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# COPPER, IRON, MANGANESE AND ZINC IN SOIL AND LEAF SAMPLES FROM SOUTHERN AND EASTERN AFRICAN SUGARCANE-PRODUCING REGIONS

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## Abstract

Copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn) are required by plants in low amounts and, depending on soil properties, may become deficient or toxic for crop growth. Limited research has been conducted on micronutrient nutrition in sugarcane, despite losses due to removals in harvests and anecdotal reports of deficiencies becoming more common. This study surveyed the distribution of these micronutrients in soils and investigated the relationships between them and key soil properties from estates in eastern and southern Africa using data from the South African Sugarcane Research Institute's Fertiliser Advisory Service from 2013 to 2017. Summary and correlation methods were used to investigate patterns. Leaf analyses were also examined for trends. Zinc was frequently below both soil and plant thresholds at all sites. There were numerous instances of soil Cu, Mn and Fe levels near or below the threshold values in all regions. However, leaf data generally did not reflect deficiencies of these nutrients. Relationships between the soil micronutrients and other soil properties were weak and could not be used to predict and recommend management for risk areas. An understanding of the impact of low Zn on cane performance is required to refine current recommendations. It also appears that dust contamination, notably for Fe, is a problem. An industry wide structured sample survey of soil and leaf material (along with production data from sampled fields) from different regions is proposed. This will permit the validity of the soil and leaf thresholds for micronutrients to be investigated and better management recommendations made.

*Keywords:* deficiency, leaf analysis, soil analysis, toxicity, trace elements

## Introduction

Micronutrients (copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)) are essential for several biochemical processes in plants and are required in low amounts from the soil and also within the plant. Generally deficiency, where supply from the soil does not meet crop growth requirements, is of greater concern than toxicity, though this may occur under some conditions. Meyer *et al.* (1999) and van Antwerpen *et al*. (2013) provide overviews of the role of these micronutrients in sugarcane as well as the general factors influencing their availability and uptake from the soil (summarised in Table 1).

Previous soil and leaf surveys, along with analysis of data from samples submitted to the South African Sugarcane Research Institute's Fertiliser Advisory Service (SASRI, FAS), have highlighted that Zn is deficient in many South African regions. In the 1999 review and analysis of the nutritional status of South African grown sugarcane, Meyer *et al.* (1999) summarised the findings of prior nutritional surveys (including Meyer *et al.*, 1971; Meyer *et al.,* 1989 and Meyer *et al.*, 1998). In that review they highlighted that Zn was the most commonly deficient micronutrient in both soil and leaf samples, typically associated with either acid soils in the rainfed regions or alkaline soils in the irrigated areas. While incidences of Mn and Cu deficiency were found, these were not widespread. The proportion of samples with Fe deficiency had increased by 5% from 1971 to 1996.

**Table 1: Summary of the role and factors affecting the availability of copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) (adapted from Meyer *et al.* (1999) and van Antwerpen *et al*. (2013)).**

| Micro-nutrient | Role in plant | Symptom of deficiency | Soil factors reducing availability | Amelioration | Toxicity notes |
|---|---|---|---|---|---|
| **Copper (Cu)** | Enzyme activity including respiration and photosynthesis | Droopy top, green blotches on leaves, eventually curling and losing colour | • High soil pH<br>• High organic matter content<br>• Cu deficient parent materials | Application of leaf spray | Not common |
| **Iron (Fe)** | Production of chlorophyll | Yellowing of leaves | • High soil pH<br>• Excess calcium, phosphorus and nitrate<br>• In acid soil, excess Mn can lead to reduced Fe uptake | Application of leaf spray | Not common, but may occur in waterlogged soils (plant “bronzing”) |
| **Manganese (Mn)** | Enzyme activator | Whitening and yellowing of leaves, mostly in the leaf middle and tips | • High soil pH | Application of leaf spray | Toxicity is more likely on acid soils (liming can alleviate) or under waterlogged conditions. Symptoms include a light brown freckling on leaves |
| **Zinc (Zn)** | Enzyme production and plant growth regulation | Development of pale green leaves changing to full chlorosis with leaf deformation with severe deficiency | • High soil pH<br>• Zn deficient materials<br>• Excess phosphorus | Application of leaf spray or soil application of Zn salts or Zn containing fertilisers | Not common |

Meyer *et al.* (1999) also summarised research, testing growth responses to several micronutrients across the sugarcane growing area in South Africa. During the 1960s several investigative trials examining soil and leaf responses to Zn were evaluated in the North Coast regions with dramatic improvements to either 55 kg $ha^{-1}$ zinc sulphate or 160 L $ha^{-1}$ of 1% solution of zinc sulphate. Responses to other micronutrients were not as dramatic and tended to be localised, if they occurred at all.

The 2010 nutritional survey by van der Laan and Miles (2010) used FAS soil data from 2007 to 2009. They reported that Zn deficiency ranged from none in the Pongola and Zululand South regions to a high of 31% in the irrigated Mpumalanga areas, while deficiencies between 10 and 15% of submitted samples were apparent for Zululand North, and the North and South Coast regions. These deficiencies were based on a threshold value of 1 mg $L^{-1}$, while the current threshold is set at 1.5 mg $L^{-1}$, suggesting the actual proportion of deficiencies was likely to have been higher. They did not report on Cu, Fe or Mn. The most recent survey of the nutritional status of soils by Mthimkhulu and Miles (2017) examined soil analyses, including Fe and Zn but not Cu or Mn, from the FAS for the period 2012 to 2016. For Zn, using the revised threshold of 1.5 mg $L^{-1}$, they reported substantially higher incidences of sub-optimal Zn levels ranging from 21 to 58% across the different growing regions. Deficiency levels were between 30 and 50% at Malelane, Pongola, Zululand North, South and Lower South Coast, while Komatipoort, Umfolozi, Zululand South, and North Coast had >50% deficiency levels. Iron deficiencies were not common with only between 10 and 17% of samples from the irrigated regions found to be deficient. There is no readily available information on the micronutrient status of soils and plants from sugarcane growing areas in other African countries.

This analysis of data from grower submitted samples provides an overview of the micronutrient (Cu, Fe, Mn and Zn) status of soils and leaf samples from sugarcane growing regions in South Africa, as well as some other southern and eastern African countries using data from the FAS commercial sample analysis laboratory. Correlation and regression analyses were used to determine whether micronutrients were predictably affected by other properties or elements assessed. Recommendations for future research are given.

