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Evaluation of Soil Test Methods for Assessing Available Manganese in Tea Soils

D M B N Dissanayake[1], G P Gunaratne[1], A K N Zoysa[1], L S K Hettiarachchi[1] and H M D N Priyantha[2]

([1]Soils and Plant Nutrition Division, Tea Research Institute of Sri Lanka, Talawakelle, Sri Lanka

[2]Department of Chemistry, Faculty of Science, University of Peradeniya, Peradeniya, Sri Lanka)

ABSTRACT

The Tea Research Institute (TRI) has identified five important micronutrients required for healthy growth of tea. Those are iron, copper, manganese, zinc and boron in decreasing order of abundance. Manganese, zinc and boron deficiency symptoms are currently being evident.

This research was conducted mainly to find out a suitable chemical solution capable of extracting most of the plant available manganese forms from tea growing acid soils.

The research was conducted as a green-house study with six different soil series namely, Mattakelle, Maskelliya, Kandy, Mahawalatenne, Malaboda and Dodangoda. Six top soils (0 - 6 cm depth) were sieved through a 6.4 riddle and 4 kg moist soil of each was transferred into plastic pots of 5 kg capacity. Manganese treatments were incorporated to the soils as 0, 10, 20 and 30 mg kg^{-1} Mn as $MnSO_4$. Perennial Tall-fescue grass (*Festuca arundinacea*) seeds were sown at the rate of 1 g per each pot.

Plant available fraction of manganese was extracted by chemical protocols namely, H_2O, 0.5M HCl + 0.05M $AlCl_3$, 0.005M DTPA + 0.1M ETA (pH = 7.0), 0.005M DTPA + 0.1M ETA (pH = 4.7), 0.002M DTPA + 0.1M ETA (pH = 4.5), 1M NH_4OAC + 0.002M EDTA, 1.5M NH_2OH . HCl + 0.1M HNO_3, 0.1M Na_2 EDTA, (pH = 6.0), 0.43M (2.5%) HOAC (pH 2.5). Plant Mn uptake was assessed in grass cuttings.

According to the regression coefficients between plant Mn uptake and extractable Mn by different solutions, 0.005M DTPA + 0.1M ETA (pH = 4.7) method appeared to be the best extractant to represent plant available Mn fraction out of three comparable extractants namely 0.005M DTPA + 0.1M ETA (pH = 4.7), 0.5M HCl + 0.05M $AlCl_3$ and 1.5M $NH_2OH.HCl$ + 0.1M HNO_3.

Key words: Available manganese, correlation, plant uptake, soil test methods

INTRODUCTION

Tea (*Camellia sinensis* L.) is a perennial crop grown in low, mid and upper elevations in Sri Lanka. As the primary base of growing medium from the nursery to the field, soil plays a vital role at all stages of the growth of the plant. Therefore, many studies were conducted during the past decades to evaluate the behavior of major nutrients in the tea growing soils in the country. Owing to the regular extensive harvesting pattern, tea plants require major nutrients such as N, P, K to be added at 3 – 4 month intervals throughout the year.

Tea plants respond significantly to added nitrogen fertilizers mainly in form of urea and sulphate of ammonia, but response to potassium and phosphorus is modest and low respectively. Consequently, as the harvest represents the vegetative part, a large amount of major and micro nutrients are removed from the soil with each harvest. Therefore, a huge demand for nutrients is created in the plant to offer harvestable leaf at 5 to 7 day intervals. In addition to the major nutrients, many micro nutrients (Zn, Mn, Cu, B and Fe) are also required for better growth and as well as to produce a harvestable crop. Unlike N, P and K micro nutrients are not added to soil regularly. Long term negligence of micronutrients application and inherent low levels of micronutrients in soil, can lead to visible deficiencies in tea fields.

Sri Lankan tea growing soils have been exposed to long term exploitation and as a consequence tea lands contain hardly any nutrient rich top soil. Under such conditions it is very difficult for the soil to accomplish the requirement of even micronutrients. At present, tea fields demand a versatile fertilizer recommendation enriched with micronutrients unlike the ordinary NPK major fertilizer mixtures (This research was carried out with Mn) in order to find out the micronutrient status in tea growing areas of the country and to acquire sufficient knowledge on its uptake .

