CHAPTER IV

RESULTS AND DISCUSSION

4.1 The physicochemical parameters

The physicochemical characteristics of 81 soil samples are summarized in Table 4.1. Their more elaborative details are, however, given in Table E-1 in the Appendix E. Sample analyses show a wide range of physicochemical properties: pH ranges from 5.79 to 8.07; organic matter content (OM) from 0.52 to 4.16 %; oxidation-reduction potential (ORP) from -291.1 to 347.9 mV. According to several previous studies, it is well aware that there is a linear relationship between soil pH and Cd uptake by plants (Manz et al., 1999 and; Singh et al., 2005). The extractable metals from the acidic soils were found to be higher than that from the neutral and near alkaline soils, implying that as the pH of soil decreases the Cd bioavailable and usually plant uptake increase (Feng et al., 2005). With increasing pH, solution Cd concentrations decrease due to increasing in the processes of hydrolysis and in adsorption density (Alloway, 1990). In this research, the corresponding effects of soil pH on bioavailability of Cd uptake by sugarcane for different soil samples was not taken into account because most of the studied soil pH was neutral and/or near alkaline with rather narrow range in soil pH.

Table 4.1 Physicochemical parameters of studied soils

Statistic Values	**Soil Properties**		
	pH	**OM (%)**	**ORP (mV)**
Min	5.79	0.52	-291.1
Max	8.07	4.16	347.9
Mean	7.26	2.38	126.5
Median	7.34	2.31	187.6

N/A= not available

Most of the studied soils are moderately reduced (400-100mV) and some are reduced (< 100 mV). From the results obtained, the negative values of oxidation-reduction potential (ORP) indicated the reducing condition of the soil condition. This is because the samples (O51-81) were collected during the rainy season (October), which in turn may caused the reducing condition of the soils.

For organic matter contents, almost half of the studied soils have intermediate high organic matter contents (2.5-4.5%) which indicate that the soils in this area are relatively fertile because organic matter content in most of the agriculture soil is between 1-3% (Kashem, 2001b).

4.2 Total metal concentrations in soil samples

Soil samples were digested using microwave digestion techniques, EPA 3052, in order to determine the total metal concentrations. The maximum, minimum, mean, and median total metal concentrations (mg/kg), and standard deviations (%) of six interested elements (Cd, Cu, Fe, Mn, Pb and Zn) obtained from determination of 81 soil samples is reported in Table 4.2. The comprehensive analytical results are given in Table F-1 of Appendix F. The unusually high concentration of Cd was observed in numbers of locations and the Cd content ranged from 0.73-172.7 mg/kg. Ranges in concentration of other elements are 5.0-27.5 mg/kg for Cu, 3,437-17,963 mg/kg for Fe, 65.27-1,222 mg/kg for Mn, 6.4-160.3 for Pb, and 26.12-3,138 mg/kg for Zn. Most of the metal concentration levels in the studied soil are in the normal range reported by Alloway (1995) and Linsay (1979); (2-100 mg/kg for Cu, 2-250 mg/kg for Pb, 7,000-550,000 mg/kg for Fe, and 20-3,000 mg/kg for Mn); except for Cd (0.01-2.0 mg/kg) and Zn (10-300 mg/kg). Soil quality standard for habitat and agriculture of Cd, Mn, Pb and in Thailand is 37, 1,800 and 400 mg/kg, respectively (PCD, 2004). All metals obviously showed wide ranges of total concentration values which can be attributed to the wide range in total metal concentrations and concentrations of soil sampling sites, based on the dividing zone previously given by National Research Center for Environmental and hazardous Waste Management (2005), as according to high (> 20 mg/kg), medium (3-20 mg/kg) and low concentration (< 3 mg/kg) of cadmium content. Thus, this would cause the deviation of the mean values of metal concentrations and standard deviations to be high.

Table 4.2 Descriptive statistics of elemental concentrations (mg/kg) in 81 soil samples by total digestion

	Metals concentrations in soil samples (mg/kg)					
	Cd	**Cu**	**Fe**	**Mn**	**Pb**	**Zn**
Max	172.7	27.50	17,963	1,222	160.3	3,138
Min	0.73	5.00	3,437	65.27	6.40	26.12
Mean	15.14	17.09	12,502	495.4	29.67	510.5
Median	2.91	16.07	12,917	442.7	21.04	170.2
SD	38.44	6.07	3,790	283.6	31.88	845.1

4.3 Uptake of metals by sugarcane

The maximum, minimum, mean, and median concentration (mg/kg) and standard deviations (%) of six interested elements (Cd, Cu, Fe, Mn, Pb and Zn) obtained from analysis of 81 samples each of root, juice and bagasse of sugarcane were shown in Table 4.3. All analytical results of metal concentrations in sugarcane samples were given in Appendix G (Tables G-1 to G-6) which also includes concentration of metals in the other additional parts of sugarcane (top, underground stem, and leaves) for end crop sugarcane samples (O 51-81) (Appendix G, Tables G-7 to G-12). Figure 4.1 shows mean metal concentration in different parts of sugarcane (root, bagasse and juice) as compared to the total metal concentration in soils. The data indicates that metal concentrations in each plant part are generally controlled by their metal levels in soil. Amount of metal uptake by plant would reveal the metal availability in soil. Environment, nutrition, growth stage and some other factors controlling plant growth may also indirectly affect the metal levels in plants (Xian, 1989).