## Materials and Methods

Soil and leaf data were sourced from the FAS laboratory for the period 2013 to 2017. For summary reporting and preliminary characterisation, data were split into the major sugarcane extension areas in South Africa (as used by Mthimkhulu and Miles (2017)) and also included several southern and eastern African countries (Table 2). Only soil and leaf analyses from sugarcane growers were included (i.e. no research samples or non-sugar crop samples). As the FAS operates as a commercial laboratory, the analyses by FAS are not replicated. However, several quality control measures (including the use of calibrated standards, quality control samples, and post-analysis data interrogation) are used during and after the analysis process to ensure quality data are produced. It is worth noting, however, no control over sample collection and handling prior to submission to FAS is possible and samples are analysed as received.

### *Soil*

In most instances samples had been collected from the 0 to 20 cm soil depth, though samples from 0 to 10, 0 to 15 and 0 to 30 cm were also included in the topsoil analysis. The FAS currently only undertakes analysis of Cu, Fe, Mn and Zn as part of the routine diagnostic package. Available amounts are extracted with an ammonium bicarbonate solution (van der Merwe *et al.*, 1984) and determined in the extract by inductively coupled plasma emission spectroscopy (Varian 720ES). Additional soil parameters considered for determination of relationships and as possible drivers of micronutrient availability included pH, exchangeable acidity and cations, resin extractable phosphorus, clay and organic matter content and derived parameters (total cations and acid saturation) (methods previously described by Mthimkhulu and Miles (2017)).

**Table 2. The regions and countries and number of sugarcane topsoil and leaf samples submitted for analysis to the Fertiliser Advisory Service between 2013 and 2017. The number of farms refers to different site locations where the samples came from in each region.**

| Region | Acronym | Topsoil | | Leaf | |
|---|---|---|---|---|---|
| | | No. farms | No. samples | No. farms | No. samples |
| **South Africa Irrigated** | | | | | |
| Komati | KT | 43 | 1 169 | 8 | 611 |
| Malelane | ML | 82 | 1 267 | 13 | 192 |
| Umfolozi | UMF | 91 | 1 427 | 13 | 60 |
| Pongola | PG | 150 | 2 362 | 13 | 62 |
| **Rainfed** | | | | | |
| North Coast | NC | 658 | 18 525 | 27 | 676 |
| South Coast | SC | 118 | 3 877 | 13 | 295 |
| Lower South Coast | LSC | 100 | 1 919 | 5 | 65 |
| Zululand North | ZN | 102 | 2 876 | 15 | 216 |
| Zululand South | ZS | 120 | 5 881 | 6 | 28 |
| Midlands North | MN | 158 | 7 511 | 21 | 567 |
| Midlands South | MS | 149 | 4 055 | 25 | 407 |
| **Southern and Eastern Africa (predominantly irrigated)** | | | | | |
| Zimbabwe | Zim | 1 | 1 745 | 1 | 1 695 |
| Swaziland | Swa | 10 | 3 243 | 6 | 1 095 |
| Mozambique | Moz | 5 | 1 196 | 4 | 821 |
| Tanzania | Tan | 2 | 650 | 2 | 1 512 |
| Zambia | Zam | 6 | 3 494 | 3 | 2 976 |
| Malawi | Mal | 3 | 3 805 | 2 | 7 177 |

Data from each region were summarised using standard summary statistics (mean, standard deviation, median and range). To identify the occurrence of potential deficiencies and toxicities for each micronutrient, samples from each region were categorised into the proportion of samples within the currently prescribed adequate range (Table 3), as well as samples below and above the lower and upper threshold values, respectively. Simple Pearson correlation coefficients were used to investigate possible relationships between micronutrient amounts and other soil properties using Genstat Version 18. Where significant ($p<0.05$) correlations with fits of >70% (positive or negative) were found (considered meaningful here), regression methods were used to investigate the relationships further using Genstat Version 18.

### *Leaf*

As with soil analysis, FAS undertakes routine analysis of Cu, Fe, Mn and Zn. The total concentrations of these and other major plant elements were determined by X-ray fluorescence spectrometry (Rigaku ZSX Primus II). Results were analysed as described for the soil data using conventional summary statistics and to examine the relationships between leaf micronutrients and with other nutrients determined. The proportion of samples from each region were determined for values below the adequate threshold, within the adequacy range, and above the high and excessively high thresholds (Table 3).

**Table 3. Topsoil and leaf threshold norms for copper, iron, manganese and zinc used by the South African Sugarcane Research Institute to determine if values are low, adequate or high, or excessively high (leaf only).**

| Micronutrient | Low | Adequate | High | Excessively high |
|---|---|---|---|---|
| | | Topsoil (mg $L^{-1}$) | | |
| **Copper** | <0.8 | 0.8 to 20 | >20 | - |
| **Iron** | <2.5 | 2.5 to 250 | >250 | - |
| **Manganese** | <2 | 2.5 to 50 | >50 | - |
| **Zinc** | <1.5 | 1.5 to 75 | >75 | - |
| | | Leaf (mg $kg^{-1}$) | | |
| **Copper** | <3 | 3 to 8 | 8 to 15 | >15 |
| **Iron** | <50 | 50 to 100 | 100 to 250 | >250 |
| **Manganese** | <15 | 15 to 100 | 100 to 250 | >250 |
| **Zinc** | <15 | 15 to 30 | 30 to 75 | >75 |

While an effort to relate soil properties to leaf uptake was undertaken, the inability to link the location of soil samples to subsequent leaf analysis from farmer fields prevented investigation as to the causal nature of the trends. In addition, the large discrepancy between numbers of samples submitted for soil analysis vs those for leaf analysis (particularly in the South African regions) along with changes in farm and field names and numbering, further hindered the ability to relate soil analysis to leaf values.

## Results

*Soil*

Summary statistics for Cu, Fe, Mn and Zn in the topsoils for the different regions are presented in Table 4 and proportional ranges for low, adequate and high values are given in Figure 1. Each element is considered in turn below.

*Copper*

In the South African regions low Cu values (<0.8 mg $L^{-1}$) were found in a high proportion of samples in the LSC (36%), NC (28%), SC (24%), ZS (18%), MS (15%) and UMF (14%). Several regions had values above the upper threshold of 20 mg $L^{-1}$, though these were relatively small proportions (maximum of 6.2% at PG) (Figure 1). In the other African regions, there were few below threshold values (<3%) or with high values (typically <1.6%). An exception was Swaziland with 7.2% of samples above 20 mg $L^{-1}$. Differences between the mean and median values were not large, suggesting that extreme low or high values were not a major influence on the distribution of results. The correlations between Cu and other soil parameters poor (r<0.7), with only a significant >0.7 correlation found with Fe in the Zim region. Regression analysis of these parameters showed a poor co-efficient of determination ($R^2$=0.49, $p$<0.001, Cu = 7.95xFe -19.5).

