BINDURA UNIVERSITY OF SCIENCE EDUCATION

EVALUATION OF MEHLICH-3 MULTI-EXTRACTANT FOR THE DETERMINATION OF EXCHANGEABLE BASES AND ESSENTIAL MICRONUTRIENTS IN THE ZIMBABWE SUGAR INDUSTRY SOILS.

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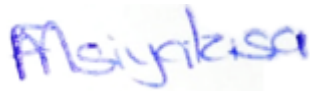
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APPROVAL FORM

The undersigned certifies that the project entitled **Evaluation of Mehlich-3 multi-extractant for the determination of exchangeable bases and essential micronutrients in the Zimbabwe sugar industry soils** was supervised, read, and recommended to the Bindura University of Science Education

Submitted by Fascinate Mateteni Siyakisa

 This work was submitted in fulfillment of the requirements of **a Bachelor of Science Honors Degree in Chemical Technology**

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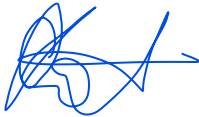**(***Student's Signature*)   (*Date*)

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ACKNOWLEDGEMENTS

DEDICATION

I dedicate this work to my first-born son Caleb Nenyasha Rambwawasvika.

ABSTRACT

The analysis of soil using single element extractants not only makes the analytical process long and expensive but also reduces the efficiency which comes with modern laboratory automation. This study was carried out to evaluate Mehlich-3 as a multi-element extractant for possible use in the determination of both soil exchangeable bases (calcium, magnesium, potassium, and sodium) and essential micronutrients (copper, iron, manganese, and zinc) in the Zimbabwe Sugar Industry soils. A total of 140 soil samples were collected across the Zimbabwe Sugar Industry covering Hippo valley, Triangle, Mkwasine, Mwenezana, and Zimbabwe Sugar Association Experiment Station. The soils were characterized for pH, texture, electrical conductivity, and organic matter. Exchangeable bases and micronutrients were extracted using conventional ammonium acetate and ethylenediaminetetraacetate extractants respectively and also by Mehlich-3 multi-element extractant. The extracted elements were quantified using Atomic Absorption Spectrometer for conventional methods and an Inductively Coupled-Plasma Optical Emission Spectrometer for the Mehlich-3 method. The Mehlich-3 method was validated by analyzing for accuracy, repeatability, intermediate precision, reproducibility, and robustness. There was a strong agreement between ammonium acetate and Mehlich-3 extractants on calcium, magnesium, potassium and sodium with R^2 values of 0.959, 0.934, 0.92,8 and 0.889 respectively. A fairly strong agreement was also observed between ethylenediaminetetraacetate and Mehlich-3 with R^2 values of 0.802, 0.835, 0.790 and 0.840 for micronutrients copper, iron, manganese, and zinc respectively. The method proved to be both repeatable and reproducible with relative standard deviations ranging from 0.72 % to 9.13 %. The recovery studies for accessing method accuracy ranged from 93% to 99%. Mehlich-3 proved to be competent and appropriate for extraction of all exchangeable base cations and essential micronutrients in the Zimbabwe Sugar Industry soils.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATION AND ACRONYMS

AGRILASA	Agri-Laboratory Association of Southern Africa
AMIS	African Mineral Standard (AMIS)
ANOVA	Analysis of variance
AR	Analytical Reagent grade
CRM	Certified reference material
EC	Electrical Conductivity
EDTA	Ethylenediaminetetraacetate
ESP	Exchangeable Sodium Percentage
FAAS	Flame Atomic Absorption Spectrometry
ICP-OES	Inductively Coupled Plasma - Optical Emission Spectrometry
LOD	Limit of detection
LOQ	Limit of quantification
M-3	Mehlich-3
MOP	Murite of Potash
OM	Organic Matter
Rpm	Rounds per minute
RSD	Relative Standard Deviation
SAR	Sodium Adsorption Ratio
SASRI	South African Sugar Research Institute
SD	Standard deviation
Std	Standard
UV-Vis	Ultra-violet Visible Spectrophotometer
ZSAES	Zimbabwe Sugar Association Experiment Station
ZSI	Zimbabwe sugar industry

CHAPTER 1: INTRODUCTION

1.1 Background

In agriculture, soil from the field is diagnosed for nutritional content by laboratory analysis to advise farmers on the appropriate types and quantities of fertilizers to be applied. Soil analysis is a long process that sometimes takes longer, causing farmers to delay farming activities. Analytical methods which are fast, convenient, and effective are requisite for improved sample turnaround time and allowing quick feedback to the farmers for profitable sugarcane farming. The conventional methods used for analyzing micronutrients and exchangeable bases took too long as they target a few nutritional elements each and thus needed several separate extractions. In the proposed Mehlich-3 (M-3), extraction is done once for the determination of both micronutrients and exchangeable bases thereby serving on time and resources.

Common classical soil extractants are ammonium acetate for the extraction of exchangeable bases and ethylenediaminetetraacetic acid (EDTA) for the extraction of micronutrients. Mehlich-3 is a multielement extractant used mostly for determining exchangeable bases (calcium, magnesium, potassium, and sodium), micronutrients (copper, iron, manganese, zinc,) and soil phosphorus (Zbíral, 2016; Fukuda et al., 2017; Seth et al., 2018). Multi-element extractants like Mehlich-3 have the advantage of increased throughput compared to classical extractants and they are also compatible with multielement instrument analyzers such as the Inductively Coupled Plasma Optimum Emission Spectrometer (ICP-OES). Although the method was first developed a long time ago by Mehlich, (1984), it had not been popular in developing countries, possibly due to the scarcity of multielement analyzers. Initially, the method was restricted to acidic soils until recently when it was also validated and approved for use in soils with neutral pH (Zhu et al., 2016).

The present study seeks to explore the possibility of using Mehlich-3 multiextractant for the simultaneous extraction of soil exchangeable bases (calcium, magnesium, potassium, and sodium) and micronutrients (copper, iron, manganese, zinc) in neutral to slightly alkaline soils of the Zimbabwe Sugar Industry (ZSI).

1.2 Problem statement

The soil extraction methods used in the Zimbabwe Sugar Industry (ZSI) for the determination of exchangeable bases and micronutrients are long and require more labour units. Mehlich-3 is a multi-extractant with the potential to reduce sample turnaround time and is compatible with both AAS and multi-element analyzer, ICP-OES. The purpose of this study is to evaluate M-3 extractant for routine use in the analysis of neutral to slightly alkaline soils of the ZSI.

1.3 Significance of the study

The current conventional extractants, ammonium acetate and EDTA target the separate extraction of exchangeable bases and micronutrients respectively while Mehlich-3 is a multielement extractant capable of extracting both at one goal. This results in reduced sample turnaround time and increased throughput. The new method will enable farmers to get their results in time, plan and budget for fertilizers requirements. Mehlich-3 has few procedures and is compatible with auto analyzers thereby reducing human errors. Classical extractants are laborious, cumbersome, and expensive as many chemicals and glassware are used.

1.4 Project Aim

To evaluate Mehlich-3 extractant on the determination of soil exchangeable bases and essential trace elements in the Zimbabwe Sugar Industry Soils.

1.5 Objectives

❖ To characterize the soils in selected sugarcane farms for texture, electrical conductivity, organic matter, and pH.

❖ To compare and establish the relationship between ethylenediaminetetraacetic acid (EDTA) and M-3 determined micronutrients (copper, iron, manganese, and zinc).

❖ To compare and establish the relationship between ammonium acetate and M-3 extracted exchangeable bases.

❖ To carry out Mehlich-3 method validation in the determination of micronutrients and exchangeable bases for possible routine use in sugarcane growing soils.

1.6 Delimitations

The research was conducted on sugarcane growing soils in the Zimbabwe Sugar Industry only. The sampling area covered sugarcane growing areas of Hippo valley, Triangle, ZSAES, Mwenezana and Mkwasine only.

CHAPTER 2: LITERATURE REVIEW

2.1 Sugarcane Plant nutrition

Sugarcane requires nutrition in the form of both macronutrients and micronutrients. Macronutrients are required in relatively higher amounts by the plant while micronutrients are required in trace amounts. Macronutrients are divided into primary elements (nitrogen, phosphorus and potassium) and secondary elements (magnesium, calcium and sulfur) (Janke et al., 2017; da Silva et al., 2020). There are also air and water-derived macronutrients (carbon, hydrogen and oxygen) obtained from the process of photosynthesis (Singh and Schulze, 2015). A combination of potassium, magnesium, calcium, and sodium can be referred to as exchangeable bases or base cations.

2.2 Exchangeable bases

The term exchangeable bases or base cations refers to cations of potassium, calcium, magnesium, and sodium. Potassium (K) is both an exchangeable base and macronutrient required by sugarcane in large quantities (McCray et al., 2017; Watanabe et al., 2019; de Andrade et al., 2021). It plays a key function in the activation of enzymes responsible for the metabolism of energy and nitrate reduction. Potassium helps in the development of strong sugarcane stalks, good root systems, and the synthesis of sugars (Ali et al., 2018).

Magnesium (Mg) is the sole metallic constituent of chlorophyll and is crucial for the process of photosynthesis. All chlorophyll molecules consist of magnesium ions at the center of their intricate structure. Although there are a lot of chemical similarities between magnesium and calcium, their behavior in plants and soil are distinct and important for soil and plant health. Exchangeable base imbalances (high Magnesium to Calcium quotients) cause calcium infertility in plants and thus the soil calcium and magnesium levels have to be monitored (van der Ent et al., 2019).

The presents of excess sodium in the soil are not good for plants due to its toxicity (Gonçalo et al., 2020). Excess sodium in soils causes the soil structure to collapse leading to increased runoff. The extent of soil sodicity is assessed in the laboratory by analyzing for sodium and calculating Exchangeable Sodium Percentage (ESP) or Sodium Adsorption Ratio (SAR). Most crops will not grow well in ESP values greater than 15 but optimum sugarcane growing requires soils of ESP below 10 (Dotaniya et al., 2016). Amelioration of sodic soils requires the infield application of lime, gypsum, or filter cake which contains calcium for replacing sodium (Leogrande and Vitti, 2019).

2.3 Micronutrients/ trace elements

Micronutrients or trace elements are critical for various metabolic processes in sugarcane and are required in trace concentrations from the soil (Titshall et al., 2018). In most cases, micronutrients are readily available in sufficient amounts for plant growth but deficiencies have been reported in some fields due to prolonged farming (Akenga, et al., 2014). The primary composition of soil micronutrients emanates from geochemical and pedochemical weathering subjected to parent rocks while the secondary composition consists of decayed plants and animals, natural waters, atmospheric gases, fertilizers, and pesticide remnants. Essential micronutrients for sugarcane growth are Iron (Fe), Copper (Cu), Boron (B), Manganese (Mn), Molybdenum (Mo), chlorides (Cl^-), and Zinc (Zn). The specific functions and symptomatic deficiencies of micronutrients Zn, Fe, Mn and Cu as described by Clowes and Breakwell, (1998), Titshall et al, (2017), and Calcino et al, (2018) are shown in Table2.1.

Table 2.1: Essential cationic micronutrients required by sugarcane crop, their functions, deficiency symptoms, and factors affecting availability in soil.

