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Effect of Calcium Carbonate Removal on the Chemical Forms of Zinc in Calcareous Soils by Three Sequential Extraction Methods

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Abstract: The mobility, transport and partitioning of Zn in soils are dependent on various soil chemical properties such as soil pH, the type and contents of clay minerals, organic matter and the carbonates. The objective of this study was evaluation and comparison of zinc fractions, in native and treated soil with three levels of zinc: 0, 5 and 10 μg Zn kg^{-1} soil as $ZnSO_4$. $7H_2O$, before and after removal of calcium carbonate by three sequential extraction methods. Calcium carbonates were removed from soil samples by the sodium acetate (NaOAc adjusted in pH = 5) and Hydrochloric Acid (HCl). Result shown that removal of carbonate led to decrease in total zinc for all samples but, there were significant differences in the Zn fractions (relative percentage) before and after carbonate removal at native soil and treated soil. Removal of calcium carbonate (in 3 methods) led to decrease in residual fraction and increase in other forms (except organic fractions and water-soluble) for rinsing with Hcl samples (for all methods) and also, led to slight decrease in residual fraction and slight increase in other forms (except organic fractions and water soluble) for rinsing with NaAOC samples. Generally, the results shown that soil chemical properties, such as calcium carbonate can influence fractions of zinc and convert soluble forms to insoluble forms.

Key words: Zinc, calcium carbonate removal, chemical fractionation, sequential extraction

INTRODUCTION

Zinc (Zn) is one of the micronutrient, which is important in growth of most plants. Further more, it is one of most necessary elements in the human nutrition and interferes in most cell activities like enzymes activities such as carbonic anhydrase, dehydrogenase, proteinase and peptidase (Marschner, 1995). Zn deficiency in soils is a global micronutrient deficiency occurring in many countries, including millions of ha of cultivated areas in Iran (Malakouti and Mashayekhi, 1995). About 60% of the arable soils in Iran are Zn-deficient. In calcareous soils, the availability of Zn is largely governed by soil pH, type of soil minerals, kind and amount of anions in the soil solution and Zn carriers (Thind *et al*., 1990). The main causes of Zn deficiency in Iran are: calcareous soils with high pH values (mainly >30% $CaCO_3$, with an actual range of 15-60% $CaCO_3$ with pH 8-8.5), high applications of phosphatic fertilizers, high concentrations of bicarbonate (HCO_3^-) in irrigation water and non-consume of Zn fertilizers. The FAO recognize calcareous soils as having major problems for agriculture. In arid zone soils, the presence of carbonate minerals effectively immobilizes heavy metals by providing an absorbing surface and by buffering pH at high values where precipitation takes place (Aydinalp and Katkat, 2004). The FAO define calcareous soils as being soils in which a high amount of calcium carbonate dominates the problems related to agricultural land use. They are characterized by the presence of calcium carbonate in the parent material and by a calcic horizon, which is a layer of secondary accumulation of carbonates in excess of 15% calcium carbonate equivalent and at least 5% more carbonate than an underlying layer (Alloway, 2003). The study of the Zn distribution in various soil fractions provides a better understanding of Zn behavior in relation to the transformation of Zn added to soil (Iyengar *et al*., 1981). After incubation of Zn with soil, Availabe forms of Zn are rapidly converted to inavailabe forms in calcareous soils. In soils, Zn of interest exists in several different forms and is associated with a range of components. Fractionation studies of Zn in soils using a sequential extraction procedure can provide an understanding of its chemical fractions and potential bioavailability. Fractionation by selective chemical extraction removes or dissociates a specific phase with the associated metal bonded to it. A

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typical sequential procedure starts with a weak solvent, followed by a stronger one to more aggressive solvents in order to sequentially solubilize various operationally defined metal fractions: exchangeable, bound to carbonates, reducible, oxidisable and residual. The water soluble, exchangeable and carbonate fractions are considered to be the most phytoavailable form of Zn, while those in the latter stages of the extraction scheme are less available to plants. Previous studies shown that native Zn in soils of Iran was mainly distributed in the residual fraction and followed by thecarbonate fraction (Yasrebi *et al*., 1994). On the other hand, carbonate fraction of Zn had significant positive correlation with uptake by plant (Yasrebi *et al*., 1994). The objective of this study was evaluation and comparison of zinc fractions, in native and treated soil with three levels of zinc as ZnSo$_4$, before and after removal of calcium carbonate by three sequential extraction methods: Sposito *et al.* (1982), Singh *et al.* (1988) and Ma and Uren (1995).

