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DETERMINATION OF CALCIUM, MAGNESIUM, ZINC AND MANGANESE IN PLANT TISSUE USING A DILUTE HCL EXTRACTION METHOD[1]

KEY WORDS: Plant analysis

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ABSTRACT

A non digestion method described earlier for K determination was tested for its ability to extract several other elements in plant tissues of sorghum, pearl millet, chickpea, and pigeonpea. The method involves shaking 0.5 g finely ground (<0.4 mm) plant sample with 40 ml of 0.5 N HCl for 5 minutes at room temperature (25° C) followed by measurement of element concentrations in the filtrate. The values of Ca, Mg, Zn, and Mn obtained by this method were in good agreement with those obtained using the triacid digestion technique. However, this was not the case for P, Fe and Cu. The precision obtained in

determining Ca, Mg, Zn and Mn by the HCl extraction method was generally comparable to that obtained by the triacid digestion method. These results suggest that the HCl extraction method can be used for routine and rapid analysis of plant tissues for Ca, Mg, Zn and Mn contents as well as for K content.

INTRODUCTION

In earlier communications[6,7] I described a non-digestion method for determining K in plant materials. The method was evaluated for estimating K in diverse plant tissue and seed materials of maize, groundnut, sorghum, pearl millet, chickpea and pigeonpea with a range in K content. The method gave K values that were in close agreement with those obtained by the triacid digestion technique. This method is simple and rapid and involves extraction of the finely ground plant materials with dilute HCl for a short time followed by measurement of K in the extract by atomic absorption spectrophotometry.

Since publication of the HCl extraction method for determining K in plant samples[6], a number of modified versions of this technique have been evaluated for estimating several cations in diverse plant materials. For example, Hunt[3] found that treatment of the finely ground plant materials with 0.5 M HCl at 30°C for 15

minutes with occasional swirling was sufficient to recover K, Ca, and Mg. The values of K, Ca and Mg obtained by this method were highly correlated with those obtained by a wet digestion method.

Hamze et al.[2] evaluated six digestion procedures to determine Ca, K, Mg and Na in citrus leaves and found that the triacid digestion was the best. However, extraction of the samples with dilute HCl or hot water digestion were found to be satisfactory for determination of K and Na. Their dilute HCl extraction method involved soaking of the ground plant materials in 0.1 N HCl for 24 hours.

In a recent study, Miyazawa et al.[5] found that a modified version of the HCl extraction method was satisfactory for determining Ca, Mg, K, Mn, Cu, and Zn in coffee, soybean, corn, sunflower and grass leaf tissue. Their method involved digesting the finely ground plant samples (500 mg) with 25 ml of 1 N HCl at 80 ^{o}C in a water bath for 15 minutes. The HCl mixture was cooled and shaken for 50 minutes on a horizontal shaker, followed by determination of the element in the extract by an atomic adsorption spectrophotometer. This method was not suitable for measuring P and Fe in the plant samples.

The HCl extraction method proposed by Sahrawat[6] is more rapid and simpler than the modifications of this method discussed above. Sahrawat[6,7] found that extraction of the finely ground plant tissue or seed materials with 0.5 N HCl for a short time (5 minutes) was sufficient for K recovery.

This brief review indicates that K and Na recovery with dilute cold HCl or hot water is good because these elements are present mainly in soluble forms within the plant. However, elements such as Ca and Mg are likely associated with the dry matter of the plant.

The objective of this work was to test the HCl extraction method[6] for determining several mineral nutrient elements other than K in plant tissue of different crops.

MATERIALS AND METHODS

Plant samples (leaf and stem) of sorghum (*Sorghum bicolor* (L.) Moench), pearl millet (*Pennisetum americanum* (L.) Leeke), chickpea (*Cicer arietinum* L.) and pigeonpea (*Cajanus cajan* (L.) Millsp.) were selected from the large number of samples received in our analytical laboratory for various analyses, to provide a range of plant material with different element concentrations for testing. The whole plant

samples were finely ground (<0.4 mm) using a Wiley mill. The ground samples were dried in the oven at 60 °C for 24 hours prior to analysis.

