

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Soil properties

Soil properties of seventy eight soil samples in this study including soil pH, organic matter content (OM) and oxidation-reduction potential (ORP) were determined and summarized in (Table 4.1). From the results obtained: pH ranged from 5.79 to 8.07; organic matter contents (OM) ranged from 0.52 to 4.16 percent; oxidation-reduction potential (ORP) ranged from -291.1 to 347.9. mV (See Appendix B)

The pH values of soil varied from slightly acid to slightly alkaline (5.79- 8.07), which probably caused by the parent material in this area is calcareous soil. Soil pH is the most important single soil property that determines heavy metal bioavailability to plants. In general, cadmium uptake by plants is usually higher in acidic than alkaline or calcareous soil. Decrease in solubility of Cd in soils is oftenly associated with the formation of carbonates and phosphates of cadmium under increasing pH, raising the pH of soil solution can lead to the formation of hydrolysis products that have a different affinity for sorption sites (Adriano, 2001). McBride (1994) also reported that the mobility and bioavailability of Cd in neutral to alkaline soils is low. In general, metals are more soluble in soils at low pH due to the dissolution of the carbonates, phosphates, and other solid phases. Andersen et al., (2002) reported that the Cd concentration of soil solution decreased as pH increased.

In the studied soil OM ranges from 0.52 to 4.16 %. This range can indicate that intermediate high of OM which meaning that fertile soil in this area. It is known that OM in soils is a reactive component and capable of retaining the metal cations. OM adsorbs metal ions by the ion exchange mechanism rendering less mobile, therefore less bioavailable. Sauve et al. (2003) found that the soils showed high sorption affinity of OM for Cd, which was as much as 30 times higher than mineral soil for sorbing Cd. Another study done in Canada (Ge and Hendershot, 2005) showed that OM is the primary sorbent for heavy metals in the boreal temperate

regions. Soils in this region often possess podzolic characteristics due to the acidic soil environment and the alleviation process.

For the oxidation-reduction potential (ORP), the values ranged from -291.1 to 347.9. The moisture content of soils influences their retention for trace metals through redox reaction. Elemental concentrations in solution extracted from sludge-treated soil indicate the decrease in solubility of Cd, Cu and Zn and increase in solubility of Mn and Fe under reducing conditions (Bingham et al., 1976).

Table 4.1 Some physical and chemical properties of soil samples in this study.

	pH	Organic matter contents (%)	Oxidation-reduction potential (mV)
Max	8.07	4.16	347.9
Min	5.79	0.52	-291.1
Mean	7.26	2.38	126.5
Median	7.34	2.31	187.6

4.2 Cd and Zn in soil

Total concentrations of Cd and Zn obtained by total digestion and the available Cd and Zn by four different single extraction procedures (the first-step of BCR (BCR1), EDTA, DTPA and CaCl₂) in seventy eight soil samples from Mae Sot district, Tak Province were determined in this study. Table 4.2 showed the result of Cd and Zn concentration including maximum, minimum, mean, median and standard deviation. (See Appendix C) Results showed the wide range from 0.8819 to 291.3645 mg/kg and 26.12 to 3,138.86 mg/kg for total Cd and Zn concentrations, respectively, the high standard deviation is unexpected due to the fact that the sampling sites chosen in this study were selected from different zones of contamination. However, from the analysis of the total Cd concentration of soil samplings, it was found that less than 10% of total samples contain total cadmium higher than 50 mg/kg. This can be seen from the difference of mean and median values.

The extractable Cd and Zn by the three extraction procedures followed the order BCR1 > EDTA > DTPA > CaCl₂. For the extraction procedures, BCR1 was the strongest extractant that can dissolve Cd into the available form. The mean Cd concentration extracted with BCR1 was 4.59 mg/kg where other three single extractants; EDTA, DTPA and CaCl₂, were 3.44, 1.39 and 0.1 mg/kg, respectively.

For Zinc, the mean Zn concentration extracted with the first-step of BCR (BCR1) and EDTA had the same trend as Cd (132.08 and 66.19 mg/kg, respectively) in which CaCl₂ showed the lowest mean concentration (2.09 mg/kg), followed by DTPA (19.51 mg/kg). According to the literature, acetic acid extracts ion-exchangeable forms of elements and forms bound to carbonates. The exchangeable fractions may indicate the forms of metals that are most available to plant uptake (Zemberyov'a et al., 2006). The results can be explained as the effect of acidity is enhanced in calcareous soils when using acetic acid extracts in the first-step of BCR (BCR1) because of a high capacity to dissolved carbonates. The elements bound to carbonates and specifically adsorbed phases can easily become mobile and become available under conditions of lower pH. Whalley and Grant (1994) reported acidic acid was able to release most of metals associated with calcium carbonates minerals which supported our results.

