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CORRELATION OF FIVE Zn EXTRACTANTS WITH PLANT RESPONSES ON HIGHLY CALCAREOUS SOILS OF DOROODZAN DAM AREA, IRAN

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ABSTRACT

Information on performance of Zn soil tests for highly calcareous soils of southern Iran is lacking. This study was conducted to obtain such information.

Zinc concentration of 18 samples from highly calcareous soils (23-58% $CaCO_3$, pH 7.9-8.5) of Doroodzan Dam area, Fars province of Iran was determined by DTPA, DTPA- NH_4HCO_3, EDTA-$(NH_4)_2CO_3$, EDTA-NH_4OAc and $MgCl_2$ extractants. Bulk samples of the soils were also used in an 8-week greenhouse experiment with corn (*Zea mays* L.) supplied with 0, 10 or 20 mg Zn kg^{-1} of dry soil as $ZnSO_4 \cdot 7H_2O$ to study the correlation of plant response with native soil Zn determined by the extractants.

Application of Zn significantly increased plant dry matter, Zn concentration and uptake at both rates of Zn but the highest plant dry matter was obtained at 10 mg Zn kg^{-1}. Regression equations relating plant responses to Zn and other soil properties showed that Zn extracted by EDTA-$(NH_4)_2CO_3$ was the best single predictor of plant dry matter (quadratic R^2=0.57) and relative yield ("broken-stick" R^2=0.72).

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Some of the other extractants also could be used as predictor of plant responses provided that additional soil properties, i.e., pH, CEC and/or % clay also be included in the equations. Graphical method of Cate-Nelson and "broken-stick" regression model showed that the "critical level" of EDTA-$(NH_4)_2CO_3$ Zn for separating deficient from nondeficient soils was 0.8 and 0.89 mg kg^{-1}, respectively.

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همبستگی پنج عصاره گیر روی با پاسخ گیاه در خاک‌های شدیداً آهکی منطقه سد درودزن ایران

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چکیده

اطلاعاتی در باره نحوه عملکرد آزمونهای روی در خاکهای شدیداً آهکی جنوب ایران در منابع علمی وجود ندارد. این مطالعه به منظور بدست آوردن چنین اطلاعاتی انجام شد.
غلظت روی در ۱۸ نمونه از خاکهای شدیداً آهکی (کربنات کلسیم ۲۳ تا ۵۸ درصد، pH ۷/۹ تا ۸/۵) منطقه درودزن استان فارس توسط عصاره گیرهای DTPA, $MgCl_2$, EDTA-NH_4OAc & EDTA-$(NH_4)_2CO_3$ AND DTPA-NH_4HCO_3 اندازه گیری شد. نمونه‌های این خاکها در یک آزمایش گلخانه‌ای هشت هفته‌ای با گیاه ذرت شامل سه تیمار روی (۰، ۱۰ و ۲۰ میلی گرم روی بصورت سولفات روی آبدار در کیلوگرم خاک) نیز بکار رفت تا همبستگی روی عصاره گیری شده با پاسخ‌های گیاه مطالعه شود.
مصرف روی سبب افزایش معنی‌دار در ماده خشک، غلظت روی و جذب کل روی گیاه در هر دو سطح روی شد ولی حداکثر ماده خشک گیاهی در سطح ۱۰ میلی گرم روی بدست آمد. معادلات رگرسیون بین پاسخهای گیاه و روی و سایر خصوصیات خاک نشان داد که روی عصاره گیری شده با EDTA-$(NH_4)_2CO_3$ بهترین معیار منفرد برای پیش بینی وزن خشک گیاه (R^2=0.57، معادله رگرسیون

درجه دوم) و عملکرد نسبی (R^2=0.72، معادله رگرسیون « خطوط شکسته ») می‌باشد. « سطح بحرانی » این عصاره گیر برای جداسازی خاکهای دارای کمبود از خاک‌های بدون کمبود روی، توسط روش نموداری کیت - نلسون معادل ۰/۸، و توسط روش « خطوط شکسته » معادل ۰/۸۹ میلی گرم روی در کیلوگرم خاک تعیین شد.
برخی از عصاره گیرهای دیگر نیز معادلات رگرسیون معنی‌داری برای پیش‌بینی پاره‌ای از پاسخهای گیاهی بدست دادند ولی این تنها در صورتی بود که علاوه بر روی، سایر خصوصیات خاک نظیر pH، ظرفیت تبادل کاتیونی و یا درصد رس نیز در معادله در نظر گرفته شوند.