The method used was the same as described earlier[6,7]. Briefly, 0.5 g of the finely ground plant material was shaken with 40 ml of 0.5 N HCl for 5 minutes in a reciprocating shaker. The suspension was filtered through Whatman No. 1 filter paper and Ca, Mg, Fe, Zn, Mn and Cu in the extract were determined using an atomic absorption spectrophotometer (Varian Techtron, Model 1200). Phosphorus in the extract was determined using the vanadomolybdate method[4]. The interfering color was removed by shaking the extract with activated charcoal.

The values of mineral nutrient elements obtained using this technique were compared with those determined by the triacid digestion method[4], as described earlier[6]. Unless otherwise stated all the data reported were from at least duplicate determinations.

The functional relationships between the HCl extraction method and the triacid digestion method were worked out[8].

RESULTS AND DISCUSSION

While the method was suitable for estimating Ca, Mg, Zn and Mn content in diverse plant tissue, it was unsatisfactory for measuring P, Fe and Cu. Concentrations of Ca, Mg, Zn and Mn in various plant tissues as determined by the HCl extraction method were generally in close agreement with those measured with triacid digestion (Table 1; Figs. 1-4).

However, there were some discrepancies between the two methods in the values measured; for example, for Ca and Mg in sorghum (Table 1). The samples tested had a wide range of concentrations of the elements measured, particularly of Ca and Mg, thus demonstrating the suitability of the technique for diverse tissues of varying element concentrations. The results are in agreement with those reported by Hunt[3] and Miyazawa et al.[5] using modified HCl extraction techniques.

The values of Ca, Mg, Zn and Mn obtained by the HCl extraction method were highly correlated with those obtained by the triacid digestion method in that most of the values except for Ca and Mg in the lower range were within the 99% confidence bounds as determined by functional relationships between the two methods (Figs. 1-4). The values of R^2 for the pooled analysis of all crops for different elements ranged from 95.2 to 99.2%.

TABLE 1

Comparison of the HCl extraction and triacid digestion methods for determination of Ca, Mg, Zn and Mn in plant samples of different crops.

Crops	No. of samples	Triacid digestion		HCl extraction	
		Range	Mean	Range	Mean
		Ca, %			
Sorghum	15	0.43-0.85	0.56	0.36-0.70	0.45
Pearl millet	10	0.24-0.59	0.34	0.30-0.67	0.40
Chickpea	15	2.22-3.01	2.62	2.29-3.19	2.71
Pigeonpea	10	0.25-1.76	0.78	0.30-1.71	0.83
All crops	50	0.24-3.01	1.18	0.30-3.19	1.20
		Mg, %			
Sorghum	15	0.18-0.39	0.25	0.13-0.29	0.18
Pearl millet	10	0.21-0.49	0.31	0.21-0.55	0.32
Chickpea	15	0.41-0.63	0.50	0.39-0.62	0.48
Pigeonpea	20	0.09-0.42	0.20	0.08-0.39	0.19
All crops	60	0.09-0.63	0.30	0.08-0.62	0.28
		Zn, mg kg^{-1}			
Sorghum	23	19-41	30	16-38	28
Pearl millet	10	32-68	44	32-67	43
Chickpea	20	33-92	56	35-98	59
Pigeonpea	20	9-33	19	8-35	19
All crops	73	9-92	36	8-98	36
		Mn, mg kg^{-1}			
Sorghum	23	47-193	115	50-216	123
Pearl millet	10	26-63	49	25-62	47
Chickpea	20	35-184	87	39-158	83
Pigeonpea	20	11-158	38	10-150	36
All crops	73	11-193	77	10-216	77

