

Evaluation of Five Extraction Methods for Predicting Available Forms of Iron in Soils of Southeastern, Nigeria

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Abstract: Available forms of iron in soils can be assessed through the use of chemical extraction methods. Soil test provide an indication of nutrient level in the soil and together with plant analysis are important agronomic tools for determining crop nutrient needs, predicting the nutrient-deficient areas and preventing the deficiency. This study was therefore conducted in laboratory and greenhouse environment to evaluate suitable extraction method for predicting available forms of iron in soils of southeastern Nigeria, using five extraction procedures (Coca-cola, 0.05M EDTA, 1N NH₄OAc + 0.05M EDTA, 1N NH₄OAc and 0.1N HCl). Iron uptake by maize (*Zea mays L.*) plants was analyzed together with the Fe content in the soils in which maize plants were grown under greenhouse conditions. Iron content in maize plants were determined by AAS. The results obtained in this study shows that Coca-cola method extracted the highest amount of the Fe (12.77 mgkg⁻¹) while, 1N NH₄OAc + 0.05M EDTA extracted the least amount of Fe (4.48 mgkg⁻¹) with an average of 7.33 mgkg⁻¹. Coca-cola-extractable Fe correlated positively and significantly with Fe concentration ($r = 0.650^{**}$) and Fe uptake ($r = 0.712^{***}$) while, 0.1N HCl- and 0.05M EDTA-extractable Fe had significant positive correlations with dry matter yield and Fe uptake. Their r values being, (0.630^{**} and 0.676^{**}) and (0.601^{**} and 0.588^{*}), respectively. Moreover, the result obtained from the regression equations corresponded with the correlations values determined. Accordingly, the study indicates that, the order of significance for the extracting solutions is as follows: Coca-cola > 0.1N HCl > 0.05M EDTA > 1N NH₄OAc > 0.05M EDTA + 1N NH₄OAc. Thus, the high correlations (r) values of extractable Fe with soil properties and yield parameters suggests the superiority of Coca-cola, 0.1N HCl and 0.05M EDTA extractants over others extractants but, the Coca-cola method was determined to be the most suitable extractant for predicting available Fe content for soils of southeastern Nigeria. However, in the absent of Coca-cola extractant, HCl and EDTA methods can equally be used in the determination of available Fe in these soils.

Keywords: Iron, extractants, maize, acid, salt, chelate, Fe uptake, acid sands.

I. Introduction

Iron is an essential plant nutrient that is required in small quantity for growth and development of plant. It is harmful when present in large quantity in soils. Iron is taken up by plants at a much higher rate than is the case with other trace elements (Sahrawat, 2003). One of the reasons Fe is rarely tested is that, the Fe availability is generally presumed to be sufficiency in soils. Ponnampereuma et al. (1967) noted that the level of available Fe in the soils is extremely pH dependent and that, at very low pH levels, Fe is reduced from its oxidized Fe³⁺ form – which is generally found in dry conditions – to its highly soluble and readily available Fe²⁺ form. Conversely, Nayak (2008) reported that, where a soil is well aerated and well drained, with good structure and porosity, Fe toxicity is unlikely to be a problem. According to Norvell and Lindsay (1982), Fe chelates aid in the movement of Fe to plant roots, but they are neither absorbed to any great extent nor do they raise the activity of Fe³⁺ or Fe²⁺ in the bulk soil solution. To be effective, Fe chelates must be stable in soil environments.

Knowledge of available forms of Fe in soil is fundamental for suitable fertilization recommendations, in other to avoid deficiency or toxicity problems. However, the deficiencies of Fe have constituted a major soil fertility problem in many parts of Nigeria (Kang et al., 1976) particularly in acid sandy soils of Southeastern Nigeria where, Fe deficiency has been widely reported (Enwezor et al., 1990). Fe deficiency partly caused by continuous and intensive farming, leading to nutrient mining (Aduayi et al., 2002 and FAO, 2010) has however led to continuous declining yields of major food crops such as maize crop (Chude et al., 2004), especially in soils where Fe deficiency occurs (Sillanpaa, 1990 and FAO, 2010).