INTRODUCTION

Zinc deficiency has been reported both in calcareous soils of southern Iran and neutral to slightly acid soils of northern Iran(22). Beneficial effects of applying Zn to calcareous soils of southern Iran have been reported under field(16) and greenhouse conditions(18). Soil tests involving chemical extractants may be used to assess Zn status of soils and to predict responses to applied Zn before planting. But any such test should be first checked for its correlation with plant responses in soils of southern Iran, which are highly calcareous(10).

DTPA(17) is perhaps the most frequently used Zn extractant for calcareous soils followed by DTPA-$(NH_4)_2CO_3$ (28). EDTA-NH_4OAc (29) and $MgCl_2$ (26) have also been used with different degrees of success. Published reports on the performance of these and other extractants in soils of Iran are limited. Present authors are not aware of any such reports for soils of southern Iran and have encountered only two research reports for other parts of Iran (4,9). Amiri and Doroodi (4) studied the relationship between concentration of Zn and Fe in the leaves of apple trees (*Malus* *pumila Mill.*) and those extractable by DTPA (17) and EDTA(1) from soils of Karaj and Ghazvin, Iran. The conclusion was that neither of the two extractants was satisfactory for prediction of plant Zn. Emami and Behbahanizadeh (9) used DTPA (17), EDTA (1), NH_4OAc (13) and HCl (30) to study the correlation of Zn, Fe, Mn and Cu uptake by corn tops with concentration of the elements in the soil extracts. Based on their results, DTPA and EDTA were recommended as the best extractants for prediction of Zn uptake, followed by NH_4OAc. Hydrochloric acid was found not suitable.

The present study was conducted to compare different extractants for evaluation of Zn status of highly calcareous soils and to obtain quantitative information about their performances.

MATERIALS AND METHODS

Bulk samples of surface soils (0-20 cm) were collected from 18 locations of the area irrigated by Doroodzan Dam, Fars province of Iran to represent major soil series of the area which is approximately 77000 ha and located 50 km northeast of Shiraz, Iran. Description of the area and soils have been given by others(23). Percent clay and chemical properties of the soils are shown in Table 1. The samples were air-dried, passed through 2 mm sieve, and mixed well, separately. Subsamples were taken for laboratory analyses and the rest were placed in plastic pots for greenhouse studies. Each pot contained 2000 g soil.

Table 1. Names of soil series and their selected physical and chemical properties†.

Soil No.	Series	1:2 pH H_2O	1:2 pH $CaCl_2$	Clay	OM	CCE	CEC
				------- %	---------		Meq $100g^{-1}$
1	Garmabad	8.5	7.8	29	2.02	23	19.0
2	Beiza	8.2	7.7	53	1.56	38	15.4
3	"	8.3	7.8	44	1.34	49	9.8
4	Hesamabad	7.9	7.7	63	1.36	49	16.3
5	"	8.0	7.6	48	2.22	53	12.5
6	Kooshkak	8.2	7.7	39	1.07	58	11.7
7	"	8.3	7.6	55	1.02	57	10.7
8	"	8.2	7.5	51	1.67	45	14.6
9	"	8.1	7.7	56	1.44	42	14.6
10	"	7.9	7.5	56	2.83	53	14.0
11	Kor	8.0	7.6	59	1.10	43	14.6
12	"	8.1	7.7	61	1.02	42	16.6
13	Sarooi	8.0	7.8	54	1.42	29	16.9
14	Takhtjamshid	8.1	7.7	60	1.56	35	15.4
15	"	8.1	7.8	65	0.94	35	16.3
16	"	8.4	7.8	48	1.41	34	12.9
17	"	8.3	7.8	52	1.61	38	13.5
18	"	8.4	7.8	26	0.80	49	7.3

