

Comparison of Some Extraction Methods for Estimating Available Mn to Maize (*Zea mays* L.) in Acid Soils of Agro-Climatic Conditions of Southeastern Nigeria

ABSTRACT

Twenty surface (0-20 cm) soil samples were used to evaluate status of soil Mn using five extraction procedures (Coca-cola, 0.05M EDTA, 0.01M HCl, 0.05M EDTA + 1N NH₄OAc and 1N NH₄OAc methods). At the greenhouse study, hydrated Mn sulphate was applied in solution, at five levels (0, 4, 8, 12 and 16 kg·ha⁻¹) in CRD while in the field studies, Mn sulphate was applied to the soils during the first year experiment only, at seventh levels (0, 2, 4, 6, 8, 8, 10 and 12 kg ha⁻¹) in RCBD. Both studies were replicated four times. The results shows that Coca-cola method extracted the highest amount of the Mn (5.61 mgkg⁻¹) while, 1N NH₄OAc extracted the least amount of Mn (1.49 mgkg⁻¹) with an overall average of 3.78 mg Mn kg⁻¹. Levels of Mn significantly ($P < 0.05$) increased maize plant height, dry matter production, concentration and uptake in maize plants. Coca-Cola method correlated positively with dry matter yield ($r = 0.875^{**}$) and Mn uptake ($r = 0.883^{**}$). 0.01M HCl method correlated negatively with dry matter yield ($r = -0.657^{*}$) and positive correlation with Mn uptake ($r = 0.687^{*}$). The 0.05M EDTA-extractable Mn had significant negative correlation with Mn concentration ($r = -0.509^{*}$), while 0.05M EDTA+1N NH₄OAc- extractable Mn had negative correlation with dry matter yield ($r = -0.586^{*}$) but correlated positively with uptake ($r = 0.546^{*}$). The critical limits were 2.82, 4.65, 5.85, 3.10 and 2.46 mg Mn kg⁻¹ for Coca-Cola, 0.01M HCl, 0.05M EDTA, 0.05M EDTA+1N NH₄OAc and 1N NH₄OAc, respectively. The results also showed that among the five extractants examined, the highest regression coefficients ($r^2 = 0.825$), ($r^2 = -0.764$) and ($r^2 = 0.633$) at ($P < 0.01$) were found between Coca-Cola and HCl, HCl and EDTA+NH₄OAc and, EDTA and EDTA+NH₄OAc-extractable Mn for Mn uptake, respectively. Accordingly, the study indicates that, the comparative extraction capacity of these extractants followed the order: Coca-cola > 0.1N HCl > 0.05M EDTA > 0.05M EDTA+1N NH₄OAc > 1N NH₄OAc. Thus, Coca-Cola, followed by 0.01M HCl extractants is highly recommended and for the determination of available Manganese to maize for acid soils of southeastern Nigeria. Similarly, the results obtained based on the two year field studies, suggests that, the recommended rates of Mn required for maximum maize grain yield production in acid soils of southeastern Nigeria, are 9.0 and 9.5 kg ha⁻¹ during the first and second cropping seasons, respectively.

Key Words: Critical limits, Coca-Cola extractant, field study, Grain yield, Laboratory, greenhouse, Mn uptake, Optimum levels.

INTRODUCTION

Maize (*Zea mays* L.) is one of the world's leading cereal grains next to rice and wheat. It is very common due to its diverse functionality as a major food source for both man and livestock (Nuss and Tanumihardjo, 2010) and it contribute greatly to the economic growth of many developing countries (FAOSTAT, 2010). It is estimated that maize together with rice and wheat provide at least 30% of the food calories to more than 4.5 billion people of developing countries (Shiferaw et al, 2011). Nigeria has great potential for the expansion of maize production beyond the present level due to its bimodal rainfall pattern, high solar radiation and favourable temperature during the growing season.

In Nigeria maize crop is widely cultivated by farmers in the tropical rainforest of southeastern Nigeria (Enwezor *et al.*, 1990; Chude *et al.*, 2004). The soils in this region are highly weathered, predominantly lateritic and mostly acidic in reaction, which often have low exchangeable bases due to intensive nutrient depletion (Ogban et al, 1999). The soils are also low in organic matter content, with low activity characteristics of the clay fraction but, high sandy in nature and leaching due to high rainfalls regimes resulting in deficient/low major and

42 minor nutrient elements specifically micronutrients (Sillanpaa, 1982). Thus, the availability of these nutrient
43 elements to maize crop is conditioned by the soils characteristic under review.

44 Among various micronutrients, manganese is especially importance owing to its typical and complex behavior
45 in soils besides its vital and indispensable role in plant growth. Manganese is a micronutrient which exists in
46 soils as insoluble oxides of trivalent and tetravalent Mn, exchangeable and water soluble divalent Mn (Shuman,
47 1988; Havlin, 2005). Uptake of Mn by plant roots is in divalent ion Mn^{2+} which is needed in small amount and
48 uptake usually is less than $1.0 \text{ kg Mn ha}^{-1}$ in cereals (Aduayi *et al.*, 2002). From the point of view of availability
49 to plants, it is an established fact that only a small fraction of total manganese in soil is made available to plants
50 in a growing season. Manganese fertilizer increases the crop yield and quality, due to improved plant nutrition
51 and increasing photosynthesis in plants (Mortvedt, 2010).

52 According to Marschner (2011), total content of manganese in surface soils ranged from 37-115 mg kg^{-1} .
53 However, 0.1 HCl extractable manganese of 8-29 mg kg^{-1} was reported by Yaro *et al.* (2006) in some soils of
54 Samaru, Zaria, Nigeria. Ibrahim *et al.* (2011) reported a ranged of available Mn in soil from 1.2 to 30.3 mg kg^{-1}
55 with mean values of 9.8 mg kg^{-1} . Kparmwang *et al.* (1995) observed that the available Mn in upper soil layer
56 ranged from 5.4 to 15.4 mg kg^{-1} which was higher than the critical limits.

57 A proper soil test is the only proven diagnostic tool that estimates plant nutrient availability in soil. It is
58 underutilized in most developing countries because of lack of rapid and reliable soil testing procedures and
59 related facilities (Porter *et al.*, 2004). Soil tests measure the quantity of nutrient element that is extracted from a
60 soil by a particular chemical extracting solution. The measured quantity of extractable nutrient in a soil is then
61 used to predict the crop yield response to application of the nutrient as a fertilizer, manure, or other amendments
62 (Menzies *et al.*, 2007).

63 In this study, the function of acid extractants (0.1M HCl and Coca-Cola) is founded on lowering the pH and the
64 outcome solubilization of some compounds containing these elements (Grava, 1980). On the other hand,
65 chelating extractant (0.005M EDTA) and neutral salt (1M NH_4OAc) has the capability of reducing the activity
66 of dissolved metals, resulting in the release of more soluble compounds in buffered pH (Gronflaten and Steines,
67 2005). The use of chelating agent such as EDTA, salt and chelate mix (NH_4OAc + EDTA) and acid such as HCl
68 and Coca-Cola as extraction methods, have longed been reported for soils elsewhere, to estimate the potential
69 availability and mobility of metals (Menzies *et al.*, 2007; Schnug *et al.*, 2001; Ivezic *et al.*, 2013; Eteng, *et al.*,
70 2014), respectively.

71 Numerous attempts have been made in the past to develop a simple and meaningful soil test method which can
72 predict manganese in soils. Such a soil-Mn-test can prove to be of great help as a diagnostic technique for
73 demarcating accurately the areas of manganese deficiency. Since the suitability of a soil test is more likely to

74 vary depending upon the extractants, soil properties and plant species, the evaluation of soil-test methods of
 75 manganese with soils of wide variability in respect of their characteristics, thus, assumes great importance.
 76 However, the information on a suitable soil-test method regarding the use of five extractants such as, Coca-Cola
 77 0.05 M EDTA, 0.1M HCl 1M NH₄OAc, NH₄OAc+ EDTA for proper prediction of available Mn for maize
 78 production is not available in respect of acid soils of southeastern, Nigeria hence, this evaluation is to develop a
 79 best suited method by comparing a number of soil test for available manganese.

80 Keeping in view the facts enumerated above, the present study was carried out with the following main
 81 objectives; assessed the status of different forms of Mn in soils, developed a soil test method for the estimating
 82 available Mn in these soils, determine critical limits of Mn in soil for maize plant, estimated response of maize
 83 (*Zea mays* L.) to manganese application and recommended Mn rate for maize production in acid soils of
 84 southeastern, Nigeria.

85 MATERIALS AND METHODS

86 2.1 Description of Sample Location and Collection

87 Soil samples were collected from maize farmlands with no history of micronutrient fertilizer application for the
 88 past 12 years for laboratory and greenhouse experiments. The sampling sites which have a high potential for
 89 dual season's maize production and suitable for production of vegetable crops, upland/swamp rice, okra, yam,
 90 citrus, oil palms, plantain/banana, pineapple genotypes (GV. III), and some new cassava varieties, was selected
 91 to cover a wide range of soil types of different properties representing, contrasting soil of southeastern, Nigeria
 92 (Table 1). The sample location lies between Latitude 4° 20' and 7° 25' North of the Equator and Longitude 5° 25'
 93 and 8° 51' East of the Greenwich meridian (Odurukwe, *et. al.*, 1995; Ojanuga, 2006). The mean minimum and
 94 maximum temperatures ranged from 21-30°C in the Coast and from 29-33°C in the interior. The rainfall pattern
 95 is bimodal and decreases from over 3000 mm in the south to 1,700 mm in the north, this has given rise to
 96 double cropping (early and late) seasons. The vegetation stretches from mangrove swamp in the coastal region
 97 through rainforest to derived savanna in the interior.