Generally, soil extractants used for predicting available forms of micronutrients in soils included the weak replacement ion salts (CaCl₂, NH₄OAc, etc.) (Whitney, 1988; Kabata-Pendias, 2001); weak acids (acetic acid and hydrochloric acid) (Shittu et al., 2010) and weak chelating agents; EDTA (Lindsay and Norvel, 1978; Agarwal and Sastry, 1995; Gronflaten and Steinnes, 2005) and DTPA (Lindsay, 1995; Aydemir, 1981 and Katyal and Shuman, 1991). Accordingly, Gronflaten and Steinnes (2005), reported that, neutral salt solutions are capable of extracting the easily soluble and exchangeable i.e. potentially accessible forms of trace elements in soils. When diluted solutions of the concentrated acids-HCl and HNO₃, was used for determining the mobile and accessible forms of trace elements in acid soils. However, due to the high active concentration of hydrogen ions

in these acids, they extracted not only the mobile, but also the steadily bound forms of the elements in the soils (Kabata-Pendias, 2001; Dahnke and Olson, 1990). Besides, chelating agents such as EDTA and DTPA have also been used to estimate plant available Zn, Cu, Fe and Mn in soils (Haddad and Evans, 1993; Agarwal and Sastry, 1995; Dolui and Chattopadhyay, 1997). Comparatively, Knezek and Ellis (1980) and Norvel (1984) reported that, of the commonly used chelated agents, only EDTA can be used successfully as extractant for estimating mobile forms of Fe in soils of different types whereas, DTPA was observed to be unsuitable for use as extractant in acid soils.

As a proper choice of an extraction method for soil test analysis, a positive correlation between the nutrient concentration determined by the method and the nutrient quantity taken up by plants is fundamental (Adiloglu, 2003; Menzies et al., 2007). The use of acid extractants is based on lowering the pH and the consequent solubilization of some compounds containing these elements (Grava, 1980). On the other hand, chelating extractants have the capacity of reducing the activity of dissolved metals, resulting in release of more soluble compounds in buffered pH (Adiloglu, 2003).

Though in Nigeria, only a few numbers of chemical extractants are being used to determine plant-available Fe in soils, the most commonly used extraction methods are the dilute acid, HCl (Kparmwang et al., 1995), and the chelating agent, DTPA (Mustapha and Loks, 2005; Tening and Omueti, 2011; Ibrahim et al., 2011; Oluwadare et al., 2013), no satisfactory extractants has so far been reported or documented for evaluating available Fe in soils of southeastern Nigeria. Moreover, a reliable soil test is essentially to determine the Fe needs of the various crops grown in this region, under various ecological conditions.

Keeping in view the important role of maize in the economy of this country and the effect of Fe on the performance and quality of maize product as well as limited information about this nutrient element condition in the soil, there is an urgent need for an accurate estimation of Fe in soils of southeastern Nigeria where, the content of Fe had been considered poorly related to Fe supply. The objective of this research was to evaluate the suitability of 5 extractants for predicting available forms of Fe in soils of southeastern Nigeria.

II. Materials And Methods

2.1. Location of study area

The sampling sites selected covered a wide range of soil types from 20 locations representing soils across Southeastern Nigeria (Table 1) which is located at Latitude 4° 20' and 7° 25' North of the Equator and Longitude 5° 25' and 8° 51' East of the Greenwich meridian (Ojanuga, 2006). The mean minimum and maximum temperatures ranged from 21-30°C in the Coast and from 29-33°C in the interior. The rainfall pattern is bimodal and decreases from over 3000 mm in the south to 1,700 mm in the north, this has given rise to double cropping (early and late) seasons. The vegetation stretches from mangrove swamp in the coastal region through rainforest to derived savanna in the interior.

