

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 CaCl₂ + 0.1 M TEA; 0.05 M HCl + 0.012 M H₂SO₄; 1 M NH₄OAc (pH: 4.8); 0.01 M EDTA + 1 M NH₄OAc; 1 M MgCl₂; 0.01 M EDTA + 1 M (NH₄)₂CO₃; 0.005 M DTPA + 1 M NH₄HCO₃ 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. At the end of the experiment, the highest correlation coefficients (r) were determined to be between the 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M TEA method and the biological indices and between the 0.005 M DTPA + 1 M NH₄HCO₃ method and the biological indices. The correlation coefficients (r) for the 0.005 M DTPA + 0.01 M CaCl₂ + 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 the 0.005 M DTPA + 1 M NH₄HCO₃ method $r=0.618^{**}$; $r=0.520^{**}$; $r=0.679^{**}$; $r=0.521^{**}$; $r=0.492^{**}$ 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.

Fe deficiency is one of the most common trace element problems in the world nowadays. Fe deficiency is frequent in high pH, high lime, low organic matter content and sandy soils. ([19]). Available Fe is inadequate in about 26.87 % of turkey's soils ([9]).

Despite the fact that several Fe extraction methods have been developed none of them was suitable to be a standard method ([16]).

Lindsay and Norvell ([18]) and Norvell ([23]) 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 the USA, because this method has produced the highest correlation with biological indices ([13]).

Hatipoglu ([12]) has determined correlation coefficients (r) between eleven extraction methods and biological indices to find out 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.

Fe deficiency is a major plant nutrition problem in Edirne region ([9]). In this research, suitable method for the determination of available Fe content of the soils of this region was investigated.

MATERIALS AND METHODS

Soil samples were taken at 0- 20 cm depth from 25 different cultivated soils in Edirne ([15]). Soil pH ([32]), lime ([17]), CEC ([31]) and texture ([10]) were determined for each sample.

Some physical and chemical properties of the soil samples are given in Table 1. The pH values of soil

samples ranged from 6.29 to 7.94; CaCO₃ contents were between 0.00 % and 15.10 %; CEC values were between 16.44 and 37.22 cmol kg⁻¹; texture of soils samples were between clay (C) and sandy loam (SL).

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

Soil no	pH (1:2.5)	CaCO ₃ ,%	CEC, cmol kg ⁻¹	Particle size distribution		
				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

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 CaCl₂ + 0.1 M TEA ([18]); 0.05 M HCl + 0.012 M H₂SO₄ ([35]); 1 M NH₄OAc ([24]); 0.01 M EDTA + 1 M NH₄OAc ([22]); 1 M MgCl₂ ([30]); 0.001 M EDTA + 1 M (NH₄)CO₃ ([33]); 0.005 M DTPA + 1 M NH₄HCO₃ ([29]) and 0.001 M EDDHA ([13]). Some properties of these extraction methods are given in Table 2.

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 hours	Lindsay and Norvell (1978)
0.05 M HCl + 0.012 M H ₂ SO ₄	1 : 4	15 minutes	Wear and Evans (1968)
1 M NH ₄ OAc (pH: 4.8)	1 : 4	30 minutes	Olson (1948)
0.01 M EDTA + 1 M NH ₄ OAc	1 : 10	1 hour	Navrot and Ravikovitch(1968)
1 M MgCl ₂	1 : 5	45 minutes	Stewart and Berger (1965)
0.01 M EDTA + 1 M (NH ₄) ₂ CO ₃	1 : 2	30 minutes	Trierweiler and Lindsay (1969)
0.005 M DTPA + 1 M NH ₄ HCO ₃	1 : 2	15minutes	Soltanpour (1991)
0.001 M EDDHA	1 : 2	10 minutes	Johnson and Young (1973)

A greenhouse experiment was designed in a randomised complete block replicated three times. 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 ([21]). Each pot was fertilized with 140 mg kg⁻¹ N (NH₄NO₃) and 80 mg kg⁻¹ P₂O₅ (KH₂PO₄), according to average application rates of N and P₂O₅ to barley in this region. Four different rates of Fe (Fe₀:0; Fe₁:10; Fe₂:20; and Fe₃:30 mg kg⁻¹) were applied to soils as Fe-EDDHA compound. Fifteen plants were left in each pot after the germination. The water content of the pots was adjusted to 70 % of field capacity during the experiment period. Barley shoots were harvested after 60 days. Harvested shoots were washed once in tap water and twice in distilled water and dried at 65 °C. Dry matter yields were determined.

Dried and ground plant materials were digested using HNO₃ + HClO₄ ([14]). The Fe concentrations of plants were determined with AA-660 Shimadzu Atomic Absorption Spectrophotometer (AAS) ([15]). Dry matter yield, Fe concentration, Fe uptake and the relative values of these biological indices were used as biological method. Relative biological indices were calculated as Fe₀ / 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 the 1 and 5 % levels ([37]).

