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Determination of Suitable Chemical Extraction Methods for Available Iron Content of the Soils from Edirne Province in Turkey

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Abstract: The aim of this research was to determine the available iron (Fe) content of the soils of Edirne Province and the most suitable chemical extraction method. Eight chemical extraction methods (0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA; 0.05 M HCl+0.012 M H_2SO_4; 1 M NH_4OAc (pH: 4.8); 0.01 M EDTA+1 M NH_4OAc; 1 M $MgCl_2$; 0.01 M EDTA+1 M $(NH_4)_2CO_3$; 0.005 M DTPA+1 M NH_4HCO_3 and 0.001 M EDDHA methods) and six biological indices (dry matter yield, Fe concentration, Fe uptake, relative dry matter yield, relative Fe concentration, relative Fe uptake) were compared. Biological indices were determined with Barley (*Hordeum vulgare L.)* grown under greenhouse conditions. The highest correlation coefficients (r) were determined to be between 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA method and biological indices and between 0.005 M DTPA+ 1M NH_4HCO_3 method and biological indices. The correlation coefficients (r) for 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA method were r:0.621**; r:0.823**; r:0.810**; r:0.433**; r:0.558** and r:0.640** and for 0.005 M DTPA+1 M NH_4HCO_3 method are r:0.618** ; r:0.520**; r:0.679**; r:0.521**; r:0.492** and r:0.641**, (**:p<0.01) respectively. These extraction methods, among all the methods tested were suggested for the determination of available Fe content of Edirne Province soils.

Key words: Fe, extraction methods, barley, biological indice

Introduction

Although required in very small amounts iron (Fe) is an essential nutrient and plays a major role in plant growth and development. The trend to more intensive crop production with higher yields and heavier use of nitrogen (N), phosphorus (P) and potassium (K) fertilizers increases the need for Fe and other trace elements in agriculture. Soil analyses are helpful in determining whether a soil can supply adequate amounts of Fe for optimal growth or not.

Fe deficiency is one of the most common trace element problems in the world now-a-days. It is frequent in high pH, high lime, low organic matter content and sandy soils (Lindsay and Schwab, 1982). Available Fe is inadequate in about 26.87 % of Turkey's soils (Eyupolu *et al*., 1998). Despite the fact that several Fe extraction methods have been developed none of them was suitable to be a standard one (Loeppert and Inskeep, 1996).

Lindsay and Norvell (1978) and Norvell (1984) suggested DTPA (pH: 7.3) method for the determination of available Fe content with regards to neutral and alkaline soils.

The 0.001 M EDDHA method was suggested for the determination of available Fe content in USA. Because this method has produced the highest correlation with biological indices (Johnson and Young, 1973).

Hatipoğlu (1981) has determined correlation coefficients (r) between eleven extraction methods and biological indices to find about the available Fe content of the soils from Central South Anatolia. The highest correlation coefficient (r) determined was between 0.001 M EDDHA method and biological indices.

In this research, suitable method for the determination of available Fe content of the soils of Edirne region was investigated.

Materials and Methods

Soil samples were taken at 0-20 cm depth from 25 different cultivated soils in Edirne (Kacar, 1995). Soil pH (Thomas, 1996), lime (Loeppert and Suarez, 1996), CEC (Sumner and Miller, 1996) and texture (Gee and Bauder, 1986) were determined for each sample.

Some physical and chemical properties of the soil samples are determined (Table 1). The pH values of soil samples ranged from 6.29 to 7.94; $CaCO_3$ contents were between 0.00 and 15.10%; CEC values were between 16.44 and 37.22 cmol kg^{-1}; texture of soil samples were between clay © and sandy loam (SL).