Trace element	Functions in plant	Deficiency symptoms	Factors affecting availability
Copper	• Enzyme activity in photosynthesis, respiration • Chlorophyll production • Metabolism of proteins and carbohydrates	• Rubbery stalks • Dark green patches on leaves • interveinal chlorosis in extreme cases the whole leaf becomes bleached • Reduced tillering and vigor • reduced internode elongation	• pH, texture • Organic matter.
Iron	• Chlorophyll production • Internal plant metabolism.	• Interveinal chlorosis along with the leaf blades • Severe cases the entire leaf becomes yellowish or white • Poor root development on stubble shoots	• High pH • Competitive inhibition with Mn in acid soils
Manganese	• Chlorophyll production • Respiration and nitrogen metabolism • Accelerates germination and maturity • Acts as a catalyst in plant processes	• Fraying and splitting of leaves in the wind. • Longitudinal pale-yellow bands from the middle to the tip of the leaf	• Require an optimum pH between 5 and 7.5
Zinc	• Plant growth regulation • Osmoregulation • Production of chlorophyll enzymes	• Stunted growth of the stool • Development of red fungal infection along with the yellowish bands in some sugarcane varieties. • Thin and elastic stalks • Appearance of broad veinal chlorotic bands on ether sides of the leaves with the leaf veins and midrib remaining green • Poor root growth	• Organic matter content • Texture • High soil pH • Liming

2.4 Common soil extractants

There are many extractant reagents targeting different components of the soil. In agriculture, important extractants are those which target available portions of the soil nutrient elements such as ammonium acetate, ethylenediaminetetraacetic acid, and Mehlich-3 as detailed below.

2.4.1 Ammonium acetate

Ammonium acetate has been commonly used for the extraction of exchangeable bases (also commonly known as soil cations) (Jaremko and Kalembasa, 2014). The availability of exchangeable base elements in the soil is influenced by their solubility and dissolution rate. Their availability is witnessed by changes in soil ionic activities over time. The direct measurements of soil ionic activity is time-consuming and the determination of exchangeable bases is an acceptable approximation that indicates available fractions for plant uptake. Neutral ammonium acetate is used to extract the base cations with the ammonium ions (NH_4^+) being exchanged into the soil colloids (Figure 2.1).
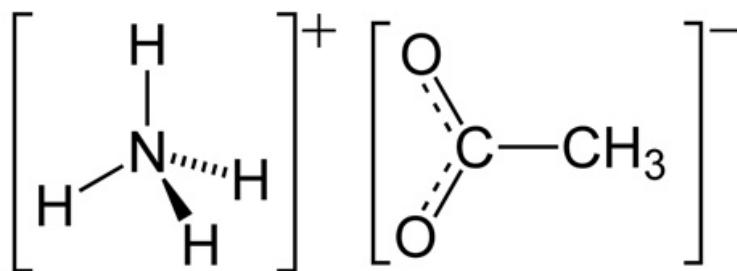
Figure 2.1: Structure of ammonium acetate used in the extraction of soil exchangeable bases

2.4.2 Ethylenediaminetetraacetic acid (EDTA)

EDTA is a common powerful extractant for micronutrients resulting from its chelating ability. The molecule is polydentate making it stronger in extraction. It is also used to remove toxic excess

micronutrients from the soil. The polydentate nature of EDTA reduces its selectivity in the extraction of micronutrients resulting in co-dissolution of other cationic elements. (Öztan and Düring, 2012; Jelusic and Lestan, 2014).
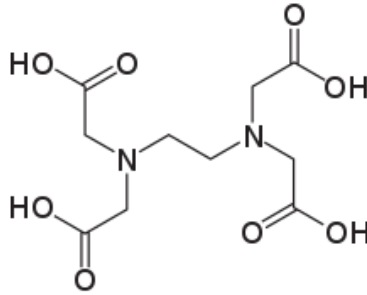
Figure 2.2: The structure of ethylenediaminetetraacetic acid used for the extraction of soil trace elements

2.4.3 Anion Exchange Resin method

The anion exchange resin method makes use of physical mediums called resins which facilitate ion exchange reactions for extracting soil elements. They are commonly used for the extraction of soil phosphates (Wuenscher et al., 2015). The reaction is reversible with dissolved phosphate ions removed from the soil solution and replaced with other ions of the same or similar electrical charge such as chlorides. The method is a good estimate of the rate of phosphate consumption by plants in diverse soil types (Freitas et al., 2013). In this method anion exchange resin and soil are suspended in water and shaken for 16 hours (overnight) after which phosphorus sobbed by resin is eluted and estimated (Fink et al., 2016).
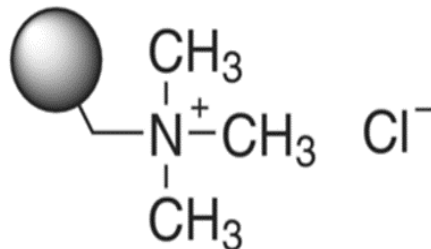
Figure 2.3: Structure of anion chloride resin used for the extraction of available phosphorus

2.4.4 Mehlich-3 extractant

Mehlich-3 (M-3) is a multi-extractant solution that extracts exchangeable bases, trace elements, and phosphorus at one goal (Seth et al., 2018). The use of M-3 was limited to acidic soils until recently when its applicability to neutral and slightly alkaline soils was discovered (Zhu et al., 2016). The method is most popular in developed countries due to its compatibility with highly automated instruments like the Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Mehlich-3 is a mixture of many reagents which target different soil nutritional elements as detailed in Table 2.2.

Table 2.2: Composition of reagents used in Mehlich-3 multi-extractant and their functions

Reagent	Function
NH_4NO_3	• Extraction of metal cations calcium, potassium, magnesium and sodium by replacement with NH_4^+ ion
NH_4F	• extraction of iron and aluminum phosphate with fluoride ion, and metal cations with NH_4^+ ion
HNO_3	• Extraction of calcium, phosphates • Acid component also extracts trace elements copper, zinc and manganese
CH_3COOH	• Acts as a buffering agent maintaining extractant pH below 2.9
EDTA	• Enhances the extraction of micronutrients copper, manganese, zinc, and iron

2.5 Instrumental analysis

2.5.1 Atomic Absorption Spectroscopy (AAS)

Atomic Absorption Spectroscopy (AAS) is a common analytical instrument used mostly for the quantification of metallic elements in solutions. The principle behind the operation of the instrument lies in the ability of elements to absorb light of specific wavelengths. Wavelength selection gives the instrument high specificity for the analysis of most elements (Paul et al., 2014). All samples should either be in aqueous or organic solution forms before analysis with solid samples having to through a dissolution process. The principle of an Atomic Absorption Spectrometer follows Beer-Lambert's law which states that absorption by elements is in proportion to the concentrations of metal elements present in the sample and calculated according to equation (2.1).

$$A = \varepsilon c l \qquad (2.1)$$

Where A= absorbance. ε = molar absorption coefficient, c = concentration of the element in solution, and l = path length of the sample (the path length of the cuvette which contains the sample).

During analysis, the sample is drawn into a flame where atomization into the gas phase occurs. Characteristic light of selected wavelength is shone on the element in the flame and the element absorbs in proportion to its concentration. (García and Báez, 2012). Hollow cathode lamps provide characteristic light for absorption by a specific element. Multielement lamps which can be used to determine more than one element are also available. The instrument can either be a double beam or a single beam. Quantification is achieved by preparing standards of known concentration which

are used to construct a calibration graph. Components of an AAS are shown on a block diagram in Figure 2.4.
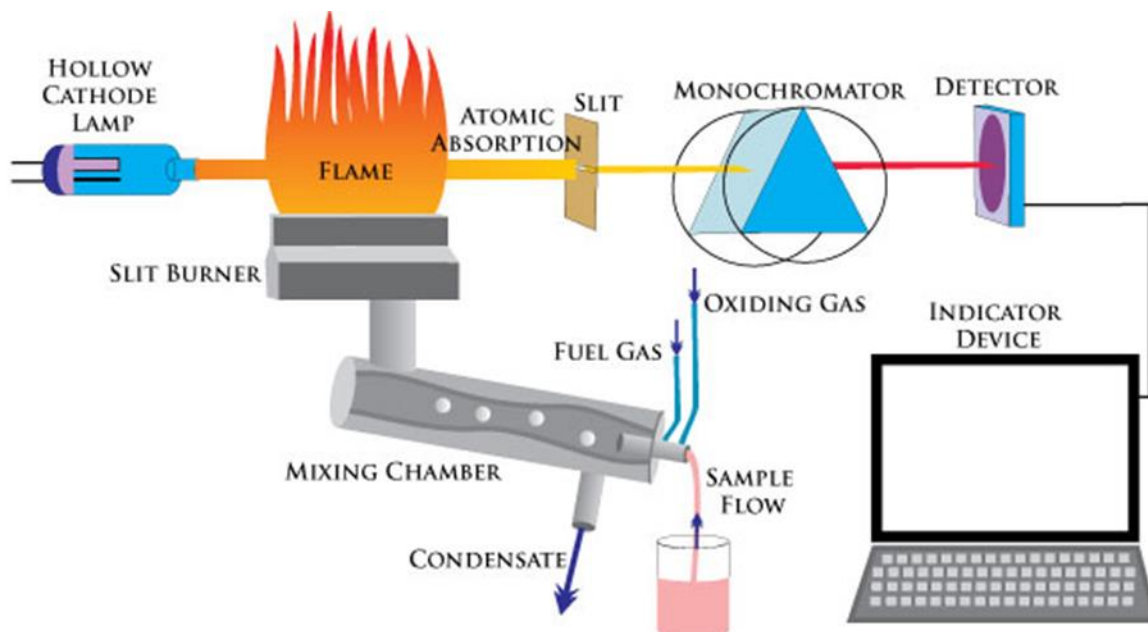
Figure 2.4: Block diagram of an Atomic Absorption Spectrometer.

(Source   https://chemistry.stackexchange.com/questions/115895/why-should-aas-use-element-lamps)

2.5.2 Inductively Coupled Plasma – Optical Emission Spectroscopy

The Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) is an analytical instrument for measuring the concentration of elements in a sample. It has several advantages over the AAS including low detection limits, high dynamic linear range, and increased throughput (Vanini et al., 2015). The instrument can be used to measure a majority of the elements in the periodic table. In an ICP-OES, argon plasma (the fourth state of matter composed of argon gas, argon ions and electrons) generates very high temperatures sometimes reaching 10 000 kelvin (Novaes et al., 2016). The energy is used to excite electrons of atoms and ions in a sample from their ground state. On their transition back to the ground state, the excited atoms release light

energy characteristic of the specified wavelength. The intensity of light energy released is a measure of the concentration of the specific element in the sample while the wavelength determines the type of element. Unlike AAS, ICP-OES has several spectral lines which can be chosen depending on perceived interference (Elango et al., 2021).