In general, EDTA is assumed to extract both carbonate-bound and organically bound fractions of metals in soils with low carbonate content, whereas DTPA buffered with triethanolamine is considered suitable for calcareous soils (Sahuquillo et al., 2003). However, the resulted showed that higher portions were extracted with EDTA in comparison with DTPA. The reason for this is probably due to EDTA is a strong chelating reagent, which has been reported to extract labile and non-labile metals, including exchangeable and weakly organically bound fractions (Bermond et al., 1998). It combines with free metal ions in solution forming soluble complexes and thereby reduces the activities of the free metal ions in solution. In response, metal ions desorbed from soil surfaces or dissolved from labile solid phases to replenish the free metal ions in solution. Although not many data are available in the literature for the same soil with both extractants, it seems that DTPA does not provide better information than EDTA. Thus, the use of EDTA would assure better analytical performance during the measurement because of its higher extraction capacity, EDTA tends to release trace metals out of the non silicate bound phases and therefore, correlate well with plant contents and with the plant available fraction.

CaCl₂ was found to be the weakest extractant for both Cd and Zn. Thus the concentration from CaCl₂ extraction shows much lower extracted Cd and Zn as compared to other extractants. This is probably caused by the amount of CaCl₂-extractable Cd and Zn depended mainly on soil pH. It decreased with increasing soil pH according to calcareous soil in this area. This corresponds to the study by Clemente and Bernal (2006) that reveals the concentration of metals in soil solution in exchangeable forms extracted with CaCl₂ were very low in this calcareous soil. The results obtained the lowest extracted Cd and Zn because CaCl₂ is mainly able to extract easily exchangeable metals (Fang et al., 2007). Extract with CaCl₂ affects only the loosely held soil Cd, such as the exchangeable and acid soluble forms (Makino et al., 2006). Gupta and Sinha (2006) showed the extraction efficiency of metals were as follows EDTA > DTPA > NH₄NO₃ > NaNO₃ > CaCl₂ which appear to be to this results.

Table 4.2 Concentrations of total and available Cd and Zn in soil samples determined by different procedures

	Cd concentration in soil (mg/kg)					Zn concentration in soil (mg/kg)				
	Total	BCR	EDTA	DTPA	CaCl ₂	Total	BCR	EDTA	DTPA	CaCl ₂
Max	291.4	100.9	41.03	13.18	1.03	3,138	2641	811.3	180.4	41.55
Min	0.8819	0.0214	0.0095	0.0053	nd	26.12	nd	0.8904	0.1676	nd
Mean	20.07	4.59	3.44	1.39	0.1031	528.5	132.1	66.19	19.51	2.09
Median	3.6185	0.4272	1.08	0.5709	0.0379	177.6	17.77	20.16	9.13	0.4170
SD	54.59	16.87	7.47	2.63	0.1796	856.5	486.5	148.8	33.69	6.23

Note: nd = non-detectable

4.3 Cd and Zn in sugarcane

Total concentration of Cd and total Zn in sugarcane samples are the direct indicate the measurement of bioavailability of Cd and Zn uptake from soil to the plant. Therefore, six parts of sugarcane; namely root, underground, bagasse, juice, leaves and top were separately determined for the amount of total Cd and Zn. Tables

4.3 and 4.4 summarize the concentration of these heavy metals (Cd and Zn) in sugarcane samples (See Appendix D). In addition, average total Cd concentration for the whole parts of sugarcane was also calculated. The mean values of Cd in each parts of sugarcane followed the descending order: root > top > underground > bagass > leave > juice (5.1919, 1.5244, 1.4081, 1.1986, 1.0991 and 0.2289 mg/kg, respectively). For Zn, the mean Zn in top is 79.7031mg/kg that showed the highest Zn concentration in this part and followed by root, underground, leave, bagasse, and juice (61.5115, 47.4327, 36.2364, 23.7670, 4.5255 mg/kg, respectively). The results showed the same descending order of Cd and Zn concentrations present in each parts of sugarcane, where the juice showed the lowest for both Cd and Zn.

