Comparison of extractability of Cd, Cu, Pb and Zn with sequential extraction in contaminated and non-contaminated soils

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ABSTRACT: Various extraction procedures were employed for measuring extractable concentrations of potential toxic elements in soil. The extractability of Cd, Cu, Pb and Zn in four contaminated and four non-contaminated soils of Japan, was compared by single extraction ($CaCl_2$, DTPA, NH_4Cl, 0.1 M HCl and 1 M HCl) and sequential extraction procedures [(six operationally defined chemical phases, viz. water soluble (F1), exchangeable (F2), carbonate (F3), oxide (F4), organic (F5) and residual (F6) fractions)]. Extractability of metals from soils samples varied depending on metals and/or extractants used. Among the extractants, 1 M HCl extracted the largest proportion of Cd (79 to 96% of total), Cu (61 to 83%), Pb (51 to 99%) and Zn (23 to 52%) from soils followed by 0.1 M HCl, NH_4Cl, DTPA and $CaCl_2$. In all the extractants, the proportion of extractability of metals was higher in the contaminated soils than the non-contaminated soils. Regardless of soils and extractants, relative extractability was higher for Cd as compared to other three metals. The use of 1 M HCl may be recommended for first-level screening of soil contamination with heavy metals. The other four weak extractants are believed to provide a better assessment of bioavailable/mobile metals content in soils than 1 M HCl extractant. However, 0.1 M HCl mobilized all four metals irrespective of soil types, therefore, might be the best choice if only one extractant is to be used. The sequential extraction procedures showed 22 to 64% of total Cd was in the mobile fraction (sum of F1 to F3), while the corresponding values for Cu, Pb and Zn in this fractions were 2 to 23% suggesting higher mobility of Cd than other three metals. The single extraction procedures are simple and easy to perform and obtained results are comparable with sequential extraction procedure.

Key words: *Contaminated soils, chemical extraction, heavy metals, single and sequential extraction*

INTRODUCTION

In many industrial countries, soils are affected by mine waste disposal, acid deposition and sewage sludge use that could provide large inputs of pollutants and especially heavy metals to the soils. The impact of contamination on the environment should be of scientific concern, in order to minimize the threat of soil and groundwater contamination (Matos, *et al*., 2001). Heavy metal contamination of arable soils through industrial activities is a serious problem in Japan. Rice fields at different locations of Japan have suffered by Cd and other heavy metals contamination. Asami, *et al*. (1994) investigated some contaminated and non-contaminated Japanese soils and found that

the total Cd, Cu, Pb and Zn concentrations were in the range of 0.7 to 8.8, 33 to 81, 31 to 237 and 118 to 738 mg/kg, respectively, which indicated large anthropogenic contribution of metals in soils. A variety of in-situ (on-site) and ex-situ remedial methods have been used/proposed for the restoration of soils contaminated with heavy metals, which warrant investigating the extractability of heavy metals in soils. Chemical extraction procedures are able to predict the changes in the heavy metal mobility or bioavailability in soils after amendments for metal remediation (Singh, 2006).

A large number of extracting solutions have been used to assess plant available trace elements (Gupta and Aten, 1993; He and Singh, 1993). Single extractants may broadly be divided into three main classes: (i) weak