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 ([1], [2], [3],[4], [8], [25], [36]).

RESULTS AND DISCUSSION

Effect of Increasing Fe Application Rates on Barley Yields, Fe Concentration and Fe Uptake

Dry matter yield of the barley plants was affected by the Fe application. While the highest dry matter yield on 18 soils was obtained from the Fe₂ (20 mgkg⁻¹), the highest dry matter yield on 7 soils was obtained with Fe₃ (30 mg kg-1) (table 3)

In general, the 18 soils, which gave the highest dry matter, yield at Fe₂ (20 mg kg⁻¹), were those with the highest levels of available Fe (Table 4). In these soils, Fe₃ appears to have caused possible toxic effects.

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, all of these values except for one i.e. 161 mg kg⁻¹, for barley and were sufficient ([26]).

In general dry matter yield using Fe₂ concentration of the barley plants was determined to be higher for the soils 1, 2, 8, 9, 11, 12, 13,14, 15, 16, 17, 18, 20, 21, 22, 23, 24 and 25 (Table 3).The reason of this result maybe the 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 ([3], [5], [7]).

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	94 a	101 b	113 c	119 d	227 a	260 b	307 c	311 c
2	2.24a	2.34b	2.48c	2.37c	83 a	94 b	98 b	110 c	186 a	220 b	243 c	261 c
3	1.91a	2.02b	2.19c	2.12c	97 a	102 b	110 c	118 d	185 a	206 a	241 b	250 b
4	3.55a	3.67b	3.80bc	3.71b	104 a	110 b	121 c	127 d	369 a	404 b	460 c	471 c
5	3.40a	3.62bc	3.70c	3.58b	108 a	113 b	118 c	122 c	367 a	409 b	437 c	437 c
6	1.98a	2.25b	2.47c	2.40c	98 a	107 b	119 c	123 c	194 a	241 b	294 c	295 c
7	2.59a	2.71b	2.83c	2.75bc	116 a	121 b	125 b	134 c	300 a	328 b	354 c	369 c
8	2.80a	3.07b	3.26c	3.15b	103 a	133 b	139 c	147 d	288 a	408 b	453 c	439 c
9	2.38a	2.58b	2.72c	2.60b	95 a	104 b	119 c	130 d	226 a	268 b	324 c	338 c
10	1.73a	1.95b	2.19c	2.10c	97 a	118 b	130 c	135 d	168 a	230 b	285 c	284 c
11	1.78a	1.97b	2.28c	2.14b	94 a	117 b	129 c	134 d	167 a	230 b	294 c	289 c
12	2.56a	2.69b	2.87d	2.72c	98 a	116 b	125 c	131 d	251 a	312 b	359 c	356 c
13	1.82a	1.95b	2.19d	2.07c	88 a	107 b	114 c	120 d	160 a	209 b	250 c	248 c
14	1.69a	1.75ab	1.94c	1.80b	97 a	113 b	127 c	138 d	164 a	198 b	246 c	248 c
15	2.87a	3.02b	3.27d	3.14c	101 a	117 b	129 c	140 d	290 a	353 b	422 c	440 c
16	2.65a	2.84b	3.18d	3.04c	92 a	110 b	127 c	139 d	244 a	312 b	404 c	423 c
17	2.48a	2.72b	2.94c	2.80b	105 a	117 b	130 c	141 d	260 a	318 b	382 c	395 c
18	1.76a	1.89b	2.04c	1.92b	93 a	110 b	129 c	134 d	164 a	208 b	263 c	257 c
19	1.94a	2.17b	2.30c	2.21bc	99 a	114 b	130 c	140 d	192 a	247 b	299 c	309 c
20	1.72a	1.92b	2.27d	2.14c	101 a	120 b	134 c	141 d	174 a	230 b	304 c	302c
21	2.86a	3.12b	3.29c	3.17b	105 a	120 b	139 c	147 d	300 a	374 b	457 c	466 c
22	3.26a	3.42b	3.64c	3.51b	103 a	117 b	132 c	145 d	336 a	400 b	480 c	509 d
23	3.40a	3.60b	3.81d	3.70c	105 a	119 b	142 c	161 d	357 a	428 b	541 c	596 d
24	2.47a	2.71b	2.90c	2.79b	116 a	127 b	139 c	150 d	287 a	344 b	403 c	419 c
25	2.56a	2.71b	2.89c	2.76b	105 a	116 b	129 c	142 d	269 a	350 b	373 c	392 c
LSD		0.10				4.50				21		
%1												

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

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 ([7], [19]).

Table 4 shows that available Fe contents of the soils 8, 9, 11, 12, 21 and 23 determined by various methods were lower than in 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 ([6], [20]).