In the present study, the accumulation and distribution of Cd and Zn in the sugarcane were found differently in their amounts. The translocation of Cd, which is classified as toxic metal, was restricted in the underground part particularly root of sugarcane, while Zn was better translocated to the above ground part particularly top. This may be the reason from the fact that Zn is micronutrient and is essential for growth of plant growth thus it may be needed to distribute to all parts of plant. The process of Cd and Zn accumulation in different parts of sugarcane may also depend on the concentration of available metals in soils, the metal mobility, and the plant species growing on these soils (Gupta and Sinha, 2006).

From the results, the concentration of Cd in root is higher than in other parts, which may be due to the metal toxicity, complexation, and structures in sugarcane that preventing the translocation to top, leave, bagasse and juice. The transport of metals from roots to shoots includes long distance translocation in the xylem and storage in the vacuole of leaf cells and these processes affected by many factors such as organic acids (Yang et al., 1997). Recently, Sinha et al. (2006) reported less translocation of metals in the upper parts of the vegetables/crops grown on contaminated soil than lower parts. Confirmation on the same trend is also revealed by Gupta and Sinha (2007) that poor translocation of metals (Cr, Fe, Mn, Cu, Pb, Cd, Ni) in *Sesamum indicum* and toxic metals (Cr, Pb, Cd) in *Chenopodium album* grown on different amendment of tannery sludge. In contrast, better accumulation of most of the tested metals (Na, K, Zn, Mn, Cu, Cr, Pb, Ni, Cd, Co) and their translocation were recorded in wild plants of *Sida acuta*, *Ricinus communis*, *Calotropis procera*, *Cassia fistula* grown on tannery sludge translocation of metals in the upper part of the plants, which supported our finding here that Zn concentrated

highest in the top may possibly due to Zn is an essential element in maintaining plants growth (Lai and Chen, 2005). Gutpa and Sinha (2006) reported that *Brassica juncea* L. Czern. (var. Vaibhav) grown on different amendment of fly ash have shown a better translocation of metals in the upper part than the lower part.

Table 4.3 Concentration of total Cd in each parts of sugarcane samples.

Cd in sugarcane (mg/kg)							
	Root	Underground stem	Bagasse	Juice	Leave	Top	Average in whole sugarcane*
Max	27.2655	6.5615	3.6585	0.8620	4.2497	3.9883	1.7095
Min	0.0662	0.0000	0.0000	0.1240	0.0000	0.5272	0.1553
Mean	5.1919	1.4081	1.1986	0.2289	1.0991	1.5244	0.5813
Median	2.9243	1.0631	0.9627	0.1870	0.8808	1.4565	0.5139
SD	5.8470	1.3433	0.9394	0.1240	0.9905	0.6424	0.3708

Note * The value obtained from calculation by weight ratio of each parts

Table 4.4 Concentration of total Zn in each parts of sugarcane samples.

Zn in sugarcane (mg/kg)							
	Root	Underground stem	Bagasse	Juice	Leave	Top	Average whole sugarcane*
Max	401.8	233.3	114.1	30.5260	176.8	282.4	57.1985
Min	14.8884	0.0000	4.3613	0.5141	13.7843	13.4137	3.6010
Mean	61.5115	47.4327	23.7670	4.5255	36.2364	79.7031	14.8488
Median	38.4623	29.9017	21.0142	2.6483	27.2763	59.8588	11.0923
SD	75.5286	50.5863	18.1877	5.2522	27.6473	55.2458	10.6078

Note * The value obtained from calculation by weight ratio of each parts

In addition to Cd and Zn concentrations as shown in Tables 4.3 and 4.4, total mass of Cd and Zn accumulated in each part of sugarcane and in the whole plant were estimated as illustrated in Tables 4.5 and 4.6 (See Appendix D). It can

been seen that although, the results show that the highest concentration for both Cd and Zn rather found in root, the total accumulated mass for both Cd and Zn, on the other hand, found in bagasse (see Tables 4.5 and 4.6), followed by the accumulated in leaves. This is due to the fact that the bagasse, juice and leaves are the main part in term of mass for the whole sugarcane body. Our results are consistent with Sai Prakash et al. (1995) who reported that the Cd was accumulated mainly in bagasse. This result indicates that for above ground parts of sugarcane contain more Cd and Zn in term of mass. However, it is still not be able to consider sugarcane as a plant used for the purpose of phytoremediation.