The available Fe contents of the soil samples were determined through eight different chemical extraction methods. These methods are 0.005 M DTPA+0.01M $CaCl_2$ +0.1 M TEA (Lindsay and Norvell, 1978); 0.05 M HCl+0.012 M H_2SO_4 (Wear and Evans, 1968); 1 M NH_4OAc (Olson, 1948); 0.01 M EDTA+1 M NH_4OAc (Navrot and Ravikovitch,1968); 1 M $MgCl_2$ (Stewart and

Table 1: Some physical and chemical properties of the soil samples

				Particle size distribution		
Soil No.	pH (1:2.5)	$CaCO_3$,%	CEC,cmol kg^{-1}	Clay,%	Silt,%	Sand,%
1	7.01	1.20	22.65	32.9	26.8	40.3
2	7.48	1.91	26.18	39.9	23.9	36.2
3	7.30	0.30	16.44	11.6	18.8	69.6
4	6.98	0.54	29.47	42.7	21.5	35.8
5	7.30	3.47	26.55	43.4	17.5	39.1
6	6.29	0.00	19.25	18.7	16.3	65.0
7	7.50	4.02	24.43	27.6	31.1	41.3
8	7.53	7.89	28.14	45.2	21.7	33.1
9	7.66	8.55	26.32	30.6	22.0	47.4
10	7.62	5.12	20.32	17.6	28.4	54.0
11	7.67	15.10	28.25	27.2	16.2	46.6
12	7.45	9.32	30.60	33.0	24.5	42.5
13	7.30	0.90	28.73	20.7	24.3	55.1
14	7.46	1.80	19.56	15.8	25.2	59.0
15	7.32	0.38	37.22	48.0	11.9	40.1
16	7.40	9.26	34.52	32.7	25.8	41.5
17	7.34	1.22	30.46	23.2	29.3	47.5
18	7.27	3.34	16.54	17.8	19.0	63.2
19	7.64	4.20	22.06	23.4	23.9	52.7
20	7.42	2.23	27.34	23.5	28.3	48.2
21	7.52	7.85	34.15	56.8	18.9	24.3
22	7.94	5.24	35.04	44.0	28.9	27.1
23	7.83	12.36	29.50	40.1	26.9	33.0
24	7.52	6.85	24.62	29.0	40.9	30.1
25	7.47	3.21	20.48	22.4	30.2	47.4

Table 2: Chemical extraction methods were used for the determination of available Fe contents of the soil samples

Methods	Soil – solution ratio	Shaking time	Reference
0.005 M DTPA +0.01 M $CaCl_2$+0.1 M TEA	1 : 2	2 h	Lindsay and Norvell (1978)
0.05 M HCl+0.012 M H_2SO_4	1 : 4	15 minutes	Wear and Evans (1968)
1 M NH_4OAc (pH: 4.8)	1 : 4	30 minutes	Olson (1948)
0.01 M EDTA+1 M NH_4OAc	1 : 10	1 h	Navrot and Ravikovitch(1968)
1 M $MgCl_2$	1 : 5	45 minutes	Stewart and Berger (1965)
0.01 M EDTA+1 M $(NH_4)_2CO_3$	1 : 2	30 minutes	Trierweiler and Lindsay (1969)
0.005 M DTPA+1 M NH_4HCO_3	1 : 2	15minutes	Soltanpour (1991)
0.001 M EDDHA	1 : 2	10 minutes	Johnson and Young (1973)

Berger, 1965); 0.001 M EDTA+1 M $(NH_4)CO_3$ (Trierweiler and Lindsay, 1969); 0.005 M DTPA+1 M NH_4HCO_3 (Soltanpour, 1991) and 0.001 M EDDHA (Johnson and Young, 1973). Some properties of these extraction methods are given in Table 2.

A greenhouse experiment was designed in a randomised complete block design replicated three times during July and August 2001. Air dried 2.5 kg soil was filled into plastic pots. Barley (*Hordeum vulgare* L.) was used as a test plant because it is sensitive to Fe deficiency (Martens and Westermann, 1991). Each pot was fertilized with 140 mg kg^{-1} N (NH_4NO_3) and 80 mg kg^{-1} P_2O_5 (KH_2PO_4), according to average application rates of N and P_2O_5 to barley in this region. Four different rates of Fe (Fe_0:0; Fe_1:10; Fe_2:20; and Fe_3:30 mg kg^{-1}) were applied to soils as Fe-EDDHA compound. Fifteen plants were left in each pot after germination. The water content of the pots was adjusted to 70% of field capacity during the experimental period. Barley shoots were harvested after 60 days. Harvested shoots were washed once tap water and twice distilled water and dried at 65°C. Dry matter yields were also determined.