MATERIALS AND METHODS

Sampling location and soil characterization: In this research, 9 surface soils (0-30 cm) were selected from the Fars, Kerman and Gilan provinces, Iran. The soil samples were taken to the laboratory and air-dried over night 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 extraction with sodium acetate (Page *et al*., 1987); percentage of Calcium Carbonate Equivalent (CCE) was measured by acid neutralization (SLS, 1954); Organic Matter (OM) content was determined using Walkley-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$_4$, HNO$_3$ and H$_2$SO$_4$) was used to determine the total contents of Zn (Ma and Rao, 1997). A summery of soil properties is presented in Table 1.

Calcium carbonate removal from soils

Removal by sodium acetate:

- Five hundred gram of soil were weighed and placed in a 800 mL beaker
- Add 500 mL of 1 N NaOAC (sodium acetate) buffered at pH 5 (with acid acetic) to the soil
- Place the beakers on the steam Table 1, maintain a temperature of 70-80°C for 30 min and stir samples occasionally until all the calcium carbonate has been removed (when CO$_2$ bubbles are no longer evident)
- Soil samples were air-dried and keeping for next stage

Removal by hydrochloric acid:

- Five hundred gram of soil were weighed and placed in a 800 mL beaker
- Add 150 mL of 0.5N HCl (hydrochloric acid) to the soil
- Stir samples occasionally until all the calcium carbonate has been removed (when CO$_2$ bubbles are no longer evident)
- Soil samples were air-dried and keeping for next stage

Incubation experiments: For the incubation study, each soil (treated with Hcl, NaOAC and untreated) was placed in plastic cups and Zn was added at the rate 0, 5, 10 μg g^{-1} as aqueous of ZnSO$_4$ solution and then each soil sample was mixed thoroughly. The soils incubated for 8 weeks at 25°C. The moisture was kept at about field capacity by adding water to a constant weight, once every 3 days. After incubation samples were air-dried. Generally, A 9×3×3 factorial experiment as a completely randomized design with 3 replications was used to study the change of chemical forms of Zn with removal calcium carbonate. Factors included 9 selected soils, 3 level of carbonate calcium removal treatment (treated with HCl, NaOAC and untreated) and 3 levels of Zn (0, 5, 10 μg g^{-1} as aqueous of ZnSO$_4$ solution). After incubation, we used three sequential extraction methods (Spozito *et al*., 1982; Singh *et al*., 1988; Ma and Uren, 1995) to extract Zn in

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

Soil No.	Sampling location	Great group	Clay	Silt	OM	CCE	pH	CEC	Zn-total	Zn-A*	Fe-A*	Mn-A*
			-------------g kg^{-1}-------------					(Cmol + kg^{-1})	-----------mg kg^{1}-----------			
1	Kerman	HXP**	153	92	0.97	327	8.5	12.70	74.65	0.834	2.84	5.50
2	Kerman	CXP	233	140	2.20	256	8.5	12.30	82.73	0.308	3.61	4.75
3	Kerman	CGD	193	360	11.90	234	8.3	14.60	102.23	0.420	4.45	9.23
4	Kerman	HXP	113	120	4.80	481	8.4	13.90	102.81	1.630	4.99	7.80
5	Kerman	HXF	273	200	1.80	364	8.1	12.80	81.31	0.306	3.55	5.60
6	Fars	CUP	151	400	39.00	148	7.2	15.34	110.89	0.658	4.96	10.68
7	Fars	CGD	378	320	32.70	164	8.0	16.20	76.62	0.222	3.88	12.41
8	Gilan	HUF	322	332	74.60	24	6.1	32.43	299.66	1.790	14.82	64.86
9	Gilan	HUF	204	254	40.60	40	5.8	29.30	192.06	1.790	18.30	58.72

A*: Extracted by DTPA; ** HXP: Haploxerepts; CXP: Calcixerepts; CUP: Calciustepts; CGD: Calcigypsids; HXF: Haploxeralfs; HUF: Haploudalfs