*Iron*

Despite the wide range for Fe soil adequacy (2.5 to 250 mg $L^{-1}$), the incidence of low or high values was high across the regions. Low Fe values were more common in the irrigated regions. In South Africa, KT, ML and PG had between 13 and 18% samples in the low range, while Swa and Mal had 22 and 13% of samples in the low range respectively (Figure 1). In Zim a particularly high proportion of samples were found to be in the low range (67%). Incidence of deficiency in the rainfed regions of South Africa, as well as at UMF, was small (<1%). Several sites had a high proportion of samples with values above the upper threshold (Figure 1) with some very high values found (>750 mg $L^{-1}$, Table 4). In South Africa, samples from the NC, SC, LSC, ZN, ZS and MS regions all had more than 40% of samples over 250 mg $L^{-1}$, with a maximum of 73% of samples found to be high in the LSC region (Figure 1). At UMF and MN, 16 and 27% of samples, respectively, were above the upper threshold, while the remaining irrigated regions (PG, KP, ML) did not show marked occurrence of high values (<1%). Median values were consistently lower than mean values, indicating the influence of very high values measured in some regions. There were almost no correlations >0.7 between Fe and other soil properties across the different regions, expect for significant positive correlation between Fe and acid saturation and the aforementioned Cu relationship (both r>0.7) in the Zim region. The Fe regression response to acid saturation was, however, weak but significant ($R^2$=0.47, $p$<0.001, Fe = 142xacid saturation -11.3)

*Manganese*

In all regions and countries, Mn tended to be within the adequate range of 2 to 50 mg $L^{-1}$, with a low incidence (<10% of samples from each area) of levels below 2 mg $L^{-1}$ (Figure 1, Table 4), while 22% of samples in the LSC were considered low. A similar trend was found for high values, where most South African regions had <10% of samples in the high range (except Zn with 17%), while Zim, Moz, Tan and Mal had between 18 and 22% of samples measured as high. The largest difference between mean and median Mn values was generally found in the rainfed regions of South Africa, where the incidence of high values was common across all sites which caused the mean values to be higher. This was not as pronounced for the irrigated regions. Manganese levels did not correlate with other soil properties (r-values <0.7).

*Zinc*

Apart from the MN and MS sugarcane growing regions in South Africa, all the other areas had a high proportion of samples with Zn levels below the 1.5 mg $L^{-1}$ lower threshold (between 38 and 56% of samples; Figure 1). The remainder of samples in each area were within the adequate range (1.5 to 75 mg $L^{-1}$) with very few samples above 75 mg $L^{-1}$. In the case of the MN and MS regions there were substantially fewer instances of sub-optimal soil Zn with 18 and 26% of samples, respectively, falling below the lower threshold. As with the other regions there were very few instances of values over 75 mg $L^{-1}$, with the majority of samples within the adequate range (Figure 1, Table 4). For the other African countries a more variable pattern was seen. In Zim and Swa about 80% of samples were below the 1.5 mg $L^{-1}$ lower threshold while Tan and Mal had 41 and 27% of samples below the threshold, respectively, and only 5 and 13% of samples from Zam and Moz sub-optimal. The high occurrence of samples below the adequate threshold and the general lack of extreme values generally resulted in mean values that were not excessively higher than the median values. In a few instances the median values were nearly half the mean (e.g. Zim, Swa, Mal), though these were also at or below the threshold value. Like for Mn, no useful correlations were found (all r-values <0.7).

**Table 4. Mean, standard deviation (StDev), median, minimum (Min) and maximum (Max) values for copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in topsoil samples submitted for analysis to the Fertiliser Advisory Service between 2013 and 2017 from sugarcane growing regions in South Africa and other African countries. Region codes are given in Table 2.**

| | Region | KT | ML | UMF | PG | NC | SC | LSC | ZN | ZS | MN | MS | Zim | Swa | Moz | Tan | Zam | Mal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cu** | **Mean** | 7.8 | 7.7 | 5.0 | 7.9 | 2.8 | 2.4 | 2.7 | 6.0 | 2.6 | 3.8 | 3.8 | 3.4 | 11.1 | 8.1 | 4.3 | 4.1 | 3.4 |
| | **StDev** | 4.6 | 5.5 | 5.7 | 6.9 | 3.9 | 2.4 | 4.3 | 5.4 | 2.5 | 3.4 | 4.2 | 1.4 | 6.3 | 4.8 | 3.1 | 4.0 | 2.0 |
| | **Median** | 7.2 | 6.8 | 4.1 | 5.5 | 1.6 | 1.7 | 1.5 | 4.8 | 1.8 | 2.8 | 2.6 | 3.1 | 10.1 | 7.0 | 3.7 | 3.3 | 3.1 |
| | **Min** | 0.3 | 0.3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.1 | 0.9 | 0.1 | 0.1 | 0.3 | 0.3 | 0.3 |
| | **Max** | 44.3 | 37.5 | 48.6 | 59.8 | 66.1 | 53.3 | 86.0 | 42.6 | 30.0 | 81.5 | 36.9 | 11.5 | 53.7 | 85.9 | 22.6 | 56.7 | 39.0 |
| **Fe** | **Mean** | 21.6 | 23.1 | 124.2 | 51.0 | 271.5 | 325.6 | 367.7 | 229.7 | 373.5 | 204.5 | 273.1 | 7.4 | 15.8 | 199.5 | 157.0 | 41.5 | 102.2 |
| | **StDev** | 36.1 | 31.6 | 162.0 | 59.5 | 184.1 | 184.8 | 176.3 | 151.0 | 199.0 | 115.7 | 180.7 | 15.9 | 27.3 | 223.4 | 127.7 | 33.3 | 122.2 |
| | **Median** | 10.7 | 11.6 | 67.5 | 28.7 | 232.8 | 296.9 | 351.4 | 216.8 | 339.3 | 180.1 | 234.4 | 1.2 | 6.5 | 114.6 | 139.4 | 36.8 | 48.0 |
| | **Min** | 0.5 | 0.5 | 0.6 | 0.5 | 0.5 | 0.5 | 10.2 | 0.5 | 2.6 | 3.2 | 2.8 | 0.5 | 0.5 | 0.5 | 1.5 | 0.5 | 0.5 |
| | **Max** | 517.5 | 350.6 | 3558.1 | 539.5 | 1728.2 | 1464.4 | 1098.5 | 1113.0 | 1363.8 | 1113.3 | 1536.1 | 162.8 | 366.8 | 1644.6 | 762.9 | 650.8 | 713.9 |
| **Mn** | **Mean** | 15.3 | 18.9 | 20.2 | 18.2 | 16.7 | 14.3 | 11.2 | 30.4 | 12.1 | 17.1 | 16.6 | 31.3 | 13.2 | 31.6 | 36.2 | 19.3 | 31.0 |
| | **StDev** | 12.9 | 21.8 | 23.3 | 17.9 | 56.9 | 15.2 | 14.5 | 29.3 | 15.7 | 20.0 | 16.6 | 23.0 | 15.8 | 23.2 | 37.3 | 14.6 | 28.9 |
| | **Median** | 12.0 | 11.9 | 13.0 | 12.4 | 7.6 | 9.3 | 6.0 | 22.0 | 6.6 | 9.7 | 11.4 | 25.4 | 8.6 | 25.2 | 26.3 | 16.3 | 23.5 |
| | **Min** | 0.7 | 0.5 | 0.6 | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.3 | 0.4 | 0.1 | 0.3 | 0.1 | 0.8 | 1.0 | 0.1 | 0.5 |
| | **Max** | 117.4 | 187.7 | 206.8 | 159.4 | 1300.0 | 160.9 | 184.8 | 252.5 | 283.4 | 240.7 | 194.8 | 195.8 | 292.4 | 139.0 | 419.4 | 107.8 | 281.2 |
| **Zn** | **Mean** | 2.3 | 3.1 | 2.0 | 2.9 | 2.2 | 2.5 | 2.5 | 2.4 | 2.5 | 4.1 | 3.6 | 1.2 | 1.4 | 3.9 | 3.3 | 11.3 | 5.0 |
| | **StDev** | 8.6 | 4.0 | 2.3 | 5.2 | 3.7 | 3.5 | 3.9 | 3.6 | 4.7 | 4.0 | 4.9 | 2.5 | 2.6 | 3.1 | 5.4 | 12.7 | 10.3 |
| | **Median** | 1.4 | 2.0 | 1.4 | 1.8 | 1.4 | 1.6 | 1.7 | 1.6 | 1.5 | 3.2 | 2.4 | 0.6 | 0.8 | 3.1 | 1.8 | 8.1 | 2.9 |
| | **Min** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| | **Max** | 286.6 | 56.8 | 32.8 | 131.1 | 129.2 | 85.8 | 126.7 | 54.4 | 148.5 | 75.0 | 130.8 | 51.8 | 80.9 | 48.6 | 67.5 | 220.7 | 412.5 |

