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Chemical Extraction of the Available Iron Present in Soils

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The available iron contents of 40 different soils were determined by using 10 different chemical extraction methods. Maize plants were grown at greenhouse conditions to compare the most appropriate method. Dry matter yields (g pot^{-1}), total and active iron contents of the plants (mg Fe kg^{-1}), total and active iron amounts taken from the soils (mg pot^{-1}) of non-applied iron pots and their relative amounts ($Fe_0/Fe_3 \times 100$) were used as biological indices. The available iron amounts of the soils found different according to the chemical extraction methods. 0.05 M EDTA (pH 7), 0.005 M diethylene triamine pentaacetic acid (DTPA) + 0.01 M $CaCl_2$ + 0.1 M triethanolamine (TEA) (pH 7) and active $Fe(COONH_4)_2.H_2O + (COOH)_2.2H_2O$ methods used for predicting available iron content of the soils gave a high degree of correlation with the biological indices. However, because of giving much more correlations with the biological indices, 0.05 M EDTA (pH 7) method suggested as the best determination method for assessing availability of iron in these soils.

Key Words: Available iron, Extraction methods, Maize, Soil.

INTRODUCTION

The soil test simply indicates the nutrients' level in the soil and together with plant analysis. They are important agronomic tools for determining crop nutrient needs, predicting the nutrient deficient areas and preventing the deficiencies. DTPA [(diethylene triamine pentaacetic acid) + $CaCl_2$ + TEA (triethanolamine)] reported by Lindsay and Norvell[1] used for the determination of available iron status of the soils in a wide range because of having critical value and also having a chance to determine zinc, copper and manganese contents with a single extractant. Otherwise, available iron determination in calcareous soils by using commonly accepted method (DTPA + $CaCl_2$ + TEA) is not found descriptive in estimating iron nutrition status of plants[2] because of the paradox similar to "the chlorosis paradox" that has been called for chlorotic leaves including as much or over total iron than green healthy ones[3]. The results of numerous analyses reports showed, visually and analytically iron chlorosis in the plants in spite of DTPA extractable soil iron concentrations above the critical concentration range[4-6].

On the other hand, a significant part of the fruit industry in Europe and especially in the Mediterranean area including Turkey is located on calcareous or alkaline soils, which favour the occurrence of iron chlorosis[7]. Many agronomic and horticultural species grown in the region also exhibit symptoms of iron chlorosis. 20-30 % reduction was reported only in peach production, which has of economic and traditional importance among the crops grown in the Bursa region[8]. Therefore, scientists proposed a series of extraction solutions to detect available soil iron. Although several chemical extraction methods[9-15] have been developed, none of them was selected as a suitable standard iron extraction method for calcareous soils[16].

The objective of this study is to compare some chemical iron extraction methods in relation with soil factors and chlorosis indicators in the plant to predict the chlorosis risk potential early in the growing season.

EXPERIMENTAL

Soil samples were collected from 0-30 cm depth from 40 different cultivated soils in Bursa province (39º35' and 40º40' N latitude, 28º10' and 30º00' E longitude) in Turkey[17]. Soil samples were air-dried in the laboratory, crushed with wooden pestle, screened through a 2 mm sieve and analyzed to determine some physical and chemical characteristics. pH and electrical conductivity (EC) determined in saturation extractant[18]. Soil texture by Bouyoucos hydrometer method[19], organic matter by modified Walkley-Black[17], lime by Scheibler calcimeter method[20]. The available iron contents of the soil samples were determined through 10 different chemical extraction methods. Some properties of these methods are given in Table-1.

TABLE-1
SOME PROPERTIES OF THE CHEMICAL EXTRACTION METHODS

Method No.	Extractants	Soil:Extract ratio (w/v)	Shaking time (h)	Ref.
M1	0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA (pH 7.3)	1:2	2.00	1
M2	1 M NH_4HCO_3 + 0.005M DTPA (pH 7.6)	1:2	0.25	21
M3	0.05 N HCl + 0.025 N H_2SO_4	1:5	0.25	22
M4	0.01 M EDTA + 1N $(NH_4)_2CO_3$ (pH 8.6)	1:2	0.50	23
M5	1 N NH_4OAc (pH 4.8)	1:5	0.50	24
M6	0.1 N HCl	1:10	0.50	25
M7	'Aktif Fe' Amonium oxalat$(COONH_4)_2.H_2O$ + Oxalic acid $(COOH)_2.2H_2O$ (pH 3.0)	1:20	2.00	26
M8	0.2M CH_3COOH + 0.25 M NH_4Cl + 0.005 M Citric acid $(C_6H_8O_7)$ + 0.05 M HCl (pH 1.3)	1:10	0.50	27
M9	0.05 M EDTA (pH 7)	1:10	1.00	28
M10	0.43 M HNO_3	1:10	2.00	26

EDTA = Ethylene diamine tetraacetic acid; DTPA = Diethylene triamine pentaacetic acid; TEA = Triethanolamine.