† Soil series as reported by Soil Institute of Iran (23); pH by glass electrode in 1:2 soil: electrolyte suspension of H_2O or 0.01 M $CaCl_2$, clay by pipet method (8) after removal of $CaCO_3$ and OM by HCl and H_2O_2, respectively; OM by Walkley-Black (2); CCE by neutralization with HCl (3); CEC by replacement of exchangeable cations by NaOAc, removal of excess Na by alcohol, exchanging Na by NH_4OAc and determining Na (7). Physical and chemical properties, other than CCE, from Karimian and Yasrebi (unpublished data).

Five different extractants namely, DTPA (17), DTPA-NH_4HCO_3 (24), EDTA-$(NH_4)_2CO_3$ (28), EDTA-NH_4OAc (29), and $MgCl_2$ (26) were used for determination of soil Zn. Concentration of Zn in the extracts was determined by Shimadzu AA-670 atomic absorption.

The greenhouse experiment was conducted as completely randomized blocks with three replicates consisting of 18 soils (Table 1) and three levels of Zn, i.e., 0, 10 and 20 mg Zn kg^{-1} as $ZnSO_4.7H_2O$. All pots received uniform applications of 150 mg N kg^{-1} of dry soil as $CO(NH_2)_2$, 50 mg P kg^{-1} of dry soil as $Ca(H_2PO_4)_2$ and 5 mg Fe kg^{-1} of dry soil as FeEDDHA. One-third of N and all of the other nutrients were added to pots as separate aqueous solutions 4 days before planting. The remainder of N was added to pots in two equal portions during the third and fifth week of plant growth. At the time of planting, soil of each pot was stirred well to ensure uniform mixture of soil and nutrients; enough distilled H_2O was added to bring the soil moisture of each pot to field capacity. Five corn seeds were planted in each pot. The pots were irrigated by adding distilled H_2O, whenever necessary. The plants were thinned to three uniform stands per pot during the second week. At the end of 8th week, the aerial parts of plants were cut, rinsed with distilled H_2O, dried at 65° C, weighed, ground and dry-ashed at 500° C. The ash was dissolved in 2N HCl and concentration of Zn was determined in the filtrate by Shimadzu AA-670 atomic absorption.

Plant dry weight, Zn concentration, Zn uptake (i.e., dry weight X Zn concentration), and relative yield (i.e., dry weight at 0 mg Zn/maximum dry weight at 10 or 20 mg Zn) were used as plant responses. The "critical level" of Zn for each extractant was estimated by the graphical method of Cate and Nelson (5,6) and the "broken-stick" regression model (14,20). Statistical analysis of data including F-test, Duncan multiple range test, and development of regression equations were performed using the facilities of Shiraz University Computer Center.

RESULTS AND DISCUSSION

The amount of Zn extracted by different extracting solutions is shown in Table 2. In general, the extractability of Zn by different extractants was in the order:

DTPA-NH_4HCO_3> EDTA-NH_4OAc> EDTA-$(NH_4)_2CO_3$> DTPA> $MgCl_2$.

Table 2. Zinc (Zn) concentration of soils determined by different extractants.