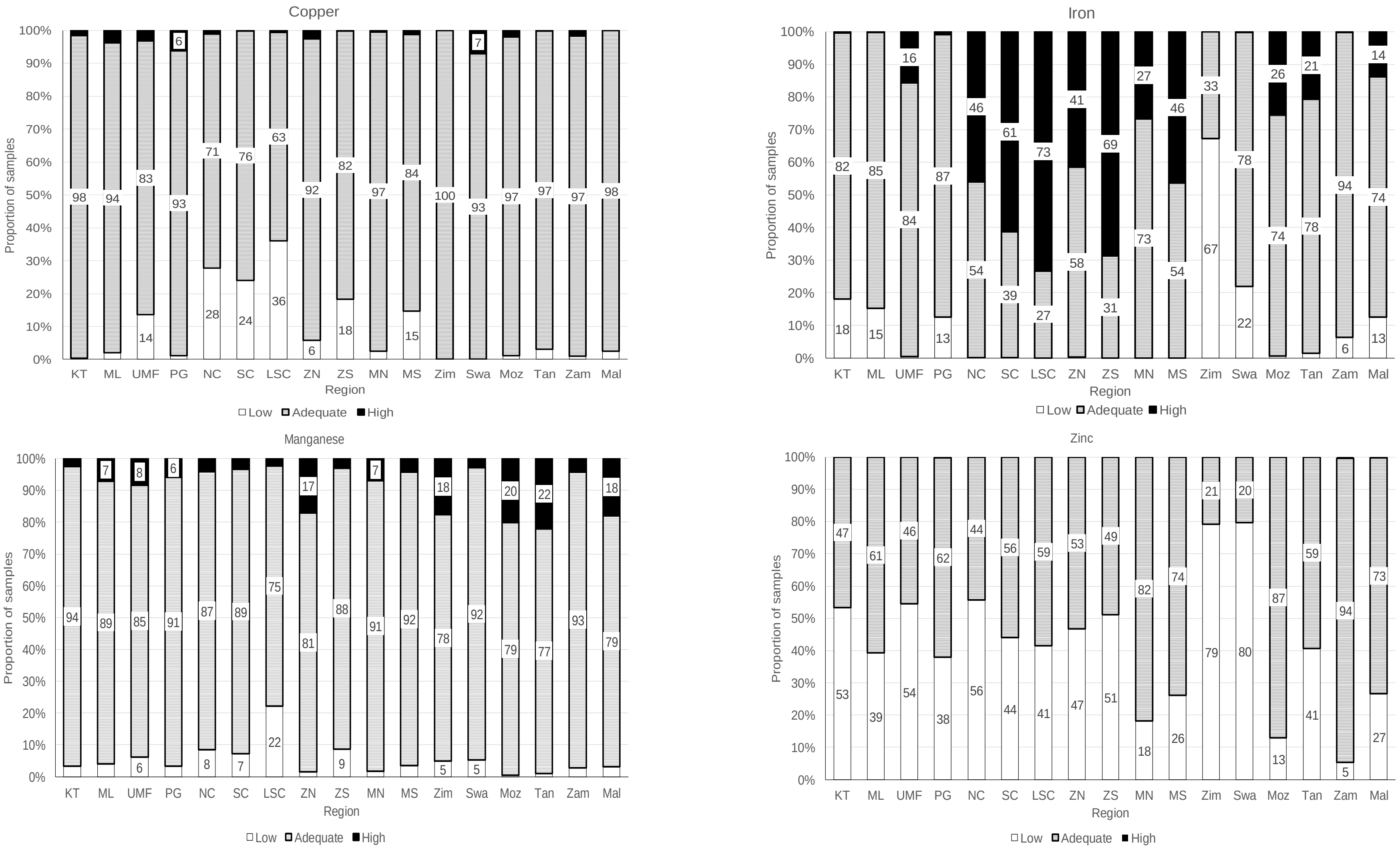

**Figure 1. Proportion of topsoil samples below, within or over the adequate range for each micronutrient. Region acronyms are given in Table 2 and threshold values in Table 3. Values given in each data column are percentages of samples within that category for a given site (only shown if >5%).**

### *Leaf*

Summary statistics for Cu, Fe, Mn and Zn in the leaf samples for the different regions are presented in Table 5 and proportional ranges for low, adequate, high and excessively high values are given in Figure 2. Each element is considered in-turn below.