A greenhouse experiment was designed in a randomized block design replicated three times during May and July 2005. Air-dried 2.5 kg soil was filled into plastic pots. Each pot was fertilized with 100 mg kg^{-1} N (NH_4NO_3) and 80 mg kg^{-1} P (KH_2PO_4). Four different rates of Fe (Fe_0: 0; Fe_1: 2.5; Fe_2: 5 and Fe_3: 10 mg kg^{-1})

were applied to soil as chelated Fe (Fe-EDDHA). Maize (*Zea mays* L.) was grown and four plants were left in each pot after germination. The water content of the pots was adjusted to 70 % of field capacity during the experiment. Maize plants were harvested after 45 d. Plant materials were washed in tap water and then twice with deionized water, dried in a forced air oven at 70 ºC for 72 h; then ground. The ground plant samples were wet digested using a HNO_3-$HClO_4$ mixture at a volume ratio of 4:1 and iron contents in digest were determined by atomic absorption spectrophotometry[29] (Philips PU 9200x, Pye Unicam Ltd. GB). Active iron contents were determined in dry plant parts incubating 24 h in 1 N HCl extraction solution (1:10) which was modified by Llorente *et al.*[30] and amounts were measured by atomic absorption spectrophotometer. Dry matter yield, Fe concentration, Fe uptake and relative values of these biological indices were used as biological method. Relative biological indices were calculated as $Fe_0/Fe_{max} \times 100$. All the analyses were conducted in triplicate. The values taken from the analyses were subjected to statistical analysis, the mean values were compared using LSD (least significant differences) multiple range test and simple correlations were measured with the computer program Tarist[31]. The extraction method that displayed the highest correlation coefficient with the biological indices was recommended for the determination of available iron[11,32,33].

RESULTS AND DISCUSSION

Some physical and chemical properties of the soils: According to the analysis, the textures of the soils were clay to sandy loam (data not shown). There is no salt problem. Organic matter contents of the soils were determined between low and medium classes[17]. pH was slightly acid to slightly alkaline. $CaCO_3$ contents of the soils generally differ between low and very high (Table-2).

Iron contents of the soils according to different extraction methods: Ten chemical extraction methods were used for the determination of available iron.

TABLE-2
SOME PHYSICAL AND CHEMICAL PROPERTIES OF THE SOILS

	Sand (%)	Silt (%)	Clay (%)	pH	EC (mS cm^{-1})	$CaCO_3$ (%)	Organic matter (%)	Total N (%)
Min.	12.44	13.13	15.85	5.47	0.14	0.11	1.10	0.072
Max.	68.86	48.00	68.34	7.84	1.72	20.13	3.93	0.210
Mean	36.16	23.75	40.08	7.30	0.66	4.39	1.95	0.120

	Available P (mg kg^{-1})	Exchangeable ions (me100 g^{-1})				Available micronutrients (mg kg^{-1})		
		Na	K	Ca	Mg	Zn	Cu	Mn
Min.	2.78	0.09	0.17	5.46	1.09	0.19	0.86	1.36
Max.	98.39	1.64	2.28	64.32	19.04	5.25	27.01	44.63
Mean	22.45	0.38	0.84	25.33	5.44	1.09	6.48	11.21

Values are minimum, maximum and average values of 40 different cultivated soils.

According to the methods, available iron amounts of the soils changed within a wide range (Table-3). The amounts varied widely not only depending on the chemicals, their concentrations, soil solution ratio, pH and shaking time of the extraction method but also depending to the soil properties[32,33]. Some physical and chemical properties of the soils affected the availability of iron to plants. The causes of low iron availability are coarse or heavy texture, high humidity, poor soil aeration and compaction, high pH and lime, low organic matter contents of the soils[4,34-37]. DTPA extractable iron contents of the soils (M1) differ between middle and high levels according to the critical values defined by Lindsay and Norvell[1] (Table-3). Although the lowest iron amounts were determined by method 4 (M4) 0.01 M EDTA + 1 N $(NH_4)_2CO_3$ (pH 8.6) and method 5 (M5) 1 N NH_4OAc (pH 4.8), method 7 (M7) which is called as 'active Fe' amonium oxalate $(COONH_4)_2.H_2O$ + oxalic acid $(COOH)_2.2H_2O$ (pH 3.0) gave the highest iron amounts.