Table 1: Location of sampling site showing soil types

Sample No.	Sample location	Soil types	Classification*
1	Bende	Shale	Aquic Hapludults
2	Okigwe	Shale	Aquic Ustifluents
3	Afikpo	Shale	Typic Ustifluents
4	Odukpani	Shale	Aquic Haplustalfs
5	Igbariam	Shale	Udic Haplustalfs
6	Abiriba	Sandstone	Psamment Paleudults
7	Etiti	Sandstone	Udipsamments
8	Uturu	Sandstone	Psammentic Paleudults
9	Nsukka	Sandstone	Typic Psammaquent
10	Ofodua	Sandstone	Typic Ustpsamments
11	Owerri	Coastal plain sand	Udic Haplustalfs
12	Umudike	Coastal plain sand	Typic Paleudults
13	Uyo	Coastal plain sand	Typic Paleudults
14	Ikot Ekpene	Coastal plain sand	Psammentic Paleudults
15	Akpabuyo	Coastal plain sand	Psammentic Paleudults
16	Oban	Basement complex	Typic Hapludults
17	Awi	Basement complex	Typic Paleudults
18	Uyanga	Basement complex	Typic Paleudults
19	Betem	Basement complex	Typic Paleudults
20	Akpet	Basement complex	Typic Hapludults

* FDALR (1995).

Source: Eteng et. al. (2014)

2.2. Sample collection and laboratory study

A laboratory study was conducted on these surface (0-20) soils to determine some physical and chemical properties (Table 3). The soil samples were air-dried and screened through a 2 mm sieve. The 2 mm sieved soils were analysed for particle size analysis by the hydrometer method (Gee and Bauder, 1986); Soil pH (soil: water ratio of 1: 2.5) was determined with a combined electrode pH meter (Thomas, 1996); Soil organic carbon was determined by the potassium dichromate wet oxidation method (Nelson and Sommer, 1996) and the content of the organic carbon was converted to organic matter (OC x 1.724) while, the effective cations exchange capacity (ECEC) was determined by the method described by Sumner and Miller (1996). For the estimation of available Fe contents in soils, the following chemical properties of the extractants as shown in Table 2 were used.

Table 2: Chemical properties of the extractants used

S/N	Extractant	Groups	pH	Soil-solution ratio	Shaking time	Reference
1	Coca-cola	Acid	2.7	1:10	10 mins.	Schnug et al. (2001)
2	0.01N HCl	Acid	4.8	1:10	1 hour	Lindsay (1995)
3	0.05M EDTA	Chelate	7.0	1:2	30 mins.	Gronflaten and Steinnes, 2005
4	1N NH ₄ OAc	Salt	7.0	1:10	1 hour	Olsen, 1992
5	0.05M EDTA+1N NH ₄ OAc	Chelate+salt	4.8	1:10	1 hour	Adiloglu, 2003

Soil samples were shaken with respective extractants of various properties (Table 2). After shaking, the soil-solution was centrifuged and filtered through Whatman filter paper No. 42. The quantity of Fe content in the soil sample was performed with an atomic absorption spectrophotometer (AAS) (UNICAM model SOLAAR 32: Fe ASTM D1068).

2.3. Greenhouse study

The greenhouse study was conducted on the same soils to assess the performance of maize parameters. One kilogramme of the air-dried soils was weighed into plastic containers of 2 liter capacity, placed on flat plastic receiver. A total number of 400 plastic containers (20 soil samples x 5 levels of Fe x 4 replications) were arranged in a complete randomized design (CRD). The plastic containers were randomly distributed on the bench in the greenhouse growth chamber and supplied with distilled-deionized water as required during the growth period. Before planting, the soils were watered to about field moisture capacity (adjusted to 70 %) with distilled water and allowed to stand for about 48 hours in the greenhouse. Maize (*Zea mays* L.) variety; Oba Supper II (yellow), was used as a test plant and sown at the rate of six seeds per plastic container and later thinned to five seedlings a week after germination.

Five levels of Fe (0, 4, 8, 12 and 16 kg ha⁻¹) converted to mg kg⁻¹ was applied as FeSO₄.5H₂O (33% Fe) in solution and recommended basal dosage of 120 kg N ha⁻¹ (mg N kg⁻¹ soil) as Urea, 60 kg P₂O₅ ha⁻¹ (mg P kg⁻¹ soil) as single super phosphate (SSP) and 60 kg K₂O ha⁻¹ (mg K kg⁻¹) as muriate of potash (MOP) respectively, as a basal N P and K fertilizer, were respectively applied uniformly to all the containers in solution form, at two weeks after planting (WAP) as recommended for this region (Enwezor et al., 1990; Aduayi et al., 2002).

