PREPARATION OF IN-HOUSE REFERENCE MATERIAL FOR THE DETERMINATION OF SELECTED HEAVY METALS IN SOILS OF MINNA, NIGER STATE

BY

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ABSTRACT

Method performance in analytical chemistry is checked using certified reference materials (CRMs). Due to their high production cost, CRMs cannot be used routinely in daily laboratory work; therefore, in-house reference materials are preferred. An in-house soil reference material was prepared in this project for use as quality control material in the determination of Fe, Zn, Cu, Pb, Mn, Cr and Cd in soil. The FUTMX7316SRM was prepared by mixing soils of road sides, mechanic workshops, dump sites and agricultural farms from different parts of Minna metropolis. The homogeneity of the bulk sample was checked before and after the soil was bottled using total nitrogen and organic carbon as homogeneity indicators. The texture of the bulked soil was also determined. Ten systematically selected samples were digested in triplicate using one of the commonly used method amongst Nigerian students and researchers, hotplate aqua regia, and the digest analyzed in four different laboratories in Nigeria for the concentrations of Fe, Zn, Cu, Pb, Mn, Cr and Cd. Certified Reference Material (CRM-989), prepared by International Soil-Analytical Exchange, Wageningen University of Environmental Sciences, Netherlands, was also analyzed for quality control and traceability of the prepared FUTMX7316SRM. The result obtained were summarized using mean, median, standard deviation and percent coefficient of variance. Analysis of variance was carried out with the results. No statistically significant differences were observed for within- or between-bottle results. Therefore, the material prepared is considered homogeneous fit for use as quality control material in the determination of Fe, Zn, Cu, Pb, Mn, Cr and Cd in soil. The property value revealed that the prepared reference material contained 0.41 \pm 0.02 % total organic carbon, 0.16 \pm 0.04 % total nitrogen, 236.21 ± 41.78 mg/kg Zn, 213.25 ± 108.63 mg/kg Mn, 17.46 ± 3.50 mg/kg Pb, 18.38 ± 7.55 mg/kg Cu, 1.35 ± 1.56 mg/kg Cd, 9071.64 ± 368.92 mg/kg Fe and 127.65± 39.95 mg/kg Cr. The values are traceable to the Certified Reference Material (CRM-989) used, and can therefore be used for inter-laboratory campaign and other quality control purposes.

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ABBREVIATIONS

ANOVA	Analysis of Variance
AQA	Analytical Quality Assurance
AQC	Analytical Quality Control
CRMs	Certified Reference Materials
CSRM	Control Soil Reference Material, FUTMX7316SRM (Prepared bulked soil sample from different environments in Minna metropolis)
CV	Coefficients of Variation
EQA	External Quality Assessment
ISO	International Organization of Standards
NAA	Neutron Activation Analysis
RSD	Relative Standard Deviation
XRF	X-Ray Fluorescence

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

In their reports, Lymperopoulou *et al.* (2014) and Zhang *et al.* (2020) defined heavy metals as a metallic element with high density, though relative (> 5 g cm⁻³) in contrast to water (1 g cm⁻³). This definition comes from the assumption that heaviness and toxicity are related. Based on the definition copper, lead, chromium, manganese, cadmium, iron, and zinc are heavy metals (Lymperopoulou *et al.*, 2014; Zhang *et al.*, 2020). Some of these Heavy Metals like nickel, selenium, zinc, molybdenum, cobalt, iron, and manganese are required by plants and animals as micronutrients for growth. The deficiency of these metals as micro-nutrients may cause varieties of deficiency diseases or syndromes (Sarwar *et al.*, 2017; Turkyilmaz *et al.*, 2018, and Zhang *et al.*, 2020). In the work of Jaiswal *et al.* (2018), heavy metals are said to cause hazards to organisms at levels above the recommended permissible limits and thus the need to prevent their bioaccumulation.

Natural sources like volcanic eruptions and anthropogenic sources like mining are sources from which heavy metals are deposited into the environment (Liatu *et al.*, 2017). Heavy metals have the characteristic that they do not leach into the soil from the topsoil which makes them present at a higher concentration in the surface soil where most plants take up nutrients for growth (Taghipour *et al.*, 2013; Rodriguez-Eugenio *et al.*, 2018).

Among the methods used for the digestion of the mass concentrations of elements in solid matrices is the use of fusion with alkali salts and acid digestion which could be in an open beaker with the source of heat from a hot plate, or in a digestion tube with the source of heat from a block-digester. Digestion bombs could also be used with the source of heat from a microwave oven (Lymperopoulou *et al.*, 2014; Turek *et al.*, 2019). After digestion, the concentration of the heavy metals in the digest are quantified through the application of several different analytical methods, grouped under the general method called atomic spectroscopy, among which is the atomic absorption spectrometry (AAS) (Helaluddin *et al.*, 2016).

Rawar and Rohman (2016) reported that AAS can be used to quantify about sixty-eight metals in solutions and the measurement sensitivity could range from parts per million (ppm) to parts per billion (ppb) levels with good precision that is better than 1% relative standard deviation (RSD). This most commonly used instrument for the analysis of metallic elements either in academic research or commercial laboratories is also said to be simple and easy to operate (Rawar and Rohman, 2016).

Materials whose property value or values are sufficiently homogeneous and well established known as Reference Materials (Shukla, 2015), are recognized and recommended as a powerful tool for quality control and quality assurance in a wide range of international, national, and professional organizations for method development and method and equipment validation and calibration (Mackey *et al.*, 2010). The lack of use of Reference Materials for quality control has resulted in the emergence of poor inter-laboratory precision in routine analysis of trace metal in soil which had led to a poor decision taken that had affected lives and the environment (A. M. C. T. B. and Analytical Methods Committee, 2013).

The production of reference materials is under controlled manufacturing procedures and based on the purpose for which it is intended for use. The purpose of reference materials could be for the calibration of values which is achieved by the use of another reference material. It could also be used for validation or control of trueness of measured values in a given laboratory, or in a group of laboratories as well as the assessment of the performance of a new measurement procedure (Wielgosz, 2004).

Reference material (RM) that is accompanied by a certificate is referred to as certified reference material (CRM) (Shukla, 2015). Several organizations produce CRM of trace metals and organic compounds among which are Community Bureau of Reference (BCR, EU), Mintek South Africa, National Institute of Standards and Technology (NIST, USA), Switzerland EMPA, National Institute for Environmental Studies (NIES, Japan), National Research Council of Canada (NRCC), Sigma-Aldrich and International Atomic Energy Agency (IAEA). However, because of their high cost of production, CRMs are not used for daily quality control in laboratories. The use of non-certified RM is cheaper than CRM and its development for different laboratories is recommended because its production also fulfills the same strict criteria of homogeneity and stability as that of CRM to assure its usefulness (ALPAC, 2018, Llaurado *et al.*, 2001).

1.2 Statement of the Research Problem

- i. For research data to be reliable, the use of CRM is important, however, there is a paucity of reference materials for the day-to-day research work.
- ii. The use of Certified Reference Materials (CRMs) for quality control checks in analytical processes like the determination of heavy metals in soils is limited in Nigeria because of the high cost (Moat *et al.*, 2020). The non-usage of this quality control measure questions the confidence that can be placed on the results obtained from many laboratories in Nigeria that determine heavy metals using the atomic absorption spectrophotometer.

iii. There is no known prepared homogeneous sample as soil reference material in the determination of heavy metals in soils of Minna or Niger State.

1.3 Justification of the study

This study provides:

- A control sample that is homogenous and can be used for inter-laboratory study, accompanied with the consensus or reference values for the concentrations of Zn, Fe, Cd, Cu, Mn, Cr, and Pb in the soil of Minna or Niger State when modified aqua regia is used as digestion method and AAS as the instrumental method.
- ii. The quality of scientific information is generally a measure of its integrity, objectivity, reproducibility, and traceability measured by internationally accepted standards like the use of Reference Material. There is considerable interest worldwide in the use of matrix reference materials as chemical analytical techniques change from the classical wet chemical techniques to sophisticated instrumental analysis requiring comparison material for calibration (Gopalakrishnan, 2005). This study will provide a large amount of matrix reference material for use in the quality control and assurance for the determination of Pb, Cu, Zn, Cr, Mn, and Cd in the soils of Minna metropolis in Niger state.

1.4 Aim and Objectives of the Study

The aim of this study is to prepare in-house reference material for the determination of total and extractible Pb, Cu, Zn, Cr, Mn, and Cd in soil.

The aim of this project was being achieved through the following objectives:

i. Preparation of homogenized composite samples of the soil for the determination of total Pb, Fe, Cu, Zn, Cr, Mn, and Cd.

- ii. Digestion of a Certified Reference Material (CRM-989) and the homogenized composite soil sample using modified aqua regia according to the standard method of EPA 3050b.
- iii. Quantification of total Pb, Fe, Cu, Zn, Cr, Mn, and Cd in the digested homogenized composite samples and CRM-989 using AAS in four laboratories in Nigeria.
- iv. Determination of the property values of the homogenized composite soil samples based on the data obtained after accuracy and precition checks using statistical analysis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heavy Metals in Soil and their Toxic Effects

Soil is a reservoir for many toxic constituents like heavy metals (Arif *et al.*, 2016). Some heavy metals like chromium, iron, cobalt, selenium, copper, chromium, iron, magnesium, manganese, iron, molybdenum, nickel, and zinc are of great importance serving as essential nutrients for different types of biochemical and physiological functions in both plants and animals. Deficiency diseases or syndromes may arise from an inadequate supply of these elements (Sarwar *et al.*, 2017; Turkyilmaz *et al.*, 2018; Zhang et al., 2020).