98 Table 1: Location of sample collection, soil classification and land use system

Sample No.	Sampling site	Classification*	Land use system
1	Abriba	Psamment Paleudults	Grasses (Andropogon)
2	Afikpo	Typic Ustifluents	Yam/Sugar cane farm
3	Akamkpa	Typic Paleudults	Gmelina Plantation
4	Akpabuyo	Psammentic Paleudults	Fallow
5	Akpet	Typic Hapludlts	Cassava/Maize farm
6	Bende	Aquic Hapludlts	Cocoa plantation
7	Betem	Typic Paleudults	Virgin forest
8	Etiti	Udic Psamment	Maize/cassava
9	Igbariam	Udic Haplustalfts	Cassava farm
10	Ikot Ekpene	Typic Paleudults	Telfairia/Water leaves
11	Nsukka	Typic Psammaquent	Cashew plantation

12	Oban	Typic Hapludts	Plantain/Banana farm
13	Odukpani	Aquic Haplustalfs	Cassava/maize farm
14	Ofodua	Typic Ustpsamments	Eggplant/Telfairia
15	Okigwe	Aquic Ustiflurents	Fallow (Pennisetum)
16	Owerri	Udic Haplustalfs	Oil palm (E. Guineensis)
17	Umudike	Typic Paleudults	Cassava (Manihot)
18	Uturu	Psammentic Paleudults	Fallow
19	Uyanga	Typic Paleudults	Cassava/Yam farm
20	Uyo	Psammentic Paleudults	Maize (Zea mays L.)

99 *USDA (2006), FDALR (1995)

100 **2.2. Laboratory Study**

101 To achieve the set objectives, a laboratory procedure was conducted on these soils to determine some physico-
102 chemical properties and status of available forms of manganese by the different extractants. Before the
103 laboratory analyses, the soil samples collected were air-dried and screened through a 2 mm sieve for particle
104 size and chemical analyses. 150 g of each sample was crushed with Agate mortar and pestle and, later sieved to
105 pass through 0.5 mm sieve for the determination of organic carbon, total nitrogen and total manganese. The 2
106 mm sieved soils were used to analyse for particle size analysis by the Bouyocous hydrometer method using
107 sodium hexametaphosphate as dispersing agent (Gee and Or, 2002); Soil pH in 1:2.5 soil-0.1 calcium chloride
108 suspensions was determined using a digital glass electrode pH meter (Thomas, 1996); Soil organic carbon was
109 determined by Walkley-Black method of wet combustion involving oxidation of organic matter with potassium
110 dichromate ($K_2Cr_2O_7$) and sulphuric acid (H_2SO_4) method described by Nelson and Sommer (1996) and the
111 content of the organic carbon (OC) was converted to organic matter by multiplying the OC values by Van
112 Bremelen factor of 1.724 based on the assumption that SOM contains 58% carbon. Available P was determined
113 by Bray P-I method (Kuo, 1996), total N was determined by Kjeldahl procedure of Bremner (1996),
114 Exchangeable bases (Ca^{+2} , Mg^{+2} , K^+ and Na^+) were extracted with 1N neutral ammonium acetate (NH_4OAc)
115 solution and amounts of K and Na in solution was measured using a Flame Photometer while Ca and Mg were
116 determined by EDTA titration method.

117 Exchangeable cations in the samples were extracted with neutral ammonium acetate and contents of K and Na
118 was determined with a flame photometer, while Ca and Mg were determined by EDTA titration method. Total
119 exchangeable acidity (TEA) was determined by method of McLean (1982). The effective cations exchange
120 capacity (ECEC) was calculated as the summation of exchangeable bases and exchangeable acidity as described
121 by Sumner and Miller (1996) while, base saturation was by calculation (dividing the sum of exchangeable bases
122 by the ECEC then multiplied by 100).

123 The total Mn contents in the soil samples were determined after tri-acid digestion of samples in Teflon crucible
124 heated on a hot plate according to Shuman (1985). To compare the use of the different extractants (Coca-Cola,
125 EDTA, NH_4OAc +EDTA, 1N NH_4OAc and 0.01N HCl) for assessment of available manganese of the soils
126 were carried out. 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 manganese in each soil was determined with an atomic absorption spectrophotometer (AAS) (UNICAM model SOLAAR 32: Mn ASTM D1068).

Table 2: Chemical properties and procedures of extraction used in the study

S/No	Extractant	Groups	pH	Soil-solution ratio	Shaking time (min)	Reference
1	Coca-cola	Acid	2.7	1:10	10	Schnug <i>et al.</i> (2001)
2	0.01N HCl	Acid	4.8	1:10	60	Lindsay (1995)
3	0.05M EDTA	Chelate	7.0	1:2	30	Gronflaten and Steinnes (2005)
4	1N NH ₄ OAc	Salt	7.0	1:10	60	Olsen (1992)
5	0.05M EDTA+1N NH ₄ OAc	Chelate+salt	4.8	1:10	60	Adiloglu (2003)

2.3. Greenhouse Study

The greenhouse experiment was conducted at the National Root Crop Research Institute (NRCRI), Umudike, Nigeria (Latitude 05°29'N and Longitude 07°33'E, and at an elevation of 122m above sea level), to evaluate the influence of manganese on dry matter yield, concentration and uptake of nutrients in maize plants, having observed in the laboratory study that, this nutrient was marginally sufficient in the soils.

Before the study, the soil samples were air-dried, crushed and passed through 2 mm sieve. 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 Mn 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.

Five levels of Mn (0, 4, 8, 12 and 16 kg ha⁻¹) converted to mg kg⁻¹ was applied as MnSO₄.5H₂O in solution. Based on previous studies in the region (Aduayi *et al.*, 2002), a 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).

Maize var. Oba Supper II yellow was used as test crop during the dry season. Six maize seeds were sown in each pot and the stand was thinned to four plants, two weeks after germination. The crops were irrigated with tap water as and when required throughout the growing period. The reason for the small quantity of soil and the number of maize seedlings planted per pot was for the soils to be depleted of the nutrients so that, the levels of manganese applied will be able to establish a response curve.

2.4. Plant Sample Collection and Analysis

Plant shoots were harvested at 42 days (6 WAP) growth period, by uprooting the entire maize plant (shoots and roots) from the soil, washed in an acidified detergent solution and rinsed with deionized water, pre-dried under shade to remove excess water and later packed in large brown envelopes. Plant materials were dried in oven at 70°C for 48 hrs to constant weight and the dry matter yield (DMY) were recorded. The dried plant tissues were then cut into small pieces, grind to pass through a 0.5 mm sieve and digested in Teflon crucible, heated on a hot plate using a tri-acid mixture of sulphuric acid (H₂SO₄)-nitric acid (HNO₃)-perchloric acid (HClO₄) at a ratio of 1:2:1 (Shuman, 1985). The content of manganese in the dilute aqua regia, were determined by Unicam model 939 AAS. Dry matter yield, Mn concentration and Mn uptake values from the experiment were used as yield parameters of maize plants. Nutrient uptake (mg·plant⁻¹) of maize plant was determined by multiplying the values of dry matter yield (g·plant⁻¹) and concentrations of manganese (mg kg⁻¹) in plant shoots (Whitney, 1988).

2.5. Field Calibration Studies

2.5.1. Location of the field study

The field study was sited at a community farm land in Odukpani LGA of Cross River State, in the southern part of the tropical rain forest zone of Nigeria (Latitude 4°N' and 7°N', and Longitude 8°E' and 8.30°E'), where soil samples were previously collected and used for the laboratory and greenhouse studies (Table 1). The field calibration studies for the maize plant were conducted after the greenhouse studies at two cropping seasons to evaluate the effects of various rates of Mn on maize grain yield and some other yield components. The field was fairly flat and had been under continuous and intensive cultivation without the micronutrient fertilization for over 15 years. The site experiences the southwesterly and northeasterly winds which is associated with the warm humid Maritime Tropical (MT) air mass respectively. As a result of the movement of these air masses winds, the region is characterized by two seasons-the wet season and the dry season. The wet season starts about March and last till October. This region has 2 - 3 months of dry seasons during which the total rainfall is less than 60 mm. The annual rainfall of the area was recorded as 3063 mm (NIMETS, 2009). The field study was conducted during the two cropping seasons.

2.5.2. Experimental Design, Field Plan, and Treatments

Seven levels of Manganese (0.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 Kg Mn ha⁻¹) as MnSO₄·5H₂O, were arranged in a randomized complete block design (RCBD) and replicated 4 times to give 28 (7 levels of Mn × 4 replications) experimental plots. Each experimental plot received a basal application of N, P and K at 120, 90 and 60 Kg·ha⁻¹, respectively and were applied uniformly as Urea, SSP, MOP to all the plots (Chude et al., 2004). The NPK were applied at planting as band placement covered while, the Mn treatments were applied two weeks after planting as side dressing. The reason for the selection of such high range of rates of Mn was to observe the response curve mainly for academic research purposes.

The dimension of each experimental plot was 6 m × 10 m (60 m²), with inter-block and inter-plot spacing of 2.5 and 2.0 m, respectively. A 2-m wide pathway was maintained around the entire experimental area. Maize seeds were sown at the spacing of 75 by 25 cm. Four seeds of Oba Supper II maize cultivar were sown manually and were thinning to two seedlings, 14 days after sowing, which gives a plant population of 106,666 plant·ha⁻¹.

Seven core soil samples were obtained at the 0-20 cm depth from the site, bulked together and mixed. The samples were air-dried and mixed through a 2 mm stainless steel sieve. The soil sample taken was subjected to the same analysis as described for the greenhouse study.