The liquid sample is introduced into the instrument by a peristaltic pump into the nebulizer where it is mixed with argon gas to produce a fine aerosol. The aerosol is directed into a spray chamber where heavy droplets are eliminated into the waste drain while the fine aerosol is directed into the plasma torch. Plasma heat is generated by an electric field around an induction coil just above the torch. On top of the plasma torch, plasma heat evaporates the sample solvent and breaks the sample into atoms and ions, and simultaneously excites them. Upon dropping to ground level, the emission spectrum produces photons which are measured by the spectrometer detector (Figure 2.5).
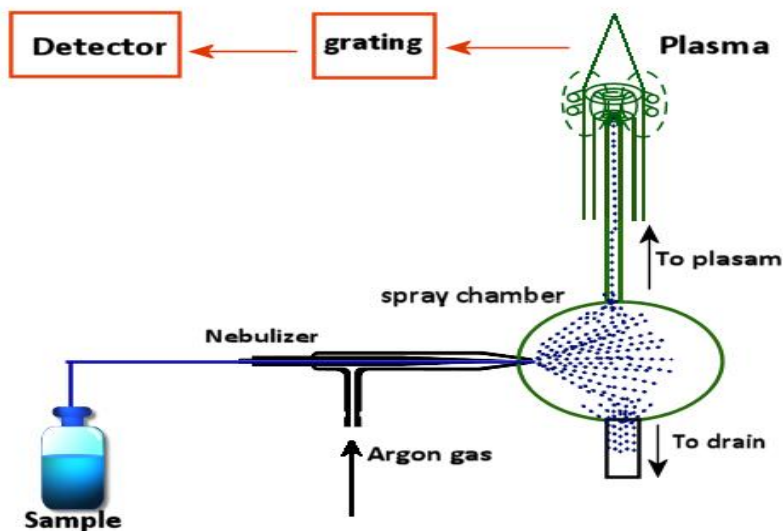
Figure 2.5: Schematic diagram showing the movement of the sample during analysis with an ICP-OES *(Source https://lab-training.com/which-is-a-better-choice-icp-oes-or-icp-ms/)*

2.6 Method Validation

Analytical method validation is done to examine and evaluate the suitability of a method for its intended purpose. The purpose will be to have confidence in the results obtained by the method. Analytical method validation is done to new methods or modified methods (commonly referred to as method verification. Revalidations can also be done when a new situation arouse in the laboratory such as changing machinery or reagents (Ravisankar et al., 2015). A schematic description of validation parameters tested on a method is shown in Figure 2.6.
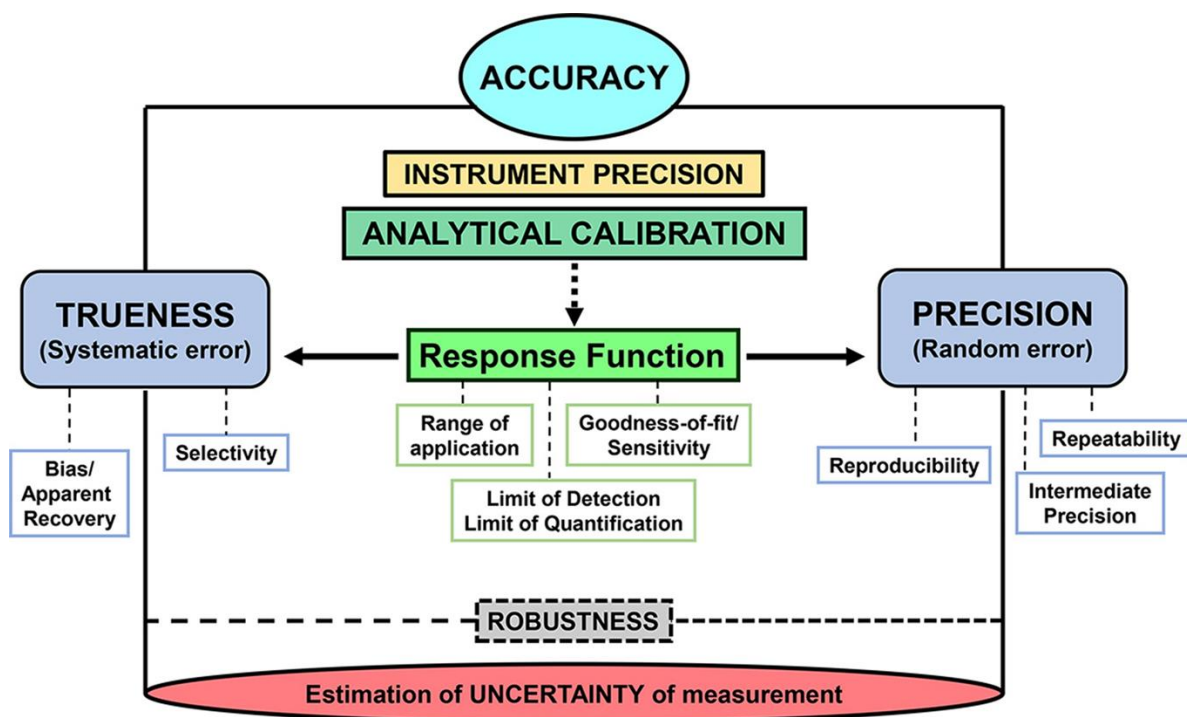
Figure 2.6: Summary of analytical method validation parameters (Raposo and Ibelli-Bianco, (2020)

Accuracy

The method accuracy refers to the closeness of an obtained result (actual concentration) to the actual value (theoretical concentration). It is an assessment of the difference between the measured

value and the real value. Accuracy determines the total error of analysis. Recovery studies are normally used to determine accuracy such that accuracy and recovery can be used interchangeably (Lavanya et al., 2013). By definition, recovery is defined as how much was recovered from the initial concentration using the purposed method and is usually expressed as a percentage.

Precision

The precision of a method is the measure of the nearness (closeness) of agreement among several experimental runs obtained from multiple sampling of a homogenous population under a similar analytical environment (Raposo and Ibelli-Bianco, 2020). Precision is measured in three categories which are reproducibility, intermediate precision, and repeatability.

Repeatability measures precision in the same operating environment, and same analyst in a short time frame (Ravisankar et al., 2015). In repeatability studies, the same analyst can repeat an analytical measurement of the same sample at least nine times without changing the environment. Results are often expressed as percentage relative standard deviation (RSD %) or standard deviation (SD). The smaller the RSD % or the SD, the greater the repeatability and the better the method. Intermediate precision is measured by testing the precision of the method after changing parameters like analyst, time, or instrument. The results are also collated and reported as RSD %.

Reproducibility measures the precision of the method in different laboratories. The laboratory can prepare a big homogenous sample and send portions to other participating laboratories together with the procedure. Reproducibility results can also be expressed as RSD % (Raposo and Ibelli-Bianco, 2020).

Linearity Range:

The method's linearity refers to the analyte concentration range where an increase in concentration results in a proportional increase in signal/ response. It is a range of concentrations from minimum to maximum which has been established to show linearity within acceptable precision and accuracy (Rao, 2018). A range of standard alone or spiked in sample matrix can be used to determine linearity range.

Robustness:

Robustness is a measure of the method's ability to withstand small deliberate alterations introduced during its execution. It is a measure of the method's ability to give accurate results despite the small and deliberate changes. The small deliberate changes can range to changes in shaking speed, shaking time, or operating temperature.

Specificity and selectivity:

The method's specificity refers to the unequivocal ability to make accurate measurements in the presence of matrix components (Okur et al., 2016). The matrix should be constituted of substances that are commonly present together with the analyte. A method with good specificity will result in its matrix not amplifying or reducing the signal. On the other hand, selectivity refers to the method's ability to measure the analyte when mixed with other components in general (Sonawane et al., 2014). In some literature, specificity and selectivity were used to refer to the same thing but they are different.

Limit of detection

The limit of detection (LOD) is the analyte's lowest concentration which is identifiable and detected within a given degree of certainty (Ravisankar et al., 2015). It can also be defined as the

lowest concentration distinguishable from the background noise with a definite degree of confidence (Lavanya et al., 2013). Common methods of determining LOD made use of instrument blank readings and sometimes the evaluation of signal to noise ratios. LOD can be affected by minor changes in the analytical system like instrumental conditions, temperature, purity of reagents, and many more

Limit of quantification

Limit of Quantification (LOQ) refers to the lowest concentration which can be quantified with acceptable accuracy and precision by a method (González et al., 2014; Sonawane et al., 2014). Like LOD, LOQ can be determined based on blank measurements or signal-to-noise ratios.

Inter-laboratory proficiency testing

Proficiency testing or inter-laboratory precision is an exercise done by laboratories to measure their analytical competencies by analyzing the same portions of a sample and comparing their results (Chakravarty et al., 2016). Normally there will be an administrator who coordinates the preparation of samples and ensure that the samples are sent to participating laboratories within a specified period. After analysis, the results are sent to the administrator who will compute the results for any deviations from the expected reference value and send the results back to the laboratories. There are two ways commonly used for determining the reference value; the first one being the use of a reference laboratory and the use of averages for acceptable results (after removing outliers). Laboratories chosen to give reference values are those accredited for the measurand by ISO/IEC 17025 (Szewczak et al., 2016).

The laboratory participated in the Agri-laboratory Association of Southern Africa (AgriLASA) proficiency testing scheme which is coordinated and headquartered in South Africa. The

proficiency testing scheme specializes in the analysis of leaf, water, soil, fertilizers, and feed samples. A total of 60 laboratories in Southern Africa participate in various disciplines. The ISO/IEC 17025 emphasises the need to participate in proficiency testing schemes before a laboratory can be accredited (Chakravarty et al., 2016).

CHAPTER 3: MATERIALS AND METHODS

3.1 Reagents

All chemicals used were of analytical grade (AR) and the following were obtained from Merck:

Ethylenediaminetetraacetic acid disodium salt (disodium EDTA), potassium dichromate (iv), ammonium acetate, ammonium nitrate, ascorbic acid, Sodium hydroxide,4-nitrophenol indicator, sulphuric acid, ammonium vanadate, calcium chloride, iron ferrous sulfate, ammonium molybdate, ammonium acetate, Diphenylamine-2-carbons are and sodium hexametaphosphate, ammonium fluoride.

Certified Reference Materials (CRM) for copper, zinc, iron, magnesium, sodium, potassium, calcium, and manganese were obtained from Spectrascan (South Africa), Bromocresol green, methyl red, lanthanum nitrate, hydrogen peroxide were obtained from Glassworld.

3.2 Apparatus and Instruments

- Atomic Absorption Spectrometer (AAS) Varian Spectra AA50 for the determination of exchangeable bases and micronutrients using classical extraction methods.

- Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) for the analysis of micronutrients and exchangeable bases using the M-3 method.

- Orbital shaker (Tecnal TE-145, Brazil) for shaking samples during extraction.

- Mettle Toledo Seven excellence multiparameter was used for the measurement of soil electrical conductivity and pH.

- Mettler Toledo analytical balance (XPE 504, maximum weight 540 g) for weighing soil samples.

- Dutch auger was used for taking soil samples to a depth of 0- 30 cm

- Soil hydrometer (S 300) was used for the determination of soil texture

- Mill for crushing soil lumps (hard clays) (Tecnal TE-330, Brazil).

- Wooden mallet and try for crushing alluvial soils.