Soil No.	Zn concentration, mg Zn kg^{-1} soil				
	DTPA	DTPA-NH_4HCO_3	EDTA-$(NH_4)_2CO_3$	EDTA-NH_4OAc	$MgCl_2$
1	0.8	9.5	1.7	4.0	0.30
2	0.2	8.2	1.0	2.6	0.25
3	0.6	8.4	1.3	3.0	0.25
4	1.0	10.0	0.8	2.7	0.30
5	0.4	9.4	1.0	3.1	0.50
6	0.4	8.4	1.3	2.0	0.12
7	0.3	7.4	1.0	1.8	0.50
8	0.5	10.6	0.9	1.4	0.15
9	0.4	8.4	1.0	1.8	0.60
10	0.8	8.4	1.3	3.5	0.55
11	0.4	7.6	0.9	3.0	0.65
12	0.3	8.2	0.6	2.4	0.50
13	0.2	7.4	0.6	2.0	0.12
14	0.4	7.4	1.1	2.7	0.15
15	0.3	7.6	0.9	1.3	0.60
16	0.5	9.5	1.1	2.5	0.15
17	0.6	7.4	1.1	1.6	0.20
18	0.5	9.6	0.9	1.6	0.12

The DTPA-extractable Zn of soils is lower than those reported for soils of Karaj and Ghazvin, Iran (4,9). This is believed to be due to differences in soil properties, among them, the higher calcium carbonate equivalent (CCE) of soils of Doroodzan Dam area (Table 1).

Application of Zn significantly increased the plant dry weight, Zn concentration, and Zn uptake (Table 3). Soils were different in this regard but the overall effect was that application of 10 mg Zn kg^{-1} of dry soil produced the highest dry weight of plant tops. Application of 20 mg Zn kg^{-1} of dry soil, although significantly increased the dry weight relative to plants with no applied Zn, produced significantly lower plant dry matter than the 10 mg Zn kg^{-1} of dry soil treatment. Gupta and Singh (11) also reported that the highest dry matter of corn was obtained at the Zn rate of 10 mg kg^{-1} and that the 20 mg kg^{-1} rate had a decreasing effect on yield. However, in soils of Karaj (9), Zn rates as high as 40 mg kg^{-1} produced higher dry weight of corn tops. Maftoun and Karimian (18) reported the difference in response of corn to $ZnSo_4$ in two different soils from Fars province of Iran. Differences in soil properties as well as the change in P/Zn ratio(27) and interaction of other nutrients, such as Fe (19), may be the reason for differences observed in plant response to Zn. Increase in plant Zn concentration and uptake was significant at all rates of applied Zn (Table 3).

Relative yields of corn tops in untreated soils ranged from 49 to 102% (Table 4). Graphical method of Cate and Nelson (5,6) and "broken stick" regression model (14,20) were used to partition the soils into "deficient" and "nondeficient" groups and to estimate the "critical level" of Zn for different extractants (Fig.1,2; Table 5) based on the relative yields. The only significant regression equation obtained by "broken-stick" model (14-20) was for EDTA -$(NH_4)_2CO_3$- extractable Zn. The left-hand portion of the equation was found to be:

Table 3. Effect of applied Zn on dry matter weight, plant Zn concentration, and Zn uptake by plant tops (each figure is average of 54 pots).

Zn rate mg kg^{-1}	Dry matter g pot^{-1}	Zn conc. mg kg^{-1}	Zn uptake µg pot^{-1}
0	16.19 c†	16.1 c	262 c
10	17.74 a	27.1 b	475 b
20	17.00 b	34.7 a	579 a

† In each column, means without common letter are statistically different at 0.05 level of significance.

Table 4. Dry matter, Zn concentration, Zn uptake and relative yield in pots with no applied Zn (each figure is average of 3 pots).

Soil No.	Dry matter g pot^{-1}	Zn conc. mg kg^{-1}	Zn uptake µg pot^{-1}	Relative yield %
1	14.2	20	276	88
2	16.3	12	189	97
3	19.0	25	483	102
4	16.8	22	365	93
5	21.3	22	463	102
6	17.6	14	248	101
7	17.8	14	255	94
8	19.3	11	202	97
9	17.9	15	278	95
10	19.3	12	230	89
11	18.0	14	248	89
12	14.0	14	186	76
13	7.8	10	81	49
14	16.3	19	299	92
15	15.8	17	292	91
16	15.5	16	238	94
17	8.7	19	164	54
18	15.9	15	230	93