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replacement of ion salts (MgCl$_2$, CaCl$_2$, NH4NO$_3$), (ii) dilute solutions of either weak acids (acetic acid) or strong acids (HCl, HNO$_3$) and (iii) chelating agents (DTPA, EDTA). The first type of extractants are able to release into solution metals which are associated with the exchange sites on the soil solid-phase and hence can be considered as bioavailable (McLaughlin, *et al.*, 2000). The chelating agents, such as DTPA and EDTA, form complexes with free metal ions in solution and thus reduce the activities of the free metal ions in solution. The unbuffered salt solutions, (CaCl$_2$ and NH$_4$Cl), is rapid and simple procedure to extract bioavailable metals (Beckett, 1989). In some cases, salt solution does not represent plant available metals (Gupta and Aten, 1993). Use of 0.1 M HCl solution (CSTPA, 1980) may reflect bioavailability of metals. ANZECC and ARM-CANZ (2000) recommend the use of 1 M HCl-extractable metal content for first-level screening-the rational being that total metal analysis include all non-residual metals as well as metals in the silicate mineral matrix, which are not available. We assume 1M HCl will not attack the silicate matrix, results from such extraction procedure may be closer to bioavailable fraction than total analysis. The procedures of sequential extraction define more precisely the individual fractions of elements in soil. This procedure requires three to seven steps. Consequently, it requires skilled personal, adequate analytical facilities and is also time consuming. However, complementary use of both single and sequential extraction methods may provide information useful for both short-term and long -term biological uptake of heavy metals (Singh, 2006).

In the other part of the study (Kashem, *et al.*, 2006) the chemical fractionation of Cd, Cu, Pb and Zn, is investigated but in the present study we made a comparison of individual fractions of sequential fractionation procedure with single extractants. Therefore, this study was designed to: (i) investigate the extractability of Cd, Cu, Pb and Zn in four contaminated and four non-contaminated soils from Japan, and (ii) compare the metal extractability by single extractants with the fractions of sequential extraction procedure.

MATERIALS AND METHODS
Soil samplings
Four contaminated and four non-contaminated surface soil samples (0 to 15 cm) used in this study were collected from different locations of Japan in November 2005. Four contaminated soil samples were Miyagi-1 & 2, Akita and Gumma and non-contaminated soils were Takizawa, Rokuhara, Hachirogata and Isawa. The Miyagi-1 & 2, and Akita soils were contaminated with heavy metals from irrigation with river water passing through mines after different types of industrial operations. The source of Gumma soil was the Annaka Zn mining industry The Gumma soil was located at the Gumma Prefecture of Kanto district and other seven soils were from different Prefectures of Thohoku district (Table 1). Rice and vegetables are the common agricultural crops. A part of each sample was air-dried, ground and passed through a 2 mm stainless steel screen before use. Some important characteristics of the soils used in this study are presented in Table 1. All analytical work of this study was performed at the Laboratory of Plant Physiology and Nutrition, Iwate University, Japan.

Soil analysis
Soil pH was measured in a 1:2 soil/water suspension. Soil organic carbon (OC) was determined by wet oxidation methods of Walkley and Black (1934). Cation exchange capacity (CEC) was determined by extraction with 1 M NH$_4$OAc (pH 7.0) (Soil Survey Laboratory Staff, 1992). The hydrometer method (Bouyoucos, 1962) was used for the particle size distribution. Total concentration of metals (Cd, Cu, Pb and Zn) in the soils was determined by the HITACHI 170-30 atomic absorption spectrophotometry (AAS) after digestion with nitric-perchloric acid mixture (3:1).

Five different extraction procedures were used to establish the extractability and to predict the phytoavilability of heavy metals in soils (Table 2). Each extraction procedure was performed in duplicate for each sample in 50 mL polycarbonate centrifuged tubes except 1 M HCl extract. All the extracts were centrifuged for 10 minutes at 3000 rpm, and the supernatant filtered. In case of 1 M HCl, the supernatant was passed through a membrane filter (0.5 µm). A brief summary of the sequential extraction procedure used (Kashem and Singh, 2001) is discussed here. Two grams of soil, 2-mm sieve fraction, was placed in a 50 mL poly carbonate centrifuge tube and following extractions were performed sequentially.
(F1): Sample extracted with 20 mL of deionized water for 1 h at 20 °C on a rolling table.

(F2): The residue from F1 extracted with 20 mL of 1 M NH_4OAc, pH 7 for 2 h at 20 °C on a rolling table.

(F3): Residue from the F2 extracted with 20 mL of 1 M NH_4OAc, pH 5 for 2 h at 20 °C on a rolling table.