3.3 Study area

This research was conducted in the sugarcane growing soils of the South Eastern Lowveld of Zimbabwe covering Triangle (21.02433º S, 3144536º E), the Hippo Valley (21.15736º S, 31.54139º E), Mkwasine (20.80851º S, 31.88138º E), ZSAES (21.03594º S, 31.61731º E) and Mwenezana (21.42289º S, 30.72369º E). The place is in the Ecological Region (V) of Zimbabwe which experience very low rainfall averaging 450 mm per annum and very hot summers with mean maximum daily temperatures of 30 ºC and a minimum of 8 ºC. Due to low rainfall in this region, sugarcane is grown under canal irrigation. A total of 140 soil samples representing the sugarcane growing soils were collected randomly in Mkwasine (16), Triangle Estate and private growers (48), Mwenezana (21), Hippo Valley Estate, and private growers (50), and ZSAES (15) from November 2020 to March 2021. The map showing sugarcane growing areas is shown in figure 3.1
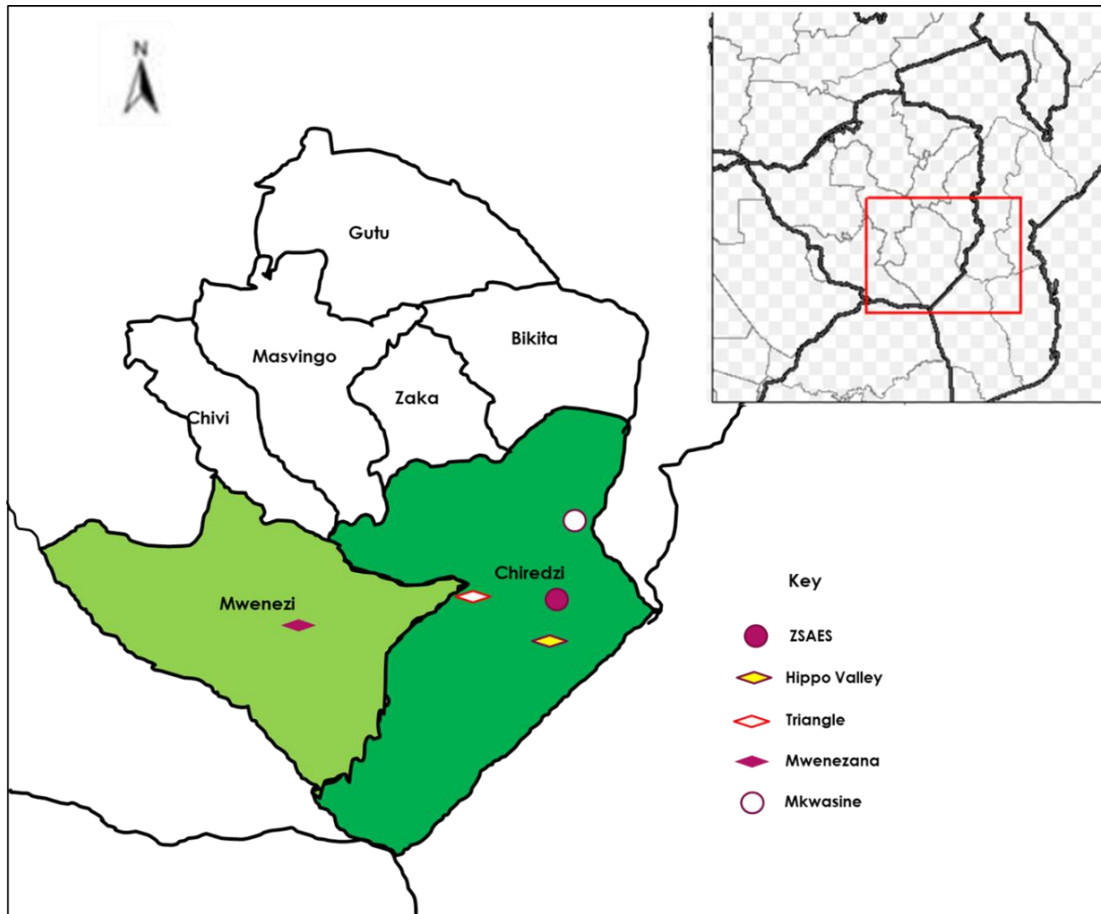
Figure 3.1: Map showing the sugarcane growing areas in Mwenezi and Chiredzi in the South Eastern Lowveld of Zimbabwe

3.4 Soil sampling and sample preparation

Soil samples were collected from ZSAES, Triangle estate, and Hippo Valley estates. Samples were collected from a depth of 0-30 cm using a Dutch auger. 30 cores were taken in a 1-hectare field as illustrated in the following diagrams

a) Field with cane rows

b) On plough out fields / virgin fields

Key

Cores ● Field ▨
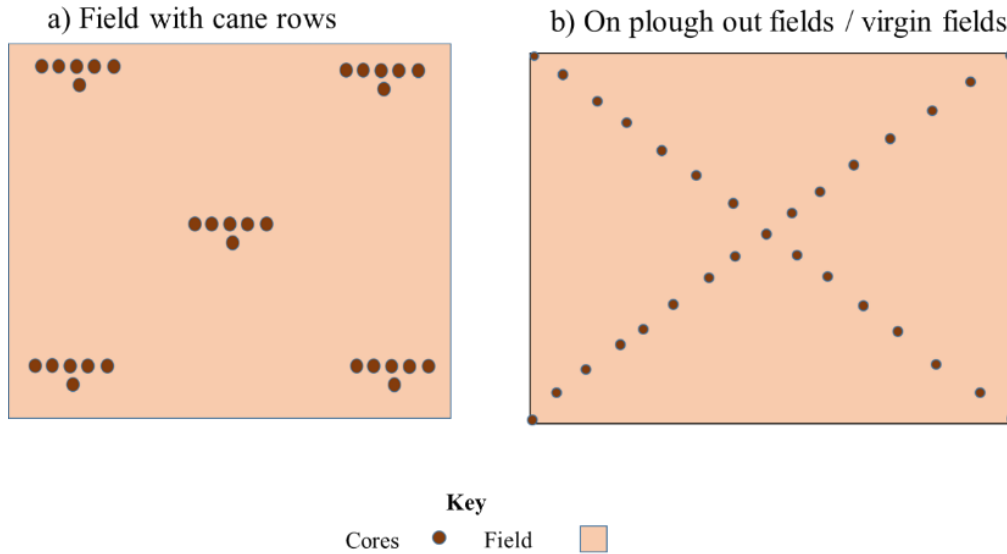
Figure 3.2: Sampling plan for fields with cane rows (a) and plough out field (own creation)

The soil samples were air-dried to drive away moisture. After drying the samples were sieved using a 2 mm sieve aperture to get the fine soil particles for the analysis of exchangeable bases and micronutrients. Each soil sample was placed in well-labeled plastic bags.

3.5 Soil characterization

The soils from across the ZSI were characterized for EC, texture, pH, organic matter, and (OM) according to the methods described below.

3.5.1 Electrical Conductivity (EC) determination

Electrical conductivity (EC) was measured on each of the prepared soil samples. A 10 g of the soil sample was weighed and placed into a 500 ml plastic bottle. A solution of 50 ml of deionized water was then added to the measured soil sample and the bottles were closed tightly. The samples were placed on an orbital shaker for an hour, shaking at 120 rounds per minute (rpm). After an hour of shaking the sample were taken for readings using the EC meter.

3.5.2 pH determination

Soil pH was measured on all samples using the 0.01 M CaCl$_2$ scale (Rambwawasvika et al., 2021). A 10 g portion of each sample was weighed and placed into a 500 ml plastic bottle followed by the addition of a 50 ml solution of 0.01 M CaCl$_2$. The bottles were closed tightly and the samples were shaken on an orbital shaker for an hour at a speed of 120 rpm. After an hour of shaking the sample were taken for readings using the pH meter

3.5.3 Organic matter (OM) determination

Organic matter (OM) was determined using the Walkley-Black chromic acid oxidation method described by Gelman et al. (2012). A 1 g portion of the soil sample was weighed and placed in a clean conical flask followed by the addition of 20 ml of 1 N K$_2$Cr$_2$O$_7$ solution was added to the measured soil sample. Also, 25 ml of concentrated sulphuric acid were added to the sample and left to boil for a minute using a hot plate. The sample was left to cool before the addition of 250 ml of deionized water. Approximately 3 drops of N- phenylanthranilic acid indicator were added and the solution was titrated with 0.4 N ferrous sulphate on a back titration to determine the unreacted 1 N K$_2$Cr$_2$O$_7$. Blank determination was done by mixing all the reagents procedurally but without the soil sample.

3.5.4 Soil texture determination

The soil texture was analyzed by the Bouyocus hydrometer method described by Huluka and Miller, (2014). A mass of 50 g soil sample was mixed with 5 ml of dispersing agent-Calgon (40 g sodium hydroxide + 100 g hexametaphosphate) and 200 ml of water. The suspension was hand shaken and left overnight followed by a further 30 minutes of shaking at 120 rpm on a mechanical shaker. The shaken suspension was poured into a 1-liter measuring cylinder which was filled with deionized water and the density was determined immediately using a soil hydrometer. The sample

was agitated with an agitating rod to allow mixing and the first hydrometer reading was taken within 5 minutes. The highest and lowest temperature readings of samples (a batch of 40 samples) were recorded. The first hydrometer and temperature values were used to calculate the silt and clay composition of the soil. The hydrometer and thermometer readings were repeated after 4 hours and 55 minutes to determine soil clay content. The first and second readings were combined to calculate sand and silt composition. The compositions were used to determine soil texture on a textural triangle.
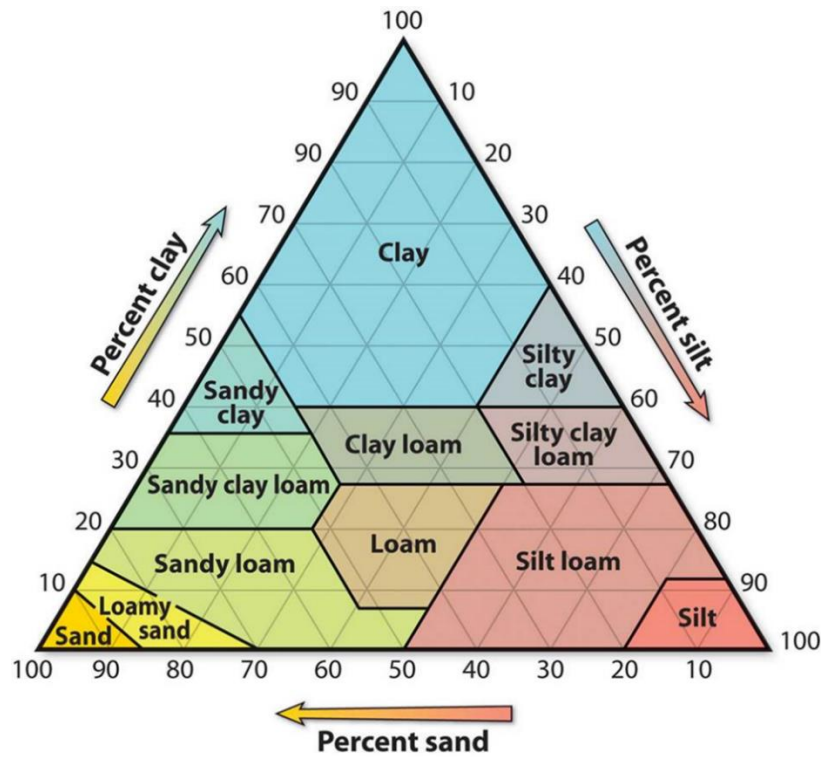
Figure 3.3: Textural triangle used to determine soil texture

3.6 Extraction and analysis of exchangeable bases using ammonium acetate

Extracting solution (1 M ammonium acetate) made by mixing in equal volumes concentrated ammonia and acetic acid in a 1:1 v/v ratio followed by adjusting the pH to 7 using either acetic acid or ammonium solution. A mass of 2.5 g strontium chloride releasing agent was added to the solution. Extraction of exchangeable bases was done by measuring 10 g of soil sample into a glass

shaking jar and then adding 50 ml of extracting solution. The suspension was shaken for an hour on a Tecnal TE-145 orbital shaker made in Brazil at 120 rpm. The suspension was filtered using filter paper Whatman No 91 (15 cm diameter) followed by reading the concentration on an AAS (Varian Spectra AA50). Working standards for making calibration curves were prepared by diluting 1000 ppm Spectroscan Certified Reference Material (CRM) of each of the exchangeable bases (calcium, magnesium, potassium, and sodium) to desired concentrations. The standard blank constituted the extracting solution only (ammonium acetate).