The estimation of Mn was mainly done by recognizing the principal components in the soil (Campbell *et al.*, 1988). The first component is a water soluble fraction, which is dissolved in pure water. The extractant is therefore pure water and among different procedures, it varies only with respect to soil solution ratio, shaking method and time. Second component is the exchangeable fraction. Unlike the major cations, exchangeable metals are held by electrostatic forces on colloidal surfaces. Third component of manganese is oxide fraction. To solubilize the Mn oxides, a reducing agent that will reduce Mn, is required. Dion *et al.* (1947) suggested hydroxylamine for extracting Mn. The last component of soil Mn is organic fraction. The procedures for extraction of organic fraction are difficult to choose because they often dissolve other fractions as well. The usual approach is the oxidation of materials to release the metals. Some procedures use chelating agents to extract the metals chelated by the soil humic material, such as those used by of Mathur and Levesque (1988) and Goldberg and Smith (1984).

This study was conducted as the method for estimating available Mn in soils of tea growing regions in Sri Lanka has not been validated by TRI.

MATERIALS AND METHODS

Experiment methodology

This experiment was carried out in the green-house using A_p horizons of six soils, sampled from six tea estates (Ury, St. Coombs, Mahavilla, Shannon, Kottawa and Balangoda located at Passara, Talawakelle, Kandy, Watawala, Galle and Balangoda) in different agro-ecological regions namely IU3c, WU2b, WM2a, WU1, WL2a and IM2a respectively. The above locations represent six different soil series namely Mattakelle, Maskelliya, Kandy, Mahawalatenne, Malaboda and Dodangoda respectively. From these soil series, only the topsoil (0 - 15 cm) was collected for the green-house experiments.

The six top soils were sieved through a 6.4 riddle and 4 kg of moist soil of each were transferred into plastic pots of 5 kg capacity. The moisture content of each soil was determined at the time of potting, to facilitate direct comparison between soil analysis and plant Mn uptake and/ or yield to interpret on a common basis. Moisture contents of different soil series Mahawalatenne, Mattakelle, Kandy, Maskelliya, Dodangoda and Malaboda were 15%, 27%, 11%, 22%, 13%, 18% respectively.

The experimental layout was RCBD factorial design with four levels of manganese, 0, 10, 20 and 30 mg kg^{-1} in the form of manganese sulfate incorporated to the soils at the time of filling. Meanwhile, at the time of potting, only 0.33 g N and 0.36 g P as $(NH_4)_2HPO_4$, 0.16 g K as K_2SO_4 and 12 mg Mg as $MgSO_4.7H_2O$ were added to each pot as the basal fertilizer mixture. During the period of four months 22 mg N and 12 mg Mg were added as a mixed solution of NH_4NO_3, $Ca(NO)_3$ and $MgSO_4.7H_2O$ to balance the N uptake and maintain constant soil pH. Throughout experiment, water was added to the soil to equalize the moisture content, based on the percentage moisture of in each pot.

A total of 72 pots were used in combination of 6 soils x 4 levels x 3 replicates. Perennial Tall-fescue grass (*Festuca arundinacea*) seeds were sown at the rate of 1 g per each pot at half inch depth from the soil surface. Meanwhile, six fallow pots were kept by treating all the basal and other necessary fertilization except the treatments.

Sampling procedure

The soils collected from each soil series were air dried and sieved using 2 mm and 0.5 mm aperture to obtain homogeneous sample for determination of chemical parameters for preliminary analysis and all the determinations were done in three replicates. During the final dismantling, all the soils in pots were air dried and representative samples were obtained after passing through a 2 mm sieve.

Sampling of leaves of grass was done in every three weeks. All the foliage of the grass was taken off by cutting at the height of 2.5 cm from the surface of the soil. After cutting, all vegetative parts were weighed using an analytical balance and samples were dried in an electric oven and kept for 24 hours at 80 °C. Then the dry weights of the samples were taken and were ground using a grinder to obtain fine powdered samples. Meanwhile, at the final dismantling, all the above ground parts and roots were separated from the soil and subjected to the above process. From these samples, 0.2 g was taken for the determinations.