Heavy metals do not degrade, a characteristic that makes them persistent and toxic (Jain *et al.*, 2017). Chen *et al.* (2015) reported that human exposure to heavy metals had increased dramatically in recent years because heavy metals or materials that contain them are used in several industrial, agricultural, domestic and technological applications. They can get into humans causing mild to serious damage to organs and tissues causing conditions like carcinogenesis, or apoptosis (Khan *et al.*, 2015; Hou *et al.*, 2019). Among the heavy metals, five (arsenic, cadmium, chromium, lead, and mercury), because of their high degree of toxicity rank among the priority metals of great concern in public health (Tchounwou*et al.*, 2012; Vodyanitskii, 2016).

2.2 Determination of Heavy Metals in Soil

The measurement of the concentration of elements in the soils is the first step in the management of soils (Palma *et al.*, 2015). This determination involves processes like drying of the sample after collection, digestion of the sample matrix, and quantification of the metals (Machado *et al.*, 2020).

2.2.1 Sample preparation for heavy metal determination in soil

There are several methods used for the extraction of heavy metals from soils. which are generally a combination of digestion procedures to enhance dissolution and measurement of the elements. The extraction may be partial or total depending on the digestion method used (Lima *et al.*, 2016).

2.2.2 Sample digestion for heavy metal determination in soil

It is important to evaluate digestion methods to know the best method for the extraction of total elements from soil (Lima et al., 2016). Some digestion methods used in the determination of the mass concentration of elements in solid matrices are the use of Fusion with alkali salts and digestion with acids in open beakers heated on hot plates, digestion tubes in a block-digester, or digestion bombs placed in microwave ovens (Lymperopoulou et al., 2014; Wang et al., 2019). However, hotplate aqua regia, microwave aqua regia, and microwave aqua regia combined with Hydrofluoric (HF) acid are the three most common digestion methods (Helmeczi et al., 2018). Aqua regia is more common to extract environmental matrices than nitric acid alone because it is more aggressive and increases the leaching fraction of certain elemental groups. It also contains a complexing agent that can significantly increase extraction efficiency. However, it does not extract elements that are bound to silicate structures in soil because these elements are not mobile in the environment. HF acid is added to aqua regia if absolute total digestion is required but because of the hazard associated with HF, it is usually avoided in laboratories where adequate facilities are not available for use (Das and Ting, 2017).

All the reagents used in analytical processes must be of analytical grade or equivalent and free from any contaminant that may interfere with the analysis. The laboratory wares that are used for the sample digestion and solubilization must be clean with dilute acids and then rinse with distilled and de-ionized water (Leśniewska *et al.*, 2016).

Lima *et al.* (2016) evaluated the digestion capacity of three soil digestion methods, USEPA 3051 (HNO₃), USEPA 3051A (3 HNO₃ :1 HCl), and Aqua Regia (1 HNO₃ : 3 HCl) in the determination of Ni, Mn, Ba, Cr, Fe, Pb, Cu, and Zn and found that the methods were statistically similar for the extraction of Fe, Cu, and Mn from soils and aqua regia method was found to have highest levels of extraction of Cr, Ni, Pb, and Zn.

Santoro *et al.* (2017) used the aqua regia extraction method among other digestion methods in an inter-laboratory comparison study. It was found that the extraction of Hg, Cu, As, Cu, Hg, Fe, Pb, and Zn by total and aqua regia methods were comparable while the result obtained for Co, Cd, and Cr showed that total extraction using HF were 10% lower than aqua regia extraction method. It was also found that high organic matter content, low SiO₂, and refractory aluminum and iron oxide, and small particle size affected the digestion capacity of aqua regia.

2.2.3 Quantification of heavy metals

Soil samples can be analyzed for metals after digestion by applying several analytical methods which are grouped under the general method called atomic spectroscopy (Almasoud *et al.*, 2015; Akinyele, and Shokunbi, 2015; Helaluddin *et al.*, 2016). Atomic spectroscopy, an important tool in analytical chemistry measures analytes at concentrations in parts per million to parts per trillion levels. These levels of detection can be achieved when the concentration of metals in the sample is reduced by dilution. These instruments have a precision of 1-2 % (Dumicius *et al.*, 2011; Helaluddin *et al.*, 2016; Lawler *et al.*, 2019).

In atomic spectroscopy, the liquid sample is aspirated into a high-temperature heat source where the liquid evaporates and the solid particles containing the analyte metals are broken into individual atoms (atomized). The heat source (like the flame) serves as the curvet. In the hollow-cathode lamp (which is different for each element of interest), the cathode is irradiated with high-energy ions which excite the metals at the cathode and the excited metal atoms vaporize and emit light which is absorbed by analyte metal in the flame. The amount of light absorbed is measured by a detector (Dumicius *et al.*, 2011; Helaluddin *et al.*, 2016; Lawler *et al.*, 2019).

2.3 Types of Atomic Spectroscopy

There are two major types of atomic spectroscopy, which are atomic absorption spectroscopy and atomic emission spectroscopy (Dumicius *et al.*, 2011; Lawler *et al.*, 2019).

2.3.1 Atomic Absorption Spectrophotometry (AAS)

The AAS is one of the most commonly used instruments for elemental analysis in academic research and commercial laboratories. This could be because of their wellestablished principle, long history, and versatility (Helaluddin *et al.*, 2016). The atomic vapor of the sample in the heat source (like the flame in flame atomic absorption spectrophotometry) absorbs light from a hollow cathode lamp. The amount of light absorbed is directly proportional to the concentration of the analyte (Rawar and Rohman, 2016).

The first commercial AAS became available in 1959 and the 1960s. L'vov (1961), Massman (1968), and others developed the electro-thermal atomizer which finds its use in graphite furnace atomic absorption (GFAA) techniques today (Helaluddin *et al.*, 2016; Rawar and Rohman, 2016).



Figure 2.1: Atomic absorption experiment (Source: Richter et al., 2016)

2.3.2 Atomic Emission Spectroscopy (AES)

Atomic fluorescence spectroscopy (AFS), inductively coupled plasmas atomic emission spectroscopy (ICP-AES) are examples of atomic emission spectroscopy (Dumicius *et al.*, 2011).

a. Atomic fluorescence spectroscopy

In Fluorescence Spectroscopy, atoms in the flame are irradiated by a laser that promotes them from their ground state to an excited electronic state. In the excited state, they fluoresce and return to the ground state. The figure below shows Atomic Fluorescence from 2 ppb of lead in tap water (Zheng *et al.*, 2019).



Figure 2.2: Atomic fluorescence from Pb at 405.8 nm (Source: Zheng et al., 2019).

The water sample containing colloids of Pb in the concentration of parts per billion (ppb) ejected from a capillary tube and then exposed to a 6 ns pulse of 1064 nm laser radiation which was focused on the drop. A plume of vapor moving toward the laser was created by the pulse. Excited Pb atoms whose fluorescence was measured with an optical system were created after exposure to a 193 nm pulse. The resolution of the signal created was 0.2 nm. The calibration curve for the colloidal Pb standards is shown on the figure as well as that of the water containing 2 ppb Pb. The figure shows a calibration curve constructed from colloidal standards and the signal from tap water containing 2 ppb Pb (Dumicius *et al.*, 2011, Zheng *et al.*, 2019).



Figure 2.3: Absorption, emission, and fluorescence by atoms in a flame (Source: Zheng *et al.*, 2019).

In atomic absorption, atomized atoms in the flame absorb part of the light from the source, hollow cathode lamp, and the unabsorbed light reaches the detector, where it is measured and recorded as absorbance. Atomic emission arises from atoms that are excited because of the high thermal energy of the flame. To observe atomic fluorescence, atoms are excited by an external lamp or laser. When the excited atom falls to a lower state, it emits radiation. Atomic fluorescence is potentially a thousand times more sensitive than atomic absorption, but the equipment is not common (Zheng *et al.*, 2019).

Another way to determine the microelement composition of samples is the use of X-ray Fluorescence Analyzers (XRF). The principle of these techniques is based on the measurement of fluorescence and dispersion of chemical elements excited by X-ray. The concentrations of chemical elements in the sample are determined by basic physical identification characteristics. This technique differs from other fluorescence techniques because it does not require chemical treatment of the sample. The samples are only finely ground, similar to laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) (Zheng *et al.*, 2019).

b. Inductively Coupled Plasmas Atomic Emission Spectroscopy (ICP-AES)

The heat source of this spectroscopy is inductively coupled plasma with heat twice as hot as a combustion flame. The high temperature, stability, and relatively inert Argon environment in the plasma eliminate much of the interference encountered with flames. The technique allows simultaneous multi-element analysis which makes it to replaced Flame Atomic Absorption. The limitation of the plasma instrument is the costs of purchase and operation (Zheng *et al.*, 2019).

Inductively coupled plasma burner cross-sectional view has two turns of 27 or 41 MHz induction coils that are wrapped around the upper opening of the quartz apparatus. High-purity Argon gas is fed through the plasma gas inlet. After a spark, the Tesla coil ionizes Argon, and freed electrons are accelerated by the radio-frequency field. Electrons collide with atoms and transfer their energy to the entire gas, maintaining a temperature of 6000 to 10000 K. The quartz torch is protected from overheating by Argon coolant gas. The concentration of analyte needed for the adequate signal is reduced by an order of magnitude with an ultrasonic nebulizer, in which sample solution is directed onto, a piezoelectric crystal oscillating at 1 MHz. The vibrating crystal creates a fine aerosol that is carried by a stream of Argon gas through a heated tube, where the solvent evaporates and stream then passes through a refrigerated zone

where the solvent condenses and is removed. Analyte reaches the plasma flame as an aerosol of dry, solid particles. Plasma energy is not needed to evaporate the solvent, so more energy is available for atomization. Also, a larger fraction of the sample reaches the plasma than with a conventional nebulizer (Dumicius *et al.*, 2011; Zheng *et al.*, 2019).