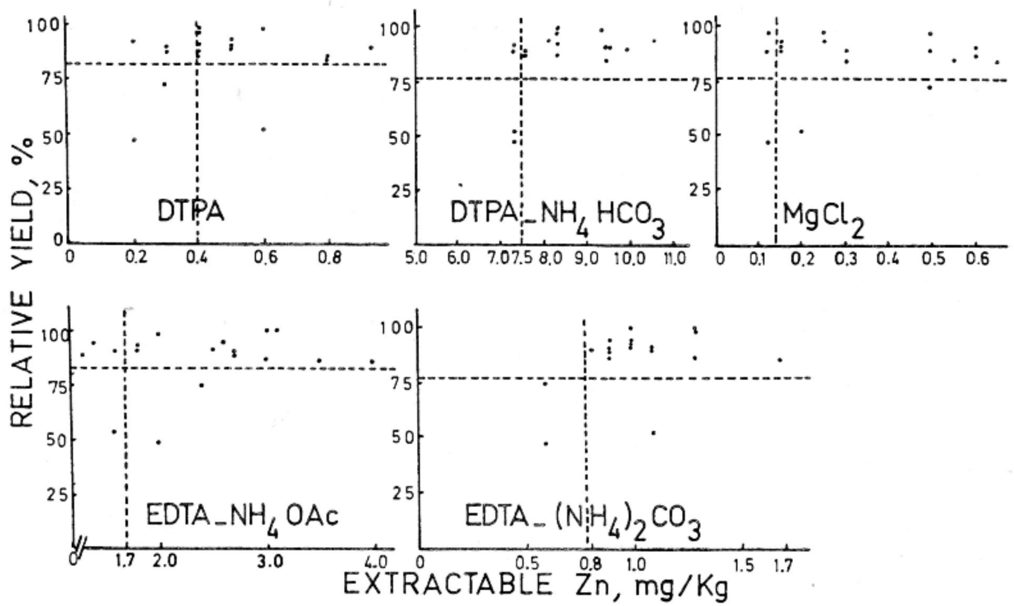

Fig. 1. Cate–Nelson scatter diagrams showing the estimated "critical level" for different extractants.

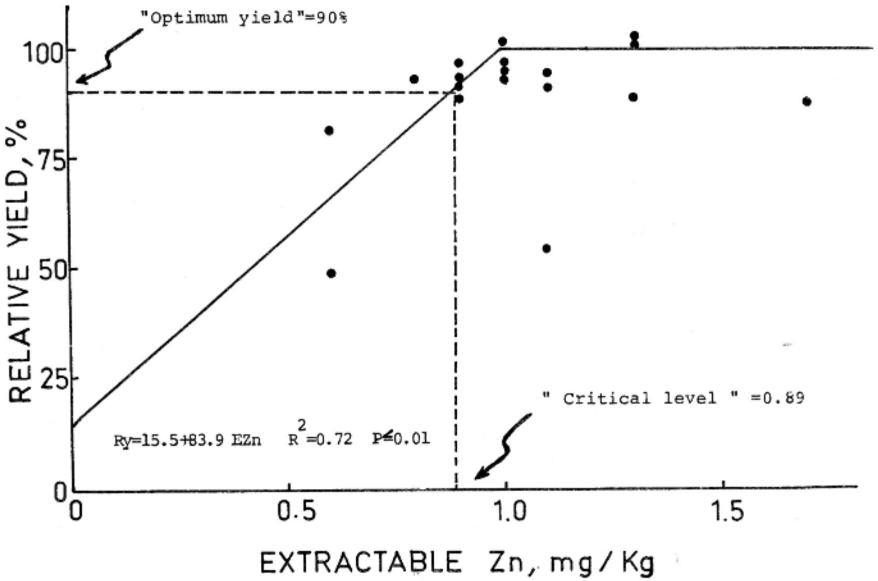

Fig. 2. "Broken-stick" regression model showing the estimated "crtitical level" for EDTA -$(NH_4)_2CO_3$ extractant.