Analytical procedure

Determination of total Mn (Fiskell, 1965)

One gram of 0.05 mm sieved and air dried soil was placed in a 100 ml Teflone beaker. 5 ml of H_2O_2 was added and the solution was evaporated to near dryness at 90 °C. The process was repeated until the sample no longer effervesces on addition of H_2O_2. 2 drops of H_2SO_4 and 5 ml of HF were added and the beaker was kept on the sand bath. Bath's temperature was slowly raised to about 200 °C and evaporated to dryness. Then 10 ml of conc. HNO_3, 1 ml of conc. H_2SO_4, and 3 ml of $HClO_4$ were added and heating was continued until strong white fumes of SO_3 are produced.

Finally, the beaker was cooled and washed down the side of the beaker with 25 ml of distilled water. The content was transferred to 50 ml volumetric flask through a 542 Whatman filter paper and volume was marked with distilled water. Determination was done using Atomic Absorption Spectrophotometer AAS (GBC Avanta P model, Australia). Working standards were prepared using BDH brand (1000 mg/ l) stock solution for Mn ion. Linear working range of standards was prepared according to the manufacture's method and for all the elements the choice of flame type was air-acetylene.

Determination of available Mn in tea soils

The following nine (09) extraction protocols were selected for the study according to the literature, because they had been screened in many instances in different parts of the world for different soils to estimate plant available fraction of different micronutrients. Extraction protocols are summarized in Table 1.

Determination of total Mn in plant materials

From each ground sample 0.200 g was obtained to temperature tolerant glass ashing tubes. Then the samples were kept inside the muffle furnace overnight at 470 °C and were dissolved in a mixture of 30% H_2O_2 and HNO_3 and heated on a hotplate till the solution was evaporated to dryness without allowing charred. Thereafter, 10.00 ml of 0.05 M HNO_3 were added to each tube to dissolve the content. Finally the content was thoroughly mixed using a cyclone mixer. In order to determine the specific elemental concentration of each sample, the content was directly aspirated into the flame of AAS.

Table 1. Different extraction protocols used

Extraction Protocol	Reference
Water	Sherman *et al.* (1942)
0.5M HCl + 0.05M $AlCl_3$	Mehlich and Bowling (1975)
0.005M DTPA + 0.1M ETA pH 7.0	Lindsay and Norvell (1978)
0.005M DTPA + 0.1M ETA pH 4.7	Lindsay and Norvell (1978) modified
0.002M DTPA + 0.1M ETA pH 4.5	Lindsay and Norvell (1978) modified
1M NH_4OAC + 0.002M EDTA	Borggaard (1976)
1.5M NH_2OH . HCl + 0.1M HNO_3	Chao (1972)
0.1M Na_2 EDTA, pH 6.0	Clayton and Tiller (1979)
0.43M (2.5%) HOAC, pH 2.5	Mitchel *et al.* (1957)

Calculation of crop uptake

Each of the leaf value, analyzed for Mn, was converted to its respective mg kg^{-1} soil value. The actual weight of each soil series in a pot was ascertained after determination of the moisture fractions. Dry weight of leaf samples was determined by heating to 105 °C in an oven for five hours. The relevant uptake values were calculated for each cut using the following equations.

Total removal of Mn (µg) = Leaf dry weight (g) x Mn concentration (µg g^{-1})

Crop uptake (mg kg^{-1} soil) = Total removal of Mn (µg)/1000 x weight of soil (without moisture) kg

Interpretation of data

The data collected were statistically analyzed using the Statistical Analysis System (SAS), version 6 (Anon, 1995) and Microsoft Excel (Anon, 2000) package. The coefficient of determination (r^2) was used to select the best-fitted model. The best extraction protocol was selected by correlating available Mn for crop uptake presented as mg Mn per kg soil with extracted manganese using each extraction solution.