### *Copper*

While most sites had some leaf samples with deficient Cu levels (<3 mg kg$^{-1}$), the proportion of samples tended to be low (most <10%) (Figure 2). Exceptions included UMF (31%), PG (15%), and notably, Zim (57%). The mean and median values across the regions were generally within the lower end of the satisfactory range (3 to 8 mg kg$^{-1}$) (Table 5). The proportion of samples with levels >8 mg kg$^{-1}$ was also low at <6% in most regions, though Swa was notable with 16% of samples being higher (Figure 2). There were few samples considered excessively high, with the highest value measured in Mal (42 mg kg$^{-1}$) (Figure 2, Table 5). No meaningful correlations were found (all r-values <0.7)

### *Iron*

Very few incidences of deficient leaf Fe values were found, though between 40 and 85% of samples from each region (except ZS) had values >100 mg kg$^{-1}$ (Figure 2), while in the ZS region this was 21% of samples. Most regions (except ZS and SC) also had between 5 and 15% of samples with leaf Fe being excessively high (>250 mg kg$^{-1}$), with >20% of samples from PG and Mal in that category. The mean values were consistently higher than median values in all regions; this attributed to the high proportion of samples with high and very high values. The only significant correlations found was with Si in ZN (r=0.78) and Zam (r=0.75). regression analysis showed that these were significant, though the regression fits were weak (ZN: $R^2$=0.62 p<0.001; Fe = 257xSi-63.5 and Zam: $R^2$=0.56 p<0.001; Fe = 150xSi-9.7).

### *Manganese*

The proportion of sub-optimal (<15 mg kg$^{-1}$) levels of Mn was negligible at all sites while high and excess levels (>100 mg kg$^{-1}$) were not common either (typically <10% of samples), with the exception of ZN (15%) and Tan (45%). There was a high variation between the mean values across the regions, though for each region the mean and median values were similar, reflecting the lack of extreme outliers. The lowest means were measured for KT, Zim, Swa and Zam (32 to 35 mg kg$^{-1}$), with most other areas in the 50 to 90 mg kg$^{-1}$ range. The highest values measured were at MS (563 mg kg$^{-1}$), Tan (419 mg kg$^{-1}$) and Mal (312 mg kg$^{-1}$) with several other regions above 200 mg kg$^{-1}$ (ML, NC, SC, ZN, and Swa). No meaningful correlations were found between Mn and other leaf nutrients.

### *Zinc*

Similar to the soils, all regions and countries had samples with below threshold Zn levels (Figure 2), though mean and median values were all within the lower end of the satisfactory range (15 to 30 mg kg$^{-1}$; Table 5 and Figure 2) and similar to each other. In the South African regions sub-optimal levels were typically found in between 40 and 50% of the samples from a region. The exceptions were ZN and ZS in the low 30% range, LSC with 22% and the SC region with 57% of samples below optimal. In the other countries Zim had the highest proportion of deficient samples (60%) followed by Mal and Tan (about 50%), while Swa only had 16% of samples below optimal. There were a few regions where high and excessive values were found, but in all areas this occurred in <10% of samples. Occurrence of high and very high values were limited. No useful correlations were found either.

**Table 5: Mean, standard deviation (StDev), median, minimum (Min) and maximum (Max) values for copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) in leaf samples submitted for analysis to the Fertiliser Advisory Service between 2013 and 2017 from sugarcane growing regions in South Africa and other African countries. Region codes are given in Table 2.**

| | Region | KT | ML | UMF | PG | NC | SC | LSC | ZN | ZS | MN | MS | Zim | Swa | Moz | Tan | Zam | Mal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cu** | **Mean** | 4.5 | 4.7 | 3.9 | 4.8 | 5.1 | 4.8 | 5.0 | 4.9 | 4.2 | 5.0 | 4.5 | 3.1 | 6.0 | 5.2 | 5.1 | 5.0 | 4.6 |
| | **StDev** | 1.4 | 1.5 | 2.3 | 1.4 | 1.8 | 0.9 | 1.5 | 1.1 | 0.4 | 1.7 | 1.0 | 1.1 | 2.0 | 1.1 | 1.5 | 1.4 | 1.4 |
| | **Median** | 4.2 | 4.4 | 4.2 | 5.0 | 4.6 | 4.7 | 5.0 | 5.0 | 4.1 | 4.6 | 4.5 | 2.9 | 5.9 | 5.1 | 5.0 | 4.9 | 4.4 |
| | **Min** | 2.0 | 2.5 | 0.0 | 1.4 | 1.3 | 2.7 | 2.4 | 1.4 | 3.5 | 2.3 | 0.1 | 1.3 | 1.7 | 2.8 | 1.9 | 0.1 | 0.0 |
| | **Max** | 21.6 | 11.6 | 9.9 | 7.6 | 18.3 | 9.0 | 8.1 | 7.5 | 5.5 | 14.9 | 8.4 | 20.0 | 13.4 | 8.9 | 12.2 | 18.9 | 42.3 |
| **Fe** | **Mean** | 176.6 | 175.9 | 154.9 | 176.5 | 143.9 | 113.9 | 161.0 | 169.9 | 84.1 | 133.8 | 119.3 | 124.2 | 152.7 | 124.0 | 170.3 | 138.0 | 205.0 |
| | **StDev** | 149.2 | 90.5 | 97.3 | 111.4 | 93.1 | 111.5 | 84.4 | 203.0 | 16.5 | 65.0 | 65.5 | 79.5 | 73.0 | 54.4 | 101.9 | 173.3 | 188.0 |
| | **Median** | 145.9 | 157.3 | 117.3 | 147.9 | 118.0 | 95.6 | 136.6 | 116.3 | 79.5 | 116.1 | 102.0 | 104.1 | 136.3 | 112.7 | 142.3 | 112.1 | 140.3 |
| | **Min** | 51.2 | 67.9 | 59.9 | 64.3 | 48.1 | 56.3 | 47.8 | 55.7 | 58.2 | 50.5 | 42.0 | 46.0 | 61.4 | 42.4 | 45.3 | 32.2 | 33.0 |
| | **Max** | 2711.2 | 603.4 | 643.9 | 766.5 | 934.7 | 1808.7 | 492.9 | 1750.0 | 133.8 | 446.5 | 620.3 | 1839.6 | 877.3 | 583.4 | 1083.7 | 7949.2 | 2303.8 |
| **Mn** | **Mean** | 33.0 | 50.8 | 40.0 | 47.7 | 48.6 | 52.5 | 60.0 | 71.3 | 42.0 | 52.7 | 59.3 | 35.0 | 33.4 | 63.1 | 90.6 | 32.6 | 50.3 |
| | **StDev** | 11.6 | 27.8 | 19.3 | 17.9 | 21.5 | 28.4 | 30.4 | 33.2 | 8.1 | 21.3 | 36.0 | 10.0 | 11.3 | 22.3 | 69.3 | 13.5 | 22.4 |
| | **Median** | 30.6 | 41.7 | 36.0 | 43.4 | 44.3 | 45.4 | 52.5 | 65.5 | 41.9 | 47.3 | 53.4 | 33.6 | 31.8 | 60.2 | 83.1 | 29.9 | 45.1 |
| | **Min** | 15.9 | 14.7 | 17.6 | 21.8 | 12.4 | 16.4 | 15.9 | 17.9 | 29.4 | 17.9 | 6.0 | 15.1 | 14.4 | 21.5 | 10.4 | 9.1 | 12.8 |
| | **Max** | 111.3 | 209.9 | 120.7 | 109.3 | 203.6 | 231.2 | 178.2 | 237.0 | 65.3 | 141.1 | 563.0 | 96.7 | 212.7 | 169.3 | 417.8 | 152.4 | 311.7 |
| **Zn** | **Mean** | 15.6 | 18.0 | 15.4 | 14.9 | 16.4 | 15.3 | 17.5 | 16.1 | 15.5 | 17.2 | 17.6 | 19.4 | 17.9 | 17.9 | 15.3 | 17.2 | 16.0 |
| | **StDev** | 3.7 | 10.1 | 5.5 | 3.9 | 4.2 | 4.1 | 4.3 | 4.5 | 2.1 | 7.5 | 10.7 | 21.5 | 3.4 | 3.9 | 3.6 | 4.4 | 6.7 |
| | **Median** | 15.3 | 15.7 | 15.0 | 14.3 | 16.6 | 14.4 | 17.7 | 15.2 | 15.3 | 16.0 | 15.9 | 14.2 | 17.7 | 17.6 | 15.0 | 16.7 | 15.0 |
| | **Min** | 8.0 | 6.0 | 5.5 | 6.5 | 8.3 | 7.4 | 7.9 | 7.5 | 11.8 | 9.7 | 0.5 | 8.0 | 9.2 | 9.0 | 7.0 | 7.4 | 7.7 |
| | **Max** | 30.3 | 104.9 | 28.5 | 26.8 | 36.2 | 44.4 | 38.3 | 29.0 | 20.3 | 145.1 | 146.6 | 271.9 | 71.2 | 31.3 | 41.9 | 82.0 | 224.1 |

