

## Evaluation of Three Sequential Extraction Methods for Fractionation of Zinc in Calcareous and Acidic Soils

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**Abstract:** Zinc (Zn) is essential micronutrient for crop growth. Deficiency of zinc has been frequently detected in soils in Iran. Generally, available form of Zn can be rapidly converted to unavailable form in calcareous soils. The objective of this study was to evaluate three methods for fractionation of Zn in nine surface (0-30 cm) calcareous and acidic soils of Fars, Kerman and Gilan provinces, Iran. The results showed that a major proportion of Zn was present in the residual and carbonate-bound fractions. Zn distribution pattern obtained by Sposito method follow the order residual>>carbonate-bound>organic-bound>exchangeable>adsorbed fractions. For Singh's method, the order was residual>>carbonate-bound>Fe crystalline oxides-bound>Fe amorphous oxides-bound>organic-bound>Mn oxides-bound>exchangeable. For Ma and Uren method the order was residual>>carbonate-bound>Fe-Al oxides-bound>Mn oxides-bound>organic-bound>adsorbed>water soluble. Correlation analysis between soil properties and the Zn fractions showed that Zn fraction distribution pattern depends on soil pH, CCE, OM, CEC and texture.

**Key words:** Sequential extraction, fractionation, calcareous, acidic soils, zinc

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### INTRODUCTION

Zinc (Zn) is an essential metal for the natural growth and reproduction of plants, animals and humans. When the supply of plant-available Zn is insufficient, crop yields are reduced and the quality of crop products is frequently impaired (Alloway, 2003). There are >300 enzymes involved in key metabolic processes in humans which contain Zn and therefore, an adequate Zn intake is essential for normal healthy growth and reproduction (FAO/WHO/IAEA, 1996). Zn in soils is distributed over 5 pools: water soluble pool, present in the soil solution, exchangeable pool, ions bound to soil particles by electrostatic interactions, organically bound pool, ions adsorbed, chelated or complexed with organic ligands, pool of Zn sorbed non-exchangeability into clay minerals and insoluble metallic oxides and pool of weathering primary minerals (Alloway, 2003). Kiekens (1995) reported a typical range of 10-300 mg Zn kg<sup>-1</sup> in soils with a mean of 50 mg Zn kg<sup>-1</sup>. The distribution of Zn in calcareous soil is dependent upon many factors, such as soil pH, minerals, cations and anions in soil solution and Zn carriers (Maftoun and Karimian, 1989).

Kabata-Pendias and Pendias (1992) reported the concentration range of soluble Zn in soils from 4-270 µg L<sup>-1</sup>, much lower than the average total concentrations of 50-80 mg kg<sup>-1</sup> in soils. More than 60%

of arable soils in Iran are zinc shortage with an average yield stagnation of around 50%. The main causes of zinc deficiency in Iran soils are: calcareous soils (mainly >30% CaCO<sub>3</sub>, with an actual range of 16-58% CaCO<sub>3</sub>) with high pH 7.9-8.5, widespread applications of phosphatic fertilizers, high concentrations of bicarbonate (HCO<sub>3</sub><sup>-</sup>) in irrigation water and non-consume of Zn fertilizers.

More than 80% of farmable soils in Iran have <1 mg kg<sup>-1</sup> DTPA extractable Zn (0.7-1.0 mg kg<sup>-1</sup>). Current knowledge suggested that the total content of Zn in soils does not represent available Zn to plants (Symeonides and McRae, 1977) and cannot help to explain the behavior of Zn in the environment either (Tack and Verloo, 1995). In soils, elements of interest may exist in different forms or fractions (Cottenie *et al.*, 1982). Fractionation of metals in soils using a sequential extraction procedure can provide some information, which helps understand their environmental behavior and bioavailability. Usually, the fractions are experimentally, defined as exchangeable, organically complexes, carbonate bound, iron and manganese hydroxides linked and residual fractions (Kabala and Singh, 2001; Kersten and Förstner, 1995). In arid zone soils, carbonate minerals effectively immobilize metals due to large absorption capacity and buffering by high pH, where precipitation takes place (Aydinalp and Katkat, 2004).

Sequential extraction is now a well-established fractionation approach (Ure and Davidson, 2001). Sequential extraction procedures provide important knowledge about the fraction distribution of metals in the soils. In the last few years, several sequential extraction procedures have been developed (McLaren and Crawford, 1973). Sequential extraction usually necessitates from three to seven steps (Tlustos *et al.*, 2005). Different fractions of soil metal vary considerably in bioavailability. The water-soluble and exchangeable forms of metals are considered to be the most available to plants (Shuman, 1991) and metals bound to organic matter are also, found to be potentially available to plants (Iyengar *et al.*, 1981). In recent years, the experimentally defined fractionation procedures have been applied to identify plant-available fractions of trace metals in soils (Sims and Kline, 1991). Filgueiras *et al.* (2002) comprehensively reviewed the sequential extraction schemes for metal fractionation, each reagent involved in these schemes is looked at critically and guidelines for their selectivity and capacity are given. A large variation of fractions with the extraction reagents and operating conditions of sequential extraction producers is evident. The researchers emphasized that small changes in the experimental conditions (e.g., pH, temperature, contact time, solid to extractant volume ratio, particle size and sample pretreatment) can lead to large variations in the fractionation (Tlustos *et al.*, 2005).