RESULTS AND DISCUSSION

Comparison of extractability of soil available Mn

The mean amounts of available Mn extracted by nine different extractants are given in Table 2. Amounts of Mn in these soils varied from 0.07 mg kg^{-1} (water) to 124 mg kg^{-1} (0.1M Na_2EDTA (pH = 6.0). Consequently, 0.002M DTPA + 0.1M ETA (pH = 4.7), 0.005M DTPA + 0.1M ETA (pH = 7), 1M NH_4AC + 0.002M EDTA and water have extracted comparatively lower amounts of Mn viz 1.01, 1.57, 1.55 and 0.07 mg kg^{-1} respectively. But, out of three DTPA extractants highest range was obtained by 0.005M

Table 2.Mean, ranges and standard deviations of soil available Mn as obtained with different extractants after four months of cropping

Extractants	Minimum ($mg\ kg^{-1}$)	Maximum ($mg\ kg^{-1}$)	Mean	SD
Water	0.07	12	2.01	3.64
0.5M HCl + 0.05M $AlCl_3$	6.20	89	44.00	28.00
0.005M DTPA + 0.1M ETA (pH = 7.0)	1.57	19	7.30	5.13
0.005M DTPA + 0.1M ETA (pH = 4.7)	2.00	30	10.00	8.00
0.002M DTPA + 0.1M ETA (pH = 4.7)	1.01	14	6.00	4.00
1M NH_4AC + 0.002M EDTA	1.55	20	7.30	5.00
1.5M $NH_2OH.HCl$ + 0.1M HNO_3	5.35	94	47.00	31.00
0.1M Na_2EDTA (pH=6.0)	2.73	124	33.00	26.00
0.43M HOAC (pH = 2.5)	2.55	19	8.71	5.33

DTPA + 0.1M ETA (pH = 4.7) which was 2.0 – 30.0 $mg\ kg^{-1}$ of Mn. The highest and lowest value of extractable Mn was found by EDTA extractant and water respectively. Meanwhile, Beckwith (1955) has shown that EDTA was very specific for organically bound Mn.

The total dry matter production and total Mn uptake are given in Table 3 and 4 respectively and correlations between extractable manganese by different extractants with plant Mn uptake are summarized in the Table 5. At first, all the soil series were pooled together to correlate plant uptake with Mn extracted by various extractants. Thereafter, each soil series was separately correlated with plant Mn uptake and Mn extracted by using different extracting methods. Hence, two types of correlation coefficients are given. However, Salcedo *et al.* (1979) and many researchers have adopted more reliable and convenient correlation methods by separately correlating for each series.

When all the data were pooled, the highest correlations of 0.404, 0.257 and 0.202 at 99% significant level were observed for extractants 0.5M HCl + 0.005M $AlCl_3$, 1.5M NH_2OH. HCl + 0.1M HNO_3 and water respectively. However, when the soil series were separately correlated with plant Mn uptake, higher correlation coefficients were obtained.

According to the summarized data in the Table 5, when all soil series were considered, three extractants gave good correlations. Highest was with 0.5M HCl + 0.05M $AlCl_3$ at 95% significant level. However, when soil series were correlated separately, r^2 values changed inconsistently. For Kandy soil series it was 0.938 at 95% significant level. However, this extracting solution did not satisfactorily correlate with other soil series.

Table 3. Total dry matter production at four months after manganese treatments

Mn (kg ha^{-1})	Soil Series					
	Mattakelle	Maskelliya	Kandy	Mahawalatenne	Malaboda	Dodangoda
	mg kg^{-1}					
0	7.5	7.3	1.40	6.4	2.5	7.3
10	11.0	10.6	0.72	7.7	2.6	7.4
20	11.5	4.8	1.50	6.3	4.9	11.0
30	2.6	8.1	0.56	7.5	4.5	6.2

LSD P = 0.05 (Location) = 0.59, LSD P = 0.05 (Mn level) = 0.48

Table 4. Total uptake at four months after manganese treatments

Mn (kg ha^{-1})	Soil Series					
	Mattakelle	Maskelliya	Kandy	Mahawalatenne	Malaboda	Dodangoda
	mg kg^{-1}					
0	1.3	0.38	0.18	0.8	0.36	0.53
10	1.5	0.77	0.14	0.84	0.66	0.56
20	1.37	0.67	0.64	0.7	0.73	0.77
30	0.96	0.74	0.17	0.8	0.56	0.7

LSD P = 0.05 (Location) = 0.12, LSD P = 0.05 (Mn level) = 0.097

The second best value for r^2 was obtained with 0.1M $NH_2OH.HCl$ + 0.5M HNO_3 extract at 95% significant level but was unable to correlate best when individual soil series were considered. Here again, Kandy series was best correlated with 0.984 r^2 value at more than 95% significant level but other series were poorly correlated with uptake. Third highest correlation value was obtained with the water extract of water at more than 95% significant level. Although this was significant, the range of the extracted concentration was very poor and individual coefficient values were well below the acceptable level. Owing to these reasons, above three extractants were rejected.