Dried and ground plant materials were digested using HNO_3+$HClO_4$ (Kacar, 1972). The Fe concentrations of plants were determined with AA-660 Shimadzu Atomic Absorption Spectrophotometer (AAS) (Kacar,1995).

Dry matter yield, Fe concentration, Fe uptake and the relative values of these biological indices were used as biological methods. Relative biological indices were calculated as Fe_0 / $Fe_{maximum\ biological\ indice}$ X 100

Correlation coefficients (r) were measured between available Fe content of the soils according to eight different methods and biological indices (dry matter yield, Fe concentration, Fe uptake, relative dry matter yield, relative Fe concentration and relative Fe uptake) of barley plants. Significance of the correlation coefficients (r) was checked at 1 and 5% levels (Yurtsever, 1984).

The extraction method which displayed the highest correlation coefficient (r) with the biological indices was recommended for the determination of available Fe content of the soils of Edirne Province.This approach for selecting extracting methods has been used before in the determination of suitable methods for many plant nutrients (Aydemir, 1981; Aydemir and Köleli, 1996; Arriechi and Ramirez, 1997; Yildiz and Özkutlu, 1997; Akman and Yildiz, 1999; Özgüven and Katkat, 2001; Elkarim and Usta, 2001).

Results and Discussion

Effect of increasing Fe application rates on barley yiels, Fe concentration and Fe uptake: Dry matter yield of the barley plants was affected by Fe application. Dry matter yield of 18 soil samples of total 25 samples were found to be higher in Fe_2 (20 mg kg^{+}) dose while it was higher in the rest 7 soil samples in Fe_3 (30 mg kg^{-1}) dose (Table 3). The available Fe content of these 18 soil samples were higher that those of 7 samples (Table 4).

The Fe concentration and Fe uptake of the plants increased with increasing Fe application (Table 3). Fe concentration of plants determined varied between 83 and 161 mg kg^{-1} for barley and were sufficient (Pseiter and Robinson, 1986).

In general dry matter yield using Fe_2 concentration of the barley plants was determined to be higher for soils 1, 2, 8, 9, 12, 13, 14, 15, 16, 17, 18,19, 20, 21, 22, 23, 24 and 25 (Table 3). The reason of this result maybe higher available Fe content in this soils.

The effect of Fe application on the biological indices of the barley plants was determined to be significant at 1% level and the results obtained are in agreement with earlier reports (Aydemir, 1981; Elinç, 1997; Baar and Ozgumu, 1999).

The Fe contents of soils according to different extraction methods: Eight extraction methods were used for the determination of available Fe content of the soil samples (Table 4). Available Fe varied widely depending on the extraction method used, reasons for which could be pointed out as the type, concentration, pH, shaking time, soil solution ratio of the extraction solution and variability observed in the physical and chemical properties of the soils used.

Some physical and chemical properties of soils affected the availability of Fe to plants. The causes of low Fe availability are coarse texture, high pH and lime, low CEC and organic matter content in soils (Lindsay and Schwab, 1982; Elinç, 1997).

Available Fe contents of the soils 8, 9, 11, 12, 21 and 23 were lower than the rest of the soils, which may have been induced by the pH values and lime contents of the soils (Table 1). On the other hand available Fe contents of the soils 4, 6, 15 and 17 with low lime and pH levels were higher. Similarly lower available Fe content was determined in the soils 3, 10, 14 and 18 of lower clay content and CEC than the soils 4, 5, 15, 17 and 22 of high clay and CEC values, which demonstrates that available Fe content is influenced by physical and chemical properties of soils (Bloom and Inskeep, 1988; Marschner, 1995).