The plants (shoots and roots) were harvested 42 DAS, rinsed in distilled water, pre-dried under shade to remove excess water and later packed in large brown envelopes and oven-dried at 70°C for 72 hours. The oven-dried plants and ground materials were digested using sulphuric acid, nitric acid and perchloric acid (H₂SO₄:HNO₃:HClO₄) method (Shuman, 1985). The Fe contents in maize plants were determined by Atomic Absorption Spectrophotometer (AAS: Pre UNICAM model 939). Dry matter yield, Fe concentration and Fe uptake values were used as yield parameters of maize plants. Iron uptake was calculated as the product of the concentration of Fe in the plant tissue and the dry matter yield.

2.4. Statistical Analysis

The data obtained were subjected to simple correlation and regression analyses at different probability levels using general linear model of Genstat (2013) and PASW Statistics 18 for Window 7.0, to show the relationships between the different extractants and the plant Fe uptake. The extractant which displayed the highest correlation coefficient (r) with the maize uptake was recommended for the determination of available Fe content of the soils of southeastern Nigeria.

III. Results And Discussion

3.1. Soil properties of the study area

Some physical and chemical properties of the soil samples determined are indicated in Table 3. In the table, pH values of the soil samples ranged from 4.01 to 5.89 with an average of 4.62, suggesting that the soils are very strongly acidic; the percent organic matter content were between 0.71 and 2.47% with an average of 1.59%. Higher percent of the soils were low in organic matter content (Tandon, 1995). The ECEC values varied

widely which ranged between 0.78 and 14.31 cmol kg⁻¹ with an average of 7.15 cmol kg⁻¹. However, ECEC of most of the soils tested were below 12 cmol kg⁻¹ and are rated low (Melsted et al., 1969), and this is the range of critical value for soils that are dominated by oxide and hydroxide clays (Omueti and Lavkulich, 1988; Bimie and Paterson, 1991). This suggests that most of the soils may have few exchange sites. For instance, the high values of ECEC obtained in some of the soil samples could be obvious as these samples were collected from shale derived soils (a hydromorphic soil) as shown in Table 1, with high clay content which ranged from 7 and 34 %, respectively. Most of the soils samples determined were coarse texture and the textural class was in the average of sandy loam (SL).

Fig 3: Some physical and chemical properties of the soil samples (N=20).

Soil Samples	pH (1:2.5)	Org. matter %	ECEC Cmolkg ⁻¹	Particle size distribution			Textural class
				Sand %	Silt %	Clay %	
Min.	4.01	0.71	0.78	39	6	7	
Max.	5.89	2.47	14.31	85	30	34	
Means	4.62	1.59	7.15	62	18	20	Sandy loam

3.2. Iron contents of soils according to different extraction methods.

Five extraction methods were used to extract available Fe content of the soil samples (Table 4). There were substantial variations in the amount of extractable Fe in the soils. Available Fe varied widely depending on the extraction method used, reasons for which could be due to type, concentration, pH, shaking time and solution ratio of the extraction solution (Sorensen et al., 1971; Whitney, 1988) and variability observed in the physical and chemical properties of the soils used (Loeppert and Iskeep, 1996 and Elinc, 1997).

Coca-cola-extractable Fe ranged from 3.57 to 21.31 mg kg⁻¹ with a mean of 12.77 mg kg⁻¹. 0.05M EDTA-extractable Fe varied widely between 1.99 and 16.28 mgkg⁻¹ with a mean of 8.81mg kg⁻¹. 0.01N HCl-extractable Fe ranged from 3.29 to 18.63 mg kg⁻¹ with a mean of 10.16 mg kg⁻¹. While the 0.05M EDTA+1N NH₄OAc and 1N NH₄OAc extraction methods have significantly (P<0.05) lower extractable Fe relative to Coca-cola, 0.01N HCl and 0.05M EDTA methods. Thus, the quantity of available Fe by 0.05M EDTA+1N NH₄OAc method was three time lower than the amount extracted by Coca-cola method and twice lower than the amount extracted by 0.05M EDTA and HCl methods, respectively. This trend was the same in the case of 1N NH₄OAc-extractable Fe. However, the values of 0.05M EDTA+1N NH₄OAc and 1N NH₄OAc-extractable Fe have values which varied widely from 1.27-8.63 mg kg⁻¹ and 2.02-9.86 mg kg⁻¹, respectively. Similarly, the result presented in Table 4 indicates that Fe extracted by Coca-cola, 0.01N HCl and 0.05M EDTA methods had values ~~contents~~ which are relatively above the mean value. On the other hand, Fe extracted in most soils by 0.05M EDTA+1N NH₄OAc and 1N NH₄OAc had values that are less than the overall mean.