Plants were sampled at 9 weeks after planting by taking three ear leaves (4th leaf) per row from each of the net six out of eight rows, giving a total of 18 leaves per plot (Lisuma, *et al.*, 2006), when about 50% silking, after maize plants had tasseled. The ear leaf samples were rinsed with tap water, enveloped and oven dried at 65°C for 48 Hours to constant weight, cut into small pieces, and ground to pass through a 0.5 mm sieve, ashed and subjected to chemical analysis as previously mentioned to in the greenhouse study.

Plants were allowed to grow to maturity before the cobs were harvested at 120 days, after which, the cobs were shelled; grain yields were measured in kgplot⁻¹ and converted into tones·ha⁻¹ at 12.5% moisture, the values were used to determine grain dry meter yield. Grains were later ground using a Willey mill and digested using a tri-acid a mixture at a ratio of 1:2:1 (H₂SO₄: HNO₃: HClO₄) as described before, and analyzed for Mn using atomic absorption spectrophotometer (AAS). Nutrient uptake (mg·plant⁻¹) of maize plant was determined by multiplying the values of dry matter yield (g·plant⁻¹) and concentrations of manganese (mg kg⁻¹) in maize grains. Data interpretation was made by comparing the results with the critical limits recommended for each parameter.

The coefficient of determination (r²) was used to select the best fitted model. The extractant which displayed the highest correlation coefficient (r) with the maize uptake was recommended for the determination of available Mn content of the acid soils of southeastern Nigeria.

2.4. Statistical Analysis

The data obtained were subjected to analysis of variance (ANOVA) procedure with simple correlation and regression analyses at different probability levels, using the computer software; Genstat (2014) and PASW Statistics 18 for Window 7.0, to show the relationships between the different extractants and the plant Mn uptake. Significant means were separated using Fisher's Least Significant Different at 5% probability.

RESULTS AND DISCUSSION

3.1 Properties of the Study Soils

The contents of some physico-chemical properties of the study soil samples are presented in Table 3. The results showed that most of the soil samples examined for the greenhouse and the field studies were coarsed in

texture with textural classes which are sandy clay loam (SCL) and sandy loam (SL), respectively (Table 3). The soils were strongly acidic in reaction, however, the optimum soil pH range for maize production was reported to be between 4 and 7 (Aduayi *et al.*, 2002). The majority of the soils were low in organic matter content. The low organic carbon could be explained by the fact that acid soils normally have low organic carbon content (Ibanga and Udo, 1996).

The total nitrogen content of the soils was moderately low according to Landon (1991) and Enwerzor (1989). The extractable P content of the soils was rated medium according to Landon (1991) while, ECEC values of the soils varied widely with a mean of 9.85 and 84.3 cmol kg⁻¹. ECEC of the soils tested were below the average of 12 cmol kg⁻¹ and these are rated low according to Melsted *et al.* (1969), and this is the range of critical value for soils that are dominated by oxide and hydroxide clays (Omuetti and Lavkulich, 1988). This suggests that most of the soil may have few exchange sites. Aluko *et al.* (2001) noted that ECEC values below a range of 10 to 20 cmol kg⁻¹ are considered marginally adequate for crop production. This trend of results is attributed to the nature of the soil types, high rainfall, high leaching and erosion of bases and loss of organic matter as reported by Asawalam (1989).

Table 3: Mean values of some physico-chemical properties of the surface (0-20 cm) soils used for the greenhouse study and the field study

Soil properties	Unit	Greenhouse Study (N=25)	Field Study
Particle Size Distribution			
Sand fraction	gkg ⁻¹	642.37	661.53
Silt fraction	gkg ⁻¹	101.22	153.16
Clay fraction	gkg ⁻¹	256.41	185.31
Textural class		Sandy clay loam	Sandy loam
Chemical Properties			
pH (1:2.5) H ₂ O		5.51	5.19
pH (1:2.5) CaCl ₂		4.78	4.62
Org. Carbon	gkg ⁻¹	4.40	1.06
Org. matter	gkg ⁻¹	7.59	1.83
Al ⁺	Cmolkg ⁻¹	1.91	1.48
H ⁺	Cmolkg ⁻¹	3.44	1.16
Effective cation exchange capacity	Cmolkg ⁻¹	9.85	8.43
Base saturation	%	76.35	84.58
Nutrient Concentration (Available Nutrient)			
Total nitrogen N	gkg ⁻¹	0.56	0.19
Available P	mgkg ⁻¹	24.20	12.23
Calcium Ca	Cmolkg ⁻¹	4.76	3.60
Magnesium Mg	Cmolkg ⁻¹	2.46	2.04
Sodium Na	Cmolkg ⁻¹	0.09	0.15
Potassium K	Cmolkg ⁻¹	0.19	1.34
Total Mn in soil	mgkg ⁻¹	367.18	115.28

3.2. Extractable Manganese Contents in Soils by Different Extraction Methods.

Results of extractable Mn by different extraction methods are presented in Table 4. The amount of extractable Mn varied remarkably depending on and extractants used and the parent material from which the soils are

239 derived (Yusuf *et al.*, 2005). The lowest content of Mn was extracted by 1M NH₄OAc and the highest by the
240 Coca-cola solution. The amount of extractable Mn in different soils varied from 1.49 to 5.61 5.61 mg kg⁻¹
241 (Table 4).

242 In this study, higher available manganese content was extracted by the Coca-cola solution, with values which
243 varied widely from 0.92 to 18.24 mg kg⁻¹ with a mean of 9.34 mg kg⁻¹, followed by 0.01N HCl extraction
244 method with Mn values which varied widely from 0.87 to 9.81 mg kg⁻¹ with a mean of 5.44 mg kg⁻¹. One
245 normal NH₄OAc extraction method extracted the lowest content of Mn relative to the other methods, with Mn
246 values which varied widely from 0.53 to 5.32 with a mean value of 2.95 mg kg⁻¹. 0.05M EDTA-extractable Mn
247 varied widely between 0.52 and 7.26 mgkg⁻¹ with a mean of 4.01mg kg⁻¹. While the 0.05M EDTA+1N NH₄OAc
248 extraction method has significantly (P<0.05) lower extractable Mn relative to Coca-cola, and 0.01N HCl
249 methods.

250 Thus, the mean values of available Mn of the acid soils were determined to be 9.34, 4.01, 5.44, 3.79 and 2.95
251 mgkg⁻¹ (Table 4), on the basis of the mean extractable Mn content in soils, the extractants employed in the study
252 could be arranged in the following order of their Mn extraction capacity: Coca-cola>0.01N HCl>0.05M
253 EDTA+1N NH₄OAc>0.05M EDTA>1N NH₄OAc methods, respectively. Generally, the variation of extractable
254 manganese obtained in the soils is a reflection of the existing differences in the strengths of the extracting
255 solutions and the availability due to the soil parent materials.

256 Thus, the quantity of available Mn by 1N NH₄OAc method was three times lower than the amount extracted by
257 Coca-cola and HCl methods, respectively. The results is in line with those reported in previous studies by
258 Kparmwang et al (1995), who reported that 0.01M HCl extracted more Mn than EDTA. Gronflaten and Steines
259 (2005) also noted that extraction with acid or acid-salts removes “acid soluble Mn” and recommended acetic
260 acid and phosphoric acid which, is a major ingredient of coca-cola solution (Schnug et al., 1996).

261 However, the amount of extractable Mn obtained in this study, suggests that the soils contain sufficient Mn for
262 successful maize production, since the values are within the critical available range of 3-5 mgkg⁻¹ reported by
263 Lindsday and Norvell (1978) and 1-5 mgkg⁻¹ reported by Esu (1991) and Mustapha (2005) for the Ustults in
264 Bauchi State, Nigeria. Previous workers noted that soil pH has a dominant effect on the solubility, availability
265 and potential cations (e.g. Mn²⁺) in soil (Clark and Baligar, 2000). Also, Rengel (2002) noted that, the
266 availability Mn in soil solution as cations (Mn²⁺) increases with increasing soil acidity, whereas the availability
267 of Mn present as anions (MoO₄²⁻) increases with increasing pH.

268 **Table 4: Distribution of extractable Mn in soils by different extraction methods**

Sample Location	Extractable Manganese (mgkg ⁻¹)					Mean
	Coca-Cola	EDTA	HCl	EDTA+NH ₄ OAc	NH ₄ OAc	
Abriba	13.62	5.12	7.25	5.61	0.53	6.43

Afikpo	4.19	2.51	5.79	3.62	1.52	3.53
Akamkpa	16.43	5.64	8.82	5.81	1.64	7.67
Akpabuyo	13.31	5.97	6.11	6.75	1.76	6.78
Akpet	18.24	4.37	9.81	2.31	1.85	7.32
Bende	5.26	0.52	0.87	0.71	2.16	1.90
Betem	6.19	3.09	4.25	4.43	2.21	4.03
Etiti	4.18	2.48	3.14	3.06	2.59	3.09
Igbariam	8.62	2.26	8.98	5.32	2.92	3.62
Ikot Ekpene	5.26	1.43	5.18	1.68	2.95	3.30
Nsukka	11.22	5.89	3.36	1.11	3.48	5.01
Oban	9.71	3.16	7.72	4.22	3.62	5.69
Odukpani	5.38	6.61	4.77	2.57	3.03	4.48
Ofodua	2.22	2.16	2.05	1.03	4.61	2.41
Okigwe	12.51	6.52	5.83	5.72	4.97	7.11
Owerri	17.33	6.46	7.47	7.04	4.19	8.49
Umudike	6.77	3.57	4.31	2.41	3.32	4.08
Uturu	7.67	3.62	5.44	3.92	5.32	5.19
Uyanga	17.93	7.26	6.26	6.36	4.02	8.37
Uyo	0.92	1.63	1.31	2.04	2.25	1.63
Min.	0.92	0.52	0.87	0.71	0.53	1.63
Max.	18.24	7.26	9.81	7.04	5.32	8.49
Mean	9.34	4.01	5.44	3.79	2.95	5.11