Table 2: Three sequential extraction methods

Fraction	Soil:solution	Extracting solution	Shaking time (h)	Temp. (°C)	Symbol
Operating conditions required in the Sposito *et al.* (1982) scheme (A)					
Exchangeable	2:25	0.5 M KNO_3	16	20	EX
Sorbed*	2:25	Deionized water	2	20	Sor
Organic	2:25	0.5 M NaOH	16	20	Om
Carbonate	2:25	0.05 M Na_2EDTA	2	20	Car
Residual	2:25	4 M HNO_3	16	80	Res
Operating conditions required in the Singh *et al.* (1988) scheme (B)					
Exchangeable	2.5:10	1 M Mg $(NO_3)_2$	2	20	EX
Carbonate	2.5:10	1 M NaOAc (pH = 5)	5	20	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 $NH_2OH.HCl$ (pH = 2)	0.5	boiling water	Mn OX
Amorphous Fe-oxide	2.5:25	0.25 M $NH_2OH.HCl$ + 0.25 M HCL	0.5	50°C water	FeA OX
Crystalline Fe-oxide	2.5:25	0.2 M $(NH_4)_2C_2O_4$ + 0.2 M $H_2C_2O_4$ + 0.1 M $C_6H_8O_6$	0.5	boiling water	FeC OX
Residual		Total zinc- (sum of the above 6 types)***			Res
Operating conditions required in the Ma and Uren (1995) scheme (C)					
Water soluble	1:10	Deionized water	2	20	Ws
Sorbed	1:10	1% NaCaHEDTA + 1 M NH_4OAc (pH = 8.3)	2	20	Sor
Mn-oxide	1:10	0.2% quinol + 1 M NH_4OAc (pH = 7)	2	20	Mn OX
Carbonate	1:10	0.5 M NaOAc–0.5 M HOAc (pH = 4.74) soaking 15 h	3	20	Car
Organic	1:10	digested twice in30% H_2O_2 (pH = 4.74) at 85°C, extracted as for carbonate fraction (1 h)			Om
Fe&Al-oxide	1:10	0.175 M (NH_4) C_2O_4-0.1 M $H_2C_2O_4$ (pH = 3.25) soaking 15 h	3	20	Fe and Al OX
Residual		Total zinc- (sum of the above 6 ypes)***			Res

*3 times extraction; ** 2 times extraction; *** Deducting the sum of the above six types of extractable Zn from its total concentration

amended and control soils. The Zn concentrations were analyzed by atomic absorption spectrophotometer (Shimatzu AA-670G). A summary of three sequential extractions shown in Table 2.

RESULTS AND DISCUSSION

Zn distribution in native soils: The concentrations of different zinc fractions of native soils sample (untreated) by Sposito *et al.* (1982) method (A), Singh *et al.* (1988) method (B) and Ma and Uren (1995) method (C) is presented in Table 3. The sequential extraction results in three schemes in untreated soil show that Zn is strongly associated with the Residual and carbonate fractions. These results are in agreement with previous research in Iran (Yasrebi *et al.*, 1994). The greater percentage of Zn in the residual fraction probably reflects the greater tendency for Zn to become unavailable once it was in soils. Lu *et al.* (2007), Mo *et al.* (2002) and Kabala and Singh (2001) reported that Zn mainly existed in the residual fraction. In contrast, other fractions were very low compared with total concentrations of Zn in three sequential extraction methods. Xian (1989) found that the sum of the exchangeable and the carbonate-bound forms were positive correlated with Zn uptake by plants. The sum of Zn in the carbonate and exchangeable fractions in our study ranged about 12.5% for A method, 9.6% for B method and 9.8% of the total Zn, which indicates that the Zn in some of these soils may be highly available for plants. Generally, the concentration of Zn in soil components by three sequential extractions was to be in the following order, According to A scheme: Residual>> carbonate fraction>Organic fraction>exchangeable> Sorbed. According to B scheme: Residual>>carbonate fraction>Fe crystalline oxides>Fe amorphous oxides> Organic fraction>Mn oxides>exchangeable.

According to C scheme: Residual>>carbonate fraction>Fe-Al oxides>Mn oxides>Organic fraction> Sorbed>Water soluble.