Extractable Fe of the soils varied widely from 5.52 to 12.77 mg kg⁻¹ with a mean of 7.33 mg kg⁻¹ (Table 4). Coca-cola-extractable Fe was significantly higher followed by 0.01N HCl and 0.05M EDTA-extractable Fe. On the other hand, the lowest available Fe content of soil sample was determined with 1N NH₄OAc and 0.05M EDTA+1N NH₄OAc methods, respectively. This result also show that higher available Fe was determined using acid (Coca-Cola and 0.01N HCl methods) and chelate (0.05M EDTA method) in comparison to the methods using neutral salt (1N NH₄OAc method) and chelate-salt (0.05M EDTA + 1N NH₄OAc method). Meanwhile, the mean available Fe content of the soils were determined to be 12.77; 10.17; 8.81; 5.52 and 4.48 mgkg⁻¹, using the extractants; Coca-cola, 0.01N HCl, E0.05M DTA, and 1N NH₄OAc, 0.05M EDTA+1N NH₄OAc methods, respectively. In line with this study, Coca-Cola has been reported to be an excellent extractant for Fe, for many soils (Schnug et al., 1996; 1998).

Table 4: Available forms of Fe content in soils obtained by 5 extractants

Soil Sample No.	Extractable iron (Fe)				
	Coca-Cola mgkg ⁻¹	EDTA	HCl	EDTA+ NH ₄ OAc	NH ₄ OAc
1	14.46	14.05	13.90	6.77	8.78
2	16.27	6.28	14.55	3.87	5.33
3	19.93	13.59	9.24	4.07	4.39
4	17.54	15.89	17.19	3.02	3.01
5	9.41	6.37	8.33	4.54	9.86
6	4.94	4.17	4.24	3.69	4.83
7	15.7	2.08	12.29	1.99	2.15
8	10.41	1.99	9.69	2.09	2.02
9	8.70	6.20	5.49	2.03	5.16
10	14.97	13.35	3.29	7.48	8.06
11	9.87	3.57	4.73	2.83	3.06
12	3.57	6.98	3.20	6.78	3.81
13	7.32	7.59	4.33	2.76	2.88
14	8.14	3.57	9.30	2.32	2.35
15	7.44	5.71	4.36	1.27	3.59
16	15.28	9.90	18.03	6.74	7.15
17	14.24	11.38	12.05	5.86	8.98

18	18.23	11.67	13.62	8.63	7.23
19	17.6	15.42	18.63	5.23	6.38
20	21.31	16.28	16.68	7.60	9.44
Min.	3.57	1.99	3.29	1.27	2.02
Max.	21.31	16.28	18.63	8.63	9.86
Mean	12.77	8.81	10.16	4.48	5.52

3.3. Dry matter, Fe concentration and Fe uptake by maize (*Zea mays L.*) grown on soils of southeastern Nigeria:

Maize (*Zea mays L.*) yield parameters obtained from the soils of southeastern Nigeria are presented in Table 5. In the table, dry matter yield varied widely from 5.45 to 17.82 g/plant with a mean of 12.79 g/plant (Table 5). Higher dry matter were produced from soils 2, 9, 15 and 17 while lower dry matter were obtained from soils 6, 11, 13, 19 and 20, respectively. However, dry matter yield of the maize plants were found to be significantly ($P<0.05$) higher at 12 kg Fe ha⁻¹ levels of application, relative to other rates of application. Generally, dry matter yield increased from control to 12 kg Fe ha⁻¹ levels of application and thereafter declined in yield to 16 kg Fe ha⁻¹ levels, respectively in all the soil samples. This result suggests that the 12 kg Fe ha⁻¹ levels of application could be the optimum level of Fe in these soils. Similar results were reports by Adiloglu (2003); Lisuma et al. (2006) and Tening and Omuiti (2011).