TABLE-3
IRON CONCENTRATIONS OF THE SOILS OBTAINED BY CHEMICAL EXTRACTION METHODS

	Extractable iron (mg kg^{-1})									
	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Min.	1.59	1.49	0.13	0.20	0.49	0.43	107.55	7.82	4.19	4.90
Max.	42.78	65.81	50.09	12.93	9.58	101.52	2080.52	202.42	216.00	2454.98
Mean	10.17	11.02	6.93	1.32	2.54	28.22	842.90	50.19	65.89	574.61

Values are minimum, maximum and average values of 40 different cultivated soils.

Effects of increasing iron application doses on yield, iron concentration and iron uptake of maize: Dry matter yield of the maize plants was affected slightly by iron applications (Table-4). Dry matter yield of the maize plants varied between 12.15 and 49.14 g pot^{-1}. Especially first (2.5 mg kg^{-1}) and second (5.0 mg kg^{-1}) iron doses increased the dry matter but decreased at the third (10 mg kg^{-1}) application dose of iron. Iron affected the uptake of other nutrient elements due to their antagonistic effects. Especially the highest application dose of iron limited uptake of zinc. Low zinc contents of soils and their limited uptake affected plants growth and the dry matter amounts negatively. Past researches showed that zinc plays important role in carbohydrate, protein and auxin metabolism, stimulates the growth and increases the dry matter yield[38-41].

Application of increasing amounts of iron affected total iron concentrations and total iron uptake of maize plants (Table-4). Total iron concentrations of the maize plants varied between 27.33 and 133.33 mg kg^{-1} and found high at second (5.0 mg kg^{-1}) and third (10 mg kg^{-1}) doses. The highest iron uptake was also determined at third application dose and varied between 0.62 and 2.95 mg tdw^{-1}. Active iron concentrations and active iron uptake of the maize plants tend to increase with the application of iron and all the applications were found higher than control (Table-4). Several researchers[42-45] also found similar results.

TABLE-4
EFFECTS OF IRON APPLICATIONS ON DRY MATTER YIELD, TOTAL AND ACTIVE IRON CONCENTRATIONS AND IRON UPTAKE OF MAIZE

		Iron doses (mg kg^{-1})			
		0	2.5	5.0	10.0
Dry matter yield of maize, g pot^{-1}	Min	12.15	12.00	12.40	12.21
	Max	48.57	49.14	47.91	49.00
	Mean	31.23 ab	31.72 a	31.50 a	30.66 b
	$LSD_{0.01}$: 0.599				
Total iron concentrations of maize, mg kg^{-1}	Min	27.33	35.33	35.33	33.67
	Max	102.00	113.67	107.33	133.33
	Mean	53.58 b	53.53 b	55.03 b	62.91 a
	$LSD_{0.01}$: 3.831				
Total iron uptake of maize mg tdw^{-1}	Min	0.62	0.71	0.63	0.92
	Max	2.95	2.54	2.54	2.70
	Mean	1.61 b	1.61 b	1.62 b	1.73 a
	$LSD_{0.01}$: 0.088				
Active iron concentrations of maize, mg kg^{-1}	Min	17.60	21.13	21.40	20.53
	Max	57.00	58.33	61.40	95.80
	Mean	28.53 d	30.18 c	32.49 b	37.40 a
	$LSD_{0.01}$: 1.283				
Active iron uptake of maize mg tdw^{-1}	Min	0.31	0.39	0.43	0.49
	Max	1.32	1.45	1.38	1.93
	Mean	0.83 d	0.89 c	0.94 b	1.04 a
	$LSD_{0.01}$: 0.038				

Values are minimum, maximum and average values of 40 different cultivated soils.

Relations between chemical extraction methods and biological indices: To select the most appropriate available soil iron determination method; plants dry matter yield, total Fe concentration, total Fe uptake and relative values of these biological indices were used as biological method[32,33]. Past researches showed that total iron amounts of the plants were not associated with the occurrence of chlorosis and they are not a valid criterion in evaluation of iron status of plants. Active iron (Fe^{2+}) which is closely related iron form with the chlorosis, known as a better nutritional indicator than total iron[5,46-50]. Therefore, besides the total amounts, active iron amounts were also determined and used as biological indices. Not all chemical extraction methods were given significant correlations with the biological indices and neither of the methods correlated with all of the biological indices. Method 1 (M1), method 7 (M7), method 8 (M8), method 9 (M9) and method 10 (M10) were the methods correlated with the biological indices (Table-5). The highest correlation coefficients were observed between method 9 (M9) and the biological indices such as dry matter yield, relative iron concentrations of plants and relative uptake of iron from soil. The highest correlation coefficients between method 7 (M7) and the biological indices were determined only at uptake of iron from soil. Method 8 (M8) is the only method, which was correlated with active iron amounts of unfertilized