3.3 Critical limit of soil extractable Mn

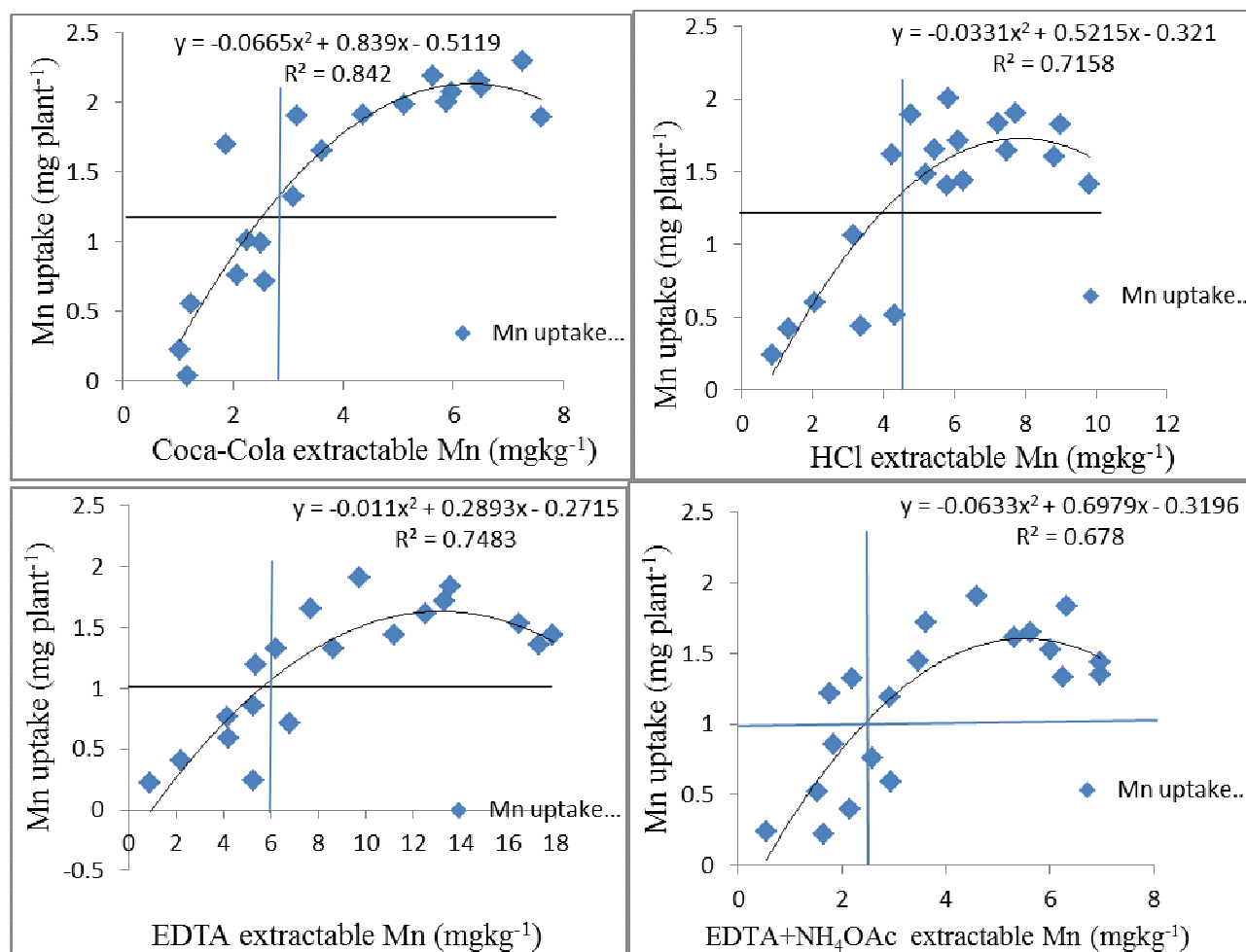
The relative suitability of different soil extractants could be judged by the statistical significance and the magnitude of R^2 -value. To estimate the critical limits (the threshold concentration below which the probability of finding an economic response to the application of a nutrient in a crop is greater) of Mn in terms of soil extractable Mn for maize plants from greenhouse study, the uptake of maize shoots was plotted against soil extractable Mn estimated by different soil extractants. The critical limits of Mn were evaluated following the statistical (R^2 -technique) and graphical procedures of Cate and Nelson (1965; 1971). The critical value divides soils as highly responsive and non-responsive to manganese application. The maximum uptake by maize (mg plant⁻¹) corresponds to the critical soil extractable Mn values (mgkg⁻¹), under which a high response of added Mn to maize crop is expected. On the basis of highest predictability (R^2) value, a population can easily be partitioned which corresponds to the postulating critical level (Fig. 1). The R^2 indicated in Figure 1 are coefficients of determination for delineation of responsive soils from non-responsive soils. Simple regression equations between soil extractable Mn (X-axis) and Mn uptake of maize shoots (Y-axis) with their respective coefficient of determination which, could be accounted by extractable Mn for a particular extractant (R^2) are shown in Figure 2.

Thus, the critical limits of Mn in soil, below which the response of maize to applied Mn could be expected, were determined as 2.82, 4.65, 5.85, 3.10 and 2.46 mg for Coca-Cola, 0.01M HCl, 0.05M EDTA, 0.05M EDTA+1N NH₄OAc and 1M NH₄OAc-extractable Mn kg⁻¹ soil, respectively. The R^2 values for Coca-Cola,

291 0.01M HCl, 0.05M EDTA extractable Mn were significant at $P>0.01$ while, NH_4OAc and 0.05M EDTA+1N
 292 NH_4OAc extractable Mn have R^2 values which are significant at $P>0.05$. The observed variation in the critical
 293 limit of soil the Mn, could be attributed partly due to contrasting soil properties and in the effectiveness of the
 294 extractant.

295 Considering the magnitude of R^2 -values (coefficient of determination for delineation of Mn responsive soils
 296 from Mn non-responsive soils), the soil extractants could be arranged in the order of reliability: Coca-Cola>
 297 HCl > EDTA > EDTA+ NH_4OAc > NH_4OAc . However, keeping in view the rapidity of the extraction procedure
 298 (Table 4) with Coca-Cola extractant and in the absence, 0.01M HCl extractants, can be used to achieve the same
 299 result. Thus, these would certainly be better extraction methods for soil Mn in acid soils of southeastern Nigeria.

300



301

302

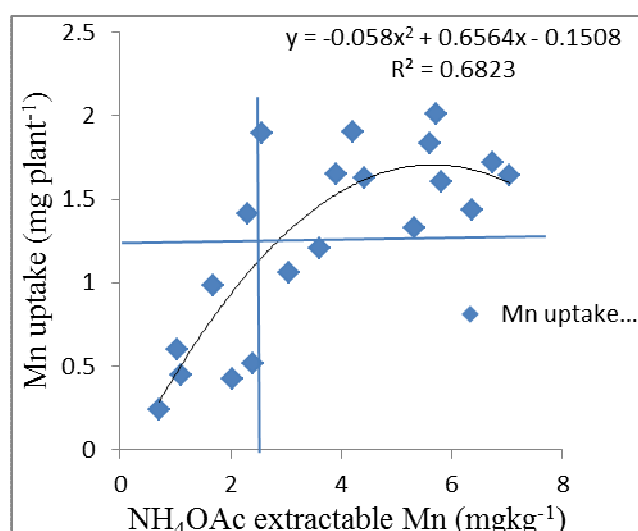


Figure 1: Scatter diagrams plotted between Mn uptakes of maize shoots versus extractable soils Mn after greenhouse experiment.

3.4 Effect of Mn Levels on Dry Matter Production of Maize shoots

Deficiency symptom of Mn was observed on maize plant shoots which appeared yellow in both intercostal during the emergence of maize tassels. The symptoms first appear on younger leaves because the dynamics of these elements in different plant tissues were limited. It was noted in previous studies by Sharma *et al.* (1991) and Marschner (2011) that, the major symptom of Mn deficiency is a reduction in the efficiency of photosynthesis leading to a general decline in dry matter productivity and yield. However, a significant result of Mn treatment on dry matter yield of maize shoots was established. In general, wide variation in the dry matter yield of maize shoots was noticed among the soils and differ significantly ($P < 0.05$) among the levels of Mn treatments, and these improved the dry matter yield of maize in some soils ranging from 12.95 to 17.03 g plant⁻¹ with a mean of 15.21 g plant⁻¹ (Figure 1).

Dry matter yields obtained at different Mn levels in soils indicated that the application of 8 kg·Mn·ha⁻¹ soil brought the highest and significant dry matter yield of maize over the 0 kg·Mn·ha⁻¹ soil level (Figure 2). The significant ($P < 0.05$) increase in DM yield in the Mn treatments over the control suggests that Mn was one of the limiting nutrients in the soils (Figure 1). Further addition of MnSO₄·5H₂O salt to 8 kg ha⁻¹, did not significantly improve DM yield of plant shoots. Although, 8 kg Mn ha⁻¹ levels of application gave the highest DM yield of maize, according to the polynomial regression equation analysis (Table 4), a maximum of 9 kg·Mn·ha⁻¹ will be required to improve dry matter production of maize in the soil, which in turn will improve maize grain yields. This finding is in agreement with previous result reported by Lisuma *et al.* (2006); Fageria (2002). The finding, suggests that 9 kg·Mn·ha⁻¹ is the critical nutrient level that will optimize maize production in the acid soils under reviewed.