Meanwhile, EDTA, extractant failed to show any significant level of correlation for all soil series together and as well as for each soil series separately. However, 1N NH_4OAc + 0.002M EDTA was able to show two acceptable coefficients with 95% confidence level for Kandy and Malaboda series only. In a similar study conducted by Randall *et al.*, (1976), it was concluded that highest correlation obtained was with 0.01M EDTA in 1N NH_4OAc extractant for 37 low and 20 high organic Wisconsin soils.

Table 5.Comparison of polynomial correlation coefficients among soil series and different extractants

Extractant	Soil Series						
	Mattakelle	Maskelliya	Kandy	Mahawalatenne	Malaboda	Dodangoda	All Soils
Water	0.178	0.246**	0.194	0.190**	0.71	0.149	0.202**
Na_2EDTA	0.468	0.682	0.266	0.297	0.409	0.155	0.107
0.002M DTPA (pH = 4.7)	0.649	0.701	0.271	0.368	0.542	0.356	0.039
NH_4AC + EDTA	0.285	0.419	0.601**	0.262	0.726**	0.497	0.153
$NH_2OH.HCl$ + HNO_3	0.349	0.568**	0.984**	0.098	0.271	0.408	0.257**
0.005M DTPA (pH =7.0)	0.303	0.084	0.507**	0.071	0.145	0.619**	0.144**
0.005M DTPA (pH = 4.7)	0.539	0.371	0.541**	0.586**	0.560**	0.590**	0.490
0.5M HCl + 0.05M $AlCl_3$	0.114	0.684**	0.936**	0.075	0.528**	0.462	0.404**
0.43M (2.5%) HOAc pH 2.5	0.250	0.360	0.285	0.559	0.414	0.359	0.065

** Significant at 95% or higher

In this study, the 0.002M DTPA (pH = 4.7) and 2.5% HOAc solutions too did not give significant correlations for the individual soil series and as well as for all soil series together. The study conducted by Mathur and Levesque (1988) using cultivated organic soils (Histosols) also found 2.5% HOAc was poorly correlated with plant Mn uptake.

When the correlation coefficients given in the Table 5, for 0.005M DTPA (pH = 7) are considered, three significant (at 95%) correlation coefficients, two coefficients were apparent as above 0.500 value Kandy and Dodangoda series. In the overall soil series, coefficient was 0.144. Nevertheless, other correlation coefficients were neither significant nor acceptable. Lindsay and Norvell, (1978) conducted a study using 35 soils of the 65 series and use of chelating agents such as DTPA for simultaneous determination of micronutrients

showed that 0.005M DTPA, 0.01M $CaCl_2$, and 0.1M TEA buffered at pH 7.3 extractants were very successful to extract plant available Mn, Zn, Fe, and Cu from near neutral and calcareous soils.

Finally, the extracting solution 0.005M DTPA (pH = 4.7) which was a modification of the above extractring solution was able to show significant and acceptable correlation coefficients for five out of six soil series and four of them were significant at 95% level. But it failed to give a better correlation for the overall soil series when correlated together. Similar observations were made by Gough *et al.*, (1980) who showed that the difference between extracted concentration of Mn using DTPA and EDTA are small. However, DTPA extractable Mn was significant while EDTA extractable Mn were never significant for the soil in the northern great plains. As the Lindsay and Norvell (1978) suggested in their study, the original pH value of the DTPA extract used was changed according to the pH of the tea growing soils. Since most of the pH of soils are within the range of 4.50 - 5.50, the extract pH was changed to 4.70 using TEA (Triehanoleamine). Furthermore, during the same study Lindsay and Norvell (1978) discovered that increasing pH from 7.0 to 7.9 had little effect on extracting amount of Zn and Cu. But the amount of Mn extracted decreased dramatically with increase in pH.