(F4): Residue from F3 extracted with 20 mL of 0.04 M hydroxylamine hydrochloride ($NH_2OH.HCl$) in 25 % acetic acid (v/v) at pH 3, reaction time 6 h in a water bath at 80 °C with occasional shacking.

(F5): Residue from F4 extracted with 15 mL of 30 % H_2O_2 (adj. pH 2), reaction time 5.5 h in a water bath at 80 °C with occasional shaking. After cooling, 5 mL of 3.2 M NH_4OAc in 20 % (v/v) HNO_3 was added; sample was shaken on a rolling table for 0.5 h at 20 °C and finally diluted to 20 mL with water.

(F6): The residue from F5 fraction, extracted with 7 M HNO_3, reaction time was 6 h in a water bath at 80 °C with occasional shaking.

Duplicates analysis was performed for each sample. Metals present in extracts were determined by HITICHI 170-30 atomic absorption spectrophotometry (AAS). The results were statistically evaluated by Pearson correlation coefficients and the descriptive statistics was performed by Minitab program (Minitab Inc. 1992).

RESULTS

Soil properties

Soil pH ranged from 4.7 in the Miyagi-2 soil to 6.5 in the Isawa soil. Relatively lower values of pH in some of these soils may be assigned to acid effluent coming from near by industrial operations as well as high humus content (Table 1). Soil textural class varied from sand to sandy clay loam. The organic matter (OM) content and cation exchange capacity (CEC) varied considerably in these soils, for example, the soil from Rokuhara had 11.0%, while Isawa soil had only 0.3% of OM. Cation exchange capacity varied from 9 to 45 cmol/kg (Table 1) and it was positively correlated ($p<0.01$) with OM content. The total concentrations of Cd, Cu, Pb and Zn in the soils varied from 0.7 to 12.5, 23 to 133, 19 to 359 and 90 to 704 mg/kg, respectively. Generally the concentrations of most metals were 5 to 18 fold higher in the contaminated soils than the non-contaminated soils (Table 1).

Metals extractability

As expected, 1 M HCl was the most efficient extractant (Table 3). The recoveries (% of total) of Cd varied from 79 to 83% in the non-contaminated soils and from 83 to 96% in the contaminated soils. There was only a small difference (<10%) in Cd extractability between 1 M HCl and 0.1 M HCl extractant in contaminated soils, however, the extractability of Cd with 0.1 M HCl was substantially lower (up to 45%) in non-contaminated soils (<1 mg/kg). Amount of Cd extracted by NH_4Cl was 21 to 90%. DTPA extracts metals that are thought to represent the plant-available fractions (Lindsay and Norvell, 1978; Mellum, *et al.*, 1998). DTPA forms soluble complexes with metals, reducing the activity in the soil solution; therefore, ions desorbed from the surface and enter into the solution.

Table 1: Sampling locations and physio-chemical characteristics of the soils used

Soils	Prefecture (Location)	District	Textural class	pH	OC (%)	CEC (cmol/kg)	[a]Total metal concentration (mg/kg)			
							Cd	Cu	Pb	Zn
Miyagi-1 (C)	Miyagi	Thohoku	Sandy loam	5.6	3.3	25.5	5.4	35	359	508
Miyagi-2 (C)	Miyagi	Thohoku	Sandy C. loam	4.7	2.4	22.4	7.2	24	238	704
Akita (C)	Akita	Thohoku	Sandy loam	5.0	5.3	19.4	5.0	133	194	470
Gumma (C)	Gumma	Kanto	Loamy sand	4.8	2.9	9.3	12.5	70	193	671
Takizawa (Nc)	Iwate	Thohoku	Loamy sand	4.9	10.3	31.4	1.0	30	19	90
Rokuhara (Nc)	Iwate	Thohoku	Sandy loam	5.2	11.0	45.0	0.9	30	37	138
Hachirogata (Nc)	Akita	Thohoku	Sandy C. loam	5.8	4.1	37.1	0.7	23	22	107
Isawa (Nc)	Iwate	Thohoku	Sand	6.5	0.3	8.6	0.7	30	34	123