3.5 Effect of Mn Levels of the Concentration of Manganese in Maize Shoots

The Mn concentration in maize shoots increased with increasing Mn levels (Fig. 1). The content of manganese concentration ranged from 69.50 to 85.61 mg kg⁻¹ with a mean of 79.73 mg kg⁻¹, and these were sufficient according to previous studies by Melsted et al. (1969), Tandon (1995), Menzies et al. (2007) and Rastija et al. (2009), respectively. Moreover, Mn application significantly (P<0.05) increased Mn concentration compared to control, indicating that Mn was one of the limiting nutrients in this soil (Figure 1). Surprisingly, lower levels of Mn also yielded higher concentration in maize shoots. It is noted in Table 3 that, 8 kg·Mn·ha⁻¹ level which gave the maximum dry matter production also gave significantly (P < 0.05) higher Mn content relative to lower levels of Mn. However, the polynomial regression equation (Table 5) determined, indicated that it will take about 10 kg·Mn·ha⁻¹ to produce optimum Mn concentration in maize plants. The wide variability in Mn concentration in maize shoots as determined in this study may be as a result of higher available Mn content and low Mn fixation in some of the soils. The effect of Mn application on the yield parameter of maize plants was determined to be significant at 5% probability level.

3.6 Effect of Mn Levels on Uptake of manganese in maize plants

The Mn uptake of the maize shoots determined at 6 WAP increased with increasing Mn levels and varied from 0.906 to 1.458 mg plant⁻¹ with an average of 1.257 mg plant⁻¹ (Figure 2). The progressive increased in uptake due to the addition of Mn, indicated that the application of 8 kg·Mn·ha⁻¹ soil yielded the highest and significant higher uptake of maize over the 0 kg·Mn·ha⁻¹ soil level (Figure 2). However, the significant result obtained at this level of Mn application, may not be unconnected to the increase in either DM yield or Mn concentration which accumulated Mn content in the various plant parts. Supporting this, the polynomial regression analysis ($Y = 0.7663 + 0.0152x - 0.0079x^2$; $R^2 = 0.7663$) computed indicated that the maximum Mn level that gave the optimum Mn uptake as shown in Figure 1, was 8 kg·Mn·ha⁻¹ of application (Table 5). In general, acidic soils with low clay contents appeared to be more responsive to Mn application (Enwerzor *et al.*, 1989). Similar results were obtained in previous studies by Fageria (2002); McBride *et al.* (2004); Lisuma et al. (2006).

Table 5: Maximum levels of Mn (kg ha⁻¹) that gave the optimum growth/yield parameters of maize shoots

Maize shoot parameter	Maximum rates of Mn (kg ha ⁻¹) required	Polynomial regression (R ²) values
Plant height	8.8	0.844
Dry matter yield	9.4	0.865
Mn content in plant shoot	10.2	0.991
Mn uptake	8.0	0.766

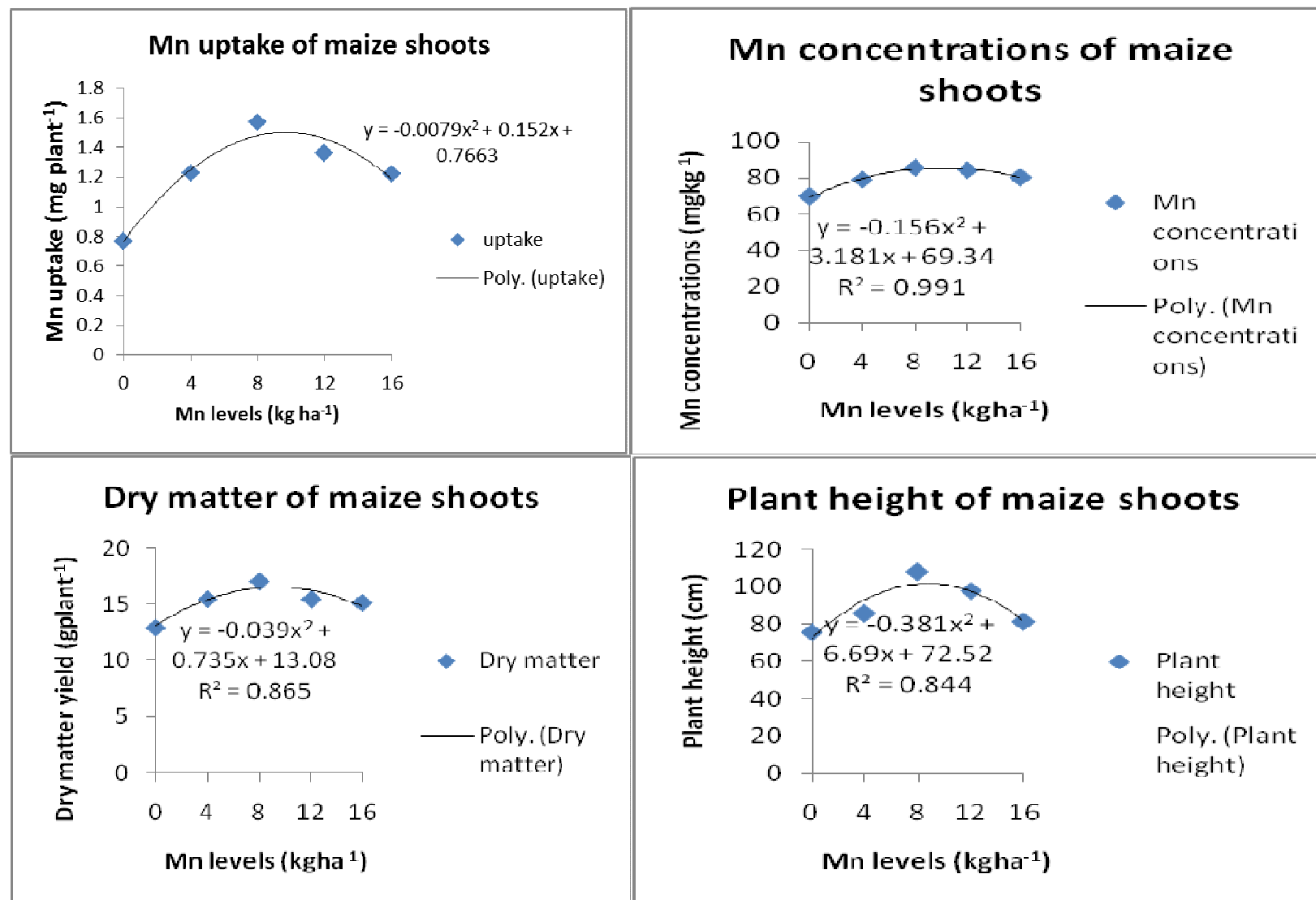


Figure 2: Graphs showing the effects of Mn levels on yield and growth parameters of maize shoots in greenhouse experiment.

3.7 Calibration and correlation for greenhouse study

The usefulness of any soil extractant to estimate the availability of micronutrient element is dependent on the ability to determine (from the extractant) the extent to which plants will accumulate a given nutrient element. Although, comparisons between various chemical extractants can be problematic due to variations in soil types, properties and treatment duration (Menzies *et al.*, 2007). An effective extractant will be able to predict the availability of these trace elements under a variety of soil conditions (McBridge *et al.*, 2004 and Menzies *et al.*, 2007).

Significant correlation coefficients (r) were determined between all the extractants and Mn uptake at $P < 0.05$ and 0.01 probability levels (Table 6). The highest significant correlation coefficients (r) were determined between Coca-Cola method which correlated significantly with dry matter yield ($r = 0.875^{**}$), and Mn uptake ($r = 0.783^{**}$) but negatively correlated with Mn concentration ($r = -0.699^{*}$) of maize, followed by HCl method which correlated negatively with dry matter yield ($r = -0.657^{*}$) and positive correlation with Mn uptake ($r = 0.687^{*}$). The EDTA-extractable Mn had significant negative correlation with Mn concentration ($r = -0.509^{*}$) of maize. While EDTA+ NH_4OAc - extractable Mn had negative correlation with dry matter yield ($r = -0.586^{*}$) but correlated positively with uptake ($r = 0.546^{*}$). These significant positive correlations with plant parameters suggest that the amount of Mn extracted by these extractants have strong association with plant uptake (Lindsay and Schwab, 1982). This may be due to Mn transformation and availability in soils which depends on various forms of this nutrient element with which Mn have significant and positive correlation (Dahnke and Olson, 1990). The results obtained from NH_4OAc method followed the above methods regarding the correlation coefficients (r) but, had no significant correlation with either of the yield parameter of maize shoots.