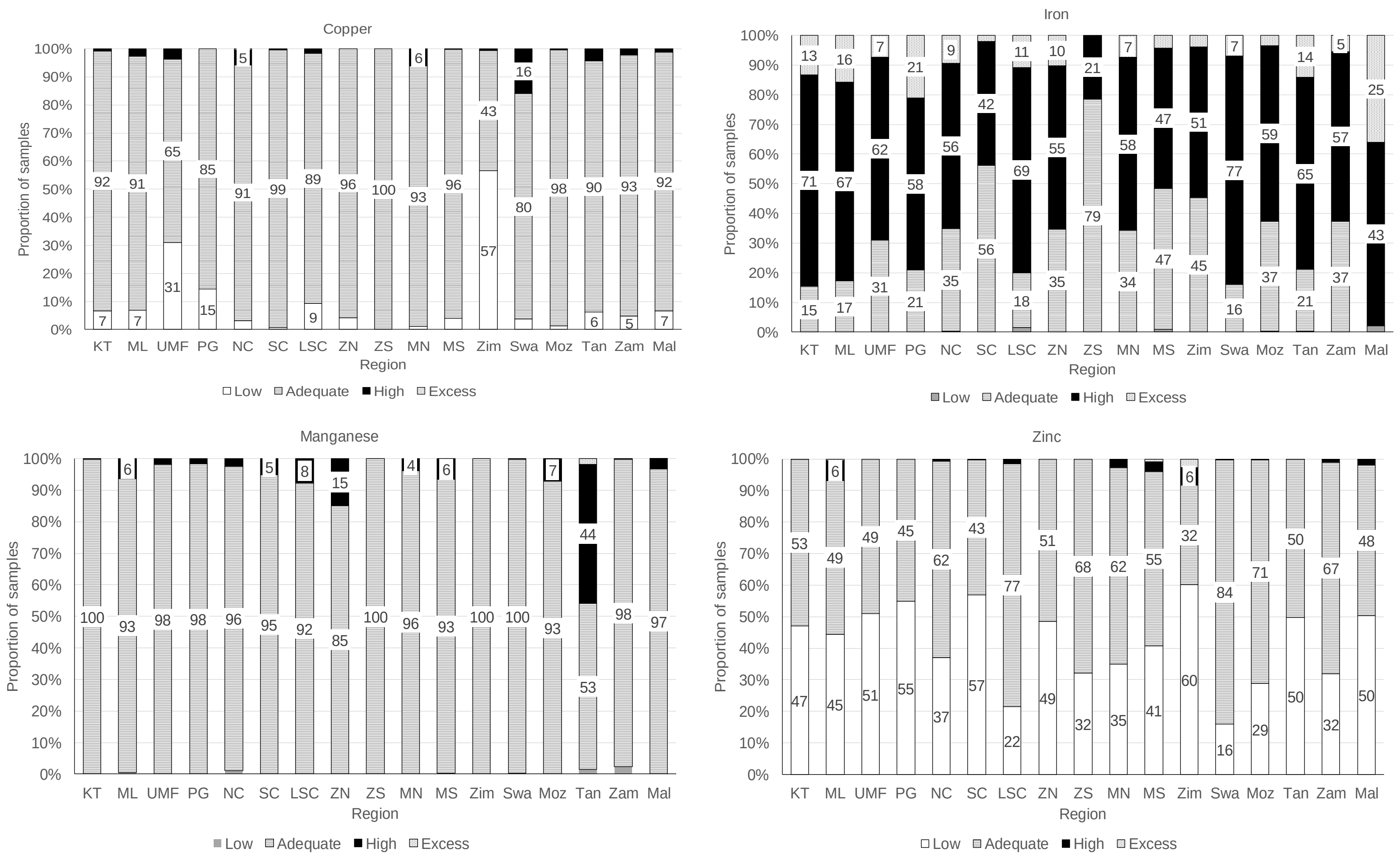

**Figure 2. Proportion of leaf samples below, within or over (split into high and excess) the adequate range for each micronutrient. Region acronyms are given in Table 2 and threshold values in Table 3. Values given in each data column are percentages of samples within that category for a given site (only shown if ≥1%).**