Table 5. Estimated "critical level" of Zn for different extractants, based on relative yields, using Cate-Nelson graphical method and "broken-stick" model.

Extractant	Critical level, mg Zn kg^{-1} of dry soil	
	Cate-Nelson	Broken-stick
DTPA	0.40	ns
DTPA-NH_4HCO_3	7.50	ns
EDTA-$(NH_4)_2CO_3$	0.80	0.89**
EDTA-NH_4OAc	1.70	ns
$MgCl_2$	0.15	ns

** Significant at 0.01 level.

ns Statistically not significant.

$$RY=15.5 + 83.9 \ EZn \quad (R^2=0.72 \ P \leq 0.01) \qquad [1]$$

in which RY is relative yield % ; and EZn is in mg Zn kg^{-1} of dry soil (Fig. 2). Assuming RY=90% as the "optimum yield" gives EZn=0.89 mg Zn kg^{-1} of dry soil as the estimated "critical level" for this particular extractant.

No published report is available on the "critical level" of Zn in soils of Iran. But the results obtained in the present study for DTPA and EDTA-$(NH_4)_2CO_3$ are lower (4, 9, 21) and that of DTPA-NH_4HCO_3 is higher (12) than those reported for soils from other parts of the world.

The only significant correlation between plant Zn concentration (in pots with no applied Zn) and soil Zn was with DTPA which showed a significant simple correlation coefficient of r=0.49. The respective regression equation was obtained as follows:

$$PZn=11.7 + 9.4\ DZn \quad (R^2=0.24\ ,\ P \leq 0.05) \qquad [2]$$

in which PZn and DZn are Zn concentration of plant tops (in pots with no applied Zn) and DTPA-extractable Zn of untreated soil, respectively, both in mg kg^{-1}. The R^2 of Eq.[2] is low but inclusion of % clay increased it considerably as shown in Eq.[3]:

$$PZn= -98.93+9.62DZn+14.35PC \quad (R^2=0.35,\quad P \leq 0.01) \qquad [3]$$

in which PC is % clay of soil by pipet method.

Dry weight of plants (in pots with no applied Zn) showed a highly significant correlation with soil Zn determined by some of the extractants as shown in Eqs.[4,5,6] provided that quadratic term of soil Zn or some chemical properties of soil was included:

$$DM=-9.18+46.05\ EZn-19.09(EZn)^2 \qquad [4]$$

$$(R^2=0.57,\quad P \leq 0.01)$$

$$DM=179.2+1.15\ BZn-21.77\ pHC-0.37\ CEC \qquad [5]$$

$$(R^2=0.63,\quad P \leq 0.01)$$

$$DM=179.7+12.0\ AZn-20.71\ pHC-0.49\ CEC \qquad [6]$$

$$(R^2=0.58,\quad P \leq 0.01)$$

in which DM is dry matter weight of plant top, g pot^{-1}; EZn, BZn and AZn are soil Zn extracted by EDTA-$(NH)_2CO_3$, DTPA-NH_4HCO_3, and EDTA-NH $_4$OAc, respectively, all in mg kg^{-1}; pHC is soil pH in 0.01M $CaCl_2$; and CEC is cation exchange capacity of soil in meq/100g.

Plant Zn uptake did not show any significant correlation with soil Zn determined by extractants under study.

Inspection of eqs. [1] through [6] clearly shows that EDTA-$(NH_4)_2CO_3$ is the only extractant that correlated reasonably well with both relative yield (Eq.[1]) and plant dry weight (Eq.[4]) in the calcareous soils under study. Thus it may be considered the best extractant under the conditions of this study. Other extractants either did not show significant correlation with plant responses or needed the inclusion of other soil chemical properties to obtain a reasonable correlation.

The relative superiority of EDTA-$(NH_4)_2CO_3$ for soils with high $CaCO_3$, which has been also reported by others (27) might be due to the fact that a large proportion of Zn in these soils are in the carbonate form(15). EDTA is the reagent used in sequential extraction of carbonate form of Zn(25).

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