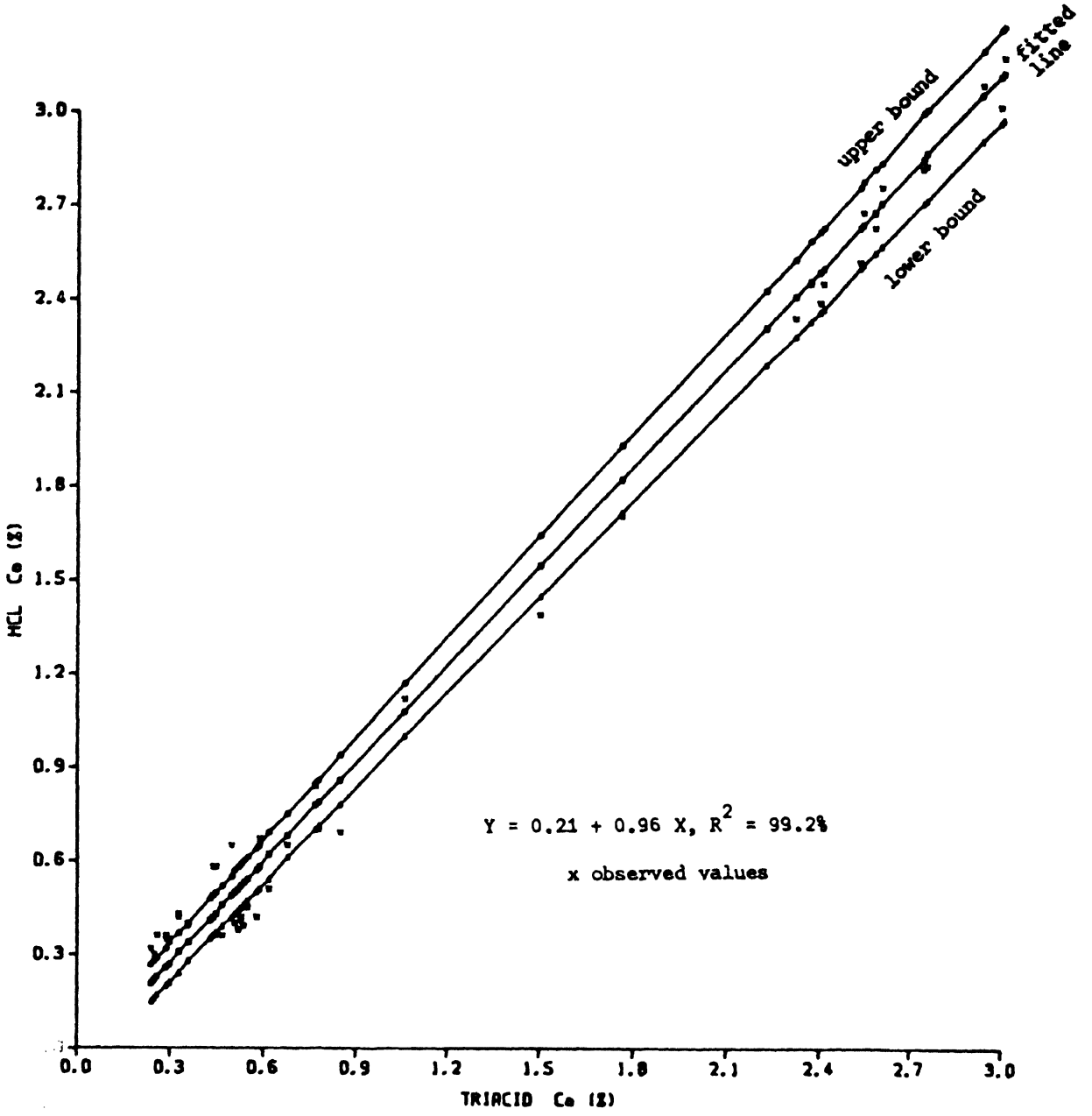

Fig. 1. Functional relationship between HCl extraction method and triacid digestion method for determination of Ca, and its 99% confidence bounds.