A comparison between sequential extraction schemes is helpful for better recognition of capability of the extraction methods and more reasonable explanation of the data (Alvarez *et al.*, 2001; Gworek and Mocek, 2003; Tlustos *et al.*, 2005; Mihaljevic *et al.*, 2003; Tokalioglu *et al.*, 2003; Van Hullebusch *et al.*, 2005; Parat *et al.*, 2003).

The objectives of this study were to compare Zn fractions determined by the sequential extraction schemes of Sposito *et al.* (1982), Singh *et al.* (1988) and Ma and Uren (1995) and to reveal the main factors controlling distribution of zinc in different fractions.

## MATERIALS AND METHODS

**Sampling location and soil characterization:** Nine surface soils (0-30 cm) were collected from the Fars, Kerman and Gilan provinces, Iran, air-dried over night, ground and passed through a 2 mm sieve. Particle size analysis was performed using Hydrometer method (Day, 1965); pH was measured in saturated paste; Cation Exchange Capacity (CEC) was determined using a method of Page *et al.* (1987); percentage of Calcium Carbonate Equivalent (CCE)

was measured by acid neutralization (Salinity Laboratory Staff, 1954); Organic Matter (OM) content was determined using Walkly and Black (1934); plant-available fraction of Zn was determined by means of atomic absorption spectrophotometer (Lindsay and Norvell, 1978). Aqua regia (mixture of HF, HClO<sub>4</sub>, HNO<sub>3</sub> and H<sub>2</sub>SO<sub>4</sub>) was used to determine the total contents of Zn (Ma and Uren, 1997). Soil properties are presented in Table 1.

### Sequential extraction procedures

**Sposito *et al.*'s method (scheme A):** The procedure of Sposito *et al.* (1982) is designed to separate zinc into five operationally defined fractions: exchangeable, sorbed, organic bound, carbonate bound and residual fractions (Table 2). A summary of the procedure is as follows:

**Exchangeable fraction (EX):** Two gram of soil were weighed and placed in a 50 mL polycarbonate centrifuge tube and extracted with 25 mL of 0.5 M KNO<sub>3</sub> for 16 h.

**Sorbed fraction (Sor):** Residue from the exchangeable fraction was extracted with 25 mL of deionized water for 2 h (3 times).

**Organic fraction (Om):** Residue from the sorbed fraction was extracted with 25 mL 0.5 M NaOH for 16 h.

**Carbonate fraction (Car):** Residue from the organic fraction was extracted with 25 mL 0.05 M Na<sub>2</sub>EDTA for 2 h.

**Residual fraction (Res):** Residue from the carbonate fraction extracted with 4 M HNO<sub>3</sub> for 16 h in 80°C.

**Singh *et al.*'s method (Scheme B):** Zn in the soils was separated into seven operationally defined fractions, exchangeable, carbonate bound, organic bound, Mn-oxide bound, amorphous Fe-oxide bound, crystalline Fe-oxide bound and residual fractions:

**Exchangeable fraction (EX):** About 2.5 g of soils were weighed and placed in a 50 mL polycarbonate centrifuge tube and extracted with 10 mL of 1 M Mg(NO<sub>3</sub>)<sub>2</sub> for 2 h.

**Carbonate bound (Car):** Residue from the exchangeable fraction was extracted with 10 mL of 1 M NaOAc (pH = 5) for 5 h.

**Organic fraction (Om):** Residue from the carbonate fraction was extracted with 5 mL 0.7 M NaOCl (pH = 8.5) for 0.5 h in boiling water bath (two cycles of extraction).

Table 1: The chemical and physical properties of the experimental soils

Soil number	Sampling location	Great group	Clay	Silt	OM g kg <sup>-1</sup>	CCE	pH	CEC (Cmol kg <sup>-1</sup> )	Zn-total	Zn-A* mg kg <sup>-1</sup>	Fe-A*	Mn-A*
1	Kerman	HXP**	153	92	0.97	327	8.5	12.7	74.65	0.834	2.84	5.5
2	Kerman	CXP	233	140	2.2	256	8.5	12.3	82.73	0.308	3.61	4.75
3	Kerman	CGD	193	360	11.9	234	8.3	14.6	102.23	0.42	4.45	9.23
4	Kerman	HXP	113	120	4.8	481	8.4	13.9	102.81	1.63	4.99	7.8
5	Kerman	HXF	273	200	1.8	364	8.1	12.8	81.31	0.306	3.55	5.6
6	Fars	CUP	151	400	39	148	7.2	15.34	110.89	0.658	4.96	10.68
7	Fars	CGD	378	320	32.7	164	8	16.20	76.62	0.222	3.88	12.41
8	Gilan	HUF	322	332	74.6	24	6.1	32.43	299.66	1.79	14.82	64.86
9	Gilan	HUF	204	254	40.6	40	5.8	29.3	192.06	1.79	18.3	58.72

\*A: Extracted by DTPA; \*\*HXP: Haploxerepts, CXP: Calcixerepts, CUP: Calciustepts, CGD: Calcigypsid, HXF: Haploxeralfs, HUF: Hapludalfs

Table 2: Comparison of three sequential extraction methods Sposito *et al.* (1982), Singh *et al.* (1988) and Ma and Uren (1995) for fractionation of Zn

