is now available (Pardo et al., 1999). Therefore, accuracy of the selected extraction schemes in this study can be further investigated directly by utilizing the CRM of lake sediment accompanied with 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.

5.7 Relationship between % clay size fraction and phosphorus with its implications

Grain size or particle size of sediment, which results from sedimentological processes, plays an important role in controlling the dispersion and trapping of contaminants (Eyre and Macconchie, 1994). The influence of grain size on the concentration of contaminants was firstly pointed out by Förstner and Wittmann (1981) that concentration of contaminant increases with the decrease size of sediment particles (higher surface areas of adsorption). In addition, Stone and English (1993) also found that the size fraction of sediment less than 8 μm is the most significant for the potential

release of bioavailable P in the water column. Therefore, % clay size (< 2 μm) fraction would be used to compare with total phosphorus and phosphorus partitioning in sediments of this study.

Due to that fact that this study investigated only three estuarine sediments from the Mae Klong, Chao Phraya and Bang Pakong estuaries, the results of the following relationships between grain size and phosphorus are just the preliminary trends. The better and clearer relationship between those two components will occur with many more replication of sediment samples.

5.7.1 Relationship between clay-size fraction and total phosphorus

From Figure 5.7.1, it was observed that the concentration of total P is strongly related to the amount of clay size fraction in sediment because its relative coefficient (r^2) is almost 1. Therefore, this can provide the answer to the question that why the Bang Pakong estuarine sediment contains the highest total phosphorus among the sediments of this study.
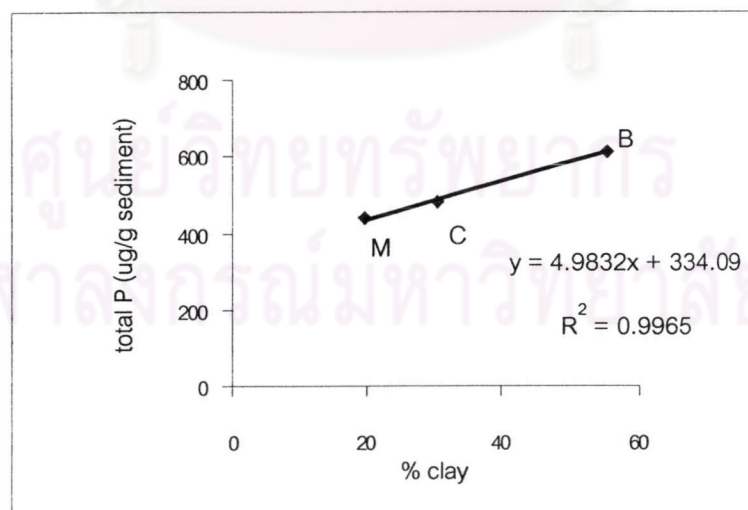

Figure 5.7.1 Relationship between % clay and total P (μg/g sediment) of
the Mae Klong (M), Chao Phraya (C) and Bang Pakong (B)
estuarine sediments

5.7.2 Relationship between clay-size fraction and phosphorus partitioning

The relationship between clay-size fraction and phosphorus partitioning is another indirect approach that can be utilized in confirming phosphorus partitioning results in the sediments obtained through the sequential extraction scheme. This relationship may help explaining how phosphorus partitioned in the sediments.

Dominant form of P, Fe-P, in the Mae Klong, Chao Phraya and Bang Pakong estuarine sediments (as discussed in the section 5.5) was confirmed by observing the relationship between clay size fraction and Fe-P. It was found that Fe-P results from all extraction schemes (Figure 5.7.2.1) strongly correlate with the amount of clay size fraction in the sediments because the relative coefficient (r^2) is near 1. Therefore, Fe-P mostly associates in the fine-grained fraction in the estuarine sediments.