Table 4.3 Descriptive statistics of elemental concentrations (mg/kg) in 81 sugarcane samples (root, bagasse and juice)

Metals concentrations in sugarcane samples (mg/kg)						
Root	**Cd**	**Cu**	**Fe**	**Mn**	**Pb**	**Zn**
Max	25.25	34.88	18,470	2,524	13.31	401.8
Min	0.41	1.86	477.9	17.57	0.63	14.89
Mean	4.03	6.48	3,013	196.4	3.70	84.14
Medium	2.10	6.08	2,225	75.83	2.75	48.49
SD	4.89	4.00	2,535	439.5	3.01	87.96
Bagasse						
Max	2.28	86.04	342.8	54.05	7.39	114.1
Min	N/D	1.02	33.68	3.57	N/D	4.36
Mean	0.61	4.94	105.1	19.41	1.49	23.12
Medium	0.52	3.73	102.8	16.51	0.89	19.74
SD	0.52	9.46	54.42	13.94	1.60	17.86
Juice						
Max	0.35	0.68	32.94	11.72	1.13	29.82
Min	N/D	0.02	1.36	0.16	N/D	0.50
Mean	0.05	0.21	7.00	1.98	0.05	4.33
Medium	0.02	0.20	6.03	1.52	0.01	2.48
SD	0.08	0.13	5.33	2.11	0.21	5.06

Note: N/D = not-detected

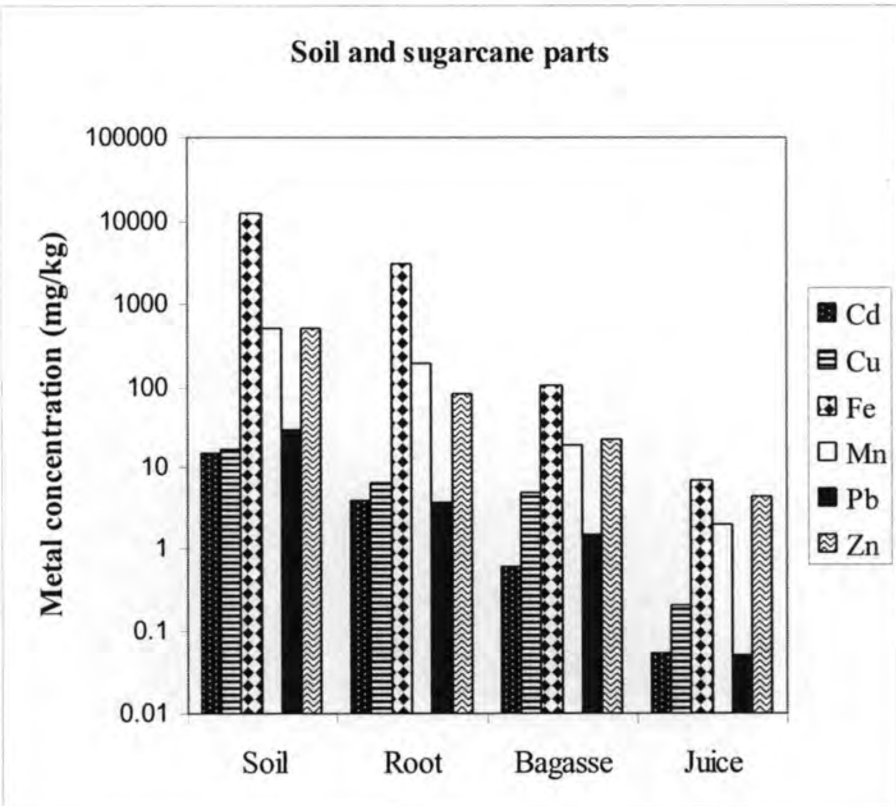


Figure 4.1 The mean metal concentrations in 81 soil and sugarcane samples (root, bagasse and juice)

There were similarities in the accumulation of metals, including Cd, Cu, Fe, Mn, Pb, and Zn, in root, bagasse and juice. Results showed the concentration of all metals was higher in root than in bagasse and juice of sugarcane. This may imply that metals were absorbed, accumulated, and retained by the roots rather than translocated to shoot. The ability of root to absorb metal may depend on its soil environment (Liu et al., 2003). Shute and Macfie (2006) reported similar patterns of distribution of Cd in soy bean of which the highest concentrations were found in roots, moderate concentrations were found in stems and leaves, and the lowest concentrations were in the pods and seeds.

The highest proportion of Cd is found in roots because of immobilization of Cd through precipitation and/or adsorption on the root surface (Tudoreanu and

Phillips, 2004). This is in accordance with the work of Angelova and Ivanov (2006) who also found that Pb, Cd and Zn were accumulated in the underground parts, mainly in roots and fruit shell. Their results showed metals in the stems of peanuts were considerably lower compared to the root system, good evidence for restricted mobility. As a general trend, Li et al (2007) also found plant roots accumulated slightly higher Cu, Cd and Pb than leaves. These results are consistent with the finding of Jutamas (2005) who found cadmium concentration in kale were accumulated mainly in the roots, and only small amounts of Cd were translocated to the stems.

Table 4.3 shows statistics results of elemental concentrations in 81 sugarcane samples (root, bagasse and juice). The average Cd concentrations are much higher in roots (4.03 mg/kg) than in bagasse (0.61 mg/kg) and juice (0.05 mg/kg). The results indicated that a great proportion of Cd taken by sugarcane was confined in roots. Most metals showed the same trend as the highest amount metal concentration present in root of sugarcane.