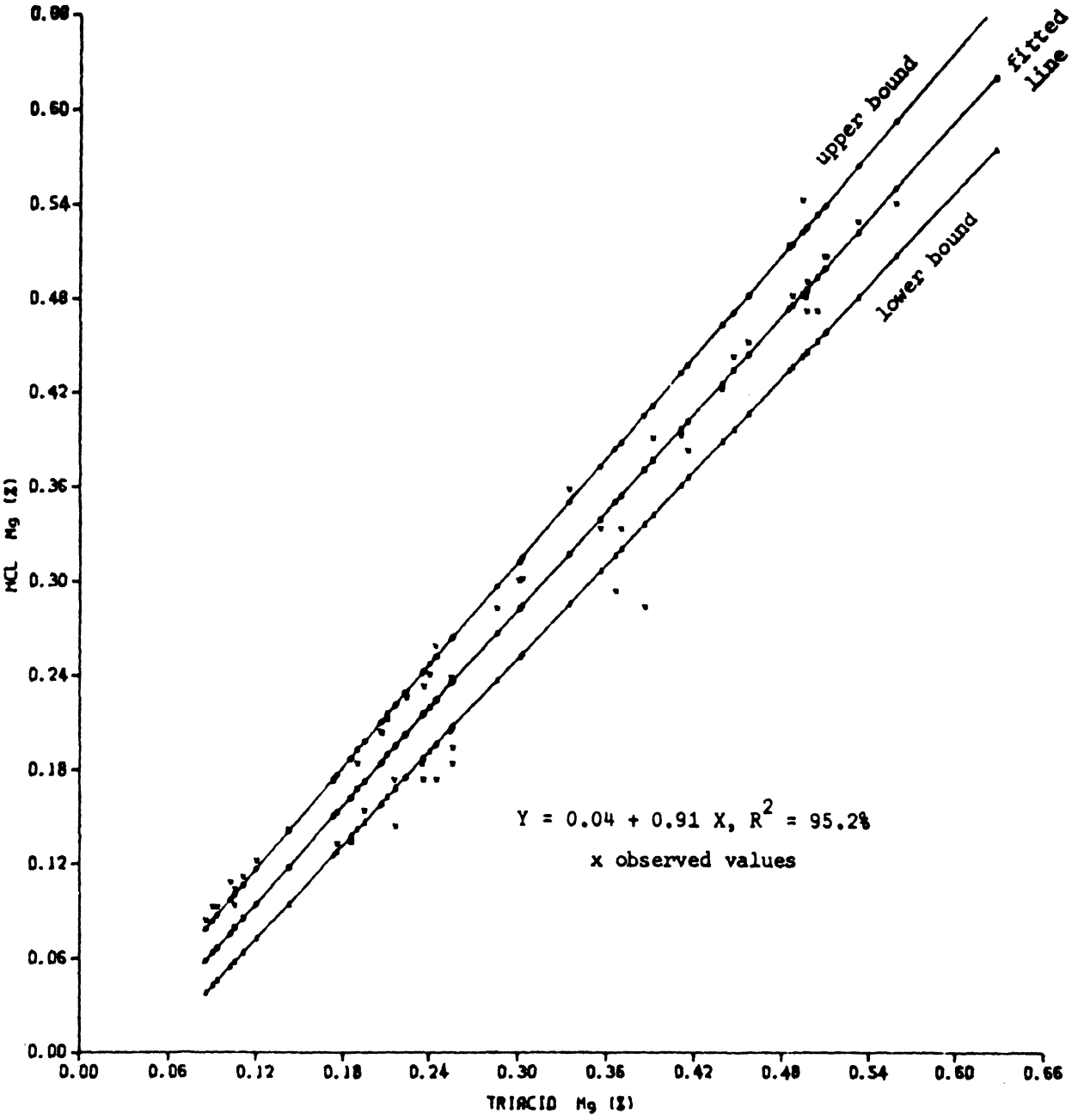

Fig.2. Functional relationship between HCl extraction method and triacid digestion method for determination of Mg, and its 99% confidence bounds.