Table 4.5 Accumulated amount of Cd in each parts of sugarcane samples

Cd accumulated in sugarcane (mg)							
	Root	Underground	Bagasse	Juice	Leaves	Top	Whole sugarcane
Max	0.2471	0.2555	1.1699	0.9010	0.5496	0.1460	2.8369
Min	0.0009	0.0000	0.0000	0.0133	0.0000	0.0072	0.0344
Mean	0.0306	0.0319	0.1638	0.1305	0.0767	0.0346	0.4642
Median	0.0138	0.0188	0.0969	0.0918	0.0686	0.0275	0.3266
SD	0.0434	0.0420	0.2092	0.1483	0.0855	0.0256	0.4831

Table 4.6 Accumulated amount of Zn in each parts of sugarcane samples

Zn accumulated in sugarcane (mg)							
	Root	Underground	Bagasse	Juice	Leaves	Top	Whole sugarcane
Max	3.5043	5.4592	20.0790	13.2392	11.6517	9.7718	1,428
Min	0.0451	0.0000	0.2653	0.2074	0.4865	0.3577	30.672
Mean	0.5318	0.9226	2.7550	1.8925	2.6034	1.7401	232.2
Median	0.3149	0.5121	2.1793	1.2172	1.6170	1.2277	137.5
SD	0.6507	1.0715	2.7553	2.0713	2.2905	1.6548	266.0

4.6 Correlation analysis

Choosing the best suitable extraction method to determine the bioavailable Cd and Zn in Mae Sot district, Tak province is the aim of this study. Therefore, the correlation analysis (r) was applied to find the relationship between the extractable Cd and Zn by different four single extractants (BCR1, EDTA, DTPA and CaCl_2) and the total Cd and Zn in soil as well as in sugarcane (accumulation of total Cd and Zn from all parts).

Prior to the analysis for the correlation coefficient (r), the data were tested whether they are normal distribution. All data were found to be not being normal distribution as shown in Tables 4.7 and 4.8 since all p values were less than 0.05. Therefore, non-parametric Spearman correlation coefficient was selected to analyze for the relationship between available Cd from four different extraction procedures and total Cd in soil and sugarcane. The correlation coefficient for total Cd concentration in soil and available Cd from different extraction methods shows in Table 4.9

Table 4.7 Analysis result of normal distribution test for Cd in soil, and sugarcane samples

One-Sample Kolmogorov-Smirnov Test

	Total Cd in soil	BCR1	EDTA	DTPA	CaCl_2	Total Cd in Sugarcane
N	77	78	78	78	78	78
Normal Parameters ^{a,b} Mean	15.6761	4.5900	3.4435	1.3889	.1031	19.5590
Std. Deviation	9.0881	6.8747	7.4743	2.6253	.17963	14.8286
Most Extreme Absolute	.419	.400	.368	.316	.283	.160
Differences Positive	.419	.400	.368	.316	.282	.160
Negative	-.351	-.393	-.323	-.299	-.283	-.114
Kolmogorov-Smirnov Z	3.678	3.533	3.252	2.792	2.500	1.406
Asymp. Sig. (2-tailed)	.000	.000	.000	.000	.000	.038

a. Test distribution is Normal.

b. Calculated from data.

Table 4.8 Analysis result of normal distribution test for Zn in soil, and sugarcane samples**One-Sample Kolmogorov-Smirnov Test**

	Total Zn in soil	BCR1	EDTA	DTPA	CaCl ₂	Total Zn in Sugarcane
N	77	78	78	78	78	78
Normal Parameters ^{c,d} Mean	528.4805	132.0832	66.1938	19.5052	2.0902	8.4566
Std. Deviation	856.4983	486.5126	148.8432	33.6889	6.2346	6.4599
Most Extreme Absolute	.335	.406	.330	.316	.396	.187
Differences Positive	.335	.406	.292	.272	.323	.187
Negative	-.279	-.393	-.330	-.283	-.369	-.160
Kolomogorov-Smirnov Z	2.938	3.589	2.918	2.499	3.256	1.653
Asymp. Sig. (2-tailed)	.000	.000	.000	.000	.000	.008

c. Test distribution is Normal.

d. Calculated from data.