Higher available Fe contents of soil samples were determined with 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA; 0.005 M DTPA+1 M NH_4HCO_3 and 0.001 M EDDHA methods in comparison with other extraction methods. On the other hand, the lowest available Fe content of soil samples were determined with 1 M NH_4OAc and 1 M $MgCl_2$ methods. These results also show that higher available Fe was determined using methods with chelate+salt (0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA; 0.005 M DTPA+1 M NH_4HCO_3; 0.01 M EDTA+1 M NH_4OAc and 0.01 M EDTA+1 M $(NH_4)_2CO_3$ methods) and chelate alone (0.001 M EDDHA) in comparison to the methods using salt (1 M NH_4OAc and 1 M $MgCl_2$ methods) and acid (0.05 M HCl+0.012 M H_2SO_4 method). Mean available Fe content of the soils were determined to be 3.77; 2.09; 1.19; 3.62; 1.67; 2.21; 3.58 and 3.14mg kg^{-1}, using the methods 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA; 0.05 M HCl+0.012 M H_2SO_4; 1 M NH_4OAc; 0.01 M EDTA+1 M NH_4OAc; 1 M $MgCl_2$; 0.01 M EDTA+1 M $(NH_4)_2CO_3$; 0.005 M DTPA+1 M NH_4HCO_3 and 0.001 M EDDHA, respectively. The acid and salt methods of HCl + H_2SO_4, $MgCl_2$ and NH_4OAc, which gave the lowest available Fe, are not recommended for the determination of Fe content in neutral and alkaline soils. The use of chelate and chelate+salt methods are suggested in these types of soil (Kacar, 1995).

The relationships between chemical extraction methods and biological indices: Significant correlation coefficients were observed between all chemical extraction methods, except 1 M NH_4OAc method and the biological indices (dry matter yield, Fe concentration, Fe uptake, relative dry matter yield, relative Fe concentration, relative Fe uptake) at 1% level (Table 5). The highest correlation coefficients (r) were determined between 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA and 0.005 M DTPA+1M NH_4HCO_3 methods and biological indices. The results obtained from 0.001 M EDDHA method followed the above methods regarding the correlation coefficients (r).

According to the results the order of significance for the extraction methods are as follows: 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA> 0.005 M DTPA+1 M NH_4HCO_3> 0.001 M EDDHA> 0.01 M EDTA+1 M NH_4OAc> 0.01 M EDTA+1 M $(NH_4)_2CO_3$> 0.05 M HCl+0.012 M H_2SO_4> 1 M $MgCl_2$> 1 M NH_4OAc.

The available Fe content of the soil samples were determined to be either insufficient or moderately