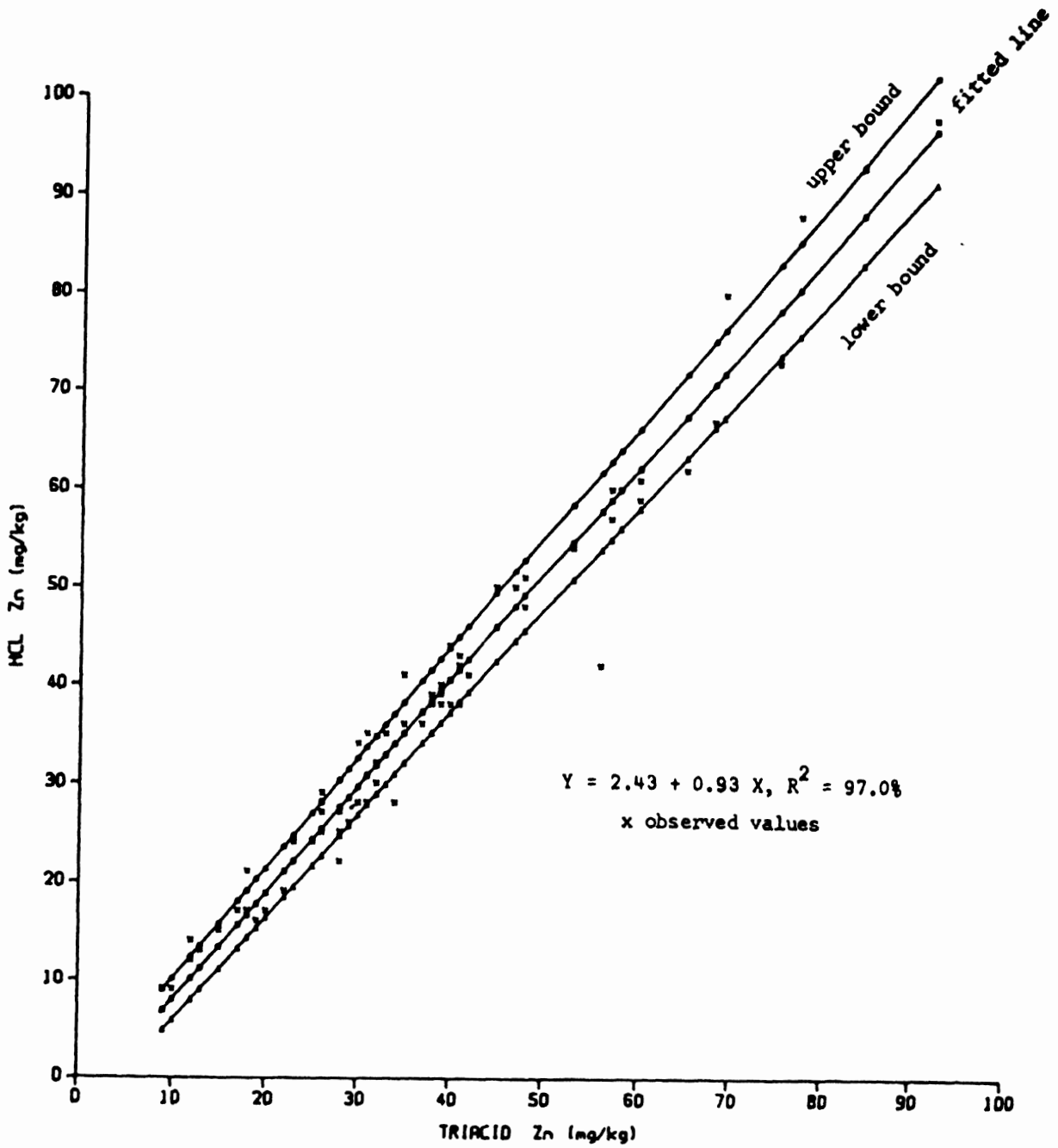

Fig. 3. Functional relationship between HCl extraction method and triacid digestion method for determination of Zn, and its 99% confidence bounds.