Fraction	Soil solution	Extracting solution	Shaking time (h)	Temperature	Symbol
<b>Operating conditions required in the Sposito <i>et al.</i> (1982) scheme (A)</b>					
Exchangeable	2:25	0.5 M KNO <sub>3</sub>	16	20°C	EX
Sorbed*	2:25	Deionized water	2	20°C	Sor
Organic	2:25	0.5 M NaOH	16	20°C	Om
Carbonate	2:25	0.05 M Na <sub>2</sub> EDTA	2	20°C	Car
Residual	2:25	4 M HNO <sub>3</sub>	16	80°C	Res
<b>Operating conditions required in the Singh <i>et al.</i> (1988) scheme (B)</b>					
Exchangeable	2.5:10	1 M Mg (NO <sub>3</sub> ) <sub>2</sub>	2	20°C	EX
Carbonate	2.5:10	1 M NaOAc (pH = 5)	5	20°C	Car
Organic**	2.5:5	0.7 M NaOCL (pH = 8.5)	0.5	Boiling water	Om
Mn-oxide	2.5:25	0.1 M NH <sub>2</sub> OH.HCl (pH = 2)	0.5	Boiling water	Mn OX
Amorphous Fe-oxide	2.5:25	0.25 M NH <sub>2</sub> OH.HCl+0.25 M HCL	0.5	50°C water	FeA OX
Crystalline Fe-oxide	2.5:25	0.2 M (NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> +0.2 M H <sub>2</sub> C <sub>2</sub> O <sub>4</sub> +0.1 M C <sub>6</sub> H <sub>6</sub> O <sub>6</sub>	0.5	Boiling water	FeC OX
Residual		Total zinc (sum of the above six types)***			Res
<b>Operating conditions required in the Ma and Uren (1995) scheme (C)</b>					
Water soluble	1:10	Deionized water	2	20°C	Ws
Sorbed	1:10	1% NaCaHEDTA+1 M NH <sub>4</sub> OAc (pH = 8.3)	2	20°C	Sor
Mn-oxide	1:10	0.2% quino+1 M NH <sub>4</sub> OAc (pH = 7)	2	20°C	Mn OX
Carbonate	1:10	0.5 M NaOAc-0.5 M HOAc (pH = 4.74) soaking 15 h	3	20°C	Car
Organic	1:10	Digested twice in 30% H <sub>2</sub> O <sub>2</sub> (pH 4.74) at 85°C, extracted as for carbonate fraction (1 h)			Om
Fe&Al-oxide	1:10	0.175 M (NH <sub>4</sub> ) <sub>2</sub> C <sub>2</sub> O <sub>4</sub> -0.1 M H <sub>2</sub> C <sub>2</sub> O <sub>4</sub> (pH = 3.25) soaking 15 h	3	20°C	Fe and Al OX
Residual		Total zinc (sum of the above six types)***			Res

\*Three times extraction; \*\*Two times extraction; \*\*\*Deducting the sum of the above six types of extractable Zn from its total concentration

**Mn-oxide bound (Mn OX):** Residue from the organic fraction was extracted with 25 mL 0.1 M NH<sub>2</sub>OH.HCl (pH = 2, adjusted with HNO<sub>3</sub>) for 0.5 h.

**Amorphous Fe-oxide bound (FeA OX):** Residue from the Mn-oxide bound was extracted with 25 mL 0.25 M NH<sub>2</sub>OH.HCl + 0.25 M HCl for 0.5 h at 50°C in water bath.

**Crystalline Fe-oxide bound (FeC OX):** Residue from the amorphous Fe-oxide bound was extracted with 25 mL 0.2 M (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub> + 0.2 M H<sub>2</sub>C<sub>2</sub>O<sub>4</sub> + 0.1 M C<sub>6</sub>H<sub>6</sub>O<sub>6</sub> for 0.5 h at in boiling water bath.

**Residual fractions (Res):** The residual fraction of Zn was calculated by subtracting the sum of the above six extractable Zn fractions from its total concentration in the soils (Total Zn was determined by 4-acid mixture) (Ma and Uren, 1997).

**Ma and Uren method (scheme C):** This method fractionates metals into six operationally defined fractions

such as: water soluble, sorbed, Mn-oxide bound, carbonate bound, organic bound, Fe and Al-oxide bound and residual fractions. The overall sequential extraction procedure is summarized as follows:

**Water soluble (WS):** One gram of soils was weighed and placed in a 50 mL polycarbonate centrifuge tube and extracted with 5 mL of 10 mL of deionized water for 2 h.

**Sorbed fraction (Sor):** Residue from the water soluble was extracted with 10 mL of 1% NaCaHEDTA + 1 M NH<sub>4</sub>OAc (pH = 8.3) for 2 h.

**Mn-oxide bound (Mn OX):** Residue from the sorbed fraction was extracted with 10 mL of 0.2% quinol + 1 M NH<sub>4</sub>OAc (pH = 7) for 2 h.

**Carbonate bound (Car):** Residue from the Mn-oxide bound was extracted with 10 mL of 0.5 M NaOAc-0.5 M HOAc (pH = 4.74) soaking 15 h and shaking 3 h (unclear!).

**Organic bound (Om):** Residue from the carbonate bound, was digested in 5 mL of 30% H<sub>2</sub>O<sub>2</sub> (pH 4.74) at 85°C and extracted by 0.5 M NaOAc-0.5 M HOAc for 1 h and this procedure was repeated in duplicate.