Table 3: Concentration ($\mu g\ g^{-1}$) of different zinc fractions in untreated soils by different methods (each figure is mean of 27 observations)

Chemical forms	Values
Sposito *et al.* (1982) method	
Ex*	0.17
Sor	ND**
Om	1.79
Car	13.41
Res	102.42
Total	117.80
Singh *et al.* (1982) method	
Ex	0.44
Car	10.88
Om	1.69
OX Mn	1.26
OXA Fe	3.2
OXC Fe	5.52
Res	101.76
Total	124.77
Ma and Uren (1995) method	
Ws	0.54
Sor	0.95
OX Mn	3.2
Car	11.44
Om	2.12
OX Al and Fe	4.53
Res	101.98
Total	124.77

**ND: Not detection

Table 4: Concentration (µg g^{-1}) of different zinc fractions by different methods in untreated soils (each figure is mean of 27 observations)

Rates of added Zn	Ex*	Sor	Om	Car	Res	Total
Sposito *et al.* (1982) method						
5	0.3	ND**	2.39	16.28	103.86	122.82
10	0.47	ND	2.82	19.16	105.27	127.72

Rates of added Zn	Ex*	Car	Om	OX Mn	OXA Fe	OXC Fe	Res	Total
Singh *et al.* (1982) method								
5	0.55	13.7	2.39	1.52	3.51	6.06	102.02	129.78
10	0.66	16	2.91	1.84	3.76	6.65	102.84	134.68

Rates of added Zn	Ws**	Sor	OX Mn	Car	Om	OX Al and Fe	Res	Total
Ma and Uren (1995) method								
5	0.81	1.07	3.58	13.88	2.86	5.66	102.15	129.79
10	1.10	1.15	3.99	16.28	3.38	6.92	102.20	134.68

**ND: Not Detecion

Fate of zinc added to native soils: The concentrations of Zn in each form in soils with Zn application are shown in Table 4. Increased rate of Zn caused significant increases in all chemical forms of Zn in three sequential extraction schemes (except for sorbed form in A method). Application of $ZnSO_4$ had the greatest effect on carbonate form in three sequential extraction schemes. Almost 57.2-57.5% of the Zn added to the soil existed as the carbonate form, for the applied 5 and 10 µg Zn g^{-1}, respectively. The other forms were 28.4-28.5% with residual, 12-10.7% with organic bound and 2.6-3% with exchangable fraction, for the 5 and 10 µg Zn g^{-1}, respectively in A method. Despite this transmutation of zinc by B method were, 56.4-51.2% with carbonate form, 10.8-11.6% with crystalline Fe-oxide bound, 14-12.6% with organic bound, 5.2-10.8% with residual form, 6.2-5.6% with amorphous Fe-oxide bound, 5.2-5.8% with Mn-oxide bound and 2.1-2.2% with exchangable fraction, for the applied 5 and 10 µg Zn g^{-1}, respectively, in B method. On the other hand in C method 48.8-48.4% of added Zn was present in the carbonate fraction and about 22.6-23.9% was present in the Fe and Al-oxide fraction.The other forms were 14.8-12.6% with organic bound, 7.6-7.9 with Mn-oxide bound, 5.4-5.6 with water soluble form, 3.4-2.2 with residual form and 2.4-2 with sorbed fraction, for the applied 5 and 10 µg as $ZnSO_4$, respectively. Diffrent amount Zn fractions in this study has shown that amount of Zn released at each step of the extracting procedure strongly influence type of reagents, the sequence in which, we were applied and extraction method. Yasrebi *et al.* (1994) reported that Zn in soils treated with zinc sulfate was mainly in the carbonate fraction. Xiang *et al.* (1995) reported that added Zn in calcareous, acidic and neutral soils of china was mostly in the residual fraction. In the other study, application of zinc sulfate increased the percentage of Zn in the EDTA-extractable fraction and in association with iron (aluminium) (Fe (Al)) and Mn oxides (Ma and Uren, 1997). Torri and Lavado (2008) shown that after treated soils with sludge for 1 year, the carbonate fraction and the iron and Mn oxides bound became the most prevalent chemical forms. McGrath and Cegarra (1992) showed that with increasing total Zn concentration with sewage sludge, a relative increase in exchangeable, organic and carbonate bound and a decrease in Res fraction was observed. Haroun reported that Zn in soils treated with tannery sludge for 50 days was mainly in the residual fraction. Illera *et al.* (2000) reported that application of different types of municipal solid waste changed the distribution of Zn chemical forms. This change caused the relative amount of residual and organic bound to increase and the relative amount of oxides fractions to increase. Generally, the relative distribution (%) of Zn in soil components after incubation with Zn sulfate by three sequential extractions was to be in the following order, According to A scheme: carbonate fraction>>Residual>Organic fraction>exchangeable> Sorbed. According to B scheme: carbonate fraction>> Organic fraction> Fe crystalline oxides>Residual>Fe amorphous oxides>Mn oxides>exchangeable. According to C scheme: carbonate fraction>>Fe-Al oxides>Organic fraction>Mn oxides>Water soluble>Sorbed>Residual> Sorbed.