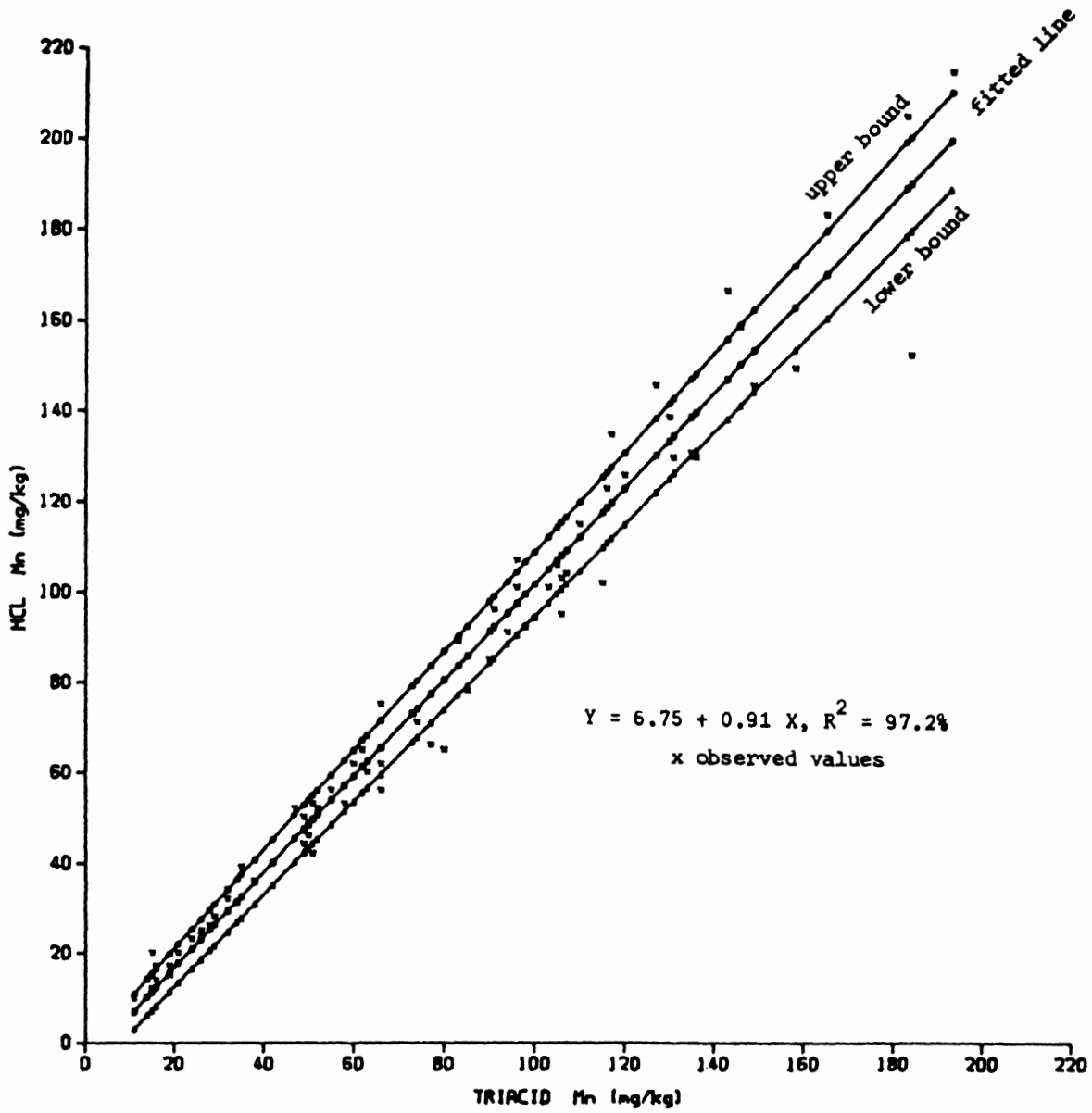

Fig. 4. Functional relationship between HCl extraction method and triacid digestion method for determination of Mn, and its 99% confidence bounds.