Emission along the length of the plasma instead of across the diameter of the plasma further enhances the sensitivity with inductively coupled plasma by a factor of 3 - 10. Additional sensitivity is obtained by detecting ions with a mass spectrometer (ICP-MS) instead of by optical emission (Dumicius *et al.*, 2011; Zheng *et al.*, 2019). Currently, Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and Laser Ablation Inductively Coupled Plasma Mass Spectroscopy (LA-ICP-MS) is often used compared to AAS (Zheng *et al.*, 2019).

c. Inductively Coupled Plasma–Mass Spectrometry (ICP-MS)

The ionization energy of Argon is 15.8 eV, higher than those of all elements except He, Ne, and F. In an Argon plasma, analyte elements are ionized by colliding with excited Argon atoms or energetic electrons. More abundant neutral atoms are usually produced in atomic emission spectroscopy but to correct this, the plasma is directed into a mass spectrometer that fragments and separates ions according to their mass-to-charge ratio. To attain accurate measurements of isotope ratios, the mass spectrometer has one detector for each desired isotope (Zheng *et al.*, 2019).

Inductively coupled plasma-mass spectrometry has a low detection limit. Therefore, it is important to ensure the cleanliness of reagents, glassware, and procedures. Solutions must be made from extremely pure water and stored in trace metal grade Teflon or polyethylene vessels protected from dust. HCl is avoided because they create isobaric interferences. The plasma-mass spectrometer interface cannot tolerate high concentrations of dissolved solids that clog the orifice of the sampling cone. The plasma reduces organic matter to carbon that clogs the orifice. Organic material can be analyzed if some are fed into the plasma to oxidize the carbon. Matrix effects on the yield of ions in the plasma are important, so calibration standards should be in the same matrix as the unknown. Alternatively, internal standards are used if they have nearly the same ionization energy as the analyte. If possible, internal standards with just one major isotope should be selected for maximum response (Dumicius *et al.*, 2011; Zheng *et al.*, 2019).



Figure 2.4: Interface between Inductively Coupled Plasma And Mass Spectrometer

(Source: Zheng et al., 2019)

The LA-ICP-MS method is simpler because sample analysis does not require the chemical extraction of microelements. The method itself allows determining the microelement composition even in very small sample amounts. Inductively coupled plasma can also be interplayed with AAS to have Inductively Coupled Plasma Atomic Absorption Spectroscopy (ICP-AAS). In ICP-AAS atoms are excited in the inductively excited high-frequency plasma (Zheng *et al.*, 2019).

d. Direct Current Electrical Breakdown Gas Atomic Emission Spectroscopy (DC Arc ES).

The essence of DC Arc ES is the registration of intensive atomic emission radiation. DC Arc ES procedures like Inductively coupled Plasma Atomic Absorption Spectroscopy (ICP-AAS) register the spectra of direct atomic emission. In the DC Arc ES excitation of atoms occur in the electric field plasma (Dumicius et al., 2011).

2.3.3 Sensitivity of Atomic Spectroscopy

One of the important factors to consider when selecting a method is sensitivity. The sensitivities of AAS, LA-ICP-MS, and ICP-MS are around 1 ppm but in the updated LA-ICP-MS, and ICP-MS, the sensitivity be very high and could detect 0.001 ppm metal concentration in a sample. The sensitivity of XRF is about 10 ppm (Dumicius *et al.*, 2011).

	Flame absorption	Furnace absorption	Plasma emission	Plasma-mass spectrometry
Detection limits (ng/g)	10-1 000	0.01-1	0.1-10	0.000 01-0.000 1
Linear range	10 ²	10 ²	105	108
Precision				
short term (5-10 min)	0.1-1%	0.5-5%	0.1-2%	0.5-2%
long term (hours)	1-10%	1-10%	1-5%	<5%
Interferences				
spectral	very few	very few	many	few
chemical	many	very many	very few	some
mass			5	many
Sample throughput	10-15	3-4	6-60	all elements
	s/element	min/element	elements/min	in 2–5 min
Dissolved solid	0.5-5%	>20%	1-20%	0.1-0.4%
		slurries and solids		
Sample volume	large	very small	medium	medium
Purchase cost	1	2	4-9	10-15

Table 2.1: Comparison of atomic analysis methods

SOURCE: Dumicius et al., 2011

2.4 Sources of Errors in Analytical Processes

The term error is referred to as the difference between the true value and measured value (Wang *et al.*, 2017; Loken and Gelman, 2019). It denotes the estimated uncertainty in measurement (Sciacchitano and Wieneke, 2016). Chemical analysis can not be performed without errors or uncertainties but it can be minimized and the size estimated with acceptable accuracy. Two terms are commonly associated with errors which are accuracy and precision (Prenesti and Gosmaro, 2015; McAlinden *et al.*, 2015; Mutwalli *et al.*, 2018). Precision is defined as the reproducibility of a measurement result while accuracy is the closeness of the measured value to the true value (Mutwalli *et al.*, 2018).

The different causes of analytical error are pre-analytical stage error, analytical stage error, and post-analytical stage error (ISO 9001, 2000). Errors could arise in the preanalytical stage as a result of faulty primary sampling in the form of an incorrect sampling device, an inappropriate container for sample storage, cross-contamination during sampling, poor adherence to sampling protocol, collection of the sample that is not representative, misidentification of sample due to incomplete or incorrect sample label, illegible label maybe because it is partly erased or no clearly written and the disparity between the label and analytical parameters on the request form and samples. Faulty sample transportation and storage could also cause analytical error arising from leakage in the storage container, incorrect storage temperature, long storage, and transportation time, and inappropriate transport container and condition. Another preanalytical stage source of error is faulty sample preparation or processing which could result from drying, cleanup, milling or grinding, mixing or homogenizing, weighing, digestion, extraction, dilution, leaching (ISO 9001, 2000).

In the analytical stage, error may arise from wrong results due to non-adherence to the principle and procedure of the test; reagents and standards are not prepared and or mixed properly and quality control (QC) materials are not used for the method and equipment validations, use of the method with unacceptably large error, an inadequate detection limit of the equipment used, faulty conversion factor, poor or inadequate calibration as a result of inadequate matrix match, an incorrect calibration procedure and range among others. None implementation of the need for modification of standard and inappropriate calibration standard could also lead to analytical stage error. Errors could also be from calculation through the use of incorrect software or calculator, use of incorrect values for the computation of results, and the use of the wrong unit. Equipment failure and servicing problems as well as wrong instrument setup can result in incorrect analytical results. Another important source of analytical stage error is the environment which could be a result of cross-contamination within the laboratory and inadequate control of ambient conditions. Human error is a major factor to contend with

as a source of error in analysis when there is a lack of trained or experienced laboratory personnel who will not follow reporting instructions, rush analysis, fail to prepare reagents accurately, and carry out proper calibration on the instrument and interpret results wrongly among others (ISO 9001, 2000).

In the post-analytical stage, error(s) is/are introduced in the analytical process when reporting, checking and verifications are not properly done. It could also arise from a wrong interpretation of test results. Quality Control programs are therefore very important to minimize these sources of error (ISO 9001, 2000).

2.5 Analytical Interferences in Atomic Absorption Processes

In the measurement of analyte concentration using AAS, the number of ground-state atoms in the flame is proportional to the amount of light absorbed. Therefore, any factor that affects the ground state atom in the flame is classified as interference. It could also be any factor that affects the instrument's ability to measure the metal accurately. There is no analytical method that is without any interference from the nature of the sample or the measurement instrument. What is important in the analytical method is to at best know the interferences that affect the determination processes and the way to remove or make up for them (Kim *et al.*, 2018; Chauhan and Mittu, 2014).

Interferences in atomic absorption can be divided into two general categories, spectral and non-spectral (Kim *et al.*, 2018). Non-spatial interferences include matrix interference, Ionization Interference, and Chemical Interference while an example of spatial interferences is background absorption (Gallhofer and Lottermoser, 2018; Oliveira *et al.*, 2018).

2.6 Analytical Quality Control and its Importance

Analytical Quality control (AQC) is the application of procedures for controlling measurement processes routinely to limit errors and obtain measurements of known and defensible quality (Varela and Pacheco, 2018). It is a unit of Analytical Quality Assurance (AQA), the overall management system that ensures an AQC program in a place is working effectively. The AQC system provides regular checks to ensure that data generated from a laboratory is of high integrity, correctness, and completeness (ISO 9001, 2000).

According to Dudzik *et al.* (2018), the integrity of analytical results depends on the adherence to Quality Control during the analytical processes. The importance of Quality Control is therefore noteworthy. It ensures that tests are not wrongly done. It also detects, reduces, and corrects any deficiencies in the laboratory analytical process; hence ensuring that quality test results are generated. It is also used to determine the precision and accuracy of analytical methods and results.

In analytical quality control, several measures are available for use among which are the use of blanks, Spiked Samples, laboratory Replicate or Split Sample, Reference, External Quality Assurance (EQA) which is also known as inter-laboratory quality control (Varela and Pacheco, 2018; Mead, 2019; Mohamed *et al.*, 2020).

2.7 Reference Material

Reference Material (RM) is a substance that the property values are sufficiently homogeneous, stable for specified properties, and well established to be fit for its intended use in the calibration of apparatus, assessment of measurement methods, identification and assay, examination of nominal properties, purity test and for assigning values to materials (APLAC, 2018; Shukla, 2015).

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2.8 Classification of Reference Materials

i. Primary reference standard

Primary Reference Standard is a class of certified standards that have the highest order of metrology. The source of this standard is well documented as well as other information like the storage, use, and traceability (International Atomic Energy Agency – IAEA, 2003).

ii. Primary reference material

Primary Reference Material is used for verification of a primary reference method that is traceable to the Primary Reference Standard (IAEA, 2003). This material may also be used for the verification of a commutable routine method. An example of this type of Reference Material is Certified Reference Materials (CRMs) which are control samples or standards used to check the quality and traceability of products, to validate analytical measurement methods, or for the calibration of measurement standards (SGS, 2012) accompanied by a document issued by an authoritative body and providing one or more specified property values with associated uncertainties and traceability, using valid procedures. Each property is associated with uncertainty at a stated level of confidence and a statement of metrological traceability (Williams, 2014).

iii. Secondary reference material

Secondary Reference Material is used for the verification of a Secondary Reference Method, traceable to the Primary Reference Standard or Material (IAEA, 2003). This material may also be used for the verification of a commutable routine method. An example of this type of Reference Material is In-House Reference Material which is Reference Material prepared for internal use (Williams, 2014).

iv. Other classes of reference materials

According to IAEA (2003), there are many other classes of Reference Material among which are:

- a. Pure substances: These are characterized for chemical purity and/or trace impurities.
- b. Standard Solutions and Gas Mixtures: These are often gravimetrically prepared from pure substances and used for calibrations.
- c. Matrix Reference Materials: These are characterized by specified major, minor, or trace chemical components. Such materials may be prepared from matrices containing the components of interest, or by preparing synthetic mixtures.
- d. Physico-chemical Reference Materials: They are characterized for physical properties such as melting point, viscosity, and optical density, and some chemical properties.
- e. Reference Objects or Artifacts: They are characterized for functional properties such as taste, odour, octane number, flash point, and hardness. This type of Reference Material also includes microscopy specimens characterized for properties ranging from fiber type to microbiological specimens.