The Tea Research Institute of Sri Lanka is currently using 0.002M DTPA extract to predict plant available Mn. But, during this study, this method failed to demonstrate its capabilities. Nevertheless, Lindsay and Norvell (1978) varied the concentration of DTPA from 0.5 to 20 x 10^{-5} M and found that increasing concentration of DTPA increase the extracted micronutrients in the order of Mn > Fe > Cu > Zn. Therefore, as a result from the study, 5 x 10^{-3} M had been selected since this concentration provides ample chelating capacity to remove measurable amounts of all plant available Mn.

CONCLUSION

According to the regression coefficients between plant manganese uptake and extractable manganese from different extracting solutions, the 0.005M DTPA + 0.1M ETA (pH = 4.7) method was selected as the best method to represent plant available Mn fraction out of three comparable extracting solutions viz. 0.005M DTPA + 0.1M ETA (pH = 4.7), 0.5M HCl + 0.05M $AlCl_3$ and 1.5M NH_2OH . HCl + 0.1M HNO_3.

Based on the findings it is possible to conclude that out of the extracting solutions tested, the 0.005M DTPA + 0.1M ETA (pH = 4.7) as best to represent plant available Mn fraction. Therefore, it is appropriate to replace the currently used method by 0.005M DTPA + 0.1M ETA (pH = 4.7) method.

REFERENCES

Anon 1995 SAS/ ETS user's guide version 6, 2nd edition, SAS institute, Cary, NC.

Anon 2000 Microsoft office 2000, Premium edition, Microsoft Corporation, USA.

Beckwith R S 1955 The use of $CaNa_2EDTA$ for the extraction of divalent Mn from soils. Aus. J. Agr. Res. Vol 6, 685 - 691.

Borggaard O K 1976 The use of EDTA in analysis. Acta Agric. Scand. 26, 144 - 149.

Campbell P G C, Lewis A G, Chapman P M, Crowder A A, Fletcher W K, Imber B, Luoma S N, Stokes P M and Winfrey M 1988 Biologically available metals in sediments. Nat. Res. Council of Canada, Div. Chem. Ottawa, Ontario.

Chao T T 1972 Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride. Soil Sci. Soc. Am. Proc. 36: 764 - 768.

Clayton P M and Tiller K G 1979 A chemical method for the determination of the heavy metal content of soils in environment studies. CSIRO Aust. Tech. Pap. 41, 1 - 17.

Dion H G, Mann P J G and Heintz S G 1947 The "easily reducible" manganese of soils. J. Agric. Sci. 37, 17 - 32.

Fiskell J G A 1965 Copper. *In* Black *et al.* (ed.) Methods of soil analysis, part 2. Agronomy. 9, 1078 - 1089. Am. Soc. Of Agron., Inc Madison. Wis.

Goldberg S P and Smith K A 1984 Soil manganese: E values, distribution of manganese - 54 among soil fractions, and effects of drying. Soil Sci. Soc. Am. J. 48, 559 - 564.

Gough L P, McNeal J M and Severson R C 1980 Predicting native plant Cu, Fe, Mn and Zn levels using DTPA and EDTA soil extractants, Nothern great plains. Soil Sci. Soc. Am. J. 44: 1030 - 1036.

Lindsay W L and Norvell W A 1978 Development of a DTPA soil test for Zinc, Iron, Manganese and Copper. Soil Sci. Soc. Am. J. 42, 421 - 428.

Mathur S P and Levesque M 1988 Soil test for copper, iron, manganese, and zinc in histosols: 2. the distribution of soil iron and manganese in sequentially extracted forms. Soil Sci. 145: 102 - 110.

Mehlich A and Bowling S S 1975 Advance in soil test methods for copper by atomic absorption spectrophotometry. Commun. Soil. Sci. Plant Anal. 6, 113 - 128.

Mitchel R L, Reith J W and Johnston I M 1957 Trace element uptake in relation to soil content. J. Sci. Food Agric. 8, 51 - 59.

Randall G W, Shulte E E and Corey R B 1976 Correlation of plant manganese with extractable soil manganese and soil factors. Soil Sci. Am. J. 40: 282 - 287.

Salcedo H I, Ellis B G and Lucas R E 1979 Extractable manganese and plant uptake. Soil Sci. Am. J. 43: 138 - 141.

Sherman G D, McHargue J S and Hodgkins W S 1942 Determination of active Mn in soil. Soil Sci. 54, 253 - 257.