Table 4.9 Spearman Correlation Coefficient between total Cd in soil and extractable Cd from four extraction procedures (BCR1, EDTA, DTPA and CaCl₂)**Correlations**

		Total Cd in soil	BCR1	EDTA	DTPA	CaCl ₂	
Spearman's rho	Total Cd in Soil	Correlation coefficient	1.000	.663**	.935**	.628**	.227
		Sig. (2-tailed)	.	.000	.000	0	.047
		N	77	77	77	77	77
	BCR1	Correlation coefficient	.663**	1.000	.736**	.499**	.223
		Sig. (2-tailed)	.000	.	.000	.000	.050
		N	77	78	78	78	78
	EDTA	Correlation coefficient	.935**	.736**	1.000	.675**	.305**
		Sig. (2-tailed)	.000	.000	.	.000	.007
		N	77	78	78	78	78
	DTPA	Correlation coefficient	.628**	.499**	.675**	1.000	.319**
Sig. (2-tailed)		.000	.000	.000	.	.004	
N		77	78	78	78	78	
CaCl ₂	Correlation coefficient	.227	.223	.305**	.319**	1.000	
	Sig. (2-tailed)	.047	.050	.007	.004	.	
	N	77	78	78	78	78	

**.Correlation is significant at the 0.01 level (2-tailed)

From the results showed, the significant positive ($p < 0.01$) correlation of total Cd in soil between BCR1, total Cd-EDTA, and total Cd-DTPA were existed ($r = 0.663^{**}$, $r = 0.935^{**}$ and $r = 0.628^{**}$, respectively), meaning that these three single extractants were correlated with the total Cd in soil. EDTA showed the highest correlation coefficients, indicating the highest correlated suitable method under this study as compared to other three extractants (BCR1, DTPA and CaCl_2). However, the correlation was non-significant and weak positive between total Cd- CaCl_2 was obtained ($r = 0.227$).

Table 4.10 Spearman Correlation Coefficient between total Zn in soil and extractable-Zn from four extraction procedures (BCR1, EDTA, DTPA and CaCl_2)

Correlations

			Total Zn in soil	BCR1	EDTA	DTPA	CaCl_2
Spearman's rho	Total Zn in Soil	Correlation coefficient	1.000	.328**	.619**	.392**	-.053
		Sig. (2-tailed)	.	.004	.000	.000	.646
		N	77	77	77	77	77
	BCR1	Correlation coefficient	.328**	1.000	.772**	.500**	.310
		Sig. (2-tailed)	.004	.	.000	.000	.006
		N	77	78	78	78	78
	EDTA	Correlation coefficient	.619**	.772**	1.000	.632**	.479**
		Sig. (2-tailed)	.000	.000	.	.000	.000
		N	77	78	78	78	78
	DTPA	Correlation coefficient	.392**	.500**	.632**	1.000	.515**
		Sig. (2-tailed)	.000	.000	.000	.	.000
		N	77	78	78	78	78
	CaCl_2	Correlation coefficient	-.053	.310**	.479**	.515**	1.000
		Sig. (2-tailed)	.646	.006	.000	.000	.
		N	77	78	78	78	78

**Correlation is significant at the 0.01 level (2-tailed)

For Zn, the correlation analysis between the total Zn concentration in soil and the extractable Zn by four single extractants (BCR1, EDTA, DTPA and CaCl_2) were also investigated and shown in Table 4.10 All three different extraction procedures showed significant positive ($p < 0.01$) correlations at 99% confidence level (except for in case of total Zn- CaCl_2). Results showed the same trend as for Cd in

which the positive significant relationships between total Zn and BCR1, total Zn-EDTA and total Zn-DTPA were obtained ($r = 0.328^{**}$, 0.619^{**} and 0.392^{**} , respectively) indicating that total Zn is correlated with these three extractants with the EDTA is the most ($r = 0.619^{**}$). However, this is not the case for total Zn concentration and CaCl_2 ($r = -0.053$) which showed non-significant and weak negative correlation, meaning that correlation has not been found between total Zn and CaCl_2 .

As mentioned earlier, the most important parameter indicating the availability of metals in soil is the accumulation of those metals in plant. Thus, the correlation analysis of the total amount of metals present in the sugarcane and available Cd and Zn concentration in soil from different extraction methods was carried out (see Table 4.11).