2.9 Importance and Uses of Reference Materials

Reference Materials find their importance in the areas of their application. They are useful as materials that support measurements concerned with chemical composition, biological, clinical, physical, engineering properties, taste, and odour (IAEA, 2003, Atkins, 2013) and hence provide:

- i. confidence in the results generated that they are the very best possible (IAEA, 2003, AIHA, 2014),
- ii. selection of the right laboratory for a project (IAEA, 2003),
- iii. a basis for correct decision making which eventually reduce loss of money, time, energy, and valuable resources (Stefanie *et al*, 2017),
- iv. means of assessing food quality and healthcare decision-making.

They may be characterized for 'identity' like chemical structure, fiber type, microbiological species or for 'property values' like the amount of specified chemical entity (IAEA, 2003). These characterized properties are useful in the under-listed areas.

2.9.5 Validation and measurement uncertainty

Estimation of bias which is the difference between the measured value and the true value is one of the most difficult aspects of method and equipment validation, but with the use of appropriate Reference Material, valuable information can be provided within the limits of the stated uncertainty level in the certificate of the Reference Material. Reference Material also provides the uncertainty of the method and equipment being validated. Reference Materials that are used for validation should be of the same matrix type as the test sample and should also contain similar analyte concentrations (Atkins, 2013, IAEA, 2003).

Uncertainty is estimated from replicate measurement of the Reference Material using the method used for validation. The uncertainty associated with a Reference Material should not be greater than one-third of that of the sample measurement (IAEA, 2003). Validation should be carried out on the initial use of the method for an analytical process or on equipment and checked annually using Reference Material. Every batch of testing activities also needs validation (AIHA, 2014).

2.9.6 Verification of the correct use of a method

The successful application of a valid method depends on its correct use, concerning both operator skill and suitability of equipment, reagents, and standards. Reference Materials can be used for training, checking infrequently used methods, and troubleshooting the method when unexpected results are obtained. This also enhances laboratory proficiency and trueness of measurement procedures (APLAC, 2018).

2.9.7 Calibration

In metrology and measurement technology, calibration is the comparison of measurement values obtained from an instrument or analytical method under test with those of the Calibration Standard with known accuracy (Simone, 2016). Simone, 2016 defined it as a set of operations used to establish the relationship between the value of quantities measured by an instrument or a process and the corresponding realized by a standard. The Calibration Standard could be a Reference Material with known property value measured by the instrument.

Summarily, calibration relates an output quantity to an input for a measurement system under a given condition. Loss of analyte, contamination, and interferences and their associated uncertainties must be addressed as part of the validation of the method (IAEA, 2003). Calibration is important to establish metrological traceability, ensure that measurement from instrument is consistent with other measurement, determine the accuracy of instrument readings and establish the reliability of the instrument (Simone, 2016).

2.9.8 Quality control and quality assurance

One of the major uses of Reference Materials is in Quality Control and Quality Assurance. This is the original purpose for the development of Reference Materials. To avoid unacceptable discrepancies in analytical results, a Reference Material is analyzed with a batch of the samples to verify the accuracy and overall performance of the analytical process. This provides traceability and establishes consensus values if more than one laboratory is involved. The chosen Reference Material should closely match the matrix of the sample and analyte concentration of interest. However, this is often difficult because of the relatively small variety of Reference Materials available (Evans and Lindsay 2002)

2.10 The Availability and Selection of Reference Materials

The demand for Reference Materials exceeds supply because of a wide range of materials that are analyzed in several fields of science. It is often difficult to have a Reference Material of exact matrix having a similar concentration range with the sample to be analyzed but the user must choose the most suitable one available. This is a limitation in the application of Reference Material. It is therefore important for laboratories to have Reference Materials suitable for their establishment (APLAC, 2018).

There are several hundred organizations producing Reference Materials worldwide among which are internationally renowned institutions such as the National Institute of Standards and Technology (NIST); collaborative government-sponsored programs such as the European Union Binding Corporate Rules (EU BCR) program, Switzerland's Eidgenossische Materialprufungs-und Forschungs Anstalt (EMPA) (Swiss Federal Laboratories for Materials Testing and Research), Germany Bundesanstalt fur Materialforschung und-proofing or BAM (Germany Federal Institute for materials research and testing); semi-commercial sector or trade associations such as the American Oil Chemicals Association (AOCA) and an increasing number of commercial organizations like Sigma-Aldrich, Mintek South Africa (IAEA, 2003, APLAC, 2018).

The regulatory body that oversees the production of Reference Materials worldwide is International Standard Organization (ISO). Guidance on the preparation of Reference Materials is given in ISO Guides 31, 34, 35, and 80. Also, guides on the preparation of working-level or In-House Reference Materials are available (IAEA, 2003).

2.11 Characteristics of Reference Materials

i. **Traceability:** It is the property of a measurement or the value of a standard that can be traced to national or international Reference Standards through an unbroken chain of comparisons, all having stated uncertainties (Miller *et al.*, 2018). AIHA (2014) defined it as the comparability and reliability of measurement results.

Reference materials are important tools for the transfer of measurement accuracy between laboratories and their property values should, where feasible, be traceable to an international or a national Standard. Traceability is, however, a relatively new concept in the field of chemical measurement and as a consequence very few Chemical Reference Materials are explicitly traceable to International Standard (SI). A hierarchy of methods is, however, used for assigning property values to materials, and even if not stated; their traceability can be described as in the Table below:
Table 2.2: Traceability hierarchy of methods

Measurement Method	Traceability
Primary method	SI
Method of known bias	SI/International standard
Independent method(s)	Results of specified methods
Inter-laboratory comparison	Results of specified methods
Source: IAEA, 2003	

ii. Stability: This is another characteristic of Reference Materials in which the property values are retained over time under the stated conditions. This implied that the material in the expected timescale and the presence of expected condition will not corrode, decompose, polymerize, burn or explode.

iii. Homogenicity: Reference Materials must be uniform in composition and characteristics. Homogeneity is classified as either in-bottle or between bottles. In-bottle homogeneity is a characteristic of Reference Materials in the same container having the property value close to the stated value within the limit of its uncertainty when different portions are analyzed for the presence of the analyte while between bottle homogeneity is that associated with the same batch of Reference Materials produced in different containers having their property values close to the stated value stated value in the certificate within the uncertainty level of the batch (Miller *et al.*, 2018).

iv. Uncertainty: This is the difference between the measured value and the true value of a sample (Miller *et al.*, 2018). It is introduced in the final measurement result to reflect errors introduced from sample pre-treatment, chemical measurement process, and instrumentation. Uncertainty can be controlled or minimized and characterize as it is impossible to eliminate it. Uncertainties need to be reported on the certificate that the analyte Reference Values are contained and they are commonly reported at a 95%

confidence level, which is sometimes referred to as a "two-sigma" uncertainty. There are varying methods for the determination of uncertainties and they depend on the data sets and associated analytical information. Practically, Reference Material uncertainty is probably not smaller than the smallest standard deviation associated with the data from which a Reference Value is derived. It should be noted that as uncertainty decreases, the assignment of appropriate uncertainties becomes increasingly accurate (Miller et al., 2018).

2.12 In-House Reference Material

In-House Reference Material, also known as Quality control (QC) sample, check sample, set up a sample, is an example of Matrix Reference Material that is prepared by a laboratory for its internal use. It is usually prepared when the matrix or concentration level of the target analyte of commercially available Reference Materials does not closely match the sample to be analyzed or when a sufficient supply is needed that will be available for a long time (Gopalakrishnan, 2005).

Several works have been done on the production of Reference Materials for metal determination. Samin and Sunanti (2018) developed an In-House zircon mineral Certified Reference Material (CRM) from Kalimantan (Tumbang Titi), and Bangka by obtaining 10 kg of the mineral dried at 90 °C for 6 hours twice, crushed with ball mill, passed through 200 mesh and homogenized with a homogenizer for 6 hours three times. They produced 40 bottles of the prototype In-House Reference Materials with a 100 g capacity each. The product was tested for homogeneity and stability by methods of ISO 13528 (2008) and ISO 35 (2006). The moisture content was done by gravimetric method and characterization for the oxides was done using X-Ray

Diffraction (XRD) method. The method's standardization was done using JCRM R 502 Certified Reference Material from Japan.

Silva *et al.* (2018) also prepared and characterized a Quality Control Material according to the specification of ISO Guide 80 for the determination of inorganic constituents present in a phosphate-bearing rock that is used for the manufacturing of phosphate fertilizers. The property values for the concentrations of Al, As, Ca, Cd, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, V, and Zn of the material were performed using microwave-assisted digestion and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The homogeneity and stability were also determined as an in-house validated technique. Phosphorus mass fraction was determined by Laser-Induced Breakdown Spectrometer. The accuracy of the measurement results was confirmed by analyzing the Certified Phosphate Rock and Fertilizer Certified Reference Materials (SRM 694 and SRM 695 respectively) prepared by NIST.