TABLE-5
CORRELATION COEFFICIENTS (r) FOR THE RELATIONSHIP BETWEEN CHEMICAL EXTRACTION METHODS AND BIOLOGICAL INDICES

	Chemical extraction method	Biological indices									
		Non-application of Fe in pots					Fe_0 / Fe_{max} × 100				
		DMY	Fe conc. of plants		Uptake of Fe amount from soil		RDMY	Relative Fe conc. of plants		Relative uptake of Fe amount from soil	
			TF	AF	TF	AF		TF	AF	TF	AF
M1	0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA	0.305†	ns	ns	ns	ns	-0.310†	0.306†	0.420‡	ns	0.322†
M2	1 M NH_4HCO_3 + 0.005M DTPA (pH 7.6)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M3	0.05 N HCl + 0.025 N H_2SO_4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M4	0.01M EDTA + 1N $(NH_4)_2CO_3$ (pH 8.6)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M5	1 N NH_4OAc (pH 4.8)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
M6	0.1 N HCl	ns	ns	ns	ns	0.327†	ns	ns	ns	ns	ns
M7	Active Fe $(COONH_4)_2.H_2O$ + $(COOH)_2.2H_2O$	ns	ns	ns	0.468‡	0.485‡	-0.341†	0.418‡	ns	0.360†	ns
M8	0.2M CH_3COOH + 0.25 M NH_4Cl + 0.005 M $C_6H_8O_7$ + 0.05 M HCl (pH 1.3)	ns	ns	0.306†	ns	0.413‡	ns	0.332†	ns	ns	ns
M9	0.05 M EDTA (pH 7)	0.497‡	ns	ns	0.422‡	0.420‡	-0.324†	0.455‡	0.446‡	0.382†	0.345†
M10	0.43 M HNO_3	ns	ns	ns	0.350†	0.399‡	ns	ns	ns	ns	ns

DMY = Dry matter yield; RDMY = Relative dry matter yield; TF = Total Fe; AF = Active Fe.
ns = non-significant; †$p < 0.05$; ‡$p < 0.01$
r = 0.05 : 0.304; r = 0.01 : 0.393
n = 40; n = 40

pots and method 10 (M10) was correlated with total and active iron amounts taken from soil. Significant correlation coefficients were also observed between method 1 (M1) and the biological indices but their coefficients were not found high. Therefore, the method 9 (M9) was found much more correlated method than the other. According to the results the order of significance for the extraction methods were as follows: method 9 (0.05 M EDTA (pH 7)) > method 7 (ammonium oxalate $(COONH_4)_2.H_2O$ + oxalic acid $(COOH)_2.2H_2O$ (pH 3.0)) > method 1 (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA (pH 7.3)) > method 8 (0.2 M CH_3COOH + 0.25 M NH_4Cl + 0.005 M citric acid ($C_6H_8O_7$) + 0.05 M HCl (pH 1.3)) > method 10 (0.43 M HNO_3 and method 6 (0.1 N HCl). The use of salt (NH_4OAc); chelate + bicarbonate salt mixes (EDTA + $(NH_4)_2CO_3$), (NH_4HCO_3 + DTPA) and acid mixed (HCl + H_2SO_4) extraction methods were found inadequate in the determination of available iron contents. Furthermore, only chelate (EDTA); chelate + salt mixes (DTPA + $CaCl_2$ + TEA); salt + acid mixes ($(COONH_4)_2.H_2O$ + $(COOH)_2.2H_2O$), (CH_3COOH + NH_4Cl + ($C_6H_8O_7$) + HCl) and acid (HNO_3, HCl) extraction methods were determined to be more suitable in determination of available iron contents for such soils. Several investigators[11,22,33,36,51] also found chelate and chelate + salt mix methods much more suitable and suggested for various regions. In agreement with present research, Danisman[13] and Turan *et al.*[52] reported 0.05 M EDTA as the most suitable method among 10 different extraction methods for the soils of Mediterranean region of Turkey. Hakerlerler *et al.*[9] also found EDTA and EDTA + NH_4OAc methods that have the highest correlations with soil iron and leaves' active iron amounts. Misra and Pande[53] concluded 0.02 N EDTA as suitable method in their researches with the soils of India, which have 7.1 to 9.1 pH values and 1.05 to 9.20 % of lime. According to the results of this work, 0.05 M EDTA method was suggested the most suitable method for determining the available iron contents of soils because of having much more and the highest correlations with the biological indices.

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