Table 4.11 Spearman Correlation Coefficient between total Cd in sugarcane (root, underground stem, bagasse, juice, top and leaves) and extractable Cd from four extraction procedures (BCR1, EDTA, DTPA and CaCl_2)

Correlations

			Total Cd in Sugarcane	BCR1	EDTA	DTPA	CaCl_2
Spearman's rho	Total Cd in Sugarcane	Correlation coefficient	1.000	.459**	.376**	.191	.146
		Sig. (2-tailed)	.	.000	.001	.094	.203
		N	78	78	78	78	78
	BCR1	Correlation coefficient	.459**	1.000	.736**	.499**	.223
		Sig. (2-tailed)	.000	.	.000	.000	.050
		N	78	78	78	78	78
	EDTA	Correlation coefficient	.376**	.736**	1.000	.675**	.305**
		Sig. (2-tailed)	.001	.000	.	.000	.007
		N	78	78	78	78	78
	DTPA	Correlation coefficient	.191	.499**	.675**	1.000	.319**
Sig. (2-tailed)		.094	.000	.000	.	.004	
N		78	78	78	78	78	
CaCl_2	Correlation coefficient	.146	.223	.305**	.319**	1.000	
	Sig. (2-tailed)	.203	.050	.007	.004	.	
	N	78	78	78	78	78	

**Correlation is significant at the 0.01 level (2-tailed)

The results showed significant positive ($p < 0.01$) correlation ($r = 0.459^{**}$) with Cd-BCR1 and Cd-EDTA was obtained ($r = 0.376^{**}$) whereas, non-significant and weak positive correlations were existed between total Cd in sugarcane with DTPA and CaCl_2 extraction procedures ($r = 0.191$ and $r = 0.146$, respectively). This suggests that the extractable Cd in sugarcane parts was correlated with BCR1 and EDTA but for other two extraction procedures (DTPA and CaCl_2) were not correlate well.

Alternatively, the extractable Zn in sugarcane parts (root, underground, bagasse, juice, top and juice) showed significant positive correlations with BCR1, Zn-EDTA, Zn-DTPA and Zn- CaCl_2 ($r = 0.554^{**}$, 0.583^{**} , 0.304^{**} and 0.364^{**} , respectively) as shown in Table 4.12.

Table 4.12 Spearman Correlation Coefficient between total Zn in sugarcane (root, underground stem, bagasse, juice, top and leaves) and four extraction procedures (BCR1, EDTA, DTPA and CaCl_2)

Correlations

			Total Zn in Sugarcane	BCR1	EDTA	DTPA	CaCl_2
Spearman's rho	Total Zn in Sugarcane	Correlation coefficient	1.000	.554**	.583**	.304**	.364**
		Sig. (2-tailed)	.	.000	.000	.007	.001
		N	78	78	78	78	78
	BCR1	Correlation coefficient	.554**	1.000	.772**	.500**	.310
		Sig. (2-tailed)	.000	.	.000	.000	.006
		N	78	78	78	78	78
	EDTA	Correlation coefficient	.583**	.772**	1.000	.632**	.479**
		Sig. (2-tailed)	.000	.000	.	.000	.007
		N	78	78	78	78	78
	DTPA	Correlation coefficient	.304**	.500**	.632**	1.000	.515**
Sig. (2-tailed)		.007	.000	.000	.	.000	
N		78	78	78	78	78	
CaCl_2	Correlation coefficient	.364**	.310**	.479**	.515**	1.000	
	Sig. (2-tailed)	.001	.006	.000	.000	.	
	N	78	78	78	78	78	

**Correlation is significant at the 0.01 level (2-tailed)

In the present study, the results revealed that the metals (Cd and Zn) extracted with BCR1 and EDTA extraction procedure have shown most efficient extractant for bioavailability of Cd and Zn contaminated in this area. Similarly, BCR1 showed good correlation between the the BCR1 extractable Cd and Cd content of wheat roots (Feng et al., 2005). While, the correlation of DTPA and CaCl_2 extraction procedures were relatively lower for assessing bioavailability of Cd and Zn contaminated in this area. Nyamanagara and Mzezewa (1999) also reported the use of EDTA-extractable metal as an indication of bioavailable fraction in sewage sludge contaminated area. Recently, Gupta and Sinha (2006) found better correlation between plant grown on soil amended with tannery sludge and EDTA extractable Cd concentration than DTPA and CaCl_2 extractable. Barzegar et al. (2003) concluded that no significant relationships were found between DTPA extractable concentration in soil and amounts of this metal in sugarcane. According to many previous studies, the use of CaCl_2 extraction procedure seems to be the most suitable method for bioavailability of metal. Although easily exchangeable metals can be extracted with CaCl_2 , it exerts a weak competition for adsorption sites of organic matter and oxide surfaces (Novozamsky et al., 1993). This may be the reason why correlation coefficient not well as support our result in which of CaCl_2 extraction procedure.