Aba and Ismaeel (2013) also prepared an In-House Reference Material from a material that contained a known amount of Uranium Ore mixed with marine sediment collected from Kuwait bay and the concentrations of ²²⁶Ra, ²²⁴Th, ²¹⁴Pb, ²¹⁴Bi, and ²¹⁰Pb radioisotopes were quantified.

Idris (2019) developed seven Certified Reference Materials of soil and biological tissues for testing of Ni, Pb, Zn, and Br for environmental, toxicological, agronomic, and nutritional purposes using Wave Dispersive X-Ray Fluorescence (WD-XRF). He used the between bottle homogeneity test method to test for homogeneity and found that the metals have relative standard deviation (RSD) >10% with the skewness and kurtosis revealing that the analytes in the materials are symmetrically distributed.

2.13 Factors Considered in Selecting Material for the production of In-house Reference Material

Primary considerations in selecting a material to be used as a Reference Material according to Gopalakrishnan (2005) and Idris (2019) include:

- the concentration of the analyte(s) in the material should be as close as possible to that of the target sample;
- ii. analyte(s) to be characterized in the material selected should be homogeneous;
- iii. material for the Reference Material production and the samples should be of the same matrix or matrix as close as possible.

2.14 Preparation of In-House Reference Material

Depending on the nature and quantity of samples to be prepared as well as on the available facilities for grinding, the following are the steps in the preparation of In-House Reference Materials (Gopalakrishnan, 2005):

- i. Feasibility study;
- ii. Selection of candidate material;
- iii. Homogeneity and stability tests;
- iv. Inter-laboratory comparison;
- v. Certification showing the production, characterization procedure, and statistical assessment.

It should be noted that the most critical stage in the preparation of In-House Reference Material is the chemical characterization, which provides the "best values" for elements of interest in the Reference Material. The term "best value" has been defined in different works to imply different degrees of confidence in the derived values. It is also called accepted value, attested value, certified value, consensus mean, consensus value, mean value, proposed value, provisional value, recommended value, usable value, and working value (Gopalakrishnan, 2005, Silva *et al.*, 2017, Idris, 2019).

2.14.8 Selection and processing of candidate material

Processing of material for chemical or physical characterization after the collection of the sample for Reference Materials production includes the following steps:

- (i) Drying in an oven at 120° C for 8 12 hours;
- (ii) Sieving by passing the material through a 20 mesh sieve;
- (iii) Ground for 20-30 hours in a quartzite lined mill and passed through 200 mesh sieve; 97% of the material should pass through 325 mesh or 99.21 to 99.97% of it should pass through 200 mesh.
- (iv)The powdered sample should then be homogenized using homogenizers like Double-cone blender, Polygonal blender, Ribbon blender, Cylindrical blender, Rotary blender, Rotary 'V' shaped mixer, or Sheet rolling (Gopalakrishnan, 2005).

2.14.2 Testing for homogeneity of the in-house reference material

Testing of homogeneity is an important stage in the preparation of Reference Material. Homogenization of bulk powdered samples is done in two stages (Gopalakrishnan, 2005).

Stage I: Preliminary testing of the bulk material to see whether the homogeneity is within acceptable limits, before bottling the sample.

Stage II: Testing of samples after homogenizing for many hours and at times few days. After the bottling of a sample, some of the bottles are randomly selected for the test of analyte(s) of interest. (Gopalakrishnan, 2005).

2.14.3 Fitness of the produced reference materials

After the production of the Reference Material, it is important to check its fitness. According to ISO Guide 33, the uncertainty in the Reference Values must be small when compared with that of the routine analytical data. The uncertainty can be stated as either the standard deviation of the population of measurement from the mean or the standard error of the mean. This usually differs depending on the confidence level at which they are stated. It was suggested that this reference value uncertainty should be at most one-third or preferably only one-tenth of the routine analytical results (Gopalakrishnan, 2005).

Kane (2001) suggested that laboratory results must estimate true values within 1% to 2% relatively. Reference Material Reference Value uncertainties need to be the order of 0.3% relatively. If, however, analytical results are within 10% of the true value the result is acceptable and fit-for-purpose, then reference value uncertainties of 1% to **3%** are entirely adequate.

2.14.4 Certification of the produced reference material

The three (3) types of procedures for certification of reference material are:

(i) Certification by inter-laboratory consensus. This is the most frequently encountered type of programme. In this method, many laboratories are involved in analyzing one or more analyte(s) of interest in the Reference Material (Gopalakrishnan, 2005). Participating laboratories may use

- (a) the method of their choice;
- (b) the same well-established widely accepted analytical method.

(ii) When it is impossible or impractical to arrive at a consensus value by a full interlaboratory programme, this second procedure is followed. A small number of "expert" laboratories analyze the Reference Material for the analyte(s) of interest and the certified value is arrived at by accepting the consensus value (Llaurado *et al.*, 2001, Idris, 2019).

(iii) In this third procedure, certification is done by a single laboratory using a 'definitive method', a method which is based on first principles, with high precision and for which the limits of uncertainty can be stated with a high degree of confidence (Emons *et al.*, 2004).

According to Gopalakrishnan (2005), a reference material certificate must contain three types of information:

- (a) Description of the material,
- (b) All information necessary for correct use and
- (c) Confidence-building information.

2.14.5 Reasons for the production of in-house reference material

The rationale for preparing quality control materials can be one or a combination of the following factors:

- i. to have a Reference Material representing as closely as possible the routine samples, suitable for quality control;
- ii. to have a suitable day-to-day Reference material to complement a commercially available Certified Reference Material;
- iii. no suitable Certified reference material exists.

It should be noted that the production of In-House Reference material may not require that the material have the full characteristics of Certified reference material like traceability and uncertainty (Emons *et al.*, 2004, Gopalakrishnan, 2005).

2.14.6 Characteristics of in-house reference material

In-house reference materials should comply with the basic requirements of any Reference Material; they should be:

- i. sufficiently homogeneous for the properties of interest (Emons et al., 2004).
- ii. have the level of their heterogeneity less than the expected standard deviation of the measurement process or an established criterion value against which the assessment of laboratory performance (Emons *et al.*, 2004).
- iii. be stable for a while, at least as long as the duration of intended use (Emons *et al.*, 2004, Gopalakrishnan, 2005).

2.14.7 Functions of in-house reference material

The principal function of In-House Reference Material is to provide laboratories with an economical means of checking their routine test procedures for precision and accuracy regularly either daily, weekly, or monthly (Gopalakrishnan, 2005). Other functions according to are:

- i. preparation of Quality Control charts,
- ii. comparison of results,
- iii. method development,
- iv. instrument performance checks,
- v. repeatability and reproducibility studies,
- vi. as check samples,
- vii. checking operator variability and
- viii. study of the impact of any changes to the environmental conditions (Emons *et al.*, 2004).

CHAPTER THREE

3.0

MATERIALS AND METHOD

3.1 Materials

3.1.1 Reagents

Analytical grade nitric acid, hydrochloric acid, hydrogen peroxide, boric acid, methyl red, bromocresol green, sodium hydroxide, copper sulphate, and sulphuric acid, manufactured by Guangdong Guanghua Sci-Tech Ltd (JHD) were used. Certified Reference Material (CRM-989) of the International Soil-Analytical Exchange, Wageningen University of Environmental Sciences, The Netherlands was used for quality control and traceability of the prepared Control Soil Reference Material.

3.1.2 Equipment

The equipment used in the study is shown in Table 3.1 below.

Equipment	Model	Manufacturer					
Hot air oven	W53C Hot air oven	Genlab Widness, England					
Digestive furnace	KDN-20C Digestive furnace with	Hinotec Instruments, Qiancheng					
	vapour condensing device	Building, Ningbo Hi-Tech Zone					
		Ningbo, China					
Atomic Absorption	210VGP AAS	Buck Scientific Instruments, 58					
Spectrophotometer		Fort Point St Norwalk , CT, 06855-1097, United States					
		00055 1077, Onice States					
	iCE TM 3500 AAS	Thermo Fisher Scientific					
	A. Analyst 200 AAS	Perkin-Elmer Inc.					
Nitrogen distillation	Glass apparatus	-					
apparatus							
Laboratory mixer	V-Tech Round 500L Drum	Lokpal Industries, India					
	capacity Laboratory Mixer						
Grinding machine	FY 130 Grinding machine	-					

Table 3.1: List of Equipment used and their Models

3.2 Sample collection and preparation

Surface soil samples were collected from agricultural farms, roadsides, mechanic workshops, and dumpsites across Minna metropolis, Niger State, Nigeria. The soil samples were bulked together to form a composite sample. It was then air-dried at room temperature for two weeks, passed through a 2 mm nylon sieve until about 80kg of the soil was obtained. The 80 kg soil sample was mixed using a laboratory mixer for 2 hours. The soil was oven-dried at 105°C until a constant weight was obtained, ground with a grinding mill, and passed through a 0.25 mm nylon sieve. The moisture content was determined using the gravimetric method by taking 10 g of the sample into pre-weighed moisture can and dried in an oven until a constant weight was obtained (Samin and Sunanti, 2018; ISO Guide 80, 2014).

The soil was again homogenized with the mixer for 2 hours. Ten sub-samples were collected at different depths and orientations from the mixer for homogeneity test. Mixing was further done for 1 hour after the homogeneity test and the sample was bottled in 500 g portions in transparent nylon bags and placed in high-density polyethylene bottles using a plastic spatula and funnel until a total of 100 bottles were obtained. One out of every ten bottled sample (10 sample bottles) were separated for within-bottle and between-bottle homogeneity test as well as the estimation of the heavy metal content (Cu, Mn, Zn, Fe, Pb, Cr, and Cd) of the analyte of interest (Magharbeh *et al.*, 2014). The composite sample was then coded FUTMX7316SRM and stored.