3. 7 Extraction and analysis of micronutrients using classical method (EDTA)

Micronutrients were extracted with a 1% EDTA solution made by dissolving 10 g of the disodium EDTA salt in a 1000 ml volumetric flask and diluting to the mark with deionized water. A mass of 5 g soil sample was mixed with 50 ml of 1% EDTA in a clean 250 ml shaking bottle. The suspension was shaken for an hour on an orbital shaker at 120 rpm. The suspension was filtered through Whatman No 542-filter paper and analyzed for micronutrients on a Varian Spectra AA50 AAS. Calibration standards for the determination of micronutrients were made by diluting 1000 ppm CRM stocks of micronutrients zinc, copper, iron, and manganese. Sample calibration curves for micronutrient analysis are shown in Appendix A.

3. 8 Analysis of exchangeable bases and micronutrients using Mehlich-3 extraction

Mehlich-3 extractant was prepared according to the method described by Ziadi and Tran, (2008). Stock solution A was made by dissolving 138.9 g of ammonium fluoride (NH_4F) with 600 ml of deionized water in a 1000 ml volumetric flask and then adding 73.5 g EDTA. The resulting solution was shaken and diluted to the mark with deionized water. The Mehlich-3 extractant was made by dissolving 200.0 g ammonium nitrate (NH_4NO_3) in about 6,000 ml of deionized water followed by the addition of 40 ml of solution A. The resulting solution was thoroughly mixed followed by

the addition of 115 ml of concentrated glacial acetic acid (CH₃COOH) and 8.25 ml of concentrated

nitric acid (HNO_3). The total final volume was brought to 10 liters and the final pH was adjusted

to 2.50 ± 0.05 using ammonia or acetic acid.

A mass of 4 g soil sample was weighed into a 250 ml shaking jar followed by the addition of 40

ml M-3 extractant. The suspension was shaken on an orbital shaker for 5 minutes at a speed of 120

rpm followed by filtration with a Whatman No 2-filter paper. The filtrate was analyzed for both

exchangeable bases and micronutrients using ICP-OES. Mixed calibration standards were made

by diluting stock CRM for each element as shown in Table 3.1. Sample calibration curves are

shown in Appendix A.

Table 3.1: The concentration of individual elements in a mixed standard for determination of calibration curve on ICP-OES

Elements	The concentration of standards (ppm)				
	Std 1	Std 2	Std 3	Std 4	Std 5
Calcium	3.75	7.50	15.00	30.00	60.00
Magnesium	1.25	2.50	5.00	10.00	20.00
Potassium	0.63	1.25	2.50	5.00	10.00
Sodium	0.31	0.63	1.25	2.50	5.00
Copper	0.63	1.25	2.5	5.00	10.00
Iron	1.8	3.75	7.50	15.00	30.00
Manganese	3.13	6.25	12.50	25.00	50.00
Zinc	0.63	1.25	2.50	5.00	10.00

Where Std is shorthand for standard

3.9 Proficiency testing

For proficiency testing results, homogeneous samples were generated by African Mineral Standard (AMIS) and distributed by the proficiency scheme coordinator called Agri-Laboratory Association of Southern Africa (AGRILASA) to participating laboratories in Southern Africa. Results from the 8 laboratories which participated using Mehlich-3 were sent to AGRILASA and shared with all participating laboratories. Z scores were calculated using equation 3.1.

$$Z\ score = \frac{x - \mu}{\sigma} \qquad\qquad 3.1$$

With x, μ, and σ representing the laboratory result, the expected result and standard deviation of all acceptable results from different laboratories respectively.

Interpretation of Z score as done by AGRILASA proficiency testing scheme is given in Table 3.2 below.

Table 3.2: AGRILASA Z score thresholds and their interpretation

Z score	Interpretation	Colour code
$-2 \leq Z \geq 2$	Satisfactory z/z' score	Not highlighted
$-3 < Z < -2$ or $3 > Z > 2$	Questionable z/z' score	Orange highlight
$-3 \geq Z$ or $3 \leq Z$	Unsatisfactory z/z' score	Red highlight

3.10 Quality control

The quality of results was assured by periodically analyzing the CRM of exchangeable base cations and micronutrients. Every batch of 50 samples constituted three check samples whose results were known. If the results of check samples were not within the expected range, the results were

disqualified and the analysis repeated. The analysts made use of repeat samples (samples previously analyzed) to check for any changes or deviations. Reagent blanks were also used to check the matrix effect when reading samples on instruments. Blank samples were read after every 10 samples to check for instrument drift.

3.11 Validation of Mehlich-3 method

The Mehlich-3 method was validated by analyzing parameters accuracy, precision (repeatability, intermediate precision, and reproducibility), robustness, dynamic linear range, LOD, and LOQ as detailed below.

3.11.1 Accuracy

The method's accuracy was tested using recovery studies (Voica et al., 2012). For each element, five standard concentrations for each element were spiked on homogenized 4 g soil samples whose concentrations for the element studied were predetermined. The samples were air-dried and analyzed to get the recovery concentrations. Recovery percentages were calculated using equation 3.2.

$$Recovery\ (\%) = \frac{obtained\ concentration\ (ppm)}{Spiked\ concentration\ (ppm)} * 100 \qquad (3.2)$$

Results were expressed as average recoveries for all the spiked concentration ranges ± standard deviation. Concentrations of spiked standard elements are shown in Table 3.3.

Table 3.3: Concentrations of single element Certified Reference used for spiking soil samples on recovery studies

	Spiked concentration for exchangeable bases (ppm)				Spiked concentration micronutrients (ppm)			
Standards	Ca^{2+}	K^+	Mg^{2+}	Na^+	Cu^{2+}	Fe^{2+} /Fe^{3+}	Mn^{2+}	Zn^{2+}
1	20	20	20	20	2	2	2	2
2	40	40	40	40	4	4	4	4
3	60	60	60	60	6	6	6	6
4	100	80	100	80	8	10	10	8
5	200	100	200	100	10	20	20	10

3.11.2 Repeatability, inter-laboratory precision, and reproducibility

The Mehlich-3 method repeatability was assessed by making 10 homogeneous representative samples of 4 g each and analyzed for all the concerned elements by the same analyst under the same conditions. The results were used to calculate RSD which was used as a measure of repeatability of the results. RSD values below 10 % were favorable.

Inter-laboratory precision studies were conducted by making 30 homogenous portions of the same sample and distributing it to 3 experienced laboratory analysts. Each analyst got 10 samples for analyzing both exchangeable bases and trace elements. The analysts were given pseudo names Analyst A, Analyst B, and Analyst C.

In reproducibility studies, homogeneous samples were generated by African Mineral Standard (AMIS) and distributed by the proficiency scheme coordinator called Agri-Laboratory Association of Southern Africa (AGRILASA) to eight laboratories in Southern Africa. Results from the lab were sent to AGRILASA and shared with all participating laboratories. The RSD % was calculated from all results from the 8 laboratories using equation 3.3.

$$RSD\ (\%) = \frac{standard\ deviation\ (SD)}{mean} * 100 \qquad (3.3)$$

3.11.3 Robustness

Small deliberate changes to test the method's robustness were changing the shaking time from 5 to 4 minutes, changing the shaking speed from 120 rpm to 115 rpm, and changing the filter paper from Whatman Number 2 to Whatman Number 91. Each change in parameter was done on 10 samples with one parameter being changed at a time while others were kept constant.

3.11.4 Limit of Detection and Limit of Quantification

The l of detection (LOD) and limit of quantification (LOQ) for each element were evaluated by taking 10 readings of the reagent blank and calculating their standard deviation (SD). LOD and LOQ calculations were done using equations 3.4 and 3.5 respectively

$$LOD = 3.3 * \frac{\sigma}{S} \qquad (3.4)$$

$$LOQ = 10 * \frac{\sigma}{S} \qquad (3.5)$$

Where σ = SD and S = slope of the calibration curve

3.12 Statistical Analysis

Where applicable, experiments were done in triplicates (n = 3) and the results were expressed as mean values ± standard deviation (SD). Descriptive statistics (mean, minimum and maximum values) were used to describe the data. Student T-test was used to compute differences between two groups ($P < 0.05$ was considered significant). Proficiency testing results were reported as Z scores. Method validation results for repeatability, reproducibility, intermediate precision, and

robustness were measured by calculating the relative standard deviation (RSD %). All computations and graphing were done using GraphPad Prism 8 and Microsoft Excel 2016 software.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Physical and chemical characterization of soils used in the study

The total number of soils used in this study were 140. These soils were taken from Mwenezana, Hippo Valley, Mkwasine, ZSAES, and Triangle. Soil pH ranged from 5.01 to 10.13 with an average of 6.70 (measured on 0.01M calcium chloride electrolyte). Soil electrical conductivity (EC) ranged from 15.00 to 216.00 µS/cm giving an average of 97.35 µS/cm. Organic matter (OM) measured from 0.78 % to 3.75 %. Various soil textural classes were used with clay content ranging from 8 to 51 %. A summarized table of the description of soils used in the study is shown in Table 4.1.

Table 4.1: Physicochemical characteristics of soils used in the study

Parameter	N	Minimum	Maximum	Mean	Median
pH	140	5.01	10.13	6.70	6.70
EC (µS/cm)	140	15.00	216.00	97.35	63.00
OM (%)	140	0.78	3.75	1.76	1.70
Sand (%)	140	31.00	85.00	66.32	69.00
Clay (%)	140	8.00	51.00	20.98	19.00
Silt (%)	140	3.00	35.00	12.63	11.74

Calcium levels across the industry soils ranged from 454 to 8950 ppm and averaged 2775.08 ± 2066.46 ppm and 2734.21 ± 2185.41 ppm for ammonium acetate and Mehlich-3 extractants respectively. Magnesium concentrations ranged from 124 ppm to 2442 ppm giving averages ± SDs of 608.25 ± 506.43 and 640.32 ± 401.51 ppm for ammonium acetate and Mehlich-3 respectively.