**Fe and Al-oxide bound (Fe and Al OX):** Residue from the organic bound extracted with 10 mL of 0.175 M (NH<sub>4</sub>)<sub>2</sub>C<sub>2</sub>O<sub>4</sub>-0.1 M H<sub>2</sub>C<sub>2</sub>O<sub>4</sub> (pH = 3.25) soaking 15 h and shaking 3 h (unclear!).

**Residual fractions (Res):** The residual fraction of Zn was calculated by subtracting the sum of the above six extractable Zn fractions from its total concentration in the soils (Total Zn was determined by 4-acid mixture) (Ma and Uren, 1997).

In three methods, after each successive extraction, the extracted solution was separated by centrifuging at 3500 rpm for definite time. The supernatant was filtered for analysis of Zn by flame atomic absorption (Shimatzu AA-670G).

**Statistical analysis:** Different statistical analysis such as relationships Zn fractions and soil properties were evaluated by correlation analysis (using Excel and SPSS softwares).

## RESULTS AND DISCUSSION

**Soil characteristics:** A brief summary of soil properties is presented in Table 1. The results show that the pH values of the soils ranged from 5.85-8.57, OM content varied from 0.97-74.63 g kg<sup>-1</sup>, clay with a range of 113.2-378.8 mg kg<sup>-1</sup>, silt with a range of 92.8-400 g kg<sup>-1</sup>, CCE and CEC with ranges 24-481.6 g kg<sup>-1</sup> and 12.37-32.43 Cmol) + (kg<sup>-1</sup>, respectively. The total concentrations of Zn in the soils varied from 74.65-299.66 mg kg<sup>-1</sup>. Plant-available form of Zn, Fe and Mn ranged from 0.222-1.79, 2.84-18.3 and 4.75-64.86, respectively.

**Distribution of the Zn fractions in three sequential extraction procedures:** The Zn fractions obtained by Sposito *et al.* (1982) method (A), Singh *et al.* (1993) method (B) and Ma and Uren method (C) are presented in Table 3-5, respectively. Figure 1-3 shows the relative proportions of Zn in each fraction according to the 3 sequential extraction procedures. The sequential extraction results show that Zn is strongly associated with the residual fraction, which agrees with the observation of many researchers (Abollino *et al.*, 2006; Lu *et al.*, 2003; Ma and Uren, 1995; Obrador *et al.*, 2003). Yasrebi *et al.* (1994) reported that zinc in calcareous soils in Iran was predominantly present in the residual fraction

Table 3: Concentration ( $\mu\text{g g}^{-1}$ ) of soil Zn in individual fractions by Sposito *et al.* (1982) method (each figure is mean of 3 observations)

Number soils	Ex*	Sor	Om	Car	Res	Total
1	ND**	ND	ND	11.44	50.70	67.15
2	ND	ND	ND	9.37	65	74.38
3	ND	ND	1.08	9.21	84.42	94.72
4	ND	ND	0.23	16.8	80.66	97.70
5	ND	ND	ND	11.97	63.83	75.80
6	ND	ND	1.59	7.43	95.54	104.56
7	ND	ND	1.65	7.97	61.87	71.5
8	0.95	ND	4.9	21.66	262.31	289.83
9	0.58	ND	6.65	24.85	152.45	184.56
Mean	0.17	ND	1.79	13.41	102.42	117.80
Min.	ND	ND	ND	7.43	50.70	67.15
Max.	0.95	ND	6.65	24.85	262.31	289.83
Srelative proportions	0.07	ND	1.16	12.39	86.36	-

\*Table 2 for abbreviations; \*\*ND: Not Detection

(87%). Dhane and Shukla (1995) reported that 95.9% of total zinc of soil belonged to residual fraction. Zinc associated with residual fraction in A, B and C schemes were 102.42 (86.36%), 101.76 (81.30%) and 101.98  $\mu\text{g g}^{-1}$  (81.21%), respectively. In B and C schemes, the residual fraction was calculated as the difference of total Zn contents (Mixture 4 acid) and sum of released fractions. However, in A method the residual fraction was extracted with 4 M nitric acid. A good recovery (the sum of the amounts of the metals removed in each step was divided by total Zn) is within 100 $\pm$ 10% (Ma and Rao, 1997). In A method, the recovery of total Zn was 94%. The carbonate bound Zn extracted by the A scheme was higher compared to that of the A and B methods. The concentrations of the carbonate bound Zn in the A, B and C schemes were 13.41 (12.4% of total Zn), 10.88 (10.9% of total Zn) and 11.44  $\mu\text{g g}^{-1}$  (11.4% of total Zn), respectively. The differences in this Zn fraction between the three sequential extraction procedures show that Na<sub>2</sub>EDTA is a stronger extractant than NaOAc (pH = 5) and NaOAc-HOAc (pH = 4.74), which maybe the reason for dissolution of other fractions (Han and Banin, 1995). On the one other hand, NaOAc-HOAc (pH = 4.74) is a stronger extractant than NaOAc (pH = 5), because Zn bound to carbonate are sensitive to pH and leached by lowering the pH (Perez-Cid *et al.*, 1999). The efficiency of the dissolution of carbonates depends on the grain size, the incipient content and nature of carbonates (Sheppard and Stephenson, 1997).