The ranges of metal concentrations in roots were 0.41-25.25, 1.86-34.88, 477.9-18,470, 17.57-2,524, 0.63-13.31, and 14.89-401.8 mg/kg for Cd, Cu, Fe, Mn, Pb and Zn, respectively. The average mean concentrations were 4.03, 6.48, 3,013, 196.4, 3.7 and 84.14 mg/kg for Cd, Cu, Fe, Mn, Pb and Zn, respectively.

The levels of Cd, Cu, Fe, Mn, Pb and Zn in the bagasse of sugarcane ranged from non detectable to 2.28 mg/kg (with an average of 0.61 mg/kg), 1.02 to 86.04 mg/kg (with an average of 4.94 mg/kg), 33.68 to 342.8 mg/kg (with an average of 105.1 mg/kg), 3.57 to 54.05 mg/kg (with an average of 19.41 mg/kg), non detectable to 7.39 (with an average of 1.49 mg/kg) and 4.36 to 114.1 mg/kg (with an average of 23.12 mg/kg), respectively.

For juice, Cd (non-detectable to 0.35 mg/kg) with the average of 0.05 mg/kg, Cu (0.02-0.68 mg/kg) with the average of 0.21 mg/kg, Fe (1.36-32.94 mg/kg) with the average of 7.0 mg/kg, Mn (0.16-11.72 mg/kg) with the average of 1.98 mg/kg, Pb (non-detectable to 1.13 mg/kg) with the average of 0.05 mg/kg and Zn (0.5-29.82 mg/kg) with the average of 4.33 mg/kg, were also observed. This indicated that all metal concentrations in juice were rather low as compared to the other parts of sugarcane (bagasse and root).

4.4 Sequential extraction

The first two steps of BCR sequential extraction procedure according to the Standard Measurement and Testing Programme (SM&T) was applied to analyze for Cd, Cu, Fe, Mn, Pb and Zn in the soil samples with ICP-OES. With regard to the BCR sequential extraction, the maximum, minimum, mean concentrations, median and standard deviations of six metals in soil samples (n = 81) in each extraction stage of BCR extraction procedure (BCR1 and BCR2) are presented in Table 4.4. The results for metal concentration partitioning by the first-two step BCR sequential extraction is also given in Appendix H, Tables H-1 to H-6.

Table 4.4 Descriptive statistics of elemental concentrations in 81 soil samples (mg/kg) by BCR1 and BCR2

	Concentration of metals in each fractions (mg/kg)					
	Cd	**Cu**	**Fe**	**Mn**	**Pb**	**Zn**
BCR1						
Max	100.9	0.65	87.92	635.7	6.13	2,641
Min	0.02	N/D	0.06	0.19	N/D	N/D
Mean	4.42	0.19	10.47	135.7	0.77	128.8
Median	0.40	0.16	4.33	94.46	0.51	16.10
SD	16.58	0.13	15.48	127.2	0.94	480.8
BCR2						
Max	114.9	5.33	5,266	986.8	52.32	1443
Min	0.02	0.08	12.79	1.01	0.60	1.46
Mean	6.28	1.17	1,103	247.1	12.84	164.9
Median	0.85	1.02	905.8	175.1	10.34	52.34
SD	19.14	0.94	1,007	237.0	11.16	298.8

Note: N/D= not-detected

Results showed that the concentrations of all metals found in the sequential extraction procedure increased generally from step 1 to step 2. The concentrations of metals in different fractions were found to vary with sites. The highest mean concentrations were found for Mn (135.7 mg/kg) in the first fraction (BCR1). For the first fraction (BCR1), Cd ranges from 0.02-100.9 mg/kg, Cu from not detectable- 0.65 mg/kg, Fe from 0.06-87.92 mg/kg, Mn from 0.19-635.7 mg/kg, Pb from not detectable-6.13 mg/kg and Zn from not detectable-2,641 mg/kg.

Fe shows the highest concentration in the second fraction (BCR2) (1,103 mg/kg). The second fraction (BCR2), the same amount of Cd (0.02-114.9 mg/kg) was released, where Cu from 0.08-5.33 mg/kg, Fe from 12.79-5,266 mg/kg Mn from 1.01-986.8 mg/kg, Pb from 0.6-52.32 mg/kg and Zn from 1.46-1,443 mg/kg were also observed. The extractable fraction of Cu and Pb, on the other hand, was very small in both fractions (0.19, 1.17 and 0.77, 12.84 mg/kg, in BCR1 and BCR2, respectively).