The low values obtained by EDTA, NH_4OAc and EDTA+ NH_4OAc methods respectively may be attributed to the soil factors such as, redox potential conditions of the soils which determined the final behavior of Mn in the soils and its availability to maize plants. Moreover, chemical properties of the soils (Table 3) suggests that they were strongly acidic, low in organic matter and ECEC, coarse in texture as reported in the previous study by Eteng *et al.* (2014). Thus, the use of salt and chelate mix (NH_4OAc + EDTA) extraction method was not adequate in the determination of available Mn, rather, the used of acid methods (Coca-Cola and HCl) was shown to be more suitable in the prediction of available Mn 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 5 and 7). Therefore, when considering the physical and chemical properties of the soils studied, the acid method can be used with satisfaction in the determination of available Mn contents in soils of southeastern Nigeria. Besides the better results obtained with the Coca-Cola method, in extracting available Mn 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 coefficients (r) between extractable Mn in soils and uptake by maize shoots (N=20)

Extraction methods	Yield parameters of maize plants (non-application of soils Mn)		
	Dry matter yield of plant (g plant ⁻¹)	Mn concentration of plant (mg kg ⁻¹)	Uptake of Mn in plant (mg plant ⁻¹)
Coca-Cola	0.875**	-0.699*	0.783**
0.005M EDTA	0.496 ^{ns}	-0.509*	0.107 ^{ns}
0.1M HCl	-0.657*	-0.153 ^{ns}	0.687*
EDTA-NH ₄ OAc	-0.586*	-0.204 ^{ns}	0.546*
1M NH ₄ OAc	0.279 ^{ns}	-0.179 ^{ns}	0.361 ^{ns}

Ns: not significant at P < 0.05, * significant at P < 0.05, ** significant at P < 0.01

3.8 Prediction of Mn uptake of maize shoots by soil extractable Manganese

Manganese uptake of maize under 0 mg Mn kg⁻¹ soil was regressed on soil extractable Mn contents estimated by different soil extractants (Table 7). The regression analysis between Mn extracted by different soil extractants (Table 7) and the Mn uptake by maize shoots showed that the Mn extracted by Coca-cola, 0.05M EDTA, 0.01M HCl, EDTA+NH₄OAc and 1N NH₄OAc extractants (Table 4) accounted for 86.6, 51.1, 77.2, 60.1 and 33.0 percent variations in Mn uptake by maize plants, respectively.

Similar to the predicted results establish from the correlation analysis (Table 6), the linear regression analysis conducted showed that, all the predicted equations regarding Mn uptake and extractants were statistically significant with exception of NH₄OAc (Table 7). In these evaluations, uptake results were very similar to those determined for dry matter yield and Mn concentration of maize plants. The linear regression equation that is not significant indicates that the material did not significantly increase Mn uptake. Though, 0.01M HCl methods performed equally well, the Coca-cola method performed better. Based on the correlation and regression analyses (Tables 6 and 7), this study has proven that Coca-cola and 0.01M HCl extraction methods which performed better among others tested, are the most suitable extractants and are therefore highly recommended for the estimation available manganese in acid soils of southeastern Nigeria. These findings are in agreement with previous studies by Aydinlal and Katkat (2004).

Table 7: Regression equations for the prediction of Mn uptake in maize by different extractants in acid soils of southeastern Nigeria (N=20).

Soil extractant (X)	Mn uptake (Y, mg pot ⁻¹) Regression equation	R ² value
Coca-Cola	Y = 243.20 + 180.74X	0.866**
EDTA	Y = 607.17 + 1592X	0.511*
HCl	Y = 245.27 + 418.18X	0.772**

NH ₄ OAc+EDTA	$Y = 348.36 - 123.75X$	0.601**
NH ₄ OAc	$Y = 446.32 + 70.16X$	0.330 ^{ns}

Ns: not significant at $P < 0.05$, * significant at $P < 0.05$, ** significant at $P < 0.01$

4.0 Field calibration study

4.1 Optimum Mn levels for maize grain production

The application of Mn fertilizer to soil significantly ($P < 0.05$) influenced maize grain yield of both the first and second cropping (Figure 3). However, the polynomial regression analysis determined for maize grain yield as a function of Mn application was also significant ($R^2 = 0.925$ and 0.921) for first and second cropping seasons, respectively. The maximum increase (150%) in maize grain yield was determined when the Mn was applied at 9.0 kg ha^{-1} , for the first cropping season, while, the second cropping season exhibited maximum increase (278%) in grain yield when Mn was applied at 9.5 kg ha^{-1} . These results imply that the second cropped maize required nutrients in greater amounts to attain full yield potential compared to that grown in the first cropping season. In the present study, maize grain yields of both seasons were reduced at the highest rate of Mn application (12 kg ha^{-1}). The reduction in maize grain yields in both cropping seasons after application at 9.0 kg ha^{-1} and 9.5 kg ha^{-1} respectively is attributed to their optimum levels of Mn requirement. These values are higher than the optimum rate of $8.0 \text{ kg Mn ha}^{-1}$ (Table 6) estimated in the greenhouse study for Mn uptake in maize shoots. Thus, the result illustrated in (Figure 3) suggests that 9.0 and $9.5 \text{ kg Mn ha}^{-1}$ respectively is the estimated optimum Mn rate for maximum maize grain yields in the study area.

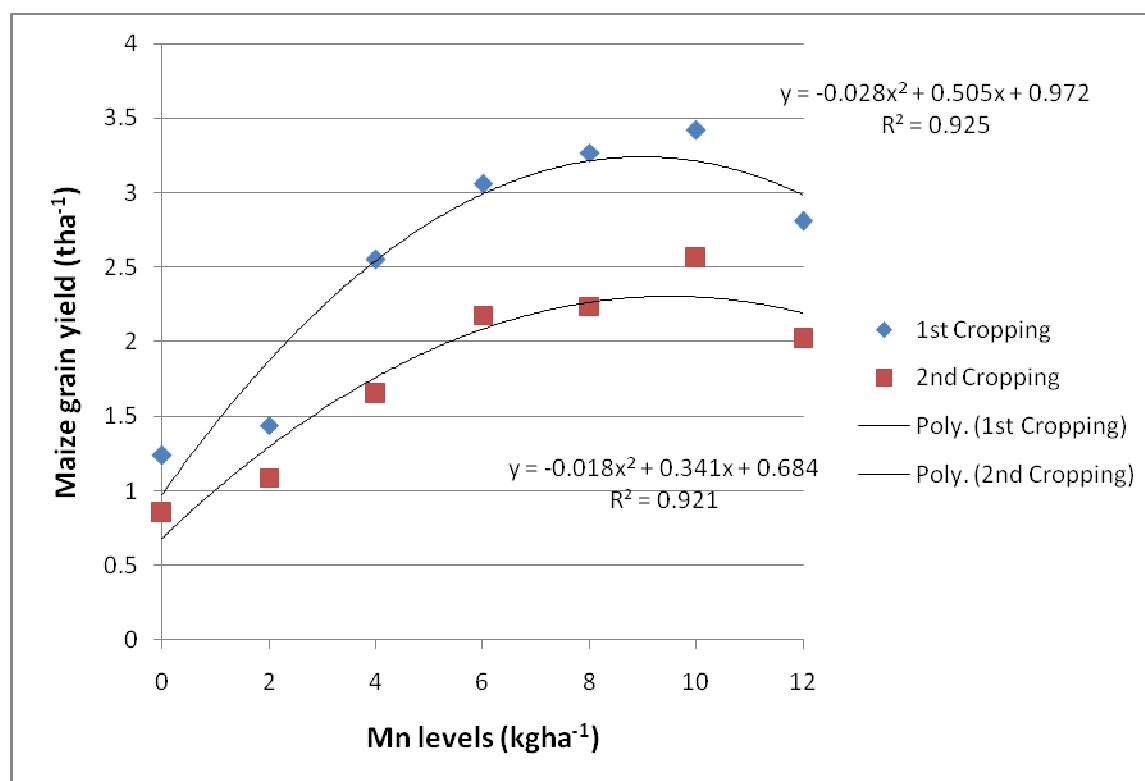


Figure 3: Optimum Mn levels for maize grain yield in field experiment.

CONCLUSION

This study demonstrated that, the soils were general sandy loams in texture, moderately to strongly acidic, low in organic matter content with low to medium contents of exchangeable bases. Available manganese was marginally supplied in soils however; deficiencies are likely to occur due to intensive cultivation of improved and high yielding crops varieties which may lead to nutrient mining.

The results obtained in this study shows that the Coca-cola method extracted the highest amount of the Mn (5.61 mgkg⁻¹) while, 1N NH₄OAc extracted the least amount of Mn (1.49 mgkg⁻¹) with an average of 3.78 mg Mn kg⁻¹. Levels of Mn applied to the soils significantly ($P < 0.05$) increased maize plant height, dry matter production, concentration and uptake in maize plants

The optimum rate of applied Mn that promoted Mn uptake in maize shoots was established at 8 kg Mn ha⁻¹. The critical limits of Mn in soil that produced responses for maize plants were determined to be; 2.82, 4.65, 5.85, 3.10 and 2.46 mg Coca-Cola, 0.01M HCl, 0.05M EDTA, 0.05M EDTA+1N NH₄OAc and 1N NH₄OAc-extractable Mn kg⁻¹ soil, respectively.

Furthermore, the results obtained based on the two year field studies, suggests that, the optimum values of Mn levels required for maximum maize grain yield production in soils of the study area were determined to be 9.0 and 9.5 kg ha⁻¹ during the first and second cropping seasons, respectively.

RECOMMENDATION

The results obtained from correlation analysis were supported by those presented in the regression equations. Accordingly, the study shows that, the comparative extraction capacity of these extractants followed the order: Coca-cola > 0.1N HCl > 0.05M EDTA > 0.05M EDTA+1N NH₄OAc > 1N NH₄OAc. However, higher correlations (r) values of extractable Mn with Mn uptake in maize plants are exhibited by the potentials of Coca-cola and 0.1N HCl extractants over others. Therefore, Coca-Cola extractant, followed by 0.01M HCl extractant, can easily be recommended and adopted as the most suitable soil testing method for the determination of available Maganese to maize in acid soils of southeastern Nigeria. Critical limits may not be required in estimating or interpreting plant Mn fertilization, as regression equations are more reliable and not empirical.