Based on average values, ammonium acetate determined potassium seems to be slightly higher than that of Mehlich-3 with averages ± SDs of 158.22 ± 94.35 ppm and 151.23 ± 86.16 ppm respectively (Figure 4.1). The differences however were not statistically significant and not constant for all the samples making it impossible to make recommendations based on them. A total of 58 samples had potassium levels below the threshold concentration of 136.5 ppm and fertilizer recommendations were given as murite of potash (MOP) to compensate for every shortfall. Sodium concentrations across the industry ranged from 2 to 1915 ppm with all samples having Exchangeable Sodium Percent (ESP %) within the recommended level below 10 % (Clowes and Breakwell, 1998). Average concentrations of exchangeable base elements across the whole industry are shown in Figure 4.1.
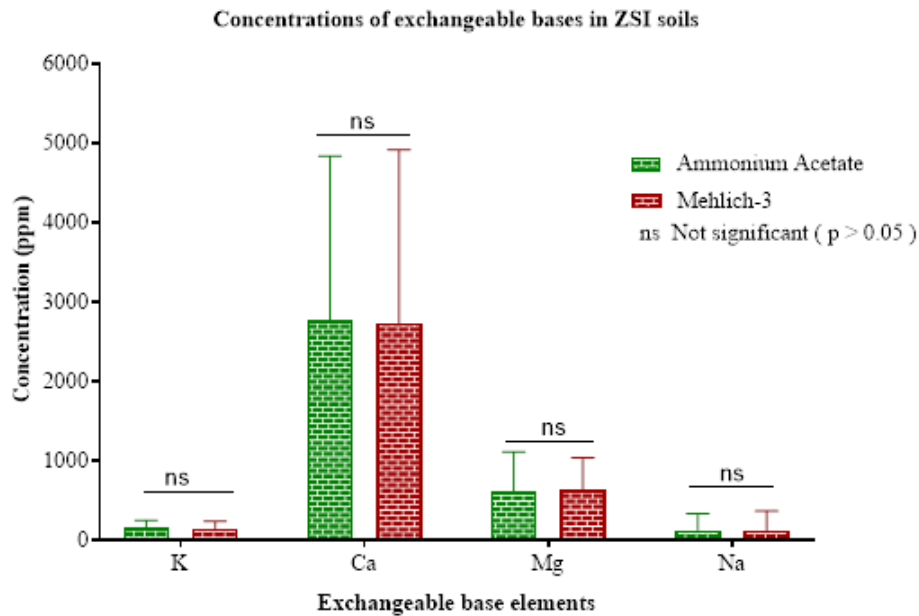
Figure 4.1: Average concentration of exchangeable base elements (K, Ca, Mg and Na) across the Zimbabwe Sugar Industry soils. Results were reported as mean values ± standard deviation of 140 samples. Results are graphed as mean values ± SD. Not significant (ns), $p > 0.05$ when comparing the conventional ammonium acetate and Mehlich-3 methods using the student t-test.

For micronutrients, the samples had higher concentrations of manganese averaging 190.06 ± 77.20 ppm and 197.56 ± 77.93 ppm as determined using EDTA and Mehlich-3 extractants respectively. There was also an abundance of iron across the soils ranging from 25.20 to 215.50 ppm and averaging 121.39 ± 28.43 ppm by the Mehlich-3 method. Average calculations indicated that Mehlich-3 determined micronutrients were generally higher than the conventional EDTA for elements zinc, and copper by 1.08 and 1.05 times respectively. EDTA determined manganese and iron were also slightly higher than those of Mehlich-3 by 1.14 and 1.11 times respectively. The differences were not consistent for all the samples not statistically significant at a 95 % confidence interval when computed using Student's t-test and could not be used to give any nutritional recommendations (Figure 4.2).
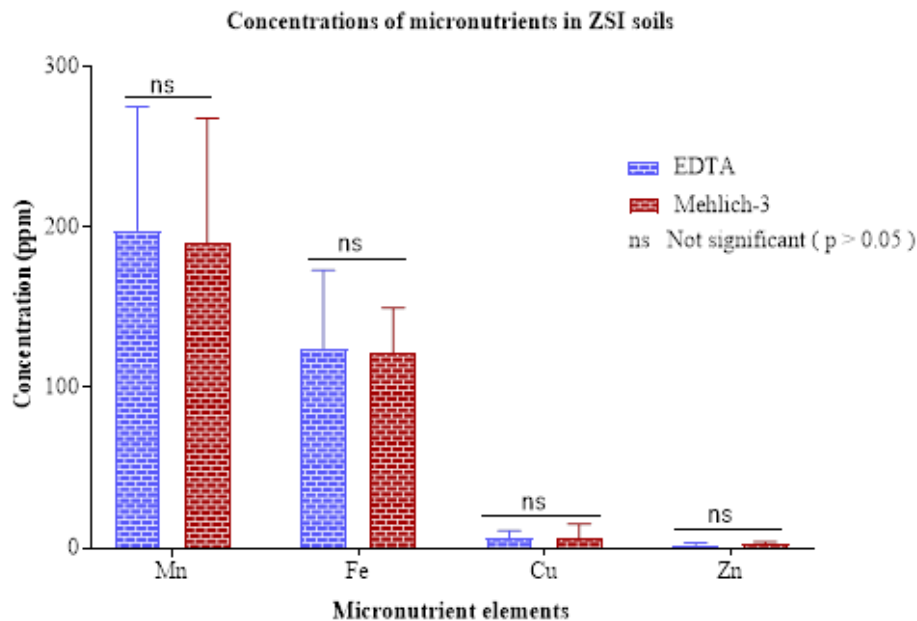
Figure 4.2: Average concentration of micronutrients (Mn, Fe, Cu and Zn) across the Zimbabwe Sugar Industry soils. Results were reported as mean values ± standard deviation of 140 samples. Results are graphed as mean values ± SD. Not significant (ns), $p > 0.05$ when comparing the conventional EDTA and Mehlich-3 methods using the student t-test.

Micronutrients Cu and Zn are found in very low concentrations requiring highly sensitive instruments like the ICP-OES for precise and accurate analysis (Hall et al., 2021). The Mehlich-3 method is arguably the most accurate as it used a highly sensitively ICP-OES method unlike AAS used for the conventional EDTA method. Despite not having micronutrient thresholds recommendation for its sugarcane cultivars, there were 15 samples (10.7 %) with zinc concentrations below the South African Sugar Research Institute (SASRI)'s critical concentration of 1.5 ppm as given by Titshall et al., (2018). There were also 3 samples (2.1 %) with copper below the critical concentration of 0.80 ppm as given by SASRI.

4.2 Relationship between Mehlich-3 (M-3) and ammonium acetate determined soil exchangeable bases

Generally, there were strong linear positive relationships between M-3 and ammonium acetate (old conventional method) determined exchangeable bases with R^2 values of 0.928, 0.959, 0.934, and 0.889 for potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) respectively. All R^2 values were significant with $p < 0.001$ and thus making M-3 an appropriate alternative to the ammonium acetate method. The findings concur with those of Fukuda et al., (2017) on Mozambiquan soils except that their results for sodium were not conclusive. Attention should be given to avoiding sodium borosilicate glassware as it can leach out sodium to the analytical solution due to the acidity of the M-3 extractant. Where precise and accurate conversions between Mehlich-3 and Ammonium acetate (AA) exchangeable bases are necessary, equations given on the graphs can be used (Figure 4.3).
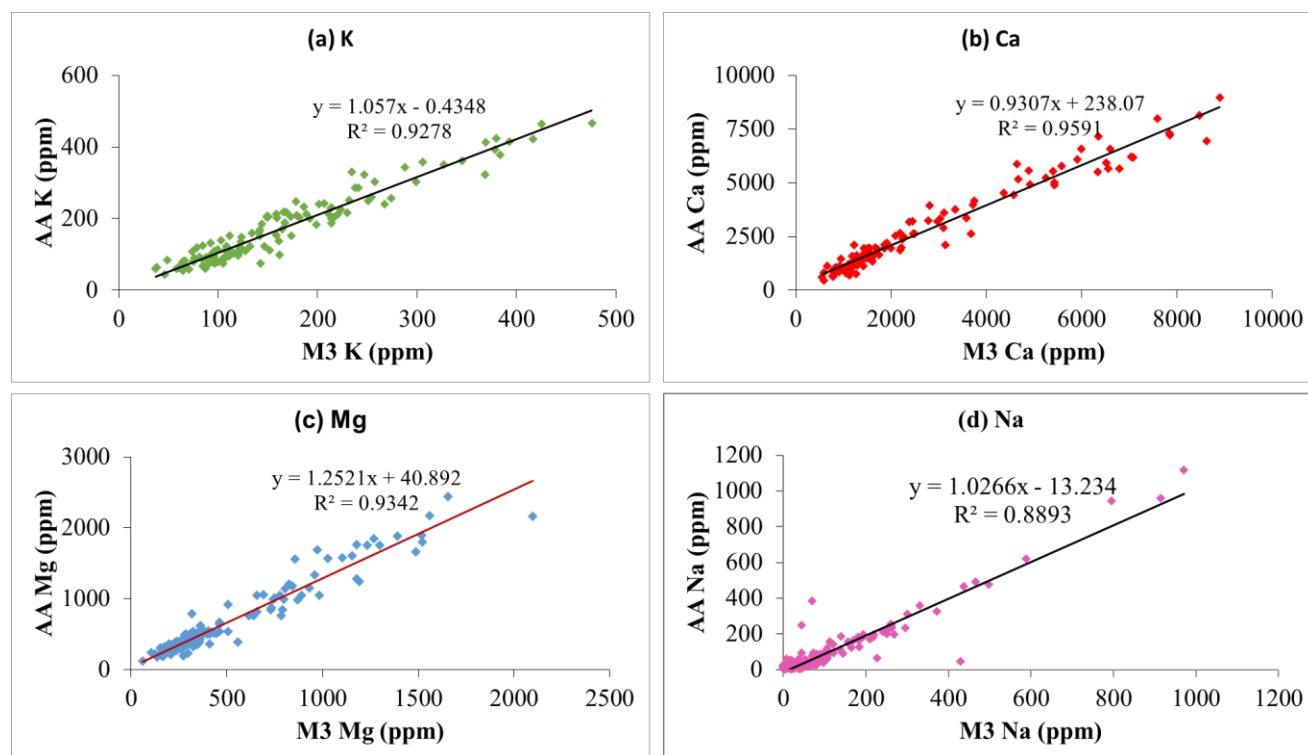
Figure 4.3: Regression graphs and equations for the relations between Mehlich-3 (M-3) and ammonium acetate (AA) determined exchangeable base elements (a) K, (b) Ca, (c) Mg and (d) Na.