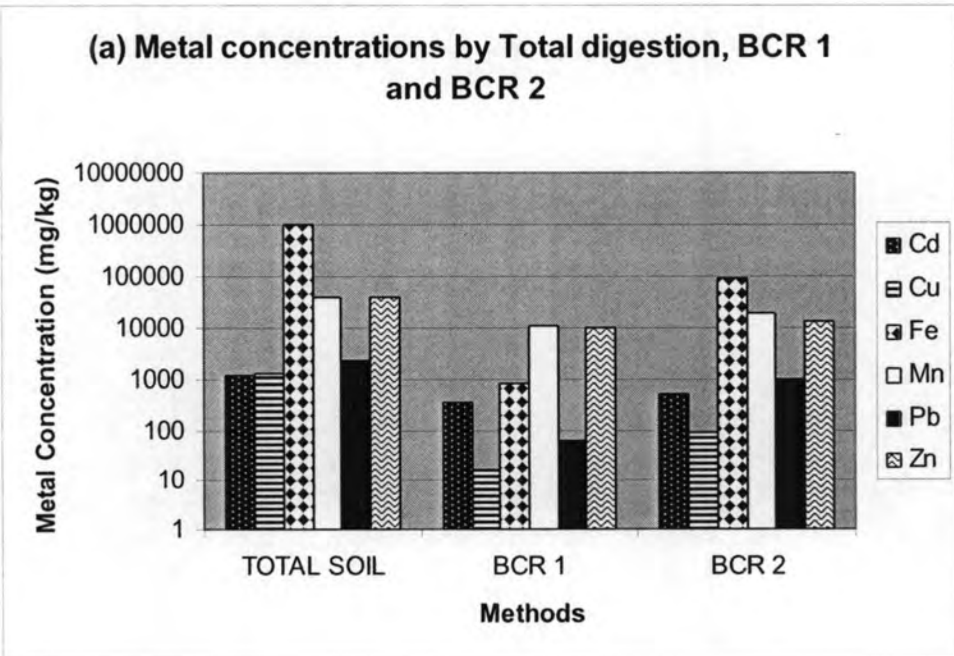
The sequential extraction procedure (BCR) permits the evaluation of the various chemical forms present in the soils. In soil, metals can be present in a number of chemical forms, and generally exhibit different physical and chemical behavior in terms of chemical interaction, mobility, biological availability and potentially toxicity (Singh et al., 2005). Results from the first-two steps of BCR sequential extraction procedures reveal principally the partitioning of Cd, Cu, Fe, Mn, Pb and Zn into two fractions: exchangeable (BCR1) and reducible (BCR2), respectively. The metal concentration associated with different phases in both BCR1 and BCR2 at different sites are shown in Figure 4.2.

The mobility and bioavailability of metals decreased approximately in the order of extraction sequence (Xian, 1989). In this study, the association of significant amount of six interested metals (Cd, Cu, Fe, Mn, Pb and Zn) with reducible fraction was observed. The results showed that the extraction yields in the second step (BCR2), with hydroxylamine hydrochloride extraction, were high for all the elements studied in most soils as compared to the first step (BCR1) extracted with acetic acid (Figure 4.2a). This can be explained by the changes in pH that occurred in each step of the BCR sequential extraction procedure cause an increase in extractable metal concentration. The role of pH in the solubilization of the different soil fractions was reported elsewhere (Narwal and Singh, 1998), i.e., the lower the pH, the more soluble of the metals.

In the second step (BCR2), the reducing agent (hydroxylamine hydrochloride) was used in the extraction procedure which released the more refractory and/or strongly attached fraction of metals from the soil matrix into the solution. The increase in amounts of metal extracted at higher extraction step may due to more effective attack on the more refractory form of metals (Rauret et al. 1999). The results are also similar to the work of Parada (2005) in which the crucial fraction of Cd in stream sediment from Haui Mae Tao, Tak Province, showed dominant Cd formed as BCR2 bounding with manganese and iron oxides.

Water soluble and exchangeable extracted fraction (BCR1) is more readily uptake solution by sugarcane. However, the next readily fraction (BCR2) can also be partially absorbed by plants. Therefore, the first two fractions (BCR1 and BCR2) can be combined and accounted for the bioavailable portions of metals uptake to sugarcane as shown in Figure 4.2b. By comparing total metal contents and the sum of BCR1 and 2, it showed that Cd, Mn, Pb and Zn are mostly at readily or near readily available forms except only the amount of Fe released in the first two fraction (BCR1 and 2), in contrast, was very low.

Some studies reported that reducible forms may transform into more soluble (exchangeable) form when the chemical and physical properties of the studied soil altered (Pérez-de-Mora et al., 2007). This may cause the change of the metal concentration during extraction period and undergo redistribution effects. It also reported that Cd and Zn are considered to be easily released and extracted because dissolved Cd and Zn are the most stable over a wide range pH range in solution and they are easily extracted without any redistribution effects. In other hand, Cu is being more strongly bounded and undergo redistribution effects (Mester et al., 1998). The extraction of Pb showed low Pb content in exchangeable fraction (BCR1), but rather high in reducible fraction (BCR2).