Effect of removal calcium carbonate in chemical forms of Zn: The changes of Zn in each fraction after removal of soil carbonates with HCl and NaOAC are shown in Table 5 and 6, respectively. Treatment with HCl and NaOAC caused a strong decrease in all chemical forms of Zn in three sequential extraction schemes. HCl and NaOAC removed large proportions of the total Zn. The decreases in total Zn were related with the losses of component soil from the lattice, again indicating the decomposition of a part of the soil clays. Generally, two reagents caused destroy of lattice silica. The sequential extraction results in three schemes in treated soil show that Zn (like before removal calcium carbonate) is most associated with the Residual and carbonate fractions. Although, soils under study in this part were without calcium carbonate and it was expected to extract negligible amounts of total soil Zn in carbonate, the concentration of carbonate was considerable in three sequential extractions. Thus, although the amounts of chemical forms of Zn are believed to correlate well with sequential extraction by aforesaid reagent, it is not critical

Table 5: Concentration (μg g^{-1}) of different zinc fractions in treated soils with HCl by different methods (each figure is mean of 27 observations)

Chemical forms	Values
Sposito *et al.* (1982) method	
Ex*	ND
Sor	ND
Om	ND
Car	7.63
Res	39.88
Total	47.52
Singh *et al.* (1982) method	
Ex	3.22
Car	6.82
Om	ND
OX Mn	0.9
OXA Fe	1.99
OXC Fe	3.51
Res	36.08
Total	52.53
Ma and Uren (1995) method	
Ws	0.02
Sor	0.96
OX Mn	2.46
Car	5.75
Om	ND
OX Al and Fe	2.93
Res	40.40
Total	52.53

Table 6: Concentration (μg g^{-1}) of different zinc fractions in treated soils with NaOAC by different methods (each figure is mean of 27 observations)

Chemical forms	Values
Sposito *et al.* (1982) method	
Ex*	0.11
Sor	ND
Om	0.62
Car	10.7
Res	79.88
Total	91.32
Singh *et al.* (1982) method	
Ex	0.45
Car	9.63
Om	0.57
OX Mn	1.17
OXA Fe	2.97
OXC Fe	5.17
Res	77.30
Total	97.25
Ma and Uren (1995) method	
Ws	0.08
Sor	0.99
OX Mn	3.27
Car	8.02
Om	0.86
OX Al and Fe	4.19
Res	79.85
Total	97.25

*ND: Not detection

to the present study that this correlation be exact. Therefore, the fractionation of the Zn usually will be reported according to the extracting reagent employed (e.g., H_2O) instead of the expected soil solid-phase fraction (e.g., sorbed). In the A method, Sorbed, exchangeable and organic fractions were lower than the detection limit of atomic absorption spectrometry in treated soils with HCl. Also in B and C methods in these soils, organic fraction was below the detection limit of the analytical method. It seems that, calcium carbonate removal led to increase in mobility fractions of zinc. This can be explained by the addition of HCl and NaOAC to soils caused dissolve mobile fractions (such as sorbed, organic form) and so led decrease immobile fractions. This result in this part could use for improve methods of human health and environmental risk assessment by improving the understanding of metal mobility and predictions of effect industrial acids and transfer into environment and consequently, environment pollutions. On the other hand, result shows that in soils treated with NaOAC similar in soils treated with HCl, decreased chemical forms of Zn in three sequential extraction schemes. The concentration of Zn after removal calcium carbonate with HCl by three sequential extractions was to be in the following order. According to A scheme: Residual>> carbonate fraction. According to B scheme: Residual>> carbonate fraction> Fe crystalline oxides> exchangeable> Fe amorphous oxides> Mn oxides. According to C scheme: Residual >> carbonate fraction > Fe-Al oxides > Mn oxides > Sorbed > Water soluble. The concentration of Zn after removal calcium carbonate with NaOAC by three sequential extractions was to be in the following order, According to A scheme: Residual>> carbonate fraction> Organic fraction> exchangeable> Sorbed. According to B scheme: Residual>> carbonate fraction> Fe crystalline oxides> Fe amorphous oxides> Mn oxides> Organic fraction> exchangeable. According to C scheme: Residual>> carbonate fraction> Fe-Al oxides> Mn oxides> Sorbed> Organic fraction> Water soluble.