Table 3: The effect of Fe application on biological indices of barley

Soil No.	Dry matter yield, g pot^{-1}				Fe concentration of plant, mg kg^{-1}				Uptake of Fe by shoots, μg pot^{-1}			
	Fe_0	Fe_1	Fe_2	Fe_3	Fe_0	Fe_1	Fe_2	Fe_3	Fe_0	Fe_1	Fe_2	Fe_3
1	2.41a	2.57b	2.72c	2.61b	94a	101b	113c	119d	227a	260b	307c	311c
2	2.24a	2.34b	2.48c	2.37c	83a	94b	98b	110c	186a	220b	243c	261c
3	1.91a	2.02b	2.19c	2.12c	97a	102b	110c	118d	185a	206a	241b	250b
4	3.55a	3.67b	3.80bc	3.71b	104a	110b	121c	127d	369a	404b	460c	471c
5	3.40a	3.62bc	3.70c	3.58b	108a	113b	118c	122c	367a	409b	437c	437c
6	1.98a	2.25b	2.47c	2.40c	98a	107b	119c	123c	194a	241b	294c	295c
7	2.59a	2.71b	2.83c	2.75bc	116a	121b	125b	134c	300a	328b	354c	369c
8	2.80a	3.07b	3.26c	3.15b	103a	133b	139c	147d	288a	408b	453c	439c
9	2.38a	2.58b	2.72c	2.60b	95a	104b	119c	130d	226a	268b	324c	338c
10	1.73a	1.95b	2.19c	2.10c	97a	118b	130c	135d	168a	230b	285c	284c
11	1.78a	1.97b	2.28c	2.14b	94a	117b	129c	134d	167a	230b	294c	289c
12	2.56a	2.69b	2.87d	2.72c	98a	116b	125c	131d	251a	312b	359c	356c
13	1.82a	1.95b	2.19d	2.07c	88a	107b	114c	120d	160a	209b	250c	248c
14	1.69a	1.75ab	1.94c	1.80b	97a	113b	127c	138d	164a	198b	246c	248c
15	2.87a	3.02b	3.27d	3.14c	101a	117b	129c	140d	290a	353b	422c	440c
16	2.65a	2.84b	3.18d	3.04c	92a	110b	127c	139d	244a	312b	404c	423c
17	2.48a	2.72b	2.94c	2.80b	105a	117b	130c	141d	260a	318b	382c	395c
18	1.76a	1.89b	2.04c	1.92b	93a	110b	129c	134d	164a	208b	263c	257c
19	1.94a	2.17b	2.30c	2.21bc	99a	114b	130c	140d	192a	247b	299c	309c
20	1.72a	1.92b	2.27d	2.14c	101a	120b	134c	141d	174a	230b	304c	302c
21	2.86a	3.12b	3.29c	3.17b	105a	120b	139c	147d	300a	374b	457c	466c
22	3.26a	3.42b	3.64c	3.51b	103a	117b	132c	145d	336a	400b	480c	509d
23	3.40a	3.60b	3.81d	3.70c	105a	119b	142c	161d	357a	428b	541c	596d
24	2.47a	2.71b	2.90c	2.79b	116a	127b	139c	150d	287a	344b	403c	419c
25	2.56a	2.71b	2.89c	2.76b	105a	116b	129c	142d	269a	350b	373c	392c
LSD 1%		0.10				4.50				21		

*: Significant differences between biological indices at p< 1 % level indicated by different letters

Table 4: Fe content in soils obtained by chemical extraction methods

Soil No.	Fe content in soils, mg kg^{-1}							
	DTPA+$CaCl_2$+TEA	HCl+H_2SO_4	NH_4OAc	EDTA+NH_4OAc	$MgCl_2$	EDTA+$(NH_4)_2CO_3$	DTPA +NH_4HCO_3	EDDHA
1	3.6	0.8	0.8	3.4	0.8	0.6	3.4	2.2
2	2.4	2.2	0.8	3.8	2.0	0.8	2.2	2.0
3	2.2	0.6	1.8	3.1	0.8	0.4	4.1	3.4
4	5.6	3.6	2.4	5.6	3.4	3.8	5.8	4.8
5	5.0	3.2	2.0	4.2	3.0	3.0	4.0	4.3
6	4.5	2.6	1.0	4.0	1.9	2.8	1.8	5.2
7	4.2	3.5	1.2	4.2	2.4	2.5	4.2	3.8
8	2.6	2.6	1.0	4.0	2.0	0.4	4.7	2.4
9	3.2	1.0	0.9	2.4	1.6	0.8	3.8	4.0
10	2.4	1.8	1.4	1.8	1.4	2.2	2.2	2.8
11	2.8	1.0	0.2	2.1	0.2	0.6	1.4	1.8
12	3.5	1.2	0.6	3.8	0.7	3.8	4.1	2.3
13	3.0	1.9	1.8	3.4	2.0	1.0	3.4	1.8
14	3.0	1.4	2.6	3.8	1.0	1.2	2.4	2.2
15	5.8	2.6	1.6	5.4	2.2	3.8	4.8	3.6
16	4.2	1.4	1.3	4.8	0.2	3.9	4.6	4.1
17	5.6	3.4	1.2	4.8	3.4	4.0	4.2	3.8
18	4.1	3.6	0.4	3.4	2.4	2.6	3.4	2.8
19	2.2	0.6	0.6	3.8	0.8	0.4	3.6	1.4
20	3.4	3.1	0.6	1.8	1.6	3.2	3.3	2.2
21	4.2	2.2	1.4	1.6	1.0	0.5	4.6	4.2
22	4.8	2.4	1.0	4.1	1.0	3.6	3.0	3.7
23	3.8	2.4	0.6	4.0	1.3	4.0	3.8	3.0
24	4.2	0.8	1.7	3.6	1.6	2.4	3.6	3.2
25	4.0	2.4	1.2	4.0	2.0	3.4	3.2	3.4
Mean	3.77	2.09	1.19	3.62	1.67	2.21	3.58	3.14