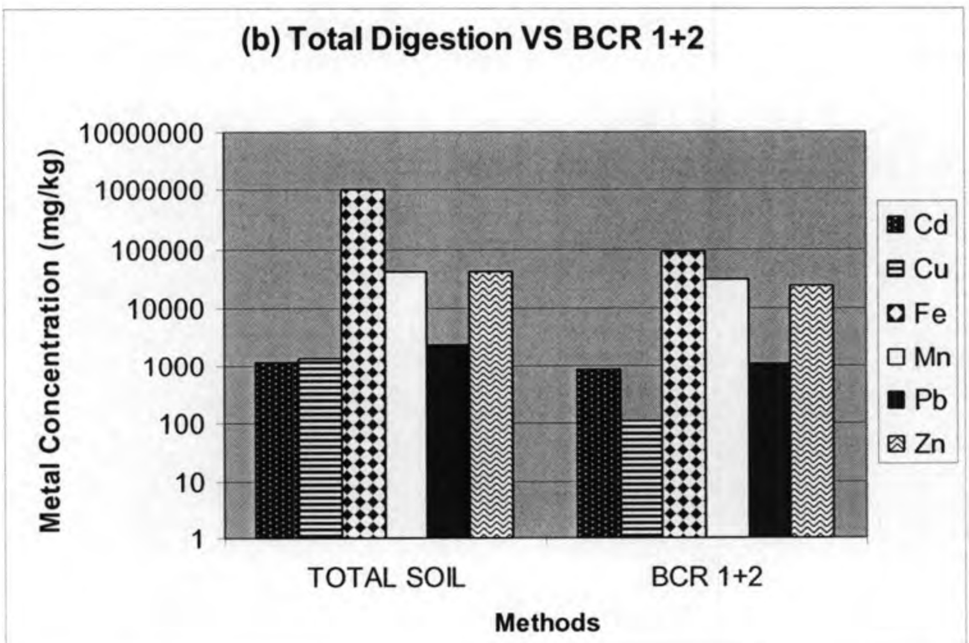


Figure 4.2 The six element (Cd, Cu, Fe, Mn, Pb and Zn) concentrations by total, BCR1 and BCR2 (a); total and the sum of BCR1 and 2 (b).

4.5 The Fractionation Patterns of the Metals

The chemical fractionations of Cd, Cu, Fe, Mn, Pb and Zn in 81 soil samples are investigated. The results of metal contents in each fraction are expressed as extraction percentage, reflecting individual fraction removal relative to the sum of the total concentration which represents 100% (Table 4.5).

According to the results, almost one third (25-30 %) of Cd, Mn, and Zn in soil was partitioned in exchangeable phases (BCR1) while Cu, Fe, and Pb were relatively low in this fraction. Portions of metals found in exchangeable fraction (BCR1) are weakly absorbed and can easily be solubilized and become readily available to plants. This may be harmful for ecosystem and for transfer of Cd in the food chain. Large amount of the reducible fraction (BCR2) was contributed by again Cd, Mn and Zn and in addition almost half of Pb content. Cd also contained relatively large concentration in this fraction which confirmable to the study of Narwal and Singh (1998). The percentage of cadmium in exchangeable (BCR1) and reducible (BCR2) fractions in soil samples are 29.58% and 42.02%, respectively. Cadmium was identified as potentially bioavailable element as it had high percentage bound to exchangeable and reducible (BCR1+2) for 71.60% of the total Cd. This indicated that Cd formed weak complex and easily removed at the initial stages of the extraction. This pattern agreed with that observed in the work of Pueyo et al. (2003), where Cd and Zn were found to be more mobile than the remaining metals (Pb, As, Tl and Bi) with the extraction yields ranging between 25 and 75%.

Table 4.5 Relative chemical distribution of six interested metals (Cd, Cu, Fe, Mn, Pb and Zn) in BCR1, BCR2 and the sum of BCR1 and 2.

% Distribution	**Cd**	**Cu**	**Fe**	**Mn**	**Pb**	**Zn**
% BCR1	29.58	1.11	0.08	27.73	2.63	25.23
% BCR2	42.02	6.94	8.94	49.88	43.81	32.70
% BCR1+2	71.60	8.05	9.02	77.61	46.43	57.93

By comparing between the first and second step of BCR sequential extraction, the copper in soil samples was associated with the reducible fraction (BCR2) (6.94 %) and only 1.11 % of Cu was released in the exchangeable fraction (BCR1). The presence of Cu in exchangeable fraction (BCR1) was almost insignificant. This indicates that Cu are not easily removed in these fractions and may be less mobilize/solubilize than Cd, as the proportions of Cu present in the first two fractions (BCR1+2) accounted for only 8.05 % from the total Cu. It is known that Cu forms specific complexes with organic matter (Christensen, 1987; Morillo et al., 2004; Narwal and Singh, 1998; Rodŕigues and Formoso, 2006). The association of significant amount of Cu with organic fraction was also observed by Narwal and Singh (1998) in which the first two factions accounted very low Cu (< 0.5 % of the total). Their results show similarity to those found in this study. The level and distribution of total and extractable Cu in the soil profile varies with soil type and parent material. Copper is specifically adsorbed or fixed in soils, making it one of the trace metals which move the least (Alloway, 1990). It is particularly interesting to note that only small fraction of Cu are presented as acid exchangeable (1.11 %) and reducible (6.94 %) fractions. The rest of Cu might be bound in more hardly metal forms.

Pb was released only 2.63 % in the exchangeable fraction (BCR1), where extraordinary large amount of 43.81 % was released in the reducible phase (BCR2). The low Pb content released in the exchangeable fraction (BCR1) indicated that Pb tends to be less mobile in the soil environment due to their limited solubility and/or high affinity for complexation with solid phase. Similar finding has been reported in the study of Pb and Zn minesoils in east China, in which low percentage of water soluble and exchangeable fraction of Pb (1.8 %) and Cu (0.2 %) were observed (Li et al., 2007). This is consistent with results obtained by several authors who found that Fe and Mn hydrous oxides are important scavengers of Pb (Morillo et al, 2004).

Interestingly, the chemical association of Fe within the soil samples is dominated by the reducible fraction (8.94 %) where in the exchangeable fraction (BCR1) Fe was partitioned only 0.08 %. These two fractions (BCR1+2) contain 9.02 % of the total Fe. The low accumulation of Fe in these two fractions is probably due to its being an element whose origin is fundamentally natural as it is one of the most common elements in the Earth's Crust (Morillo et al, 2004). For Manganese, half of Mn content in soil was bound to the reducible fraction (BCR2), accounting on average for 49.88 % of total Mn, where 27.73 % of Mn released in exchangeable fraction (BCR1). The high content of Zinc in both fraction (BCR1 and 2) was also released (25.23 and 32.70 %) which accounted for 57.93 % of the total Zn.