The Fe concentration in maize shoots increased with increasing Fe application (Table 5). Accordingly, Fe concentrations varied widely between 31.90 and 118.48 mg/kg with a mean of 102 mg/kg, and these were sufficient (Melsted et al., 1969; Tandon, 1995; Menzies et al., 2007). Higher Fe concentration were obtained in soils 6, 11, 14, 16, and 17 while lower Fe concentration were produced in soils 1, 2, 3, 5, 8, 18 and 20. The wide variability in observed in this study may be as a result of higher available Fe content in some of the soils. The effect of Fe application on the yield parameter of maize plants was determined to be significant at 5% level and the results obtained in this study are in agreement with those reported by Adiloglu (2003); Lisuma et al. (2006); Tening and Omuiti (2011). Accordingly, the Fe uptake of the maize shoots at determined at 6 WAP increased with increasing Fe application (Table 5) and varied widely from 0.23 to 2.39 mg/plant with an average of 1.38 mg/plant. However, on the average, soils 9, 14, 15 and 17 have significant ($P<0.05$) higher Fe uptake (1.538, 1.582, 1.658 and 1.752 mg plant⁻¹) relative to other soils while, soils 1, 5, 19 and 20 yielded the lowest Fe uptake (0.98, 1.298, 1.266 and 1.148 mg plant⁻¹) of maize plant shoots, respectively. Similar results were obtained by Tening and Omuiti (2011) in soils of humid zone of southeastern Nigeria and Adiloglu (2003) in soils of Edirne Province, Turkey for barley plants. Generally, the wide variability observed in maize yield parameters may be due to higher or lower available Fe content in some locations of the soils of southeastern Nigeria. However, Ponnampuruma et al (1967) reported that higher available Fe in soil is pH dependent, while Sahrawat (2008) and Nayak (2008) noted that availability of Fe content in soil depends on drainage nature of the soils. At low pH levels, Fe is reduced from its oxidized Fe³⁺ form which is generally found in dry conditions to its highly soluble and readily available Fe²⁺ form. This is confirmed by Nayak (2008) who reported that, where a soil is well aerated and well drained, with a good structure and porosity, Fe toxicity is unlikely to be a problem. Norvell and Lindsay (1982) had earlier noted that Fe chelates may or may not raise the activity of Fe³⁺ or Fe²⁺ in the bulk soil solution. Whichever is the case may have resulted to the wide variability in Fe uptake of maize plants grown in different soils of southeastern Nigeria. Similar results were obtained by Tening and Omuiti (2011) in soils of humid zone of southeastern Nigeria; Adiloglu (2003) in soils of Edirne Province, Turkey for barley plants; Lisuma et al (2006) in volcanic soil of Mpangala, Tanzania for maize and also McBride et al. (2004).