Table 5: The correlation coefficients (r) for the relationship between chemical extraction methods and biological indices

Biological indices / Chemical extraction	Non application of Fe in pots			Fe_0 / $Fe_{maximum\ biological\ indice}$ X 100		
	Dry matter yield	Fe concentration of plant	Uptake of Fe amount from soil	Relative dry matter yield	Relative Fe concentration of plant	Relative uptake of Fe amount from soil
0.005 M DTPA + 0.01 M $CaCl_2$+0.1 M TEA	0.621**	0.823**	0.810**	0.433**	0.558**	0.640**
0.05 M HCl + 0.012 M H_2SO_4	0.369*	0.528**	0.501**	0.247	0.479**	0.478**
1 M NH_4OAc	0.212	0.338*	0.294	0.083	0.194	0.184
0.01 M EDTA + 1 M NH_4OAc	0.539**	0.659**	0.692**	0.307	0.619**	0.617**
1 M $MgCl_2$	0.303	0.757**	0.531**	0.156	0.384*	0.341*
0.01 M EDTA + 1 M $(NH_4)_2CO_3$	0.460**	0.438**	0.536**	0.245	0.535**	0.451**
0.005 M DTPA + 1 M NH_4HCO_3	0.618**	0.520**	0.679**	0.521**	0.492**	0.641**
0.001 M EDDHA	0.517**	0.563**	0.643**	0.565**	0.265	0.541**

*: P< 0.05 **: P < 0.01

sufficient according to different extraction methods. It supports earlier researchs in this region (Salam *et al.*, 1997; Eyupolu *et al.*, 1998).

Chemical properties of the soils showed that they were neutral to slightly alkaline and contained medium level of lime (Table 1). Use of acid (HCl+H_2SO_4) and salt (NH_4OAc, $MgCl_2$) extraction methods were inadequate in the determination of available Fe content and chelate (EDDHA) and chelate+salt mix (DTPA+NH_4HCO_3; DTPA+$CaCl_2$+TEA; EDTA+NH_4OAc and EDTA+$(NH_4)_2CO_3$ methods) were determined to be more suitable in the determination of available Fe content for such soils (Kacar, 1995), supporting which in the present work, highest correlation coefficients (r) were obtained from the chelate and chelate + salt mix methods (Table 5). As a result, when considered the chemical properties of the soils studied chelate and chelate + salt mix methods can be used with satisfaction in determination of available Fe contents of the Edirne region soils.

The 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA; 0.005 M DTPA+1 M NH_4HCO_3 and 0.001 M EDDHA methods, among the others, can be used confidently to determine the available Fe content of the soils of Edirne region because the highest correlation coefficients (r) were determined when these methods were used (Table 5). These methods were also suggested for various regions (Aydemir, 1981; Haddad *et al.*, 1993; Elinç, 1997). The 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA method can be used in the determination of available Fe content in this region and zinc (Zn), copper (Cu) and manganese (Mn) contents can be determined in addition and this characteristic of method therefore is to be taken into consideration when selecting a method.

Consequently all of the following methods i.e. 0.005 M DTPA+0.01 M $CaCl_2$+0.1 M TEA; 0.005 M DTPA+1 M NH_4HCO_3 and 0.001 M EDDHA can be recommended in the determination of available Fe content of Edirne region soils because of the highest correlation coefficients (r) determined. On the other hand, these methods are suitable to certain physical and chemical properties of the soils in this region.

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