Finally, most of the zinc in native soils occurs in the residual and carbonate bound. These results are in agreement with many previous observations (Yasrebi *et al.*, 1994; McGrath and Cegarra, 1992). Zinc bound to organic matter extracted by the C method (2.12  $\mu\text{g g}^{-1}$ , 1.32% of total Zn) was higher than the amount extracted using the A (1.79  $\mu\text{g g}^{-1}$ , 1.16% of total Zn) and B methods (1.69  $\mu\text{g g}^{-1}$ , 1.1%). Various

Table 4: Concentration ( $\mu\text{g g}^{-1}$ ) of soil Zn in individual fractions by Singh *et al.* (1982) method (each figure is mean of 3 observations)

Number soils	Ex*	Car	Om	OX Mn	OXA Fe	OXC Fe	Res	Total
1	ND**	9.57	ND	0.74	2.97	4.50	56.85	74.65
2	0.23	7.50	ND	0.59	1.96	3.42	69.00	82.72
3	ND	7.37	1.23	0.47	1.93	3.36	87.83	102.22
4	0.18	13.44	0.26	0.99	3.38	6.10	78.42	102.80
5	0.23	9.58	ND	0.63	2.49	4.35	64.00	81.30
6	0.31	5.94	1.73	0.36	1.43	2.49	98.58	110.88
7	ND	6.37	1.8	0.38	1.53	2.68	63.84	76.62
8	2.34	15.86	4.16	3.41	6.46	11.26	256.13	299.65
9	0.68	22.30	6	3.76	6.60	11.50	141.17	192.06
Mean	0.44	10.88	1.69	1.26	3.20	5.52	101.76	124.77
Min.	ND	5.94	ND	0.36	1.43	2.49	56.85	74.62
Max.	2.34	22.30	6.02	3.76	6.60	11.50	256.13	299.65
Relative proportions	0.24	9.39	1.1	0.87	2.61	4.47	81.30	-

Table 5: Concentration ( $\mu\text{g g}^{-1}$ ) of soil Zn in individual fractions by Ma and Uren (1995) method (each figure is mean of 3 observations)

Number soils	Ws**	Sor	OX Mn	Car	Om	OX Al and Fe	Res	Total
1	0.09	0.68	2.26	9.76	ND*	3.65	58.21	74.65
2	0.26	0.86	2.48	8.00	ND	2.78	68.35	82.73
3	0.07	0.76	2.60	7.86	1.3	2.73	86.91	102.23
4	0.18	0.83	2.89	14.34	0.28	4.95	79.34	102.81
5	0.08	0.82	2.03	10.21	ND	3.53	64.64	81.31
6	0.36	1.03	2.32	6.34	1.91	2.02	96.90	110.89
7	0.07	0.67	3.32	6.80	1.99	2.17	61.60	76.62
8	2.11	1.27	4.19	18.48	5.52	9.13	258.96	299.66
9	1.64	1.66	6.73	1.20	8.1	9.85	142.89	192.06
Mean	0.54	0.95	3.20	11.44	2.12	4.53	101.98	124.77
Min.	0.07	0.67	2.03	6.34	ND	2.02	58.21	74.62
Max.	2.11	1.66	6.73	21.20	8.10	9.85	258.96	299.65
Relative proportions	0.30	0.84	2.79	9.86	1.32	3.65	81.21	-

\*Table 2 for abbreviations; \*\*ND: Not Detection

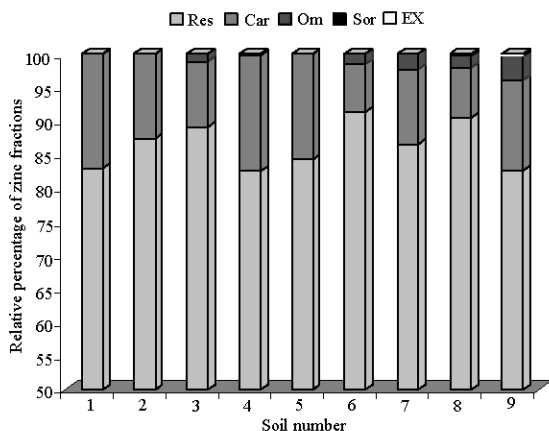


Fig. 1: Relative percentage of zinc fractions by Sposito *et al.* (1982) scheme (A)

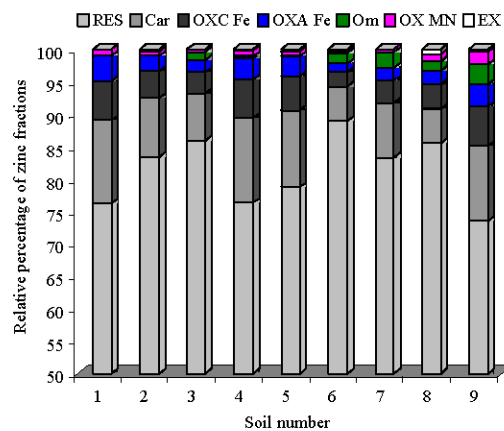


Fig. 2: Relative percentage of zinc fractions by Singh *et al.* (1988) scheme (B)

approaches for the dissolution of organic bound Zn are known: release by oxidation, release by dissolution and addition of competing ligands (Hlavay *et al.*, 2004). Sodium hypochlorite (reagent in B scheme, pH = 8.5) was able to release lower portion of Zn compared to hydrogen peroxide (reagent in C scheme, pH = 4.74), probably due to higher pH of the reagent. Also, evidence shows that acidified hydrogen peroxide, the most frequently cited method for the oxidation of organic matter and sulfides, attacks Mn oxides (Papp *et al.*, 1991). For Fe oxides and

silicate minerals (Douglas and Fiessinger, 1971), this step cannot be considered specific for Zn linked to organic matter. On the other hand, minimal degradation of Mn and Fe oxides (Shuman, 1983) and silicates have been indicated for sodium hypochlorite, suggesting its suitability for use earlier in the extraction sequence (Ahnstrom and Parker, 1999). Some schemes apply NaOH (Kersten *et al.*, 1991) to extract organically bound trace metals by dissolving organic matter. But, the use of sodium hydroxide may precipitate as hydroxides and