In this study, the distribution of the metals varied greatly among the samples. It should be emphasized that very different distribution patterns have been reported by other researchers, depending on the soil and contamination type (Kos et al., 2003). The extractability of all six tested metals in soil samples by BCR1 and BCR2 as compared to total contents were in the following orders:

BCR1: Cd > Mn > Zn > Pb > Cu > Fe

BCR2: Mn > Pb > Cd > Zn > Fe > Cu

BCR1+2: Mn > Cd > Zn > Pb > Fe > Cu

Based on the extraction yields in BCR 1+2, the metals studied can be ranked as: Mn (77.61 %) > Cd (71.60 %) > Zn (57.93 %) > Pb (46.43 %) > Fe (9.02) > Cu (8.05 %). This suggests that the mobility and bioavailability of the six metals are in the similar order. The proportion of all six interested metals in the first two fractions, based on the sum values, is 0.08-29.58 % and 6.94-49.88 %, in BCR1 and BCR2 respectively, as compared to the total metal contents. From the figures, it is obvious that the proportion of Cu in the first two steps of BCR sequential extraction (BCR1+2) is relatively low (8.05 %). The low content of the metals in the exchangeable fraction (BCR1) could signify a low available of the metals to plants, since the readily soluble form of a trace metal is often regarded as the most bioavailable (El-Demerdashe et al., 1995). This confirms that Mn, Cd, Zn and Pb have strong affinity for Fe/Mn oxides to which more that 30% of the total was adsorbed in reducible fraction (BCR2). On the contrary, Cu was found only released mainly in association with the total digestion.

4.6 Validation of the method

To validate the total digestion (EPA 3052), concentrations of Cd and Zn were determined in the certified reference material (CRM025-050, RTC). The results of the certified reference materials by the total digestion (EPA 3052) are presented in Table 4.6 which showed the good agreement with the certified values.

Table 4.6 The certified and measured reference material (CRM025-050)

Elements	Measured values (mg/kg)	Confidential Interval (mg/kg)
Cd	352.2±12.65	350±46.3
Zn	52.97±0.41	48.8±8.29

4.7 Correlation Analysis

4.7.1 Principal Component Analysis (PCA)

The high variability of the metals concentrations in soil samples obtained at various sampling sites requires a careful evaluation and interpretation. From the results presented in Tables 4.2, 4.3 and 4.4, it is difficult to draw a conclusion on which metals (Cu, Fe, Mn, Pb and Zn) and soil properties parameters (pH, OM, and ORP) influence bioavailable Cd uptake by sugarcane. Thus, the multivariate statistical approach Principal Component Analysis (PCA), a particular type of factor analysis, was chosen and conducted in an attempt to determine the relationships between available Cd extracted with the first step of BCR (BCR1) and the large number of variables (the presence of interested six metals and soil properties), which known to have the influence on available Cd uptake to sugarcane and affect both the concentrations in the soil system and possible adsorption.

Principal Component Analysis (PCA) is a powerful technique that attempts to explain the variance of a larger set of intercorrelated variables and transforming it into a smaller set of interdependent variables, so called Principal

Component or PCs (Sinha et al., 2007). In this research, PCA was performed on data matrices consisted of information on the total concentration of six interested metals (Cd, Cu, Fe, Pb, Mn, and Zn) present in soils, available metals concentration (BCR1) in soil and soil characteristics (pH, organic matter content (OM), oxidation-reduction potential (ORP)) in order to decrease the number of descriptor responsible for the highest percentage of a total variance of the experimental data (Ražić et al., 2006). Thus, total and available metals (Cd, Cu, Fe, Pb, Mn, and Zn) in soil samples were taken as variables. Soil characteristics included soil pH, organic matter content (OM) and oxidation-reduction potential (ORP) were also included in the input matrix as variables. The PCA was carried out on the correlation matrices of the data using the SPSS program package (version 11.5).

All data for Principal Component Analysis (PCA) is given in Appendix I. Loading values suggested the correlation between certain elements and soil characteristics of analyzed data. When PCA was applied to the data matrix, with corresponding scree plot obtained in eigen-analysis of correlation matrix presented in Figure I-1 (Appendix I) exhibits the appearance of four principal components (PCs) responsible for 78.813 % of variance of the data. PCA allows visualizing the information of data set in four principal components retaining the maximum possible variability within that set. Component plot in rotated space is given Figure I-2 (Appendix I) which illustrated the four groups. Four Principal Component (PCs) were extracted according to the Kaiser criteria in which only components with eigen values > 1 were included in the analysis.

Total variance explained table and component matrix are presented in Appendix I, Tables I-1 and I-2, respectively. Total Variance explained table showed the significant factors and the percent of variance explained by each of variables before undergo the rotation which accounted for 78.813 % of the total variance. Direct oblimin was used as the rotation method in the analysis of the PCA following standardization of the data in order to get a better and more explicit of variables to Principal Component (PCs). Rotation of coordinate system of factors will not affect the position of the objects relative to each other, but will simplify the factors' structure (Ražić et al, 2006). Output of the data after undergo rotation is presented in Table 4.7.