C-Contaminated soil; Nc-Non-contaminated soil; OM-Organic matter; CEC-Cation exchange capacity;

[a] Total concentration of metals extracted with nitric-perchloric acid

Table 2: Summary of the extraction methods used in this study

Extractants	Ratio (w/v), Time, Temp.	Pool	Reference
0.01 M CaCl$_2$	1:5, 16 h, room temperature	Neutral salt soluble/exchange	Ahnstrom and Parker (1999)
DTPA	1:2, 2 h, room temperature	Chelating extractable	Lindsay and Norvell (1978)
1 M NH$_4$Cl	1:6, 16 h, room temperature	Neutral salt soluble/exchange	Krishnamurti *et al.* (1995)
0.1 M HCl	1:10, 0.5 h, room temperature	Weak acid extractable	Counc. Soil Test. Plant Anal. (1980)
1 M HCl	1:33.3, 2 h, room temperature	Weak acid extractable	ANZECC and ARMCANZ (2000)

Table 3: Heavy metal concentrations in soils after individual extractions (mg/kg) and percent of total in parentheses

Extractants	Non-contaminated soils			Contaminated soils		
	Range (min. to max)	Mean	SD	Range (min. to max)	Mean	SD
			Cd			
CaCl$_2$	<0.1 (3-7)	<0.1 (5)	± 0.01 (2)	0.3-3.2 (7-30)	1.7 (21)	± 1.2 (10)
DTPA	<0.1-0.3 (6-37)	0.1 (14)	± 0.09 (16)	1.5-6.4 (29-63)	3.8 (48)	± 2.2 (14)
NH$_4$Cl	<0.1 -0.4 (21-58)	0.3 (37)	± 0.11 (17)	2.9 -8.1 (59-90)	5.4 (72)	± 2.4 (14)
0.1M HCl	0.2 -0.3 (23-45)	0.3 (33)	± 0.03 (9)	4.1 -9.1 (73-92)	6.1 (83)	± 2.3 (8)
1M HCl	0.6-0.8 (79-83)	0.7 (81)	± 0.11 (2)	4.2 -10 (83-96)	6.6 (89)	± 2.8 (6)
			Cu			
CaCl$_2$	ND	ND	ND	0.1-0.2	0.1	± 0.1
DTPA	ND -3.1 (ND -16)	1.2 (5)	± 3.4 (15)	3.3-15 (11-18)	8.6 (14)	± 5 (3)
NH$_4$Cl	ND -0.1 (ND-<1)	0.1 (0.3)	± 0.1 (<1)	0.2-0.4 (0.2-1)	0.3 (<1)	± 0.1
0.1M HCl	1.0-7.5 (3-33)	2.9 (12)	± 3.1 (14)	7.0-44 (27-33)	20 (29)	± 17 (3)
1M HCl	18-20 (61-78)	19 (67)	± 0.9 (8)	17-109 (60-83)	49 (70)	± 43 (9)
			Pb			
CaCl$_2$	ND	ND	ND	0.2-0.5	0.3	± 0.1
DTPA	0.1-2.0 (0.3-5.4)	0.9 (3)	± 0.9 (3)	0.6-6.0 (0.3-3)	3.3 (1.4)	± 3 (1)
NH$_4$Cl	ND	ND	ND	3.7-26 (2 -11))	19 (7.4)	± 11 (4)
0.1M HCl	1.2-4.1 (6-13)	2.7 (10)	± 1.2 (3)	36-76 (18-32)	60 (25)	± 17 (6)
1M HCl	11-23 (51-65)	15 (54)	± 5.4 (13)	137-239 (67-99)	185 (77)	± 43 (14)
			Zn			
CaCl$_2$	2.4-24 (2-17)	8.2 (6)	±11 (4)	4-78 (1-12)	45 (7)	± 34 (5)
DTPA	0.2-16 (0.2-12)	3.5 (4)	±7.6 (3)	17-150 (4--22)	119 (16)	± 58 (9)
NH$_4$Cl	0.5-11 (0.5- 8)	5.5 (3)	±4.7 (2)	18-185 (4-26)	101 (19)	± 72 (10)
0.1M HCl	2.1-26 (2-19)	11 (9)	±11 (4)	58-208 (12-31)	151 (25)	± 68 (8)
1M HCl	26-45 (23- 33)	34 (29)	±8.6 (2)	237-321 (45-52)	282 (48)	± 38 (3)