As shown in Table 4, higher available Fe content of soil samples was determined with the 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M TEA; 0.005 M DTPA + 1 M NH₄HCO₃ and 0.001 M EDDHA methods in comparison to other extraction methods. On the other hand, the lowest available Fe content of soil samples was determined with the 1 M NH₄OAc and the 1 M MgCl₂ methods.

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 ₂ SO ₄	NH ₄ OAc	EDTA +NH ₄ OAc	MgCl ₂	EDTA+(NH) ₄ CO ₃	DTPA +NH ₄ HCO 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

These results also show that higher available Fe was determined using methods with chelate + salt (0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M TEA; 0.005 M DTPA + 1 M NH₄HCO₃; 0.01 M EDTA + 1 M NH₄OAc and 0.01 M EDTA + 1 M (NH₄)₂CO₃ methods) and chelate alone (0.001 M EDDHA) in comparison to the methods using salt (1 M NH₄OAc and 1 M MgCl₂ methods) and acid (0.05 M HCl + 0.012 M H₂SO₄ method). Mean available Fe content of the soils was 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 CaCl₂ + 0.1 M TEA; 0.05 M HCl + 0.012 M H₂SO₄; 1 M NH₄OAc; 0.01 M EDTA + 1 M NH₄OAc; 1 M MgCl₂; 0.01 M EDTA + 1 M (NH₄)₂CO₃; 0.005 M DTPA + 1 M NH₄HCO₃ and 0.001 M EDDHA, respectively. The acid and salt methods of HCl + H₂SO₄, MgCl₂ and NH₄OAc, which gave 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 type of soils ([15]).

The Relationships Between Chemical Extraction Methods and Biological Indices

The correlation coefficients (r) determined between chemical extraction methods and biological indices are given in Table 5. Significant correlation coefficients were observed between all chemical extraction methods, except 1 M NH₄OAc method and the biological indices (dry matter yield, Fe concentration, Fe uptake, relative dry matter yield, relative Fe concentration, and relative Fe uptake) at 1 % level (Table 5). According to Table 5, the highest correlation coefficients (r) were determined between 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M TEA and 0.005 M DTPA + 1M NH₄HCO₃ methods and biological indices. These correlation coefficients (r) determined were 0.621**, 0.823**, 0.810**, 0.433**, 0.558** and 0.640** for 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 TEA method and 0.618**, 0.520**, 0.679**, 0.521**, 0.492** and 0.641** for 0.005 M DTPA + 1M NH₄HCO₃ method, respectively. The results obtained from the 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 CaCl₂ + 0.1 M TEA > 0.005 M

DTPA + 1 M NH₄HCO₃ > 0.001 M EDDHA > 0.01 M EDTA + 1 M NH₄OAc > 0.01 M EDTA + 1 M (NH₄)₂CO₃ > 0.05 M HCl + 0.012 M H₂SO₄ > 1 M MgCl₂ > 1 M NH₄OAc.

Table5: The correlation coefficients (r) for the relationship between chemical extraction methods and biological indices

Biological indices Chemical extraction methods	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 ₂ SO ₄	0.369*	0.528**	0.501**	0.247	0.479**	0.478**
1 M NH ₄ OAc	0.212	0.338*	0.294	0.083	0.194	0.184
0.01 M EDTA + 1 M NH ₄ OAc	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) ₂ CO ₃	0.460**	0.438**	0.536**	0.245	0.535**	0.451**
0.005 M DTPA + 1 M NH ₄ HCO 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

CONCLUSION

The available Fe content of the soil samples were determined to be either insufficient or moderately sufficient according to different extraction methods. Supports earlier researchs in this region ([9], [27]).

Chemical properties of the soils studied show that they are neutral to slightly alkaline and contained medium level of lime (Table 1). Use of acid (HCl + H₂SO₄) and salt (NH₄OAc, MgCl₂) extraction methods are inadequate in the determination of available Fe content and chelate (EDDHA) and chelate + salt mix (DTPA + NH₄HCO₃; DTPA + CaCl₂ + TEA; EDTA + NH₄OAc and EDTA + (NH₄)₂CO₃ methods) were determined to be more suitable in the determination of available Fe content for such soils ([15]), supporting which in the present work, highest correlation coefficients (r) were obtained from the chelate and chelate + salt mix methods (Table 5). As a results, when considered the chemical properties of the soils studied chelate and chelate + salt mix methods can be used with satisfaction in the determination of available Fe contents of the Edirne region soils.

The 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M TEA; 0.005 M DTPA + 1 M NH₄HCO₃ 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 regional soils ([3], [7], [11]). The 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M TEA method can be used in the determination of the available Fe content in this region and zinc (Zn), copper (Cu) and manganese (Mn) contents can be determined in addition and this characteristic of this 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 CaCl₂ + 0.1 M TEA; 0.005 M DTPA + 1 M NH₄HCO₃ 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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