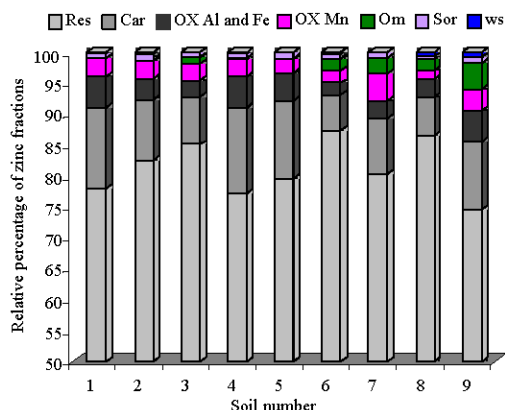


Fig. 3: Relative percentage of zinc fractions by Ma and Uren (1995) scheme (C)

attacks aluminosilicates and clays (Pickering, 1981). The amount of zinc bound to the fraction of exchangeable, determined by the B method ( $0.44 \mu\text{g g}^{-1}$ , 0.24% of total Zn), was higher compared to the results obtained by A method ( $0.17 \mu\text{g g}^{-1}$ , 0.07% of total Zn). The percentage of the exchangeable Zn is very low in soil (>0.25%). These results are in agreement with those reported by many investigators (Li *et al.*, 2001; Yasrebi *et al.*, 1994). For extraction of the exchangeable fraction, almost all possible combinations of major cations with either  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , or acetate has been used, with concentrations ranging between 0.05 and 1 mol  $\text{L}^{-1}$  and pH in the neutral range (Hlavay *et al.*, 2004). The relative exchangeability of trace metals is determined by the affinity of the exchanging cation for the soil solid phase. This affinity increases with increasing valency and decreasing radius of the hydrated cation (Pickering, 1981). The differences in this Zn fraction between the sequential extraction procedures show that  $\text{Mg}(\text{NO}_3)_2$  (B scheme) is a stronger extractant than  $\text{KNO}_3$  (A scheme), because magnesium ion has similar ionic radius to zinc ion than potassium ion. The concentration of manganese oxide in C scheme ( $3.2 \mu\text{g g}^{-1}$ , 2.79% of total Zn) was much higher than B scheme ( $1.26 \mu\text{g g}^{-1}$ , 0.87% of total Zn). The reagent of 0.2% quinol in 1 M  $\text{NH}_4\text{OAc}$  (pH = 7) used in C method has higher extraction ability than 0.1 M  $\text{NH}_2\text{OH.HCl}$  (pH = 2) applied in the B method. Chao (1972) and Jarvis (1984) suggested that a mildly reducing mixture of 0.2% hydroquinone in 1 mol  $\text{L}^{-1}$   $\text{NH}_4\text{Ac}$  would dissolve considerably less Mn than  $\text{NH}_2\text{OH.HCl}$  (Beckett, 1989). These results of this study are in disagreement with previous studies, which maybe for the reason that priority non-observance in step extraction, which is able to dissolve organic fraction (Miller *et al.*, 1986). Zn bound to Fe and Al oxides were extracted either by one step or were partitioned to two

fractions, referred to as amorphous and crystalline Fe-oxides. The B sequential extraction method obtained  $3.2 \mu\text{g g}^{-1}$  (2.61% of total Zn) and  $5.52 \mu\text{g g}^{-1}$  Zn (4.47% of total Zn) in the amorphous (extracted by 0.25 M  $\text{NH}_2\text{OH.HCl}$  in 0.25 M HCL) and in the crystalline (extracted by 0.2 M  $(\text{NH}_4)_2\text{C}_2\text{O}_4+0.2 \text{ M H}_2\text{C}_2\text{O}_4+0.1 \text{ M C}_6\text{H}_8\text{O}_6$ ) Fe-oxide bound, respectively. Chao and Zhou (1983) showed that 0.25 mol  $\text{L}^{-1}$  hydroxylamine solution in 0.25 mol  $\text{L}^{-1}$  HCl could dissolve amorphous iron oxides. The oxalate-extraction of the B and C schemes is thought to remove Zn bound to the surface of Fe and Al minerals. This reagent has high iron-complexing capacity and low reducing properties (Gleyzes *et al.*, 2002). Stanjek and Weidler (1992) reported that the solubility of iron oxides in oxalate solutions depends on the nature and the concentration of surface hydroxyl groups, so the solubility of iron oxides in oxalate solutions decreased with an increase in the degree of crystallinity. Amounts of zinc associated with Fe and Al-oxide extracted by C method (0.175 M  $(\text{NH}_4)_2\text{C}_2\text{O}_4-0.1 \text{ M H}_2\text{C}_2\text{O}_4$  adjusted in pH 3.25) was found to be  $4.53 \mu\text{g g}^{-1}$  Zn (3.65% of total Zn). In the A method, the sorbed fraction was lower than the detection limit of atomic absorption spectrometry. Furthermore, this fraction by C method was  $0.95 \mu\text{g g}^{-1}$  (0.84%), these differences can be explained by the different strength of the extractant used in the C scheme (extracted by 1% NaCaHEDTA in 1 M  $\text{NH}_4\text{OAc}$ , pH = 8.3) in comparison with A scheme (extracted by deionized water). Therefore, it is a very important property of the sequential extraction reagents that they are selected for attacking abilities to target major components of the sample matrix. Zn extracted by  $\text{H}_2\text{O}$  is relatively labile and thus may be potentially bioavailable (He *et al.* 1995), this fraction is called water soluble. This fraction is usually negligible, except in acidic and sandy soils. Water soluble Zn by C method was very low ( $0.54 \mu\text{g g}^{-1}$ , 0.3% of total Zn) compared with total Zn content. Overall, the decreasing order of Zinc fractions (relative percentage) according to A scheme was residual>>carbonate fraction>organic fraction>exchangeable>adsorbed. The order of the B scheme was residual>>carbonate fraction>Fe crystalline oxides>Fe amorphous oxides>organic fraction>Mn oxides>exchangeable. The order of the C scheme followed residual>>carbonate fraction>Fe-Al oxides>Mn oxides>organic fraction>adsorbed>water soluble.