Range = minimum to maximum; SD= Standard deviation; ND= not detected

The relative extractability of Cd with DTPA was 6 to 37% in non-contaminated soils and from 29 to 63% in contaminated soils. The CalCl$_2$ extracted relatively smaller amount (3-30%) of total Cd than those of other extractants (Table 3).

As soils were not contaminated with Cu, the extractability of Cu was relatively low compared to Cd, irrespective of the extractant used. However, 1 M HCl extracted highest proportion of Cu (60 to 83%), followed by 0.1 M HCl (3 to 33%) and DTPA (up to 18%) extractant (Table 3). Chloride salts, such as NH$_4$Cl and CaCl$_2$ were found to be very weak extractants to release Cu (~ 1%). For Pb, only two acid (HCl) extractants recovered considerable amount from all soils, while 1 M HCl dominated (51- 99%) over 0.1 M HCl (6- 32%) as

it did for Cd and Cu. The efficiency of other three extractants was not consistent and found to be unsatisfactory for non-contaminated soils (<38mg/kg) (Table 3). Zn was released with all extractants in varying amounts similar to Cd. The efficiency of Zn extraction was highest with 1 M HC (23 to 52%) and lowest with CaCl$_2$ (1 to 17%). Overall, the extractability of Zn with different extractants was in the order: 1 M HCl > 0.1 M HCl > NH$_4$Cl > DTPA > CaCl$_2$ as was the case for Cd, Cu and Pb with minor exceptions (Table 3).

Comparison of metal extractability of single extractants with sequential extraction

The chemical fractionation of Cd, Cu, Pb and Zn in contaminated and non-contaminated soil fractions was

determined by the multi step sequential extraction procedures of Kashem, *et al.* (2006). This method fractionates metals into six operationally defined fractions such as: water soluble (F1), exchangeable (F2), carbonate-bound (F3), oxide-bound (F4), organically-bound (F5) and residual (F6) fractions. Cadmium was associated mostly in first three fractions (F1-F3) (mobile), while Cu, Pb and Zn in the last three (F4-F6) fractions. The mobile fraction of metals varied from 22 to 64% for Cd, 2 – 4% for Cu, 8 – 15% for Pb and 4 – 23% for Zn with the higher values in the contaminated soils as compared to non-contaminated soils. In this part, we compared the average efficiency of single extraction procedures with element recoveries in F1, F2, F3, F4, F5 and F6 fractions of sequential extraction (Fig. 1).

Evidently, 1 M HCl was the most efficient agent for releasing Cd (85%), followed by Cu (69%), Pb (65%) and Zn (39%) from these soils. These results indicate that 1 M HCl extracted most of the non residual (sum of F1 to F5) plus part of residual (F6) fraction of Cd, Cu and Pb, while this agent was not able to dissolve quantitatively the Zn from organic (F5) and F6 fraction (Fig. 1). Metals extracted with 0.1 M HCl exceeded the mobile (sum of F1, F2 and F3) fraction for all metals. On other hand, the efficiency of NH_4Cl was found similar to 0.1 M HCl for Cd extraction. Ammonium chloride extractable Pb and Zn were lower than Cd but the extractable portions of the former elements were equivalent to their mobile fraction in the soils. Ammonium chloride had very poor extractability for Cu because Cu was mostly associated with residual and organic fraction (Kashem, *et al.* 2006). The chelating agent (DTPA) also played significant role to extract metals equivalent to mobile fractions except Pb (Fig. 1). DTPA extracted very small amount (2%) of Pb that represents water soluble and part of exchangeable fraction of Pb. Calcium chloride ($CaCl_2$) was not found suitable for Cu and Pb extraction; however it extracted 13% of Cd and 7% of Zn that represent exchangeable (F1 + F2) fraction of the sequential extraction procedure.