Table 4.7 Structure matrix

	Component			
	1	**2**	**3**	**4**
Total Cd	**.938**	.152	.229	.166
Total Pb	**.888**	.305	.229	.188
Available Cd	**.867**	-.020	.565	.221
Total Zn	**.856**	.117	-.015	.081
Avaialble Zn	**.851**	-.047	.579	.208
Available Pb	**.760**	-.149	.710	-.055
Total Fe	.033	**.945**	-.177	-.067
Total Cu	-.005	**.888**	-.373	-.019
Total Mn	.481	**.707**	.106	.204
OM	-.026	**.682**	.289	-.512
pH value	.105	**.680**	-.187	.063
Available Cu	.234	-.308	**.888**	.014
Available Mn	.207	.032	**.859**	-.223
Available Fe	.277	-.203	**.538**	.131
ORP	.103	.034	-.058	**.951**

Extraction Method: Principal Component Analysis.
Rotation Method: Oblimin with Kaiser Normalization.

Based on the PCs loadings, the four principal components (PCs) classification from PCA are listed below:

- The first component (PC1): consisted of
  - Available Cd concentration
  - Available Zn concentration
  - Available Pb concentration
  - Total Cd concentration
  - Total Zn concentration
  - Total Pb concentration
- The second component (PC2): consisted of
  - Total Fe concentration
  - Total Cu concentration
  - Total Mn concentration
  - Organic matter content (OM)

- pH

- The third component (PC3): consisted of
    - Available Cu concentration
    - Available Mn concentration
    - Available Fe concentration
- The forth component (PC4): consisted of
    - Oxidation-reduction potential (ORP)

From PCA analysis, the results showed that the first component (PC1) has the highest factor loading (4.882) where the second (PC2), the third (PC3) and the forth (PC4) are 3.403, 3.382 and 1.449, respectively. The first component (PC1), account for the most important, is strongly correlated with total Cd, total Zn, total Pb, available Cd, available Zn and available Pb as illustrated by high factor loading in PC1 (Appendix I, Table I-1). Variables that are correlated with one another are combined into factors which are though to be representative of the underlying correlations (Zhang, 2006). This suggested that available Cd is correlated to total Cd, total Pb and total Zn, available Pb, and available Zn as they are present in the same group. This implies that the presence of Pb and Zn may have the relation with the available Cd uptake to sugarcane. Lead and Zn are often found together in ore deposit (Zhang et al). Alloway (1990) reported that Pb is considered to have a synergistic effect (+,+) on Cd uptake due to it being preferentially absorbed, thus leaving more Cd in soil solution. Several studies have been found relationships most commonly observed between Cd-Pb (Liu et al., 2003; Luo and Rimmer, 1995; Kabata-Pendias and Pendias, 2001) and Cd-Zn (Alloway, 1990; Luo and Rimmer, 1995; Kabata-Pendias and Pendias, 2001).

The second component (PC2) is mostly dependent upon total Fe, total Cu, total Mn, pH and organic matter content (OM) which has 3.403 of factor loading. This component (PC2) appears to be related to the soil parent material. Iron and Mn are the two of the most common elements present in the soil (Kabata-Pendias and Pendias, 2001; Zhang et al, 2008). Very high loadings of Fe and Mn strongly relate this factor to soil parent material. Organic matter (OM) and soil pH are also factors included and appear to have a significant influence on the formation of Fe/Mn oxides (Kabata-Pendias and

Pendias, 2001). Copper retention and partitioning in soils are related to the presence of organic matter, Fe and Mn oxides, and clay minerals through exchange and specific adsorption mechanism (Balasoiu et al., 2001). Thus, the second component (PC2) can be regarded as soil parent material related as it is obvious that only the total of Mn, Fe, and Cu are included in the second component (PC2).

Alternatively, the third component (PC3) comprised only the available forms of metals namely: available Cu, available Mn and available Fe, meaning that these factors are correlated (the factor loading = 3.382). It is interesting to note that in the second and the third component (PC2 and PC3), the total and available form of metals was separately grouped, where the fourth component (PC4) oxidation-reduction potential (ORP) was standing alone.

It should be noted here that for pH and ORP, the 2 parameters that have been recognized to influent the available form of metals in soil; however, in this study they varied in the narrow range, only few samples show very different from average value. This may lead to low loading factors in PCA analysis.

4.7.2 Correlation of available Cd in soil (BCR1) and soil properties

In order to demonstrate relationship between available Cd in soil (BCR1) and the soil properties parameters including pH, organic matter (OM) and oxidation-reduction potential (ORP), correlation analysis was performed. There are number of different coefficients that measure the degree to which two variables are linearly related. The test for normality of the data was performed and checked by applying nonparametric test: 1-sample K-S (the Kolmogorov-Smirnov) from SPSS software. Significant level, α, was 0.05. Results showed that normal distribution was not existed in the data set at α of 0.05 (Appendix I, Table I-4); therefore a non-parametric measure the Spearman correlation coefficient (r) might be more appropriated and thus was used in this study by means of the BIVARIATE procedure in the SPSS. Spearman correlation coefficient (r) of available Cd (BCR1) and soil properties (pH, OM, and ORP) were investigated and listed in Table 4.8.

Table 4.8 Spearman correlation coefficients (*r*) of available Cd and soil parameters.

	Physico-chemical parameters			
	Available Cd	**pH**	**OM**	**ORP**
Available Cd	1.0			
pH	**0.206**	1.0		
OM	**0.218**	0.435*	1.0	
ORP	**0.051**	0.053	-0.309*	1.0

* Correlation is significant at the 0.05 level

The first column in the correlation matrix (Table 4.8) was only investigated to demonstrate the relationship between available Cd and soil properties (pH, OM, ORP). Results showed weak positive relationships in all cases between available Cd-pH, available Cd-organic matter (OM) and available Cd-oxidation-reduction potential (ORP) ($r = 0.206$. 0.218 and 0.051, respectively). This suggested that correlation has not been found between available Cd-pH, available Cd-OM and available Cd-ORP.