Since Agemian scheme (Agemian, 1997) provides the most promising results with higher accuracy and precision than those by SEDEX scheme (Ruttenberg, 1992) and modified SEDEX scheme (Vink et al., 1997), the relationships of other fractions were tested by using Agemian scheme's results only (Figure 5.7.2.2). Polyphosphates and refractory P strongly correlate with the amount of clay size fraction in the sediments whereas calcium bound P correlates inversely. However, trend of loosely bound P is not clear. Therefore, it might indicate that polyphosphates and refractory P associate mostly with the fine-grained fraction of sediments whereas calcium bound P associates with the coarse-grained fraction. However, the better and clearer relationship will need the more study of sediment samples.
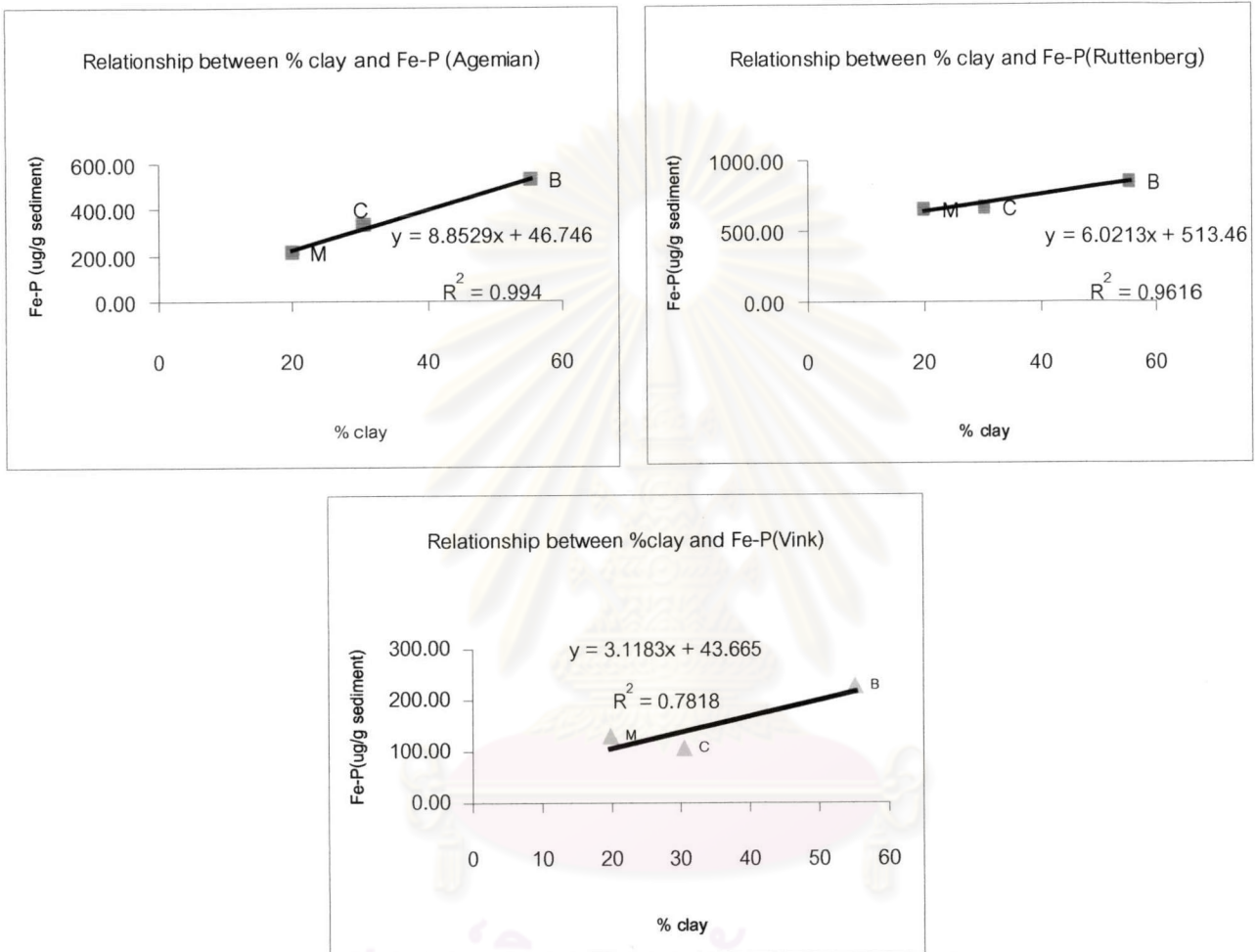
Figure 5.7.2.1 Relationships between % clay and Fe-P or Fe and Al bound P
(μg/g sediment) in the Mae Klong (M), Chao Phraya (C) and
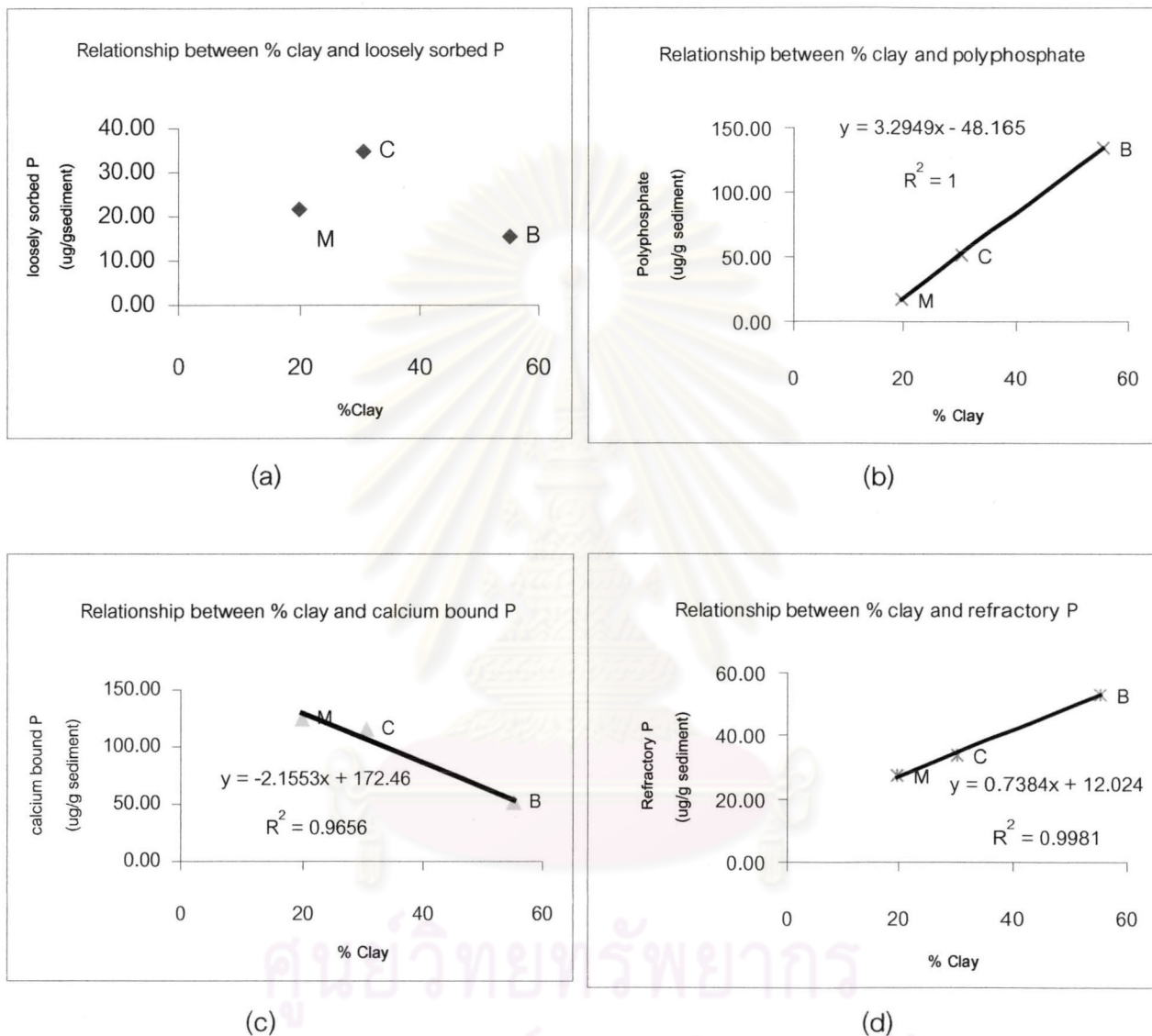Bang Pakong (B) estuarine sediments from three selected
sequential extraction methods

Figure 5.7.2.2 Relationships between % clay and (a) loosely sorbed P;

(b) polyphosphates; (c) calcium bound P; and (d) refractory P

(μg/g sediment) in the Mae Klong (M), Chao Phraya (C) and

Bang Pakong (B) estuarine sediments analyzed by Agemian scheme