Interrelationships among extractable, mobile, total metal concentrations, soil pH and CEC

A significant positive relationship ($r = 0.26$ to 0.98; $p < 0.05$ and < 0.001) between extractable, mobile and total Cd, Pb and Zn was observed (Table 4). As soil was not contaminated with Cu, hence its relationships were not found consistent and significant (Table 4). Soil pH and cation exchange capacity (CEC) showed significant negative relationships with extractable, mobile and total metal concentrations except extractable Cu. Soil organic matter did not show any significant relationship and hence the results are not reported. A similar relationship of pH and CEC with mobile fraction of sequential extraction was found in the other part of this study (Kashem, *et al.*, 2006).

Table 4: Pearson correlation coefficients between concentration of metals, pH and CEC in soil

	E.Cd	M.Cd	T.Cd	E.Cu	M.Cu	T.Cu	E.Pb	M.Pb	T.Pb	E.Zn	M.Zn	T.Zn	pH	CEC
E.Cd		0.89	0.88	0.35	0.48	0.26	0.63	0.77	0.63	0.90	0.83	0.85	-0.50	-0.61
M.Cd	0.89		0.98	NS	0.52	0.28	0.40	0.85	0.73	0.76	0.95	0.96	-0.56	-0.68
T.Cd	0.88	0.98		NS	0.62	0.38	0.38	0.86	0.68	0.71	0.88	0.92	-0.55	-0.71
E.Cu	0.35	NS	NS		0.45	0.49	0.58	NS	NS	0.50	NS	NS	NS	NS
M.Cu	0.48	0.52	0.62	0.45		0.93	0.26	0.52	0.54	0.33	0.31	0.58	-0.31	-0.58
T.Cu	0.26	0.28	0.38	0.49	0.93		NS	NS	0.28	NS	NS	0.36	-0.30	-0.45
E.Pb	0.63	0.40	0.38	0.58	0.26	NS		0.44	0.45	0.85	0.42	0.43	NS	-0.25
M.Pb	0.77	0.85	0.86	NS	0.52	NS	0.44		0.88	0.69	0.89	0.84	-0.48	-0.57
T.Pb	0.63	0.73	0.68	NS	0.54	0.28	0.45	0.88		0.64	0.80	0.84	-0.32	-.046
E.Zn	0.90	0.76	0.71	0.50	0.33	NS	0.85	0.69	0.64		0.76	0.75	-0.41	-0.44
M.Zn	0.83	0.95	0.88	NS	0.31	NS	0.42	0.89	0.80	0.76		0.92	-0.47	-0.54
T.Zn	0.85	0.96	0.92	NS	0.58	0.36	0.43	0.84	0.84	0.75	0.92		-0.55	-0.63
pH	-0.50	-0.56	-0.55	NS	-0.31	-0.30	NS	-0.48	-0.32	-0.41	-0.47	-0.55		NS
CEC	-0.61	-0.68	-0.71	NS	-0.58	-0.45	-0.25	-0.57	-0.46	-0.44	-0.54	-0.63	NS	