## Discussion

### *Copper*

Previous reports had indicated that Cu deficiency was limited to <1% of samples, based on leaf diagnostics (Meyer *et al*., 1999). Du Toit (1956) was perhaps the first to report an apparent Cu deficiency on a granite derived soil on the SC and further instances on the NC, based largely on visual symptoms. A foliar spray remedied the problem, though further investigations did not take place. Subsequently Alexander (1967) reported on a survey of leaf values on farms located on Table Mountain Sandstone and found that only 2% of farmers had incidence of Cu deficiency (using a 3 mg kg$^{-1}$) threshold. While surveys and research on Cu thresholds and ranges have been somewhat limited, those studies suggest the Cu deficiency is negligible, while toxicity is even considered. This may in part be due to the few research trials investigating responses to micronutrients that showed that there were no beneficial responses, and sometimes even negative responses, to applying Cu to the soil on cane yield (Meyer et al. 1999).

The results from this survey suggest Cu deficiency is more widespread than previously reported. Using the threshold-based comparison of optimal nutrient ranges in soil, it was found that all sites, with the exception of Zim, had incidence of sub-optimal soil levels while several sites had >10% of samples showing deficiency, particularly in the rainfed regions (NC, SC, LSC, ZS and MS), but also in the irrigated UMF region. Leaf analysis suggested a different pattern of deficiency to the soils tests, where the proportion of deficient leaf samples tended to be higher in the irrigated regions, both within SA and the other African regions, than in the rainfed regions. It was only the UMF region where the proportion of samples that were deficient in Cu, in both soil and leaf samples, was high (14 and 31% of samples for soil and leaf respectively). It was also noteworthy that, for the Zim region, despite 57% of leaf samples with sub-optimal Cu values, there were negligible soil deficiencies found.

The apparent contradiction in findings may be due to several factors. A key issue was that it was not possible to pair soil and leaf samples at the different sites, and thus not possible to establish the relationships between soil values and plant uptake. Attempts to establish relationships between the selected micronutrients in the soil and other soil properties, or leaf micronutrients and other leaf nutrients also did not provide meaningful insight into factors that may be controlling or affecting uptake of a particular micronutrient. In this regard, the only meaningful correlation detected was soil Cu with Fe in the Zim region, though the regression relationship was poor ($R^2$=0.49). This type of relationship was not observed in any other region. Other factors controlling Cu availability (e.g. clay content, OM, pH (Meyer *et al*., 1999)) were also not predictably related to Cu content. It is speculated that the high variation in soil and leaf test values and the high diversity of sites, even within a single region, may be limiting the detection of more site (farm or field) specific trends.

### *Iron*

Deficiency in soil Fe was not common, only occurring in the irrigated regions in South Africa and other African countries (notably in the Zim region), while a negligible number of incidences of leaf deficiencies were found. High values were more common, notably in the more acidic soils (rainfed areas) of South Africa and some of the African countries. This was likely due to the typically higher soil pH's measured in these regions (Mthimkhulu and Miles, 2017) as Fe availability increases with a decrease in pH and declines under more alkaline conditions (Marschner, 2012; Rutkowska *et al*., 2014). The proportion of samples with high values (>250 mg L$^{-1}$) was of greater concern, mostly in the rainfed coastal and midlands region of South Africa, and some other African countries. Correlation analysis, however, did not indicate any strong relationships between soil pH, possibly for similar reasons to the previously mentioned variability in the data. In the case of leaf analysis, Fe deficiency was also almost entirely absent while high and excess values occurred in a large proportion of the samples across all regions. This indicates that Fe toxicity may be a serious concern, though it is not a commonly reported problem in crops and is typically associated with waterlogged soils or very acidic soils (Marschner, 2012).

Given the common occurrence of Fe in the soil (as sesquioxides), it is perhaps not surprising that many of the soils found in the generally more acidic rainfed regions give high extractable values, though any negative consequences are not apparent. In the case of the leaf analysis, where proportions of high and excess were dominant in all regions, two causes are proposed. In the first instance it may be that the upper threshold for adequacy (100 mg kg$^{-1}$) is too low and should be revised to a higher value, though values reported elsewhere tend to be in a similar range as used here (e.g. McCray *et al.*, 2016, Reuter and Robinson, 1997). However, Reuter and Robinson, (1997) also indicate that there are instances where much wider optimum ranges for Fe are suggested (20-600 and 49-915 mg kg$^{-1}$). If these ranges are adopted, then the proportion of samples with high and very high values would be considerably lower than reported here. The second and more likely cause pertains to possible contamination of leaf material from dust. Several sites had samples with particularly high Fe values (often in excess of 1 000 mg kg$^{-1}$), well beyond what is commonly reported or anticipated. The primary cause of this has been attributed to dust (or applied chemicals) contamination of leaf material and is a common problem in leaf sampling (e.g. Cary *et al*., 1994; Jones, 2001; Wyttenbach and Tobbler 1998; Markert, 1995). Cary *et al*. (1994), using titanium as an indicator of particulate contamination, found that up to 70% of leaf measured Fe could be attributed to dust and soil contamination. They also indicate that dust contamination can lead to elevated amounts of aluminium and Si. This may also partly explain the weak relationships with Si found at two sites. Cary *et al*. (1994) indicate that the extent to which this contamination affects the analysis is related to the amounts that the crop takes up in relation to the amount likely to be present in the dust. For elements where dust is likely to be a major contributor (e.g. Fe, Si), this tends to mask plant uptake if their requirement is relatively low (e.g. Fe).

Generally leaf material is neither wiped down prior to sampling nor washed after collection or chemical analysis, thus samples with high levels of surface contamination are likely to have anomalous readings. Given that under these conditions it is not possible to distinguish between leaf Fe or contamination sourced Fe, it is not possible to give clear guidance on likely contamination or deficiency. A key tool in this regard would be the use of visual deficiency (or toxicity) symptoms and consideration of soil pH (where high pH (>7) can lead to reduced Fe availability and low pH (<4) can induce Fe chlorosis through Mn antagonism) (Meyer *et al*., 1999). If dust contamination is anticipated, then it may be necessary to undertake leaf wiping or rinsing during or immediately after sampling to reduce this effect. Such procedures are used in other parts of the world (e.g. McCray *et al.*, 2016), where soon after collection samples are rinsed in clean water before drying. In the case of the FAS, typically samples are delivered in a dry or semi-dry state and washing of the material is then not possible as this may leach cell contents from desiccated cell structures.