REFERENCE

- Adiloglu, A. (2003). Determination of suitable chemical extraction methods for available iron content of the soils of Edirne province in Turkey. *Pakistan journal of biological Sciences* 6(5): 505-510.
- Aduayi, E. A., Chude, V.O., Adebuseyi, B. A. and Olayiwola, S. O. (2002). Fertilizer use and Management Practices for Crops in Nigeria. Federal Fertilizer Distribution Department. Federal Ministry of Agriculture and Rural Development, Abuja, pp 126.
- Aluko, A. P., J. J. Owunubi and J. A. Fagbero (2001). Physico-chemical properties and potentials of some wetland soils of the mangrove ecosytem for sustainable forest trees establishment. In: Management of wetland soils for sustainable agriculture and environment Proceedings of the 7th Annual Conference of the Soil Science Society of Nigeria. Pp. 237-240.
- Amalu, U. C. (1991). A trace element survey of soils of coconut growing areas of southern Nigeria. *Nig. J. of Palms and Oils Seeds* 13: 78-86.
- Asawalam, D. O. K. (1989). The human element in soil erosion and the campaign for hunger in Southern Nigeria. Proc. of the 17th Ann. Conf. of the SSSN: 50-55.
- Aydinalp, C. and Katkat, A. V. (2004). The comparison of extraction methods for evaluating some heavy metals in polluted soils. *Plant soil environment*, 50 (5): 212-217.
- Bremner, J. M. (1996) Nitrogen Total. In: Sparks, D.L., Ed., *Methods of Soil Analysis Part 3: Chemical Methods*, SSSA Book Series 5, *Soil Science Society of America, Madison, Wisconsin*, 1085-1122.
- Brown, J. R. (1987). Soil testing: sampling, correlation, calibration, and interpretation. SSS Special Publication No. 21. *Soil Sci. Soc. of America, Inc. Madison, Wisconsin*. 144 p

- 481 Cakmak, I. (2002). Plant nutrition research: priorities to meet human needs for food in sustainable ways. *Plant*
482 *Soil* 247: 3–24.
- 483 Cate, R. B., Jr. and Nelson, L. A. (1965). A rapid method for correlation of soil test analysis with plant
484 response data. North Carolina Agric. Exp. Stn. Inst. Soil Test Serv. Tech. Bull., No. 1: 67-69.
- 485 Cate, R. B. and Nelson, L. A. (1971). A simple statistical procedure for partitioning soil-test correlation data in
486 two soils. *Soil Sci. Soc. Am. Proc.*, 35: 658-659.
- 487 Chude, V.O., Malgwi, W.B., Amapu, I.Y. and Ano, A.O. (2004) Manual on Soil Fertility Assessment. Federal
488 Fertilizer Department/National Special Food Programme for Security, Abuja, 89.
- 489 Clark, R.B., Baligar, V.C. (2000). Acidic and alkaline soil constraints on plant mineral nutrition. In: R.E.
490 Wilkinson (ed.) Plant-Environment Interactions. Marcel Dekker Inc, New York, pp: 133-177.
- 491 Cottenie, A., B. T. Kang, L. Kieken and A. Sajjapongse (1981). Micronutrient status. In: D. J. Greenland (ed)
492 Characterization of Soils in Humid Tropics. Oxford Univ. press New York 85 pp.
- 493 Dahnke and Olson, 1990 Dahnke, W. C. and Olson, R. A. (1990). Soil test correlation, calibration, and
494 recommendation. p. 45-71. In: R. L. Westerman (3rd ed.) Soil Testing and Plant Analysis. SSSA,
495 *Madison, Wisconsin*.
- 496 Enwezor, W.O., Ohiri, O.C., Opuwaribu, E.E. and Udo, E.J. (1990) A Review of Fertilizer Use on Crops in
497 Southeastern Zones of Nigeria (in Five Vols.) (FFDD Eds). Boboma Publ., Ibadan, 47-70.
- 498 Enwezor, W. O., Udo, E.J. and Sobulo, R. A. (1981). Fertility Status and Productivity of Acid Sands. In: *Acid*
499 *Sands of Southeastern Nigeria*, SSSN Special Publication Lagos, Nigeria, *Monograph No. 1*: 56-73.
- 500 Enwezor, W.O., Udo, E.J., Usoro, N.J., Ayotade, J.A., Chude, V.O. and Udegbe, C.C. (1989) Fertilizer Use
501 and Management Practices for Crops in Nigeria. Series 2. Federal Ministry of Agriculture, Water
502 Resources and Rural Development, 52-56.
- 503 Esu, I. E. (1991). Detailed soil survey of NIHORT farm at Bunkure Kano State, Nigeria. Institute. *Agric. Res.*,
504 *Zaria pp* 72.
- 505 Eteng, E. U, Asawalam, D. O. Ano, A. O. (2014). Total and available micronutrients in soils of contrasting
506 parent materials in Southeastern Nigeria. *Journal of Agriculture, Biotechnology and Ecology*. 7(2):1-
507 16.
- 508 Fageria, N. K. (2002). Influence of micronutrients on dry matter yield and interaction with other nutrients in
509 annual crops. *Pesq. agropec. bras.*, Brasília, v. 37, n. 12, p. 1765-1772
- 510 FAO FAOSTAT–Agriculture Database. Available: [http://faostat3.fao.org/faostat-](http://faostat3.fao.org/faostat-gateway/go/to/download/Q/*/E)
511 [gateway/go/to/download/Q/*/E](http://faostat3.fao.org/faostat-gateway/go/to/download/Q/*/E) Accessed 2013 Sep 15.
- 512 FAOSTAT (2010). FAO Production Year Book. Food and Agricultural Organization, Rome.
- 513 FDALR (1995). Federal Department of Agriculture and Land Resources. The Reconnaissance Soil Survey of
514 Cross River State, Nigeria. FDALR, Abuja, 43p.
- 515 Gee, G. W. and Or, D. (2002). Particle Size Analysis. In: Methods of Soil Analysis. *Soil Sci. Soc. Of Am. Book*
516 *Series No. 5 ASA and SSSA Madison, Wis. Pp.* 225-293.

- 517 GenStat (2004). GenStat for Windows. Release 7.2. 7th Edition, VSN International Ltd, Oxford.
- 518 Grava, I. (1980). Importance of soil extraction techniques. p.9-11. In: W. C. Dahuke et al. (ed). Recommended
- 519 soil test procedures for the North Central Region (revised). North Central Regional Publication. 221.
- 520 ND. Agric. Exp. Stn. Bull. 499 (revised).
- 521 Gronflaten, L. K. and Steines, E. (2005). Comparison of four different extraction methods to assess plant
- 522 availability of some metals in organic forest soil. Comms in soil Sci and plant anal. Vol. 36 (19 & 29):
- 523 2699-2718.
- 524 Haddad, K. S. and Evans, J. C. (1993). Assessment of chemical methods for extracting zinc, manganese,
- 525 copper and iron from New South Wales Soils. *Communication in Soil Sci. Pl. Anal*, 24: 29-44.
- 526 Havlin, J. L., Beaton, J. D., Tisdale, S. L., Nelson, W.L. Soil fertility and fertility and Fertilizers: An
- 527 Introduction to Nutrient Management 7th rd. Asoke K-Ghosh, Prentice Hall Publishers. New Delhi,
- 528 India. 2005. <http://dx.doi.org/10.1097/00010694-198507000-00003>
- 529 Ibanga, I. J. and Udo, E.J. (1996) Soil Survey and Fertility Baseline Data collection of Akwa Ikot Effangha
- 530 Farm, Akpabuyo LGA, Cross River State Nigeria. National Agricultural Land Development Authority,
- 531 Abuja.
- 532 Ibrahim, A. K., Usman, A., Abubakar, B. and Aminu, U. U. (2011). Extractable micronutrients status in
- 533 relation to other soil properties in Billiri LGA. *Journal of Soil Science and Environmental*
- 534 *Management. Vol. 3 (10):* 282-285
- 535 Ivezić, V., Lončarić, Z., Engler, Meri, D., Kerovec, D. and Singh, B. R., (2013) Comparison of different
- 536 extraction methods representing available and total concentrations of Cd, Cu, Fe, Mn and Zn in soil.
- 537 *Poljoprivreda* 15(2):53-58.
- 538 Kparmwang, T., Chude, V. O. and Esu, I. E. (1995). Hydrochloric acid (0.1N HCl) and DTPA extractable and
- 539 total iron and manganese in basaltic soil profiles of the Nigeria savanna. *Comm. Soil Sci. Plant Anal.*
- 540 26:2235-2245.
- 541 Kparmwang, T., V.O. Chude, B.A. Raji and A.C. Odunze (2000). Extractable micronutrients in some soils
- 542 developed on sandstone and shale in Benue Valley, Nigeria. *Nigerian Journal of Soil Science*
- 543 *Research* 1:42-48.
- 544 Kuo, S. (1996) Phosphorus. In: Sparks, D.L., Ed., *Methods of Soil Analysis Part 3: Chemical Methods*, SSSA
- 545 Book Series 5, *Soil Science Society of America, Madison, Wisconsin*, 869-920.
- 546 Landon, J. R. (1991). Tropical Soil Manual. A Handbook for Soil Survey and Agricultural Land Evaluation in
- 547 the Tropics and sub Tropics. Longman Scientific & Technical Publ., Harlon.
- 548 Lindsay, W. I. and A. P. Schwab (1982). The chemistry of iron in soils and its availability to Plants. *J. Plant*
- 549 *Nutr.*, 5: 821-840.
- 550 Lindsay, W. I. and Norvel, W. A. (1978). Reactions of EDTA complex of Fe, Mn, Zn and Cu with soils. *Soil*
- 551 *Science Society of America Proceedings*, 33: 86-91