4.3 Relationship between EDTA and Mehlich-3 determined micronutrients

There was a fairly strong linear relationship between Mehlich-3 and EDTA determined micronutrients as indicated by high coefficient of determination (R^2) values of 0.841, 0.791, 0.835, and 0.803 for zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu). Although Mehlich-3 is known to work best on acidic and neutral soils, this study proved that it can be applied to neutral to slightly alkaline soils common in the Zimbabwe Sugar Industry (Rambwawasvika et al., 2021). The obtained relationships concur with the successes of the method as reported by other researchers (Zbíral, 2016; Mylavarapu et al., 2014). There were always poor correlations ($R^2 < 6$) between soil physicochemical properties pH, EC, texture, and OM and availability of

micronutrients hence no recommendations were made. Where precise and accurate conversions between Mehlich-3 and EDTA- micronutrients are necessary, equations given on regression plots can be used (Figure 4.4).
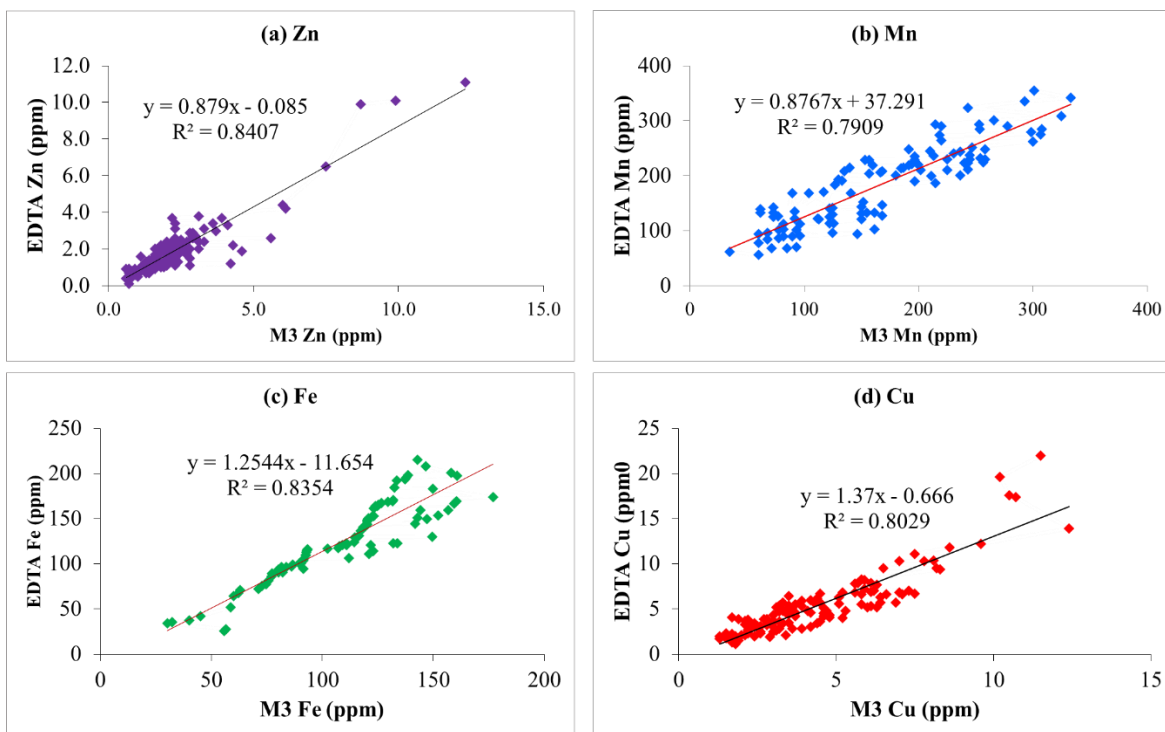
Figure 4.4: Regression graphs and equations showing the relationship between Mehlich-3 and EDTA determined micronutrients (a) Zn, (b) Mn, (c) Fe and (d) Cu.

Mehlich-3 was previously known for the extraction of multi-elements on acidic to neutral soils (Ostatek-Boczynski et al., 2012; Mylavarapu et al., 2014). The present findings concurred with the findings of Iatrou et al (2015) and Kumawat et al (2017) that Mehlich-3 is also applicable to neutral and slightly alkaline soils as prominent in the Zimbabwe Sugar Industry. The buffering effect of acetic acid keeps the pH low making the extractions efficient even at higher soil pH.

4.4 Proficiency testing scheme results

The Z score results for exchangeable base cations potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) were always within the satisfactory range ($-2 \leq Z \geq 2$) for the four samples analyzed. Sodium (Na^+)'s Z scores for the first two samples for cycle 3 round 4 (C3R4) were out of acceptable specification giving values of 4.32 and 2.71 for samples A and B respectively. The researcher did not analyze samples for round 5 (C3R5) with the new method but all results for round 6 (including sodium) were within specification. The high Z scores for the first sodium results could have emanated from using sodium borosilicate glassware by the analyst resulting in the leaching of sodium, adding to the prepared sample. Later, care was taken to avoid sodium borosilicate glassware when analyzing sodium and Z scores were within a satisfactory range (Table 4.2).

Table 4.2: Proficiency testing scheme results reported as Z scores for exchangeable base cations potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium (Na^+).

Sample	Z score			
	K^+	Ca^{2+}	Mg^{2+}	Na^+
C3R4 Sample A	0.34	0.47	0.45	4.32
C3R4 Sample B	0.91	0.74	0.19	2.71
C3R6 Sample A	0.22	1.23	0.14	1.65
C3R6 Sample B	1.07	0.11	-0.35	-0.41

Note: C3R4 represents cycle 3 round 4 and C3R6 represent cycle 3 round 4

The performance of the Mehlich-3 method on the analysis of micronutrients was perfect for copper and iron with all the Z scores within the satisfactory range. Manganese had an initial Z score of -4.87 which was far below the expected value. This only happened once and could not recur as the analyst becomes more careful and experienced in the analysis. In cycle 3 round 5 (C3R5) zinc had

a Z score of 3.45, way above the satisfactory range. This only happened once as all the other results were within satisfactory range and could not be used as the basis for disqualifying the method. Results of the micronutrient proficiency tests are shown in Table 4.3.

Table 4.3: Proficiency testing scheme results reported as Z scores for micronutrients copper (cu^{2+}), iron (Fe^{3+}/Fe^{2+}), manganese (Mn^{2+}) and zinc (Zn^{2+})

Sample	Z score			
	Copper	Iron	Manganese	Zinc
C3R4 Sample A	0.47	0.00	-4.87	0.00
C3R4 Sample B	0.95	0.03	N/A	0.12
C3R5 Sample A	0.20	-0.35	0.83	3.45
C3R5 Sample B	0.00	-0.45	0.52	1.41
C3R6 Sample A	0.18	0.08	2.31	---
C3R6 Sample B	0.63	0.16	-1.22	1.28

Note: C3R4 represents cycle 3 round 4, C3R5 represents cycle 3 round 5 and C3R6 represents cycle 3 round 4.

4.5 Validation of the M-3 method for the determination of exchangeable bases and micronutrients

4.5.1 Accuracy

The Mehlich-3 method proved to be accurate based on the high percentage of recoveries obtained. The percentage recoveries for exchangeable bases were slightly higher compared to those of trace elements ranging from 93.56 ± 4.77 % for calcium (ca) to 99.21 ± 5.11 % for magnesium (Mg). Trace elements recoveries ranged from 93.54 ± 4.33 % for iron (Fe) to 95.82 ± 3.22 % for copper

(Cu). The higher recoveries obtained on exchangeable base cations might be attributed to higher concentrations of standards spiked (20 – 200 ppm for each base cation) unlike in micronutrients where lower concentrations ranging from 2 to 20 ppm were spiked. The choice of spiked concentrations was determined by the natural abundance of the respective elements in the soil. The linearity ranges of recovery concentrations are shown in Appendix B while the average recoveries for each element are shown in Table 4.4.

Table 4.4: Average recovery results for all elements expressed as mean values of all spiked standards ± standard deviation

Element	Range of spiked CRM (ppm)	Average recovery (%)
Mg	20-200	99.21 ± 5.11
Ca	20-200	93.56 ± 4.77
Na	20-100	95.84 ± 6.11
K	20-100	97.66 ± 4.32
Zn	2-10	95. 43 ± 7.43
Cu	2-10	95.82 ± 3.22
Mn	2-20	93.63 ± 5.13
Fe	2-20	93.54 ± 4.33

4.5.2 Repeatability and reproducibility

All elements passed repeatability studies as indicated by RSD (%) values less than 10%. The most repeatable exchangeable base element was calcium as shown by a lower RSD % of 0.72. Potassium was the least repeatable on exchangeable bases with an RSD % of 2.60. On micronutrients, zinc was the least repeatable with an RSD % of 5.98. The reason for zinc's lower repeatability could

be emanating from the lower concentrations in which it is found in the soils and the lack of uniform distribution.

Reproducibility studies had greater RSD (%) values compared to those of repeatability and intermediate precision as they involve different laboratories and different analysts. RSD (%) values for reproducibility studies ranged from 5.34 % to 9.34 % (Table 4.5).

Table 4.5: Laboratory repeatability results for exchangeable bases and trace elements as measured by an RSD %

Element	Repeatability (RSD %)	Reproducibility (RSD %)
Calcium	0.72	7.14
Magnesium	1.67	5.34
Sodium	1.12	6.71
Potassium	2.60	7.88
Copper	3.49	7.17
Zinc	5.98	9.14
Manganese	2.63	7.38
Iron	4.27	5.44

4.5.3 Intermediate precision

The lowest RSD values in intermediate precision studies were found on exchangeable bases with calcium having the lowest RSD value of 4.20 % and the highest RSD was sodium a value of 6.74 %. The highest RSD value on trace elements was iron with 7.67 % while the lowest was copper with 3.67 %. Generally, intermediate precision RSD values were higher compared to those found in repeatability studies that made use of one analyst thereby reducing human error (Table 4.6)

Table 4.6: Comparison of RSD (%) values of three analysts in intermediate precision studies done under the same conditions

Analysts	RSD (%)							
	Cu	Fe	Zn	Mn	Mg	Ca	Na	K
A vs B	3.67	7.67	6.21	4.36	4.72	4.20	2.94	4.75
A vs C	5.07	5.53	5.82	5.17	3.83	5.28	5.02	3.75
B vs C	5.30	9.13	6.14	6.78	4.57	6.03	6.74	4.38
Average	4.68	7.44	6.06	5.44	3.47	5.17	4.90	4.29

4.5.4 Limit of detection and limit of quantification

For exchangeable bases calcium had the lowest LOD and LOQ of 0.04 ppm and 0.12 ppm respectively while potassium had the highest LOD of 0.07 ppm and LOQ of 0.20 ppm. On micronutrients, copper had the lowest LOD and LOQ of 0.11 ppm and 0.33 ppm respectively while iron had the highest LOD and LOQ of 1.26 ppm and 3.82 ppm respectively. The low LOQ obtained indicated that the method is reasonably suitable for the diagnosis of exchangeable base elements and micronutrients at low concentrations in the Zimbabwe Sugar Industry soil.