Application of zinc in soil treated with HCl and NaOAC: The concentrations of Zn in each form in soils without calcium carbonate (treated with HCl and NaOAC) with Zn application are shown in Table 7 and 8, for treated with HCl and NaOAC, respectively. With Zn addition, the concentrations of added Zn in all fractions increased (except organic fractions in three methods and sorbed bound in A method). About 48.5-51.5% of added Zn in the HCl treatment was present in the carbonate fraction, about 37-38% was present in the residual fraction and 9.5-13.8% was present in the exchangeable fraction in A method. On the other hand in the NaOAC treated soils, 38.5-44% of added Zn was present in the carbonate fraction, 38.5-41% was in the residual fraction, 14.2-15.5% was present in the organic fraction and 4.5-5.5% was present in the exchangeable fraction in A method. Added Zn in HCl treated soils by B method predominated in the residual

Table 7: Effects of removal calcium carbonate (with HCl) and Zn rate on the chemical forms of Zn (µg g^{-1}) by different methods (each figure is mean of 27observations)

Rates of added Zn	Ex*	Sor	Om	Car	Res	Total
Sposito *et al.* (1982) method						
5	0.69	ND	ND	10.06	41.75	52.5
10	0.95	ND	ND	12.78	43.71	57.44

Rates of added Zn	Ex	Car	Om	OX Mn	OXA Fe	OXC Fe	Res	Total
Singh *et al.* (1982) method								
5	4.84	7.20	ND	1.06	2.18	3.76	38.46	57.5
10	5.91	7.48	ND	1.24	2.32	4.09	41.41	62.45

Rates of added Zn	Ws	Sor	OX Mn	Car	Om	OX Al and Fe	Res	Total
Ma and Uren (1995) method								
5	0.08	1.02	2.79	7.55	ND	3.56	42.49	57.5
10	0.12	1.10	3.22	9.91	ND	4.3	43.8	62.45

Table 8: Effectsof removal calcium carbonate (with NaOAC) and Zn rate on the chemical forms of Zn (µg g^{-1}) by different methods (each figure is mean of 27observations)

Rates of added Zn	Ex*	Sor	Om	Car	Res	Total
Sposito *et al.* (1982) method						
5	0.39	ND	1.34	12.62	81.94	96.29
10	0.57	ND	2.18	15.10	83.75	101.60

Rates of added Zn	Ex	Car	Om	OX Mn	OXA Fe	OXC Fe	Res	Total
Singh *et al.* (1982) method								
5	0.97	10.01	1.33	1.41	3.24	5.58	79.68	102.21
10	1.12	11.75	2.23	1.63	3.52	6.20	81.07	107.52

Rates of added Zn	Ws	Sor	OX Mn	Car	Om	OX Al&Fe	Res	Total
Ma and Uren (1995) method								
5	0.55	1.05	3.64	9.55	1.61	5.25	80.56	102.21
10	0.81	1.18	4.04	10.56	2.62	6.32	81.99	107.52