Figure 3.1: Steps in the Inter-Laboratory Study

3.3 Determination of Textural Class of the Collected Soil Sample

The particle size distribution of the collected soil sample was determined using bouyoucos hydrometer method. The proportions of sand, silt, and clay particles were obtained using the steps below:

- Into a 250 cm³ conical flask was weighed 50 g of 2 mm sieved air-dried soil followed by the addition o 100 cm³ of sodium hexametaphosphate (Calgon) solution and left for 30 mins.
- ii. The soil suspension was transferred to a dispersion bottle and stir on a mechanical shaker for 15mins.

- iii. The soil suspension was then transferred to a 1 dm³ capacity measuring cylinder and water was added to the 1 L mark.
- iv. The soil suspension was stirred with a wooded plunger for 2 mins until all the particles were in suspension.
- v. A soil hydrometer was inserted and a reading at 40 secs was taken.
- vi. Immediately, the thermometer was inserted and the temperature of the suspension was taken.
- vii. It was left on a stable surface undisturbed and after 2 hrs the soil hydrometer was inserted as well as the thermometer and readings at 2 hrs were taken.
- viii. A blank was prepared by adding 100 cm³ Calgon into a 1 L capacity measuring cylinder and made it to a 1 L mark with water. The hydrometer and thermometer reading at 40 secs and 2 hrs were also taken as that of the sample.

Calculation

% silt + Clay =
$$\frac{(S1 - BI) + ((STI - 20^{\circ} C)X \ 0.36))}{50} * \frac{100}{1}$$
 (3.1)

% Clay =
$$\frac{(S2-B2) + ((ST2-20^{\circ}C)X 0.36))}{50} * \frac{100}{1}$$
 (3.2)

$$Sand = 100 - (\% silt + Clay)$$
 (3.3)

$$\% \text{ silt} = (\% \text{ silt} + \text{clay}) - \% \text{ clay}$$
 (3.4)

Where: Sample Hydrometer reading at 40 secs = S_1 Sample Thermometer reading at 40 secs = ST_1 Sample Hydrometer reading at 2 hrs = S_2 Sample thermometer reading at 2 hr = ST_2 Blank Hydrometer reading at 40 secs = B_1 Blank Thermometer reading at 40 secs = BT_1 Blank Hydrometer reading at 2 hrs = B_2 Blank Thermometer reading at 2 hrs = BT_2

3.4 Assessment of Homogeneity of the Prepared Control Soil Reference Material

Before bottling the ten (10) portions from several parts, at different depths and orientations of the mixer were analyzed for the concentration of the total nitrogen using the macro-Kjeldahl method. This was chosen as the index because it is poorly distributed in soil. The soil samples were also analyzed for total organic carbon (Idris, 2019).

After bottling, ten (10) bottles of the packaged materials were selected and analyzed for total nitrogen and total organic carbon to check for the between-bottle homogeneity in triplicate. The triplicate data were used to establish the within-bottle homogeneity. The prepared Control Samples were stored in a dry place at room temperature (Idris, 2019).

Number of test unit =
$$3 \times \sqrt[8]{n}$$
 (Magharbeh *et al.*, 2014) (3.5)

Where n = total number of prepared unit

3.5 Determination of Organic Carbon and Organic Matter

The total organic carbon was determined by Walkley Black wet oxidation method as described in the report of Ramamoorthi and Meena (2018). The procedure is highlighted below.

- One (1) gram of the soil sample was weighed into a 250 cm³ conical flask and to it was added 5 cm³ of 1 N K₂Cr₂O₇ and the flask was swirled gently to disperse the soil in solution
- ii. Ten (10) cm³ of concentrated H_2SO_4 was added and mixed with the soil and the reagents thoroughly. It was then allowed to stand on an asbestos pad for 30 minutes to cool down and 200 cm³ of distilled water was added.

- iii. Four (4) drops of ferroin indicator solution were added and the contents of the flask were titrated against 0.4 N (NH₄)₂FeSO₄. The endpoint was approached when the solution first assumes a greenish appearance which gradually changed to green and finally to brown.
- iv. A blank determination was done without the soil sample
- v. The total organic carbon content of the soil sample was calculated using equations 3.6 to 3.8 below:

Organic carbon
$$(g kg^{-1}) = \frac{(BT-ST)*N*10}{W}$$
 (3.6)
Where:
BT = Blank Titre value
ST = Sample Titre value

 $N = Normality of (NH_4)_2 FeSO_4$

W = Weight of soil sample

N was obtained by:
$$\frac{\text{mL of } K_2 \operatorname{Cr}_2 \operatorname{O}_7 (5 \text{ mL})}{\text{BT}}$$
(3.7)

Soil organic matter
$$(g kg^{-1}) = OC * 1.72$$
 (3.8)

3.6 Determination of Total Nitrogen

The total nitrogen of the soil sample was determined by the Kjeldahl Method according to the steps in the report of Mádlíková *et al.*, 2018. The steps are highlighted below:

- i. One (1) gram of the soil sample was weighed into a digestion flask and 0.5 g of catalyst (CuSO₄) and 15 cm³ of concentrated H_2SO_4 were added.
- ii. The sample was then digested at 360 °C until a clear digest was obtained. It was allowed to cool and about 25 cm³ of distilled water was gradually added and

the flask swirled and mixed well to bring every material into suspension. More distilled water was added to bring up to the 100 cm³ mark in the flask.

- iii. Five (5) cm³ of 4 % H₃BO₃ mixed with indicator solution (methyl red and bromocresol green prepared with ethanol) were measured and transferred into a 50 cm³conical flask marked to indicate the 35 cm³ mark. The flask was placed under the condenser of the distillation apparatus. Ten (10) cm³ of the digest and 20 cm³ of 40% NaOH were transferred into the distillation apparatus through the funnel of the apparatus.
- iv. The stem of the bypass tube was closed and distillation was started immediately.
 When the distillate reached the 35 cm³ mark on the flask, the stem of the bypass tube was opened and the end of the condenser was rinsed with distilled water.
- v. The distillate was titrated with 0.01 M HCl.
- vi. A blank was done along with the sample from digestion to distillation which contained no soil sample.
- vii. The concentration of total nitrogen in the soil was then calculated using equation

$$3.9$$
 below:

% Total N =
$$\frac{(T_1 - T_2) * N * V_1 * 14.01 * 100}{1000 * W * V_2}$$
 (3.9)

Where:

T1 = Titre value for sample

- T2 = Titre value for blank
- N = Concentration of the acid (HCl)

W = Weight of soil sample

- V1 = Final volume of the digest
- V2 = Volume of digest taken (aliquot used) for distillation

3.7 Digestion of Soil Samples

The digestion method, EPA 3050b, used was found in the report of Bonsucesso *et al.*, 2018. Below is the digestion steps:

- Two (2) grams of the soil samples each of the separated FUTMX7316SRM was weighed into digestion tubes and 10 cm³ of aqua regia (1:1 HNO₃ and HCl) mixture was added and the slurry was mixed and covered with a vapor recovery device.
- The sample slurry was heated to 95 °C and reflux for 15 minutes without boiling.
 The sample was cooled and 5 cm³ of concentrated HNO₃ was added and the vapour recovery device was replaced and refluxed for 30 minutes.
- iii. Three consecutive 5 cm³ of concentrated HNO₃ was added until brown fume (indicating oxidation) stops which indicated the complete reaction with the HNO₃. The vapour recovery device was removed and the digest was heated again to 95 °C without boiling and maintained for two hours until the solution had evaporated to approximately 5 cm³.
- iv. The sample was cooled and 2 cm³ of water and 3 cm³ of 30 % H₂O₂ were added. The tube was then covered with the vapour recovery device and heated carefully to start the peroxide reaction. Care was taken to ensure that losses do not occur due to excessive vigorous effervescence. The reaction mixture was heated until effervescence subsides and the tube was cooled. The addition of 30 % H₂O₂ in 1 cm³ aliquot was done with warming until the effervescence was minimal but none exceeded a total volume of 10 cm³.

- v. The tube containing the acid-peroxide digest was then covered again with the vapour recovering device and heated at 95 °C without boiling for another two hours.
- vi. 10 cm³ of concentrated HCl was added to the sample digest, covered with the vapor recovery device, and heated to reflux at 95 °C for 15 minutes.
- vii. The digest was filtered through Whatman No. 41 filter paper into a 100 cm³ volumetric flask and the residue in the filter paper was washed with hot 0.1 M HCl in 10 cm³ portions three consecutive times. The filtrate was allowed to cool and made up to volume with distilled water and transfer into a polythene bottle.

3.8 Analyses of the Digested Samples

Six laboratories in Nigeria that have functional Atomic Absorption Spectrophotometer (AAS) were identified which included the Central Research Laboratory of the Federal University of Technology Minna (LAB 1). Other laboratories were assigned codes as LAB 2 to LAB 6. The digests were taken to each of these laboratories for the quantification of Cu, Mn, Zn, Fe Pb, Cr, and Cd using their Flame Atomic Absorption Spectrophotometer (FLAAS) irrespective of the model.

3.9 Data Analysis

The results obtained from total nitrogen and organic matter determinations were used to study the homogeneity of the prepared Control Samples. Mean, standard deviation, and coefficient of variance (CV) were computed for results obtained. Analysis of Variance (ANOVA) was used to check if there are significant differences in the mean total nitrogen and organic carbon between and within the ten sampled bottles of the Control Sample. This was used to test for between and within-bottle homogeneity. Significant means were separated into homogeneous subsets using Duncan Multiple Range Test (DMRT) (Zhao *et al.*, 2019).

The recoveries of the metals from the CRM used were calculated by adopting the formula stated in the work of Parvizi *et al.* (2018):

$$\operatorname{Recovery}(\%) = \frac{\operatorname{Xtest}}{\operatorname{XCRM}} * 100 \tag{3.10}$$

Where: X_{test} = Concentration of metal in the CRM from the experiment and X_{CRM} = Actual concentration of metal in the CRM.