The precision and accuracy of the proposed method is further borne out from the data on multiple independent extractions and analyses of the same sample (Table 2). The data show that the HCl extraction method generally provided precision comparable to that obtained by the triacid digestion method except in case of Mn in pearl millet and pigeonpea where the standard error (SE) for the proposed method was higher than that obtained by the triacid digestion method.

When a new method is compared with the standard method, it is desirable that the relationship responses by the two methods, both contributing to their own errors should be obtained as functional relations in addition to usual regression which provides biased estimates of slope and intercept of the linear functional relation between measurement of the two methods [8].

The coefficient of slope and intercept for Ca, Mg, Zn and Mn for all crops combined were therefore estimated using the approach described in Kendall and Stuart [8]. Table 3 shows the coefficients of functional linear relationships between the two methods alongwith their 95% confidence intervals. It is clear that these intervals for intercept enclose zero and those for

TABLE 2

Precision of the HCl extraction and triacid digestion methods fo determination of Ca, Mg, Zn and Mn in plant tissue, based on te independent extractions and analyses of each sample.

Crop	Triacid digestion			HCl extraction		
	Range	Mean	SE(±)	Range	Mean	SE(±)
			Ca, %			
Sorghum	0.33–0.36	0.343	0.001	0.32–0.34	0.330	0.001
Pearl millet	0.29–0.36	0.305	0.002	0.31–0.32	0.314	0.001
Chickpea	2.43–2.67	2.577	0.008	2.21–2.80	2.492	0.023
Pigeonpea	0.33–0.48	0.421	0.004	0.49–0.53	0.512	0.001
			Mg, %			
Sorghum	0.19–0.20	0.196	0.001	0.20–0.20	0.200	0.000
Pearl millet	0.23–0.25	0.237	0.001	0.22–0.23	0.227	0.000
Chickpea	0.49–0.51	0.501	0.001	0.47–0.49	0.478	0.001
Pigeonpea	0.15–0.16	0.159	0.000	0.16–0.16	0.160	0.000
			$Zn, mg\ kg^{-1}$			
Sorghum	52–55	53.0	0.094	43–45	43.9	0.088
Pearl millet	34–36	35.1	0.074	38–40	38.6	0.097
Chickpea	53–55	53.8	0.092	45–47	46.4	0.070
Pigeonpea	16–17	16.5	0.053	18–19	18.4	0.052
			$Mn, mg\ kg^{-1}$			
Sorghum	39–41	39.6	0.070	38–42	39.6	0.117
Pearl millet	52–54	53.5	0.085	51–57	54.3	0.226
Chickpea	120–125	121.5	0.158	132–134	133.1	0.074
Pigeonpea	11–11	11.0	0.000	11–13	11.3	0.067

TABLE 3

Coefficients of functional linear relationships between HCl extraction method and triacid digestion method for determination of Ca, Mg, Zn and Mn in plant tissue.

Element	Intercept	Slope
Ca	0.21 (-0.144, 0.186)	0.96 (0.85, 1.08)
Mg	0.044 (-0.081, 0.170)	0.91 (0.55, 1.47)
Zn	2.432 (-4.713, 9.578)	0.93 (0.76, 1.13)
Mn	6.752 (-6.309, 19.813)	0.91 (0.75, 1.09)

[a] Values are for all crops combined.
Values within parentheses are the 95% confidence limits

slopes enclose unity in all cases, indicating that the two methods are providing the same measurement except for random fluctuations.

It thus appears that the HCl extraction can be used as a simple and precise method for determining Ca, Mg, Zn and Mn, as well as K, in plant samples. This technique allows greater speed of analysis and thus permits processing of larger number of samples per unit time. However, when using this technique for plant tissue other than that used in this study, it is

suggested that at least some samples be calibrated against standard digestion techniques to ensure that the extraction is complete.

Though Na analysis was not attempted using the proposed method its extraction should be complete as in the case of K and it should be possible to determine Na in plant samples using the HC1 extraction technique.

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