All values in the Table are significantly different at p d" 0.05 probability level; NS = non significant; E = extractable metal concentration measured by single extraction procedures; T=total metal concentration extracted with nitric-perchloric acid: M= mobile fraction of metal (sum of F1 to F3) extracted from Kashem, *et al.,* (2006); CEC= cation exchange capacity.
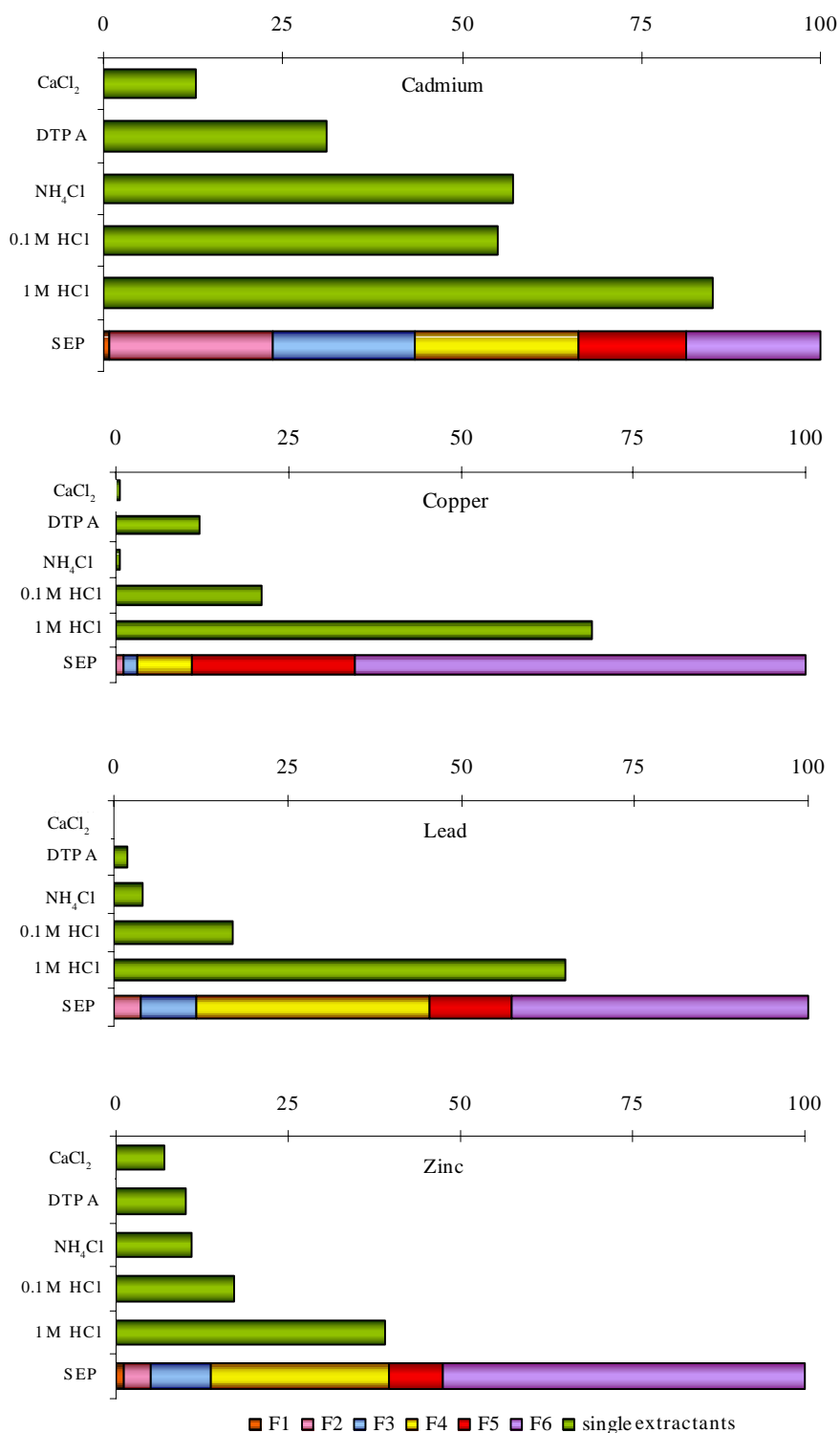
Fig. 1: Mean proportion of Cd, Cu, Pb and Zn in each single extractants compared with various fractions (% of total) of soils. F1=water soluble, F2=exchangeable, F=carbonate-bound, F4=oxide-bound, F5=organic-bound, F6=residual. SEP=sequential extraction procedure