### *Manganese*

Generally there was a low incidence of Mn deficiency found in the soil samples while almost no incidences were found for the leaf samples, suggesting that this is not a particular concern, even in the more alkaline areas, where it was expected the high pH would result in reduced availability and consequently leaf deficiencies. High or excess Mn levels were a more common occurrence in both soil and leaf samples, though correlation analysis did not provide any explanation for this. This may in part be linked to dust contamination, and as in the case of Fe, lead to elevated values that may otherwise not be expected. Jones (2001) suggests that where Fe contamination from dust is a problem, it is likely that Mn (and to a lesser extent other elements) may also be elevated. This may also explain some of the high values found for these other elements, though the distribution was not consistent between the Fe and Mn.

### *Zinc*

Previous surveys have suggested a lower proportion of samples with deficient soil Zn (Meyer *et al*. 1999 (12%), van der Laan 2010 (typically <15%), using a 1 mg $L^{-1}$ threshold. The change to 1.5 mg $L^{-1}$ lower threshold increased the number of samples with deficiency as reported by Mthimkhulu and Miles (2017), with their proportion of deficient samples being similar to those reported here. This is also reflected in the leaf analysis where low values were found across all regions, with between 16 and 55% of sample being deficient. While potential Zn deficiency has been acknowledged before, these data suggest the problem may be far greater than previously found. Unfortunately, the lack of relationships between Zn and other soil or leaf properties makes it difficult to use these as indicators of risk for reduced Zn availability and it appears necessary to monitor sites regularly to ensure that adequate levels are maintained. It is also noteworthy that several past experiments have demonstrated excellent responses to soil and foliar applied Zn where it was conclusively demonstrated as being growth limiting (Meyer *et al.,* 1999). However, no recent research has been undertaken to improve the understanding of soil test values and crop uptake or remedial recommendations. Marschner (2012) and Rutkowska *et al.* (2014) indicate that liming and phosphorus application may lead to localised and short-term Zn deficiencies in soils, which may explain some of the deficiencies reported here as these are commonly used management practices in sugarcane production.

## Conclusions and Recommendations

This survey of soil and leaf micronutrients (Cu, Fe, Mn and Zn) using analysis data from grower submitted samples to FAS over the past five years has highlighted key patterns and concerns regarding micronutrient levels in the sugarcane growing sector:

- Copper deficiency does not appear to be a major limiting factor for crop production with occurrence of deficiency in leaf analysis largely localised to irrigated regions. Of notable concern was that Zim had a very high proportion of deficient samples, suggesting that further investigation of this may be required in affected areas. The apparent high incidence of deficiency in soils from the rainfed regions seemingly does not translate to similar levels of plant deficiency, suggesting the current soil test is not an adequate predictor of likely plant deficiency.
- Iron deficiency was not widespread. While several regions had incidence of low soil Fe levels (notably irrigated regions with generally higher soil pH than rainfed regions), leaf analysis did not support this where few incidence of deficiency were found. Of greater concern was high extractable soil Fe in several regions (primarily rainfed, attributed to typically lower soil pH of those regions), and high incidence of high and very high Fe leaf values across all regions. For the leaf analysis it is speculated that the results are skewed by dust contamination of leaf material submitted for analysis. As such it is not possible to separate contamination from true Fe levels.
- Incidence of Mn deficiency in soils was generally not a major concern, with only the LSC region with high incidence of deficiency. High values were a more common occurrence across all regions, but this did not seem to translate to more frequent incidence of high leaf values. Leaf values were largely in the adequate range across most regions, a notable exception being in Tan where 44% samples were over the upper adequate threshold.
- Zinc deficiency presents the greatest concern across all regions. There were high proportions of samples in all regions that had sub-optimal soil and leaf values, suggesting that many regions may benefit from supplemental Zn fertilisation. Use of Zn-fortified fertilisers or direct Zn salts such as zinc sulphate or zinc hydroxide, is likely to reduce the incidence of sub-optimal leaf values.

There were several limitations that constrained how the data could be used, and thus reduced the interpretative value of the trends observed:

- Data was obtained from grower submitted samples where there was no control over sampling methods and handling (with possible contamination) and could not be accounted for in the study. The impacts of sampling location, contamination or mixing are thus unknown.
- From within different regions there was high variability in sample values which may have masked general trends that might be expected for certain properties. It is suspected that this variability was the main driver of the poor relationships found between the parameters investigated.
- Linking of soil samples to leaf samples was not possible. While an effort to cross reference farms and fields, within and across years was attempted during early phases of data analysis, there were very few samples where this was possible. It is suspected that many growers attempt to reduce sample analysis costs thus split sample collection across their farms over several crop cycles. Changes in field numbering may also contribute to the problem.
- Furthermore, considerably fewer leaf samples are taken than for soil samples in the South African regions, further hindering linking of soil and leaf analysis. However, for the other African countries where soil and leaf analysis are sourced from few farms or estates, there were still no clear relationships established, suggesting that understanding the localised site specific drivers within a field may be necessary to explain the ranges and drivers reported.

An additional concern was typically a soil analysis is undertaken to identify a potential nutrient concern, with remedial action applied after this which should then reflect in crop uptake. Given that it is not possible to ascertain what actions were taken by a farmer after soil analysis, it then becomes more difficult to link a soil value to a crop value. Thus, this survey of grower submitted samples was useful to provide some insight into patterns and magnitude of possible deficiencies and toxicities in regions, thus guiding future focus areas and highlighting possible changes required to sample collection and handling procedures (such as washing of freshly collected leaf samples). However, with the available data it was not possible to refine existing thresholds, develop a link between soil tests and leaf uptake, or clearly identify other factors that may be used to improve the identification of sites that are likely to exhibit a deficient or toxic condition.

In this regard, to gain a better understanding of the magnitude of potential deficiency (or possibly toxicity) of micronutrients it is necessary to undertake an industry-wide survey involving paired soil and leaf sampling and analysis (such as previously reported by Meyer *et al.,* 1999). However, unlike in previous surveys, this sampling should also consider collection of production data to evaluate the true merit of current micronutrient thresholds and their relationship to yield as well as the soil tests used. The study would need to consider possible dust contamination of leaf material and use appropriate sampling and analytical protocols to account for this confounding issue. Such a survey and analysis would help identify high risk areas, establish site specific drivers for observed responses and establish if current thresholds are appropriate based on crop performance. Such information will also help focus future research efforts to identify specific management intervention to alleviate detected problems.

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