Soil sample No.	Dry matter yield (g plant ⁻¹)					Fe concentration of plant (mg kg ⁻¹)					Uptake of Fe by shoots (mg plant ⁻¹)				
	Fe ₀	Fe ₄	Fe ₈	Fe ₁₂	Fe ₁₆	Fe ₀	Fe ₄	Fe ₈	Fe ₁₂	Fe ₁₆	Fe ₀	Fe ₄	Fe ₈	Fe ₁₂	Fe ₁₆
1	8.97	10.27	14.52	16.25	14.17	65.2	75.6	76.5	078.4	082.2	0.58	0.78	1.11	1.27	1.16
2	6.91	13.80	15.72	17.79	15.67	55.5	80.2	88.0	104.6	124.5	0.38	1.10	1.38	1.86	1.95
3	8.18	11.60	13.75	15.22	14.30	43.1	67.9	115.0	126.9	130.8	0.35	0.79	1.58	1.93	1.87
4	6.46	10.56	12.11	16.73	15.35	55.5	88.3	112.5	116.7	127.9	0.36	0.93	1.36	1.95	1.96
5	7.32	13.07	15.68	16.25	14.99	31.9	82.0	113.0	116.1	102.2	0.23	1.07	1.77	1.89	1.53
6	6.64	09.97	12.38	14.42	13.09	86.8	111.4	98.8	129.0	132.4	0.58	1.11	1.22	1.86	1.73
7	9.23	10.89	13.36	15.01	14.26	50.3	94.6	118.1	130.8	135.3	0.46	1.03	1.58	1.96	1.93
8	8.69	12.77	14.97	16.74	16.08	31.9	65.5	107.7	113.0	123.6	0.28	0.84	1.61	1.89	2.02
9	7.30	13.27	15.03	17.82	16.30	66.2	74.4	105.3	134.0	137.8	0.48	0.99	1.58	2.39	2.25
10	8.04	10.70	15.62	16.39	14.61	54.0	92.8	115.7	128.4	134.0	0.43	0.99	1.81	2.10	1.96
11	6.64	09.52	10.35	14.79	14.06	66.2	88.8	121.1	133.6	140.5	0.44	0.85	1.25	1.98	1.98
12	10.23	12.85	13.50	15.83	14.73	45.8	99.5	116.6	121.4	126.1	0.47	1.28	1.58	1.92	1.86
13	8.38	09.03	13.32	14.51	14.01	33.4	104.1	116.3	129.4	139.4	0.47	0.94	1.55	1.88	1.95
14	9.33	12.37	13.81	16.69	14.94	39.2	114.6	133.9	133.9	136.9	0.37	1.42	1.84	2.23	2.05
15	10.63	13.71	15.98	17.03	16.24	34.4	106.8	115.5	135.4	141.7	0.37	1.46	1.85	2.31	2.30
16	7.96	08.59	14.43	15.94	14.32	66.7	106.3	125.8	135.8	144.7	0.53	0.91	1.82	2.16	2.07
17	10.45	12.58	15.67	16.83	16.00	69.6	114.4	129.9	136.6	141.9	0.73	1.44	2.04	2.28	2.27
18	8.20	12.05	13.70	15.63	13.66	54.1	71.6	110.2	125.9	130.3	0.44	0.86	1.51	1.97	1.78
19	7.30	11.14	12.64	13.22	11.74	62.3	93.4	123.4	127.1	136.4	0.45	1.04	1.56	1.68	1.60
20	5.45	10.39	12.42	13.59	13.12	55.5	57.6	114.8	125.3	130.2	0.30	0.60	1.43	1.70	1.71
Mean	8.14	11.46	13.95	15.83	14.58	54.38	89.49	112.87	124.16	130.04	0.435	1.022	1.572	1.961	1.897
LSD (0.05)			1.03					7.89					0.14		
CV (%)			14.1					21.5					24.4		

Table 5: The effect of Fe application on dry matter yield, Fe concentration and Fe uptake by maize grown on soils of southeastern Nigeria

3.4. Prediction of Iron availability in the soils

The usefulness of any soil extractant to predict the availability of micronutrient element is dependent on the ability to predict (from the extractant) the extent to which plants will accumulate a given nutrient element. Although, comparisons between various treatments can be problematic due to variations in soil types, properties and treatment duration (Menziez et al., 2007), an effective extractant will be able to assess the availability of these trace elements under a variety of soil conditions (McBride et al., 2004 and Menziez et al., 2007). Significant correlation coefficients (r) were observed between all the extractants and Fe uptake at 5 % level (Table 6). The highest correlation coefficients (r) were determined between Coca-Cola and HCl methods and Fe uptake of maize. However, Coca-Cola-extractable Fe had significant positive correlations with Dry matter yield, concentration and uptake of maize. Their r values are 0.528*, 0.650** and 0.712***, respectively. HCl-, EDTA-, and NH₄OAc-extractable Fe had significant positive correlations with Dry matter yield and uptake of maize plants. Their r values are (0.630** and 0.676**), (0.601** and 0.588*), (0.508* and 0.475*), respectively. The EDTA+NH₄OAc-extractable Fe had significant positive correlations with uptake (0.463*). The significant positive correlation with plant parameters suggests that the amount of Fe extracted by these extractants has strong association with plant uptake (Lindsay and Schwab, 1982). This may be due to Fe transformation and availability in soils which depends on various forms of this nutrient element with which Fe have significant and positive correlation (Dahnke and Olson, 1990). The results obtained from EDTA method followed the above methods regarding the correlation coefficients (r). Accordingly this study indicates that, the order of significance for the extracting solutions is as follows: Coca-Cola > HCl > EDTA > NH₄OAc > EDTA+NH₄OAc.