In earlier study in Iran, Yasrebi *et al.* (1994) reported that native Zn mainly existed in the residual and the carbonate fractions (residual>>carbonate bound> sorbed>exchangeable>organic bound). Ma an Uren (1995) reported that Zn fractions in soils of Australia decreased in the following order: residual>sorbed>Mn-oxides bound>Fe, Al-oxides bound>organic bound>carbonate

bound water soluble. Abollino *et al.* (2006) reported that Zn fractions in contaminated soil decreased in the following order: Res>OX Mn>Car>Om>EX. Gworek and Mocek (2003) concluded that different methods give us different answer. Tlustos *et al.* (2005) also indicated that individual soil fraction is strongly dependent on the extracting agent and/or procedure applied within individual extracting schemes. A similar study, Tokalioglu *et al.* (2003) elucidated three sequential extraction procedures for partitioning of heavy metals in car park dusts and reported that amount of metal released at each step depended both on the type of reagents used and the sequence in which they were applied. Francisco *et al.* (2008) suggested that it is imperative to put forward an agreed-established by consensus-sequential extraction scheme for each soil class within a particular range of physicochemical and chemical characteristics.

**Correlation analysis between soil properties and Zn fractions:** Correlation analysis between selected soil properties and the Zn fractions obtained by three sequential extractions were established for all soils (Table 6). Correlation analysis between soil properties and different Zn fractions by A scheme show that EX fraction of Zn had significant positive correlation with CEC, Zn-DTPA and OM, whereas the changes in part showed significant negative correlations with pH and CCE. This is similar to the results of Chahal *et al.* (2005). On the other hand, the relationship of B method was similar to the result of A method, except CCE, which has insignificant

correlation. Xiang *et al.* (1995) reported that the Zn EX fraction has a significant positive correlation with pH. The correlation of Om fraction with pH and CCE by A method was significantly negative and with OM, Zn-DTPA and CEC was significantly positive, similar to result of EX fraction. B method has similar correlation to the C and B methods. However, Zn-DTPA in B method has insignificant correlation. The high correlation coefficients between the SOM content and the Om fraction are ascribed to the strong chelating power of SOM with Zn as well as to the surface adsorption of Zn onto SOM (Chahal *et al.*, 2005). Chahal *et al.* (2005) reported that the Om fraction of zinc significantly correlated with clay, OM, silt and Zn-DTPA. Prasad *et al.* (1995) found that significant positive correlation between this fraction with clay, CEC and OM. The Car fraction was negatively correlated with pH, but positively correlated with CEC and Zn-DTPA in all three methods. To be necessary to point out that the negative correlation between the CCE and the Car fraction is related to the high concentration of Car fraction in two acidic soils (numbers 8 and 9). Yasrebi *et al.* (1994) found that significant positive correlation between Car Zn with pH, CEC and OM. OX Mn fraction in B method was correlated positively with CEC, OM and Zn-DTPA. Whereas, was correlated negative with pH. These results are in accordance with the findings of Jeng and Singh (1993). On the other hand, C method offered similar results to the B method, except Zn-DTPA and OM were insignificantly correlated. Jeng and Singh (1993) reported that concentration of OX Mn increased with high OM and low pH.