The results collected from the four participating laboratories for each of the metals were first represented on a graph to observe the trend of the obtained result. Boxplot was used to check for outliers and evaluation of the data was done by the computation of the median and median average deviation (MAD) of each metal from each laboratory. The average median value for each metal from the four laboratories was compared with each other using ANOVA and significant averages were separated using DMRT (Tasker *et al.,* 2019). The software SPSS 22.0, 2013 Version was used for the analysis of the results (Konieczka and Namiesnik, 2018; Hineman *et al.,* 2018; Velychko and Gordiyenko, 2019).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Particle Size Distribution of the Prepared In-House Soil Reference Material (FUTMX7316SRM)

The amount of sand, silt, and clay of ten (10) systematically collected soil samples from FUTMX7316SRM before pulverizing and their average is shown in Table 4.1 below. The sample contained an average of 5 %, 8 %, and 86 % clay, silt, and sand respectively. The textural class of the soil sample is Loamy sand.

Sample	Clay (%)	Silt (%)	Sand (%)
1	6	6	88
2	6	8	86
3	6	8	86
4	5	9	86
5	5	8	87
6	6	8	86
7	5	8	87
8	5	9	86
9	6	8	86
10	5	8	87
Average	5	8	87

Table 4.1: Particle Size Distribution of FUTMX7316SRM

Textural class: Loamy sand

4.2 Homogeneity of FUTMX7316SRM

The homogeneity test, using total nitrogen and organic carbon as indicators, of the FUTMX7316SRM before and after bottling is shown in Tables 4.2 and 4.3 respectively.

Sample	Total Nitrogen (%)	Organic Carbon (%)
1	0.185 ± 0.078	0.419 ± 0.013
2	0.087 ± 0.033	0.421 ± 0.002
3	0.195 ± 0.078	0.407 ± 0.005
4	0.067 ± 0.075	0.408 ± 0.011
5	0.165 ± 0.035	0.413 ± 0.004
6	0.150 ± 0.028	0.414 ± 0.020
7	0.185 ± 0.007	0.412 ± 0.059
8	0.185 ± 0.021	0.366 ± 0.019
9	0.170 ± 0.042	0.406 ± 0.023
10	0.165 ± 0.078	0.432 ± 0.003
Mean	0.155 ± 0.044	0.410 ± 0.017
Range	0.067 - 0.195	0.366 - 0.432
Significance (95%CI)	0.340NS	0.413NS

 Table 4.2: Total Nitrogen and Organic Carbon as Indicators of Homogeneity of the FUTMX7316SRM before Bottling

NS: Not significantly different (p < 0.05) Means are \pm Standard deviation

Sample bottle number	TN (%)	OC (%)
1	0.161 ± 0.035	0.377 ± 0.044
2	0.145 ± 0.004	0.380 ± 0.037
3	0.145 ± 0.008	0.378 ± 0.023
4	0.149 ± 0.015	0.382 ± 0.030
5	0.159 ± 0.025	0.378 ± 0.034
6	0.170 ± 0.004	0.377 ± 0.045
7	0.156 ± 0.021	0.381 ± 0.063
8	0.158 ± 0.012	0.359 ± 0.018
9	0.194 ± 0.042	0.382 ± 0.035
10	0.152 ± 0.004	0.377 ± 0.050
Mean	0.159 ± 0.015	0.377 ± 0.007
Range	0.145 - 0.194	0.359 - 0.382
Significance (95%CI)	0.255NS	1.000NS

Table 4.3: Total Nitrogen and Total Organic Carbon Content of FUTMX7316SRM as Indicators for the Between Bottle Homogeneity Test after Bottling

NS: Not significantly different (p < 0.05) Means are \pm Standard deviation

The concentration of total nitrogen and total organic carbon used as indicators for the homogeneity test before bottling the prepared FUTMX7316SRM ranged from 0.063 - 0.250 % and 0.373 - 0.454 % respectively (Table 4.2). The Analysis of Variance (ANOVA) test of the data obtained showed that there were no significant differences in both the total nitrogen and total organic carbon contents between the ten (10) samples collected at different points and depths within the laboratory mixer.

The between-bottles homogeneity test, using total nitrogen and total organic carbon as indicators for FUTMX7316SRM after bottling (Table 4.3) revealed that the total nitrogen and total organic carbon ranged from 0.126 - 0.238 % and 0.344 - 0.454 % respectively, and the ANOVA test of the data showed that there were no significant differences in these indicators between the ten (10) bottles of the prepared FUTMX7316SRM separated for analysis. This showed that there is between bottle homogeneity of the prepared FUTMX7316SRM.

4.3 Accuracy of the Method

The percentage recoveries of some heavy metals in the CRM- 989 from four laboratories in Nigeria using flame atomic absorption spectrophotometer are shown in Table 4.4. This was used for quality control check of the digestion method used as well as the instrument (Atomic Absorption Spectrophotometer) used by the four (4) laboratories in Nigeria.

Table 4.4: Recoveries in Percentage of Some Heavy Metals in the CRM	989
obtained from Four Laboratories in Nigeria	

Metal	LAB 1	LAB 2	LAB 3	LAB 4
Zn	33.05	10.74	8.28	45.59
Fe	78.25	51.46	0.56	-
Mn	73.81	95.24	3.60	304.95
Pb	65.78	110.11	11.31	94.15
Cu	88.56	93.46	15.16	93.14
Cr	-	83.15	-	-
Cd	102.19	128.35	-	88.20

ISO Guide 80, 2014 stated that the recovery of a metal from a sample should be within the range of 75 - 125 % before the method used in a determination can be acceptable. The recoveries of Fe, Cu, and Cd in the CRM – 989 from LAB 1 (78.25 %, 88.56 %, and 102.19 % respectively) were within 75 - 125 % but those of Pb, Mn, and Zn (65.78 %, 73.81 %, and 33.05 % respectively) were low. The recoveries of Mn, Pb, Cu, and Cr from the CRM obtained from LAB 2 were within the range of 75 - 125 % (92.24, 110.11, 93.46, 83.15, and 128.35 % respectively) but that of Cd (128.35 %) was slightly above 125 % while those of Fe (51.46 %) and Zn (10.74 %) were low. The recoveries of Cu, Pb, and Cd obtained from LAB 4 were within the range of 75 - 125 % (93.14 %, 94.15 %, and 88.20 % respectively) but that of Mn (304.95 %) was very high and that Zinc (45.59 %) was low. The recoveries of all the metals from LAB 3 were very low; they were all less than 16 %; Cr and Cd were below the detectible limit (BLD).

The recoveries of metals that fell within the specified range of 75 - 125 % (ISO Guide 80, 2014) were Fe from LAB 1, Mn from LAB 2, Pb from LAB 2 and LAB 4, Cu from LAB 1, LAB 2, and LAB 4, Cr from LAB 2 and Cd from LAB 1 and LAB 4. These recoveries were comparable to those obtained in the work of Chan and Ma (2001) with recoveries above 74 % with the use of aqua regia as a digestion method.

4.4 Precision of the measurements

4.4.1 Zinc

The concentration of Zn in FUTMX7316SRM obtained from four laboratories in Nigeria is shown in Table 4.5 below.

Sample	LAB 1				LAB 2			LAB 3			LAB 4		
			CV			CV						CV	
	Mean	SD	(%)	Mean	SD	(%)	Mean	SD	CV (%)	Mean	SD	(%)	
1	256.92	0.98	0.38	34.25	14.50	42.32	142.72	166.90	116.95	162.50	22.75	14.00	
2	263.92	10.07	3.81	22.00	0.00	0.00	183.43	220.09	119.99	205.50	67.82	33.00	
3	261.60	10.44	3.99	39.50	31.11	78.77	25.50	0.00	0.00	211.50	76.14	36.00	
4	272.52	20.41	7.49	48.00	33.23	69.24	133.29	153.43	115.11	200.00	34.00	17.00	
5	268.18	10.22	3.81	39.75	23.69	59.59	166.86	204.43	122.52	217.50	71.78	33.00	
6	271.73	10.62	3.91	60.75	19.45	32.01	217.45	270.61	124.45	197.00	67.97	34.50	
7	252.85	14.83	5.87	35.00	14.85	42.43	110.44	126.06	114.14	211.00	33.76	16.00	
8	260.08	10.01	3.85	24.25	34.29	141.42	161.63	193.22	119.55	214.00	40.66	19.00	
9	267.92	10.09	3.77	68.25	15.20	22.28	30.80	0.00	0.00	241.00	19.28	8.00	
10	281.75	14.20	5.04	79.75	12.37	15.52	23.10	0.00	0.00				
Mean	265.75	11.19	4.19	45.15	19.87	50.36	119.52	133.47	83.27	206.67	48.24	23.39	
Median	265.92	10.33	3.88	39.63	17.32	42.37	138.00	160.16	116.03	211.00	40.66	19.00	
Range	252	.85 – 281	.75	22	2.00 – 79	0.75	23	23.10 - 217.45			162.50 - 241.00		
95% Confidence level		0.228			0.289			0.925			0.861		

 Table 4.5: Descriptive Statistics of the Concentration of Zinc in the Prepared FUTMX7316SRM Obtained from Four Laboratories in Nigeria

The mean \pm Standard deviation (SD) and median \pm median average deviation (MAD) concentration of Zn in mg/kg of the FUTMX7316SRM were 265.75 \pm 11.19 and 265.92 \pm 10.33 from LAB 1, 45.15 \pm 19.87 and 39.63 \pm 17.32 from LAB 2, 119.52 \pm 133.47 and 138.00 \pm 160.16 from LAB 3 and 192.25 \pm 43.63 and 208.58 \pm 37.33 from LAB 4. The mean Coefficients of Variance (CV) in the percentage of the concentration of Zn obtained from the laboratories were 4.19, 50.36, 83.27, and 21.40 for LAB 1, LAB 2, LAB 3, and LAB 4 respectively; the CV of the concentration of Zn from LAB 1 was low while those of the other laboratories were high. Evident from the high CVs of LAB 2, LAB 3, and LAB 4 are the presence of extreme values in the data obtained diminishing the reliability of the data because according to (Konieczka and Namiesnik, 2018), large uncertainties (variations) distinguish reliable and unreliable measurements. The concentration of Zn from LAB 1 with low uncertainty (CV) showed more reliability compared to other laboratories. The decreasing order of the reliability of the obtained concentration of Zn from these laboratories, therefore, was LAB 1 > LAB 4 > LAB 2 > LAB 3.