The low values obtained by NH₄OAc and EDTA+NH₄OAc may be attributed to the soil factors due to redox potential conditions of the soils which determined the final behavior of Fe in the soils and its availability to maize plants. Moreover, chemical properties of the soils showed that they were strongly acidic, low in organic matter and coarse in texture (Eteng et al., 2014). Thus, the use of salt and chelate mix (NH₄OAc+ EDTA) extraction method was not adequate in the determination of available Fe, but the used of acid method (Coca-Cola) was shown to be more suitable in the prediction of available Fe content in the soils. The result obtained in this study with Coca-Cola method is similar to the result reported by Schnug et al. (2001) in soils on the Island of Ruegen. This is supported by the result with higher correlation coefficients (r) which were obtained from acid methods (Table 6) as a result, when considered the physical and chemical properties of the soils studied; the acid method can be used with satisfaction in the determination of available Fe contents in soils of southeastern Nigeria. Besides the better results obtained with Coca-Cola method, in extracting available Fe fractions from these soils, the correlations with plant uptake was superior to the other extracting solutions. Further advantages of Coca-Cola as an extractant are its ubiquitous availability and readiness for us but also its easy and safe handling and the fact that the procedure has no harmful impacts as compared to the other extracting solutions (Schnug et al., 1996; 1998).

Table 6: Correlation of extractable Fe with yield parameter of maize (N=20)

S/No.	Extraction methods	Yield parameters of maize plants (non-application of Fe in soils)		
		Dry matter yield of plant	Fe concentration of plant	Uptake of Fe from soil
1.	Coca-cola	0.578*	0.650**	0.712**
2.	0.01N HCl	0.630**	0.281	0.676**
3.	0.05M EDTA	0.601**	0.131	0.588*
4.	1N NH ₄ OAc	0.508*	0.029	0.475*
5.	0.05M EDTA+ 1N NH ₄ OAc	0.313	0.274	0.462*

Linear regression analysis conducted on Fe uptake revealed that all the equations were statistically significant with exception of EDTA+NH₄OAc (Table 7). In these evaluations, uptake results are very similar to those for dry matter yield of maize plants. The equation that is not significant indicates that the material did not significantly increase Fe uptake. Though, EDTA and HCl methods performed equally well but, the Coca-cola method performed better. The order of importance for uptake are Coca-cola> HCl> EDTA> NH₄OAc. However, regression analysis carried out in this study suggests that plant available Fe extracted by Coca-cola method is highly recommended.

Table 7: Regression of extractable Fe with Fe uptake (N=20).

Regression equation	R ²
Fe uptake = 174.27 + 36.0 Coca-Cola-Fe	0.675***
Fe uptake = 209.51+47.34 HCl-Fe	0.602**
Fe uptake = 95.33 + 21.44 EDTA-Fe	0.574**
Fe uptake = 110.89 + 25.52 NH ₄ OAc-Fe	0.406*
Fe uptake = 185.89 + 16.45 NH ₄ OAc+EDTA-Fe	0.313 ^{ns}

Ns = not significant at $P < 0.05$. * = significant at $P < 0.05$. ** = significant at $P < 0.01$
*** = significant at $P < 0.001$

IV. Conclusion And Recommendation

4.1. Conclusion

Soil test provide an indication of nutrient level in the soil and together with plant analysis are important agronomic tools for determining crop nutrient needs, predicting the nutrient-deficient areas and preventing the deficiency. In this study, substantial variations in the amount of available Fe in soils of southeastern Nigeria were determined. Coca-Cola method was determined to be the most suitable extractant among others in the determination of available Fe in the soils. However, in the absent of Coca-cola extractant, HCl and EDTA methods can equally be used in the determination of available Fe in the soils. This is supported by the higher correlations coefficients (r) exhibited by the nature of these three extractants with maize yield parameters (Table 6) and the regression equations for Fe uptake (Table 7). As a result, when considering the chemical properties of the soils studied, the used of acids and chelate methods were found to be satisfactory for the prediction of available Fe contents in southeastern Nigeria soils. Nevertheless, these methods are suitable to certain physical and chemical properties of the soils in this region.

V. Recommendation

Further studies are expected to be carried out in field experiments for at least two years to determine critical limit and optimum levels of iron for fertilizer recommendation, in soils of southeastern Nigeria.

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