DISCUSSION AND CONCLUSION

Elevated concentrations of total and extractable metals in the Miyagi, Akita and Gumma soils were due to anthropogenic sources of metal additions to these soils. The sources of metal contamination for Miyagi 1 and 2, and Akita soils were from irrigation with river water passing through mines after different types of industrial operations, while for the Gumma soil, the Annaka zinc mining industries contributed to metal contamination. The proportion Cd and Zn measured by different single extractants represented more than one soil metal fraction determined by the sequential extraction procedure (Kashem, *et al.*, 2006). The total metal analysis (HNO_3:$HClO_4$) include all non-residual as well as metals present in silicate mineral matrix, the later fraction (silicate bound) is not considered bioavailable. In our study, 1 M HCl dissolved all non-residual and part of residual Cd, Cu and Pb and hence it overestimated the bioavailability because the residual fraction is not considered as plant available (Sims and Kline, 1991; Narwal and Singh, 1998). The exchangeable fraction is generally considered immediate nutrient reservoir for plants (Narwal and Singh, 1998). Metals associated with carbonates would be susceptible to pH changes, may be regarded as potentially phytoavailable. Heavy metals present in other chemical forms, such as oxides, organic and residual with very low solubility and high stability for biological activity, would not have direct bearings on their uptake by plants (Xian, 1989). On the other hand, 1 M HCl showed less variability among soils in the proportion extracted as compared to other extractants, such as 0.1 M HCl, NH_4Cl, DTPA and $CaCl_2$. The later extractants may provide more realistic estimate of bioavailability than 1 M HCl. Although metal extractability by 1 M HCl in the sediment samples from Sydney Harbour was high (>80% for Zn and Pb; >60% for Cd and Cu) even then McCready, *et al.*(2003) concluded that 1 M HCl may have application in evaluating potential bioavailability differences for these metals among sediments. The significant positive relationship between metals extracted with single extractants and mobile fraction of metals from sequential extraction procedure suggests that both sequential and single extractants are useful to predict metals bioavailability (Table 4). The strong negative relationship of extractable metal concentration and soil pH indicates that metal extractability decreased with increasing soil pH, as observed by many other investigators (Singh, *et al.*, 1995; Evans, *et al.*, 1995). They found that DTPA and NH_4NO_3 extractable metals decreased with increasing soil pH. In general, soil pH and CEC was found to be an important predictors of

metal extractability (Rieuwerts, *et al.*, 2006), retention and movement in soils (Matos, *et al.*, 2001; Udom, *et al.*, 2004). Strong positive correlations of total Cd, Pb and Zn, with their extractable and mobile fractions and each other suggest overloading of geochemical system by contamination with these metals from anthropogenic sources. There was a large variability in metal extraction depending on extractant, metal type and nature of the soil. Weak extractants showed larger variability among soil types with respect to metal content than did by 1 M HCl, and hence they may be used to assess metals bioavailability. Nature of individual metals also need to be considered for selecting extractants as they are not equally suitable for all metals. Metal extractability is higher in the contaminated soils as compared to non-contaminated soils, irrespective of the extractant used indicating greater potential bioavailability of metals in the contaminated soils. The results obtained from sequential extraction indicated that most Cd was associated in the mobile fraction,,while Cu in the organic and residual, Pb and Zn in the oxides and residual fractions. Metals associations with oxides and residual fractions are considered least extractable. It may be stated here that the comparsion of single and sequential extraction procedure provided an interesting insight in extracting mobile/ bioavialble fractions of heavy metals in contaminated and non-contaminated soils in this study.

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