Table 4.7: Mehlich-3 limit of detection and limit of quantification for exchangeable bases and micronutrients

Element	LOD (ppm)	LOQ (ppm)
Calcium	0.04	0.12
Magnesium	0.04	0.14
Sodium	0.05	0.18
Potassium	0.07	0.20
Copper	0.11	0.33
Zinc	0.26	0.80
Manganese	0.84	2.55
Iron	1.26	3.82

4.5.5 Robustness

Slight changes in shaking time and shaking speed could not alter the results very much as indicated by lower RSD (%) ranging from 0.69 % to 8.22 %. It was noted however that change in filter paper from Whatman number 2 to Whatman number 91 resulted in very high RSD (%) and thus changing filter paper should not be regarded as a robustness parameter. The determination of the method's robustness clearly indicates that the parameters must not be changed especially for micronutrients as proven by higher RSD (%) values ranging from 4.10% to 6.31 % when the shaking time was changed from 5 minutes to 4 minutes of shaking for the trace elements. For exchangeable bases, there was little effect as shown by a small RSD ranging from 0.69 % to 3.30 % when the shaking time was changed. The same happened when the shaking speed was changed resulting in high RSD (%) depicted in Table 4.8.

Table 4. 8: The results of method robustness after deliberate small changes in shaking time, shaking speed and filter paper. Results are expressed as RSD (%)

| Parameters | RSD (%) | | | | | | | |
	K^+	Ca^{2+}	Mg^{2+}	Na^+	Cu^{2+}	Zn^{2+}	Fe^{2+}/Fe^{3+}	Mn^{2+}
5 min Vs 4 min	0.69	3.30	1.69	0.80	6.31	4.94	7.71	4.10
120 rpm Vs 115 rpm	2.04	1.21	1.24	4.24	8.22	4.94	6.21	4.35
Filter paper No 2 Vs No 91	9.27	0.12	1.23	2.4	7.67	14.92	11.03	8.27

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Mehlich-3 multi-extractant have been found suitable for the analysis of exchangeable bases calcium, magnesium, potassium and sodium as well as micronutrients copper, iron, manganese and zinc in the ZIS soils. There was a good agreement between Mehlich-3 results and those of conventional methods, EDTA and ammonium acetate for the extraction of micronutrients and exchangeable bases respectively. The difference between results of conventional methods and Mehlich-3 were insignificant to warrant any change in nutrient thresholds used for fertilizer recommendations. This study clearly proved that M-3 can be applied for the analysis of neutral to slightly alkaline soils which are common in the Zimbabwe Sugar Industry. This means Mehlich-3 method can inherit same nutrient thresholds used by ammonium acetate for potassium fertilization as well as calculations of exchangeable sodium percent (ESP %) and sodium adsorption ratio (SAR) for the determination of soil sodicity. The study has successfully evaluated and validated Mehlich-3 as a suitable substitute for use in the analysis of soil exchangeable bases and micronutrients in the Zimbabwe Sugar Industry soils.

5.2 Recommendations

Since Mehlich-3 has proved to work well on diverse soils found in the Zimbabwe Sugar Industry, recommendations are hereby made for its adoption in the analysis of exchangeable bases and trace elements. Since this study has demonstrated the suitability of Mehlich-3 multi-nutrient extractant for the determination of exchangeable bases (calcium, magnesium, potassium and sodium) and micronutrients (copper, iron, manganese and zinc) in the Zimbabwe Sugar Industry soils, it is worthy assessing its suitability for the extraction of other micronutrients boron and molybdenum

which were not part of this study. Extraction of many elements will increase the method's efficiency as well as cutting down on the cost of analysis and sample turnaround time.

Research should be done to establish thresholds of micronutrients on different sugarcane cultivars in order to ascertain deficiencies. Currently the industry does not apply micronutrient fertilizers assuming that the soils has enough for the crops however this study revealed that 15 % of the soils had zinc levels below the South African threshold of 1.5 ppm and 2.1 % had copper levels below the threshold of 0.8 ppm. Deficiencies can be a source of hidden hunger resulting in yield reduction and consequently loss of revenue to sugarcane growers.

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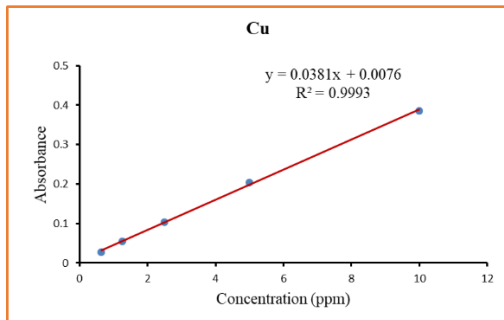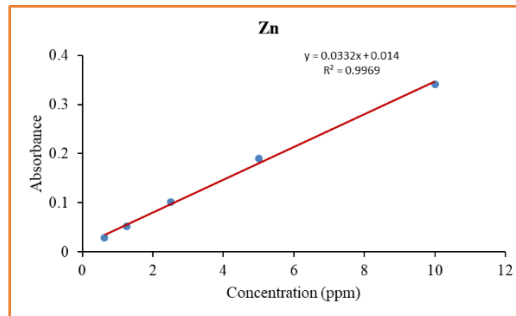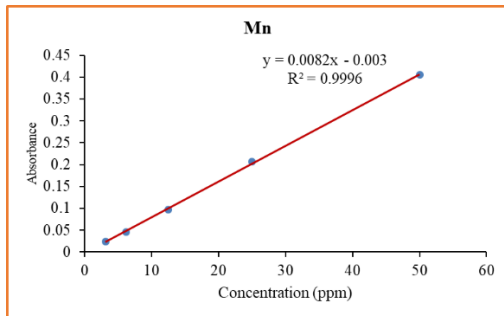
APPENDICES

Appendix A: Sample calibration graphs used in spectroscopy analysis

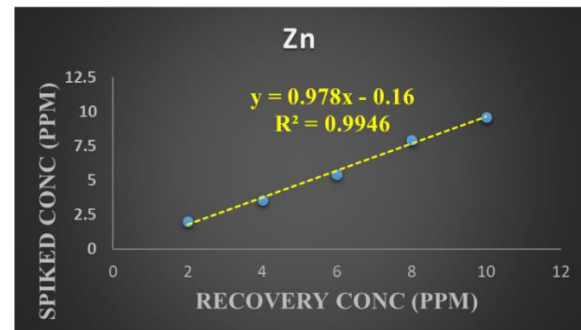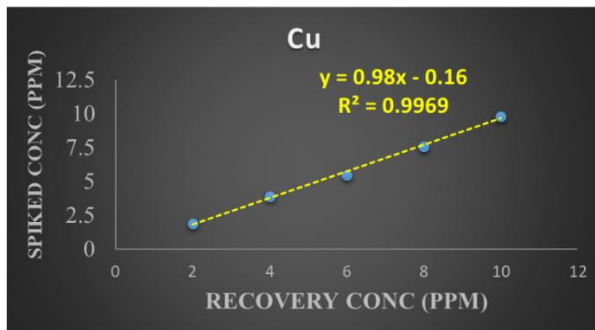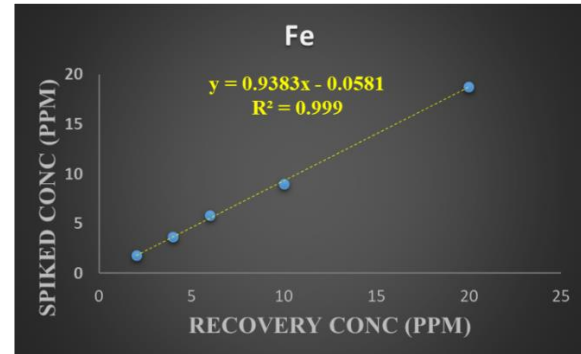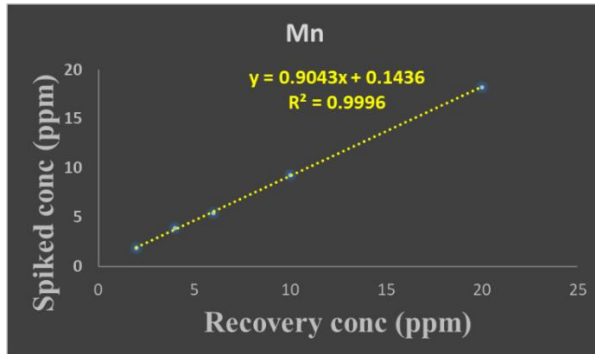A1: Sample calibration graphs for the determination of exchangeable bases
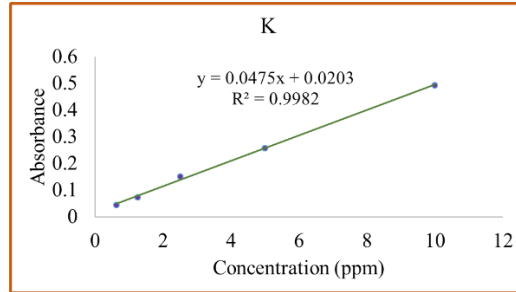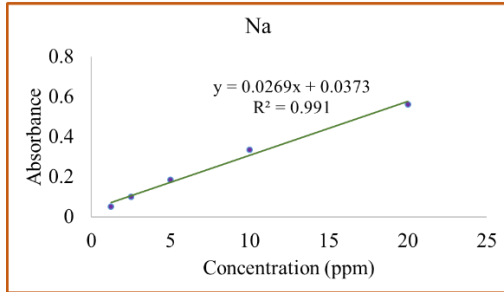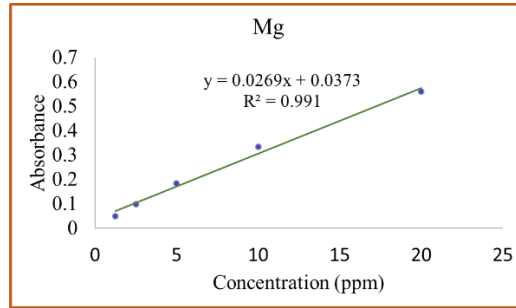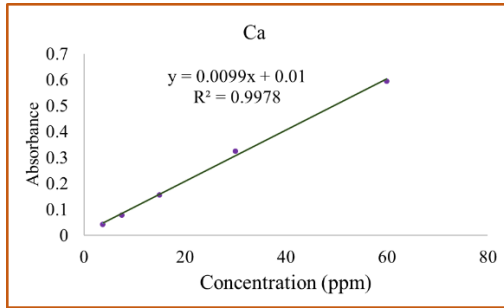
A2: Sample calibration graphs for the determination of micronutrients
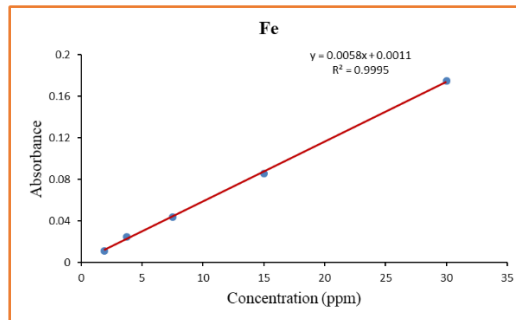
Appendix B: Recovery studies for CRM for both exchangeable bases and micronutrients

B1: Micronutrients

B2: Exchangeable bases