Table 6: Correlations analysis between soil properties and Zn fractions by three sequential extraction methods Sposito *et al.* (1982), Singh *et al.* (1988) and Ma and Uren (1995)

	pH	CCE	Clay	Silt	OM	CEC	Zn-DTPA
<b>Sposito <i>et al.</i> (1982) scheme (A)</b>							
EX	-0.87**	-0.70*	0.31 <sup>ns</sup>	0.27 <sup>ns</sup>	0.83**	0.96**	0.74*
Sor	-	-	-	-	-	-	-
Om	-0.96**	-0.82**	0.34 <sup>ns</sup>	0.48 <sup>ns</sup>	0.79**	0.95**	0.67*
Car	-0.76*	0.35 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.11 <sup>ns</sup>	0.49 <sup>ns</sup>	0.81**	0.90**
Res	-0.83**	-0.70*	0.27 <sup>ns</sup>	0.40 <sup>ns</sup>	0.87**	0.92**	0.72*
<b>Singh <i>et al.</i> (1988) scheme (B)</b>							
EX	-0.74*	-0.60 <sup>ns</sup>	0.34 <sup>ns</sup>	0.29 <sup>ns</sup>	0.81**	0.84**	0.63 <sup>ns</sup>
Car	-74.8*	-0.34 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.13 <sup>ns</sup>	0.42 <sup>ns</sup>	0.77*	0.87**
Om	-0.93**	-0.83**	0.24 <sup>ns</sup>	0.46 <sup>ns</sup>	0.80**	0.91**	0.62 <sup>ns</sup>
OX Mn	-0.87**	-0.49 <sup>ns</sup>	0.16 <sup>ns</sup>	0.10 <sup>ns</sup>	0.68*	0.94**	0.81**
OXA Fe	-0.80**	-0.63 <sup>ns</sup>	0.09 <sup>ns</sup>	-0.04 <sup>ns</sup>	0.59 <sup>ns</sup>	0.89**	0.88**
OXC Fe	-0.81**	-0.48 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.08 <sup>ns</sup>	0.60 <sup>ns</sup>	0.88**	0.87**
Res	-0.79**	-0.69*	0.29 <sup>ns</sup>	0.42 <sup>ns</sup>	0.87**	0.91**	0.69*
<b>Ma and Uren (1995) scheme (C)</b>							
Ws	-0.91**	-0.75*	0.24 <sup>ns</sup>	0.27 <sup>ns</sup>	0.83**	0.97**	0.76*
Sor	-0.93**	-0.68*	0.03 <sup>ns</sup>	0.25 <sup>ns</sup>	0.64 <sup>ns</sup>	0.84**	0.71*
OX Mn	-0.81**	-0.65 <sup>ns</sup>	0.16 <sup>ns</sup>	0.17 <sup>ns</sup>	0.56 <sup>ns</sup>	0.84**	0.28 <sup>ns</sup>
Car	-0.74*	0.33 <sup>ns</sup>	-0.09 <sup>ns</sup>	-0.10 <sup>ns</sup>	0.47 <sup>ns</sup>	0.81**	0.91**
Om	-0.93**	-0.81**	0.23 <sup>ns</sup>	0.41 <sup>ns</sup>	0.77*	0.93**	0.67*
OX Fe and Al	-0.79**	-0.49 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.07 <sup>ns</sup>	0.57 <sup>ns</sup>	0.89**	0.86**
Res	-0.80**	-0.67*	0.38 <sup>ns</sup>	0.38 <sup>ns</sup>	0.87**	0.91**	0.70**

The OXA Fe and OXC Fe fractions in B scheme gave very high negatively correlation coefficients with pH, whereas the changes in parts showed significant positive correlations with CEC and Zn-DTPA. Hazra *et al.* (1993) found that there was a significant positive correlation between OXA Fe with OM. The coefficients of correlation of OX Fe and Al fraction with CEC and Zn-DTPA were positively significant by C scheme. The result showed that Sor fraction in C scheme was positively significantly correlated with CEC and Zn-DTPA. The highly correlation between adsorbed fraction and clay content is related to the adsorption and ion exchange of zinc onto clay particles. Whereas, the changes in part showed significant negative correlations with pH. In A method, Sor fraction was below the detection limit of the analytical method, we were unable to correlate with this part. Res Zn in all three schemes was significantly positively correlated with CEC, OM and Zn-DTPA, but negatively correlated with pH and CCE. Rivero *et al.* (2000) reported that the residual soil Zn significantly correlated with clay and carbonate content in soil, but not with total Zn content. Yasrebi *et al.* (1994) found that significant positive correlation between Res fraction with Clay. Therefore, the correlation of Zn fractions with soil properties showed that soil pH and CEC are the dominate factors controlling the distribution of Zn in soils. In general, the fraction distribution of Zn in this area is dependent many factors, such as pH, CCE, OM and CEC.

### CONCLUSION

The results obtained from three sequential extractions indicated that most Zn was associated with the residual fraction. The Zn fractions followed the different orders for different sequential extraction approaches. Sposito *et al.* (1982). A scheme: residual>>carbonate fraction>organic fraction>exchangeable>adsorbed. B scheme: residual>>carbonate fraction>Fe crystalline oxides>Fe amorphous oxides>organic fraction>Mn oxides>exchangeable. C scheme: residual>>carbonate fraction>Fe-Al oxides>Mn oxides>organic fraction>adsorbed>water soluble. Thus, Zn released at each step of the extracting procedure was strongly dependent on the type of reagents, the sequence in which we were applied and extraction method. In fact the results obtained from different sequential extraction procedures may give very different answers about the fractionation of elements. Yasrebi *et al.* (1994) reported that Sposito *et al.* (1982) method was the most suitable methods for prediction and mobility of Zn fractions in soils of Iran. The correlation of Zn forms with some soil properties showed that soil pH and CEC are the most

important factors controlling the distribution of Zn in soils although other factors such as CCE and OM content should also be considered. Further study, is needed to standardize the sequential extraction method, which might have a promising future for estimate of mobility and bioavailability of metals in soils to plants.

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