ND: Not detection

fraction 47.5-53.3%, followed by the exchangeable fraction 26.9-32.4%, the carbonate fraction 6.6-7.6%, the Fe crystalline oxides fraction 5-5.8%, the Fe amorphous oxides fraction 3.3-3.8% and the Mn oxides fractions were 3.2-3.4%. In the NaOAC treated soils by B scheme, added Zn existed in the residual fraction 37.7-47.5%, the organic fraction 15.2-16.6% and the Fe crystalline oxides fraction 8.2-10.3%. The carbonate, exchangeable, Fe amorphous oxides and Mn oxides fractions were 7.6-17.8, 6.7-10.4%, 5.5 and 4.6-4.8%, respectively. In C method, most of the Zn occurred in the carbonate fraction 36-41.6% and the residual fraction 41.8-34% in treated with HCl. followed by, the Fe-Al oxides, Mn oxides, Sorbed and Water soluble fractions were 12.6-13.7, 6.6-7.6, 1.2-1.4 and 1.2-4.1%, respectively. On the other hand in NaOAC treated soils by C method 30.6-25.4% of added Zn was present in the carbonate fraction and about 21.2% was present in the Fe and Al-oxide fraction.The other forms were 14.4-21.5% with residual bound, 15-17.6% with organic fraction, 9.4-7.3% with water soluble form, 7.4-7.7 with Mn-oxide bound and 1.2-1.9 with sorbed fraction, for the applied 5 and 10 µg as $ZnSO_4$, respectively. To seem, the application of Zn in soils without calcium carbonate to reduce the carbonate fraction and to increase the others fractions. Generally, Even though soils under study in this part were without calcium carbonate (removal calcium carbonate with HCl and NaOAC) and it was expected to extract negligible amounts of total soil Zn in carbonate, the concentration of carbonate form was considerable in three sequential extractions, which maybe for the reason that dissolves the other fractions, excluding carbonate form. The accurate extraction of chemical forms is difficult and sometimes impossible. Sposito *et al.* (1982) in fractionation of heavy metals reported. Although, the amounts of exchangeable, sorbed, organic, carbonate and sulfide forms of trace metals are believed to correlate well with sequential extraction by KNO_3, H_2O, NaOH, EDTA and fiNO_3, it is not critical to the present study that this conelation be exact. Therefore, the fractionation of the five trace metals usually will be reported according to the extracting reagent employed (e.g., KNO_3) instead of the expected soil solid-phase fraction (e.g., exchangeable). To seem, the chemical extractants used in the present investigation are not selective and specific. Thus, the so-called extractant name-extractable could be a good than chemical form nominate.

CONCLUSION

Zinc (Zn) is essential for the natural growth and reproduction of plants, animals and humans. About 60% of the arable soils in Iran are Zn-deficient. One of main cause of Zn deficiency in Iran is calcareous soils with high pH. Availabe forms of Zn are rapidly converted to unavailable forms in calcareous soils. Previous studies have shown that native Zn in soils of Iran is mainly distributed in the residual fraction, followed by the carbonate fraction. On the other hand, carbonate fraction of Zn had significant positive correlation with plant uptake. The objective of this study was to evaluate and compare Zn fractions in native and the soil treated with three levels of Zn (0, 5, 10 $\mu g\ g^{-1}$ as $ZnSO_4$ aqueous solution) before and after removal of calcium carbonate (with sodium acetate and hydrochloric acid) by three Sequential Extraction Methods (SEM): Sposito *et al.* (1982), Singh *et al.* (1988) and Ma and Uren (1995). The results showed that in untreated soils (native soils) Zn was strongly associated with the residual and carbonate fractions. The correlation of Zn forms with selected soil properties showed that soil pH, CEC, CCE and OM are dominant factor of controlling the distribution of Zn in soils. Application of $ZnSO_4$ to soils had the greatest effect on carbonate form in three SEM. Calcium carbonate removal with HCl and NaOAc caused a strong decrease in all chemical forms of Zn in three SEM. The sequential extraction results in three schemes in treated with HCl and NaOAc soil shows that Zn (similar before removal of calcium carbonate) is mostly associated with the residual and carbonate fractions. Application of $ZnSO_4$ in soil treated with HCl and NaOAc caused significant increases (even carbonate form) in all chemical forms of Zn in three SEM. Even though soils under study in this part were without calcium carbonate (removal calcium carbonate with HCl and NaOAc) and it was expected to be negligible in carbonate Zn, the concentration of carbonate form was considerable in three SEM. Generally, the complete extraction of chemical forms is difficult and sometimes impossible. The results indicated that, the chemical extractants used in the present investigation are not selective and specific. Thus, it is better to call each fraction by the extractant used rather than call it by a chemical form.

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