Numerous studies have been reported the correlation between the availability of metals and soil pH. However, no such correlation was seen in the studied soils which can be explained by the same reason described in PCA analysis and loading factor. The narrow range of the soil pH may cause insignificant change in the availability of metals and no such effect has been seen. In addition, the variation of OPR with the negative values may be overlooked as only few existed and this may cause no effect on available Cd in soil.

4.7.3 Correlation of available Cd in soil (BCR1) and factors in the first component (PC1)

The first component (PC1) obtained from the Principal Component Analysis (PCA) was selected to further investigate the relationships because of the highest factor loading. The Spearman correlation coefficients (*r*) were selected as the measurement for the correlation between variables in order to make sure that variables classified in the first component (PC1) shared good correlation. To determine the relationship between available Cd (BCR1) and the factors in the first component (PC1) of principal component analysis (PCA), correlation analysis was conducted using the BIVARIATE procedure in the SPSS. For this purpose, a correlation analysis was performed to evaluate the relationship between the available Cd (BCR1) in soils and each factors present in the first component (PC1) namely; available Zn, available Pb, total Cd, total Zn and total Pb, respectively. The correlation matrix is presented in Table 4.9 and only the first column in the correlation matrix was determined.

Table 4.9 Spearman correlation coefficients (*r*) of available Cd and factors in the first component (PC1) of principal component analysis (PCA).

	First component (PC1) of principal component analysis (PCA)					
	Available Cd	**Available Pb**	**Available Zn**	**Total Cd**	**Total Pb**	**Total Zn**
Available Cd	1.0					
Available Pb	**0.296***	1.0				
Available Zn	**0.946***	0.397*	1.0			
Total Cd	**0.554***	0.120	0.480*	1.0		
Total Pb	**0.321***	-0.083	0.277*	0.789*	1.0	
Total Zn	**0.468***	0.133	0.382*	0.890*	0.644*	1.0

* Correlation is significant at the 0.05 level

According to the results, all metals pairs revealed significant positive relations (significant at 95%) between available Cd-available Pb; available Cd-available Zn; available Cd-total Cd; available Cd-total Pb, and available Cd-total Zn (r = 0.296, 0.946, 0.554, 0.321, and 0.468, respectively) with the pair of the available Cd-available Zn showed the highest (r = 0.946) indicating that available Cd is a function of the available and total metals content Cd, Pb and Zn.

4.7.4 Correlation of available (BCR1) and total metals in sugarcane

One of the most important applications of chemical fractionation of metals in soils is to determine bioavailability to plants (Yan et al, 2007). Table 4.9 showed the correlation of metals in the soil, however, this section is demonstrated the correlation of metals in sugarcane. For this purpose, a correlation analysis was performed to evaluate the relationship between the available metal (BCR1) in soils and total metals accumulated in all parts of sugarcane (root, underground stem, bagasse, juice, top and leaves) for all six interested metals (Cd, Cu, Fe, Mn, Pb and Zn). If the accumulation of an element by sugarcane correlated significantly with the available metal in soil (BCR1), it can be assumed that the extractable fraction (BCR1) is readily available to plant. Table 4.10 showed the correlation coefficients (*r*) investigated for available metals concentration in soil (BCR1) and the total metals concentrations uptake to sugarcane in all parts included root, underground stem, bagasse, juice, top, and leave for the end crop soil samples (O 51-81). Since, the estimation of total metal in sugarcane (root, underground stem, bagasse, juice, top and leaves) has been carried out for the end crop (O 51-81; 10 months sugarcane) sugarcane samples only (Appendix G, Tables G-7 to G-12). Therefore, only thirty sugarcane samples were used for the analysis.

Table 4.10 Spearman correlation coefficients (*r*) of available (BCR1) and total metal concentrations in sugarcane.

	Total metals accumulated in sugarcane samples (all parts)					
	Total Cd	**Total Cu**	**Total Fe**	**Total Mn**	**Total Pb**	**Total Zn**
Available Cd	**0.473***					
Available Cu	-0.337	**0.004**				
Available Fe	-0.103	0.357	**-0.093**			
Available Mn	-0.099	-0.393*	-0.387*	**0.176**		
Available Pb	0.222	-0.077	0.008	0.205	**0.022**	
Available Zn	0.469*	0.175	0.108	0.046	-0.015	**0.431***

* Correlation is significant at the 0.05 level

Results showed significant positive correlations were existed between total Cd in sugarcane-available Cd in soil (r = 473); and total Zn in sugarcane-available Zn in soil (r = 431). Weak positive correlations were obtained for total Cu in sugarcane-available Cu in soil (r = 0.004); total Mn in sugarcane-available Mn in soil (r = 0.176); and total Pb in sugarcane-available Pb in soil (r = 0.022). However, negative correlation was observed for total Fe in sugarcane and available Fe in soil (r = -0.093).

Again, the results are consistent with the finding from the first component (PC1) of the Principal Component Analysis (PCA) which the correlation of each metals pair (between bioavailable and the total) of Cd and Zn were existed. This finding may confirm that the presence of Zn may have the influence on available Cd uptake by sugarcane.