- 552 Lindsay, W. L. (1995). Chemical reactions in soils that affect iron availability to plants. A quantitative
553 approach. *Iron Nutrition in Soils and Plants*, Kluwer academic Publishers. 7-14pp
- 554 Lisuma, J. B., Semoka, J. M. R. and Semu, E. (2006). Maize yield response and nutrient uptake after
555 micronutrient application on a Volcanic Soil. *Agronomy Journal. Madison, W. I.*, 98: 402-406.
- 556 Marschner (2011). Mineral nutrition of higher plants, 3rd edn. Academic, London. Elsevier science Ltd.
557 Academic Press. New York. 123p.
- 558 McLean, E.O. (1982). Soil pH and lime requirement. In A.L. Page, R.H. Miller & D.R. Keeney (Eds),
559 Methods of soil analysis. Part 2. Chemical and microbiological properties. 2nd ed. (pp 199–224).
560 Madison, Wisconsin, USA.
- 561 McBride, M. B., Richards, B. K. and Steenhuis, T. (2004). Bioavailability and crop uptake of trace elements in
562 soil columns amended with sewage sludge products. *Plant and soil*, 262:71-84.
- 563 Melsted, S. w., H. L. Motto, and T. R. Peck (1969). Critical plant nutrition composition values useful in
564 interpreting plant analysis data. *Agronomy Journal*. 61: 17-20.
- 565 Menzies, N. W., Donn, M. J. and Kopittke (2007). Evaluations of Extractants for estimation of the
566 phytoavailable trace metals in soils. *Environ. Pollut.* 145: 121-130.
- 567 Mortved, T. J. (2010). Micronutrients in crop production, Overview of micronutrients and their sources.
568 Colorado State Univ., Available at: [http://www.back-to-](http://www.back-to-basics.net/archives/articles/pdf/micronutrients.pdf)
569 [basics.net/archives/articles/pdf/micronutrients.pdf](http://www.back-to-basics.net/archives/articles/pdf/micronutrients.pdf)
- 570 Mustapha, S. and Loks, N. A. (2005). Distribution of available Zn, Cu, Fe and Mn in Fadama soils from two
571 District Agro-ecological zones in Bauchi State, Nigeria. *J. Environ. Sci.* 9 (2): 22-28
- 572 Nelson, D.W. and Sommers, L.E. (1996) Total Carbon, Organic Carbon and Organic Matter. In: Sparks, D.L.,
573 Ed., *Methods of Soil Analysis Part 3: Chemical Methods*, SSSA Book Series 5, Madison, Wisconsin,
574 *Soil Science Society of America*, 961-1010.
- 575 Nuss E.T. and Tanumihardjo, S.A. (2010). Maize: a paramount staple crop in the context of global nutrition.
576 *Compr Rev Food Sci F* 9: 417–436
- 577 Odurukwe, S. O., F. A. Anuebunwa, A. W. Iloka, A.Udeabor and M. A. Ibedu M (1995). Physical
578 environmental of Southeastern Nigeria. I: Indigenous fallow and multipurpose tree and shrub species
579 in the farming system of southeastern zone of Nigeria. A report of diagnostic survey. NRCRI, Umdike
580 Publications.
- 581 Ogban, P.I., Akwue, I.P. and Mauako, O. (1999). Properties and Management of Catenary Soils on Coastal
582 Plain Sands in Southeastern Nigeria. In: Babalolu, O., Omoti, U. and Isenmila, A.E., Eds.,
583 *Management of the Soil Resources of Nigeria for Sustainable Agricultural Production in the 21st*
584 *Century. Proceedings of the 25th Annual Conference of the Soil Science Society of Nigeria*, Benin, 45-
585 52.
- 586 Ojanuga, A.G. (2006). Agroecological Zones of Nigeria Manual. In: Berding, F. and Chude, V.O., Eds.,
587 *National Special Programme for Food Security (NSPFS) and FAO*, 124.

- 588 Olsen, S. R. (1992). Micronutrients interaction. In: Micronutrients in Agriculture. Mortvedt , J. J., Giordano, P.
589 M. and Lindsay, W. L. (eds). *Soil Science Society of American, Madison, W*, 248.
- 590 Omuetti, J. A. I. and Lavkulich, L. M. (1988). Identification of clay minerals in soils: the effect of sodium-
591 pyrophosphate. *Soil Sci. Soc. Am. J.* 52: 285-287.
- 592 Osiname, O.A., Schulte, E.E. and Core, R.B. (1973). Soil Tests for Available Copper and Zinc in Soils of
593 Western Nigeria. *Journal of the Science of Food and Agriculture*, **24**, 1341-1349.
594 <http://dx.doi.org/10.1002/jsfa.2740241105>
- 595 Porter, G.S., Bajita-Locke, J.B., Hue, N.V., Strand, D. (2004). Manganese solubility and phytotoxicity affected
596 by soil moisture, oxygen levels, and green manure additions. *Comm. Soil Sci. Plant Anal.* 35, 99-116.
597 *Press. New York.* 48-50 pages.
- 598 Rastija, M., Rastija, D., Simic, D. and Mihaljevic, I. (2009). Yield and concentration of maize Mn and Zn in
599 grain of maize parameter inbred and hybrids. *Poljoprivreda* 15(2):20-25
- 600 Rengel, Z. (2002). Role of pH in availability of ions in soil. In: Z. Rengel (ed.) Handbook of Plant Growth. pH
601 as a Master Variable in Plant Growth. Marcel Dekker, New York, pp: 323-350.
- 602 Schnug E, Fleckenstein J, Haneklaus, S. (2001). Coca-Cola extractant for the Evaluation of available
603 micronutrient concentrations in soils for oil seed rape. Institute of Plant Nutrition and Soil Science,
604 Fed Agric. Research Centre, Germany; 2001.
- 605 Schnug, E., Fleckenstein, J and Haneklaus, S. (1996). Coca-Cola is it! The ubiquitous extractant for
606 micronutrients in soils. *Commun. Soil Sci. Plant Ana.* 27: 1721-1730.
- 607 Schnug, E., Fleckenstein, J and Haneklaus, S. (1998). Factors affecting the determination of available
608 micronutrient concentrations in soils using Coca-cola as extratant. *Commun. Soil Sci. Plant Ana.* 29
609 (11-14): 1891-1896.
- 610 Sharma, P. C. Sharma, P. N. Chatterjec, C. and Agarwald, S. C. (1991). Manganese deficiency in maize effect
611 on Pollen Viability. *Plant and Soil.* 138: 139-147.
- 612 Shiferaw B, Prasanna B, Hellin J, Bänziger M (2011). Crops that feed the world 6. Past successes and future
613 challenges to the role played by maize in global food security. *Food Sec* 3: 307–327.
- 614 Shuman, L. M. (1985). Fractionation method for soil micronutrients. *Soil Sci. GEOBASE* 140:11-22.
- 615 Shuman, L. M. (1988). Effects of organic matter on the distribution of manganese, copper, iron and zinc in soil
616 fractions. *Soil Sci.* 146:192-198.
- 617 Shuman, L. M. (1991). Chemical forms of micronutrients in soils. In: Mortvedt, J. J., Cox, F. R., Shuman, L.
618 M., Welch, R. M. (Ed.), Micronutrients in Agriculture, 2nd ed, *Soil Sci. Soc. Am. Madison, WI*, pp.
619 113-140.
- 620 Sillanpaa, M. (1982). Micronutrients and the Nutrient Status of Soils: A Global Study. FAO Soil Bulletin No.
621 48, FAO/Finnish International Development agency, Rome.

- 622 Šimić, D., Zdunić, Z., Jambrović, A., Ledenčan, T., Brkić, I., Duvnjak, V., Kovačević, V. (2009): Relations
623 among six micronutrients in grain determined in a maize population. *Poljoprivreda* 15(2):15-19.
- 624 Sumner, M.E. and Miller, W.P. (1996). Cation Exchange Capacity and Exchange Coefficients. In: Sparks,
625 D.L., Ed., *Methods of Soil Analysis Part 3: Chemical Methods*, SSSA Book Series 5, Soil Science
626 Society of America, Madison, Wisconsin, 1201-1230.
- 627 Tandon, H.L.S. (1995). Micronutrients in Soils, Crops and Fertilizer: A Source Book-Cum-Directory.
628 Fertilizer Development and Consultation Organ, New Delhi.
- 629 Thomas, G.W. (1996). Soil pH and Soil Acidity. In: Sparks, D.L., Ed., *Methods of Soil Analysis Part 3:*
630 *Chemical Methods*, SSSA Book Series 5, Soil Science Society of America, Madison, Wisconsin, 475-
631 490.
- 632 USDA (2006). Keys to Soil Taxonomy. 10th Edition, United States Department of Agriculture, NRCS Soil
633 Conservation Service, Washington DC.
- 634 Welch, R. M. (1995). "Micronutrient nutrition of plants" Critical Reviews in Plant Science. 14 (1): 49-82.
- 635 Whitney, D.A. (1988) Micronutrient Soil Test Zinc, Iron, Manganese and Copper. In: Dahuke, W.C., Ed.,
636 *Recommended Chemical Soil Test Procedures for the North Central Region*, North Dakota Agric. Exp.
637 Stn. Bull. 499 Review, 20-22.
- 638 Yaro, D. T, Kparmwang, T, Aliyu, S. M., Wuddivira, H. N, Tarfa, B. D. (2006). Status of DPTA and HCl
639 extractible cationic micronutrient in soils of the long term DNPk plot at Samaru Zaria. *Nigerian*
640 *Journal of Soil research*,; 3: 27-32.