There were no significant differences (p > 0.05) in the mean concentration of Zn between the subsamples obtained from each of LAB 1, LAB 2, and LAB 3. The concentration of Zn between the subsamples from LAB 4 showed some level of significant differences (p < 0.05). This showed the there were present in the data some extreme value(s).

4.4.2 Iron

The concentration of Fe in the FUTMX7316SRM obtained from three laboratories in Nigeria is shown in Table 4.6.

Sample		LAB 1			LAB 2			LAB 3			
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV		
1	7838.93	495.79	6.32	9500.00	919.24	9.68	201.41	104.08	51.68		
2	8058.10	60.10	0.75	15775.00	1661.70	10.53	224.81	62.50	27.80		
3	8303.10	788.42	9.50	7950.00	1909.19	24.01	278.00	0.00	0.00		
4	7913.10	364.16	4.60	8200.00	5939.70	72.44	211.81	114.83	54.21		
5	8402.27	927.18	11.03	9625.00	3146.63	32.69	234.31	80.18	34.22		
6	8720.60	1665.89	19.10	9550.00	6293.25	65.90	261.78	17.29	6.60		
7	7863.10	1028.84	13.08	6675.00	813.17	12.18	280.62	-	-		
8	8025.60	1516.81	18.90	9775.00	8237.79	84.27	200.86	120.41	59.95		
9	7970.60	862.67	10.82	5775.00	2368.81	41.02	306.00	0.00	0.00		
10	7623.93	825.02	10.82	10500.00	7495.33	71.38	291.00	0.00	0.00		
Mean	8071.93	853.49	10.49	9332.50	3878.48	42.41	249.06	55.48	26.05		
Median	7998.10	843.85	10.82	9525.00	2757.72	36.86	248.04	62.50	27.80		
Range	7623.	93 - 8720.	60	5775.0	0 - 15775	.00	200	200.86 - 306.00			
95% Confidence level		0.974			0.727			0.814			

Table 4.6: Descriptive Statistics of the Concentration of Iron in FUTMX7316SRM Obtained from Four Laboratories in Nigeria

The mean \pm SD and median \pm MAD concentration of Fe in mg/kg of ten subsamples of FUTMX7316SRM were 8810.77 \pm 1848.01 and 8944.77 \pm 1851.47 for LAB 1, 9332.50 \pm 3878.48 and 9525.00 \pm 2757.72 for LAB 2 and 273.08 \pm 83.90 and 248.04 \pm 71.34 for LAB 3. The percentages CV for the mean values were 20.31, 42.41, and 29.97 for LAB 1, LAB 2, and LAB 3 respectively. These uncertainty levels were high which reveals the possible presence of extreme values (Konieczka and Namiesnik, 2018). The ANOVA result revealed the there were no significant differences (P > 0.05) in the mean concentrations of Fe between the ten subsamples of FUTMX7316SRM from three laboratories.

4.4.3 Manganese

The concentrations of manganese in the subsamples of FUTMX7316SRM obtained from four laboratories in Nigeria are shown in Table 4.7.

Sample		LAB 1		LAB 2				LAB 3		LAB 4			
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
1	156.65	5.47	3.49	356.25	132.58	37.22	2571.43	3620.56	140.80	143.90	34.54	24.00	
2	155.22	29.40	18.94	360.00	162.63	45.18	2188.25	3079.80	140.74	122.50	28.18	23.00	
3	157.51	19.23	12.21	382.50	95.46	24.96	11.90	0.00	0.00	150.95	54.34	36.00	
4	158.46	14.78	9.33	335.00	141.42	42.22	2792.35	3933.85	140.88	172.00	29.24	17.00	
5	154.75	7.31	4.72	295.00	49.50	16.78	2393.10	3365.26	140.62	166.30	54.88	33.00	
6	144.18	8.58	5.95	243.75	8.84	3.63	2112.35	2967.23	140.47	131.60	19.08	14.50	
7	157.60	14.74	9.36	285.00	10.61	3.72	1848.70	2595.79	140.41	167.65	26.82	16.00	
8	154.08	14.95	9.70	275.00	28.28	10.29	1675.00	2350.85	140.35	136.65	28.70	21.00	
9	156.46	20.14	12.87	246.25	47.73	19.38	14.30	0.00	0.00	138.85	24.99	18.00	
10	142.84	13.68	9.58	265.00	-	-	15.10	0.00	0.00	142.85	47.85	33.50	
Mean	153.78	14.83	9.62	304.38	75.23	22.60	1562.25	2191.33	98.43	147.33	34.86	23.60	
Median	155.84	14.76	9.47	290.00	49.50	19.38	1980.53	2781.51	140.44	143.38	28.97	22.00	
Range	142.	84 – 158	.46	243	.75 – 382	.50	50 11.90 - 2792.35				122.50 - 172.00		
95% Confidence level	0.944			0.802			0.934			0.778			

 Table 4.7: Descriptive Statistics of the Concentration of Manganese in FUTMX7316SRM Obtained from Four Laboratories in Nigeria

It was observed that there were differences in the mean and median of the data collated which revealed the presence of extreme values in the data (Tan *et al.*, 2018). The mean \pm SD and median \pm MAD concentration of manganese in mg/kg of the FUTMX7316SRM from the four laboratories were respectively 153.78 \pm 14.83 and 155.84 \pm 14.76, 338.63 \pm 116.14 and 315.00 \pm 72.48, 1562.25 \pm 2191.33 and 1980.53 \pm 2781.51 and 147.33 \pm 34.86 and 143.38 \pm 28.97. The percent CV of LAB 1 was less than 10 % (9.62 %) but those of the other laboratories were greater than 20 % (28.31 %, 98.43 %, and 23.60 % for LAB 2, LAB 3and LAB 4 respectively). The large variances also confirmed the presence of outliers (Konieczka and Namiesnik, 2018). The ANOVA report which compared the concentrations of Mn between the ten subsamples of the FUTMX7316SRM from each of the laboratories revealed that there were no significant differences (p > 0.05) between the subsamples.

4.4.4 Lead

The concentration of lead in the ten subsamples of the FUTMX7316SRM obtained from four laboratories in Nigeria is shown in Table 4.8. The mean \pm SD and median \pm MAD concentrations were 13.42 \pm 3.59 and 12.75 \pm 3.33, 19.55 \pm 5.02 and 19.13 \pm 3.01, 16.58 \pm 12.21 and 9.28 \pm 3.71 and 17.99 \pm 4.00 and 20.12 \pm 4.96 and the CVs of the data were high. The respective CVs of the concentration of Pb from LAB 1, LAB 2, LAB 3 and LAB 4 were 26.30, 24.70 %, 64.74 %, and 21.21 %. The wide differences observed between the mean and the median concentration values revealed the presence of extreme values (Cezera *et al.*, 2009; Hineman *et al.*, 2018). There were no significant differences (p > 0.05) in the mean concentration of Pb between the ten subsamples for each of the four laboratories.

Sample		LAB 1			LAB 2			LAB 3		l	LAB 4		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
1	16.67	1.61	9.64	19.75	2.47	12.53	8.10	11.46	141.42	21.49	4.73	21.99	
2	11.83	3.33	28.13	18.75	1.06	5.66	8.20	11.60	141.42	18.24	2.38	13.02	
3	13.67	3.33	24.36	16.75	1.77	10.55	14.50	0.00	0.00	13.70	3.57	26.03	
4	12.50	4.00	32.00	11.25	1.06	9.43	9.50	3.82	40.19	20.07	5.42	27.01	
5	10.75	0.35	3.29	12.00	-	-	12.70			20.16	6.25	31.00	
6	14.00	1.32	9.45	27.50	3.54	12.86	8.51	2.39	28.08	20.80	5.20	24.98	
7	12.00	1.00	8.33	10.75	6.72	62.49	7.80	11.03	141.42	21.27	5.53	26.00	
8	13.00	3.61	27.74	26.25	4.60	17.51	9.05	3.61	39.85	24.88	5.22	20.98	
9	11.67	2.93	25.11	17.50	0.00	0.00	14.30	0.00	0.00	14.12	2.55	18.03	
10	12.00	4.27	35.60	19.50	7.07	36.26	17.80	0.00	0.00	-	-	-	
Mean	12.81	2.57	20.37	18.00	3.14	18.59	11.05	4.88	59.15	19.41	4.54	23.23	
Median	12.25	3.13	24.74	18.13	2.47	12.53	9.28	3.61	39.85	20.16	5.20	24.98	
Range	10	10.75 - 16.67		10.7	10.75 - 27.50		7.	7.80 - 17.80			13.70 - 24.88		
95% Confidence level		0.549			0.325			0.827			0.159		

 Table 4.8: Descriptive Statistics of the Concentration of Lead in the Prepared FUTMX7316SRM Obtained from Four

 Laboratories in Nigeria

4.4.5 Copper

The concentration of Cu in the prepared FUTMX7316SRM obtained from four laboratories in Nigeria is shown in Table 4.9 below. The mean \pm SD and median \pm MAD of the concentration of Cu in mg/kg in the Control Soil were 13.57 \pm 5.40 and 12.58 \pm 5.10 for LAB 1, 27.08 \pm 20.90 and 16.88 \pm 6.19 for LAB2, 23.28 \pm 30.98 and 9.78 \pm 13.83 for LAB3, and 20.50 \pm 4.47 and 15.50 \pm 3.41 for LAB 4. The CVs for the respective laboratories were 34.36 %, 42.57 %, 100.55 % and 20.60 %.The CVs were high and showed the presence of extreme values (Tan *et al.* 2018).

Sample		LAB 1			LAB 2			LAB 3	6		LAB 4		
	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	
1	13.85	4.66	33.67	16.75	4.60	27.44	7.16	8.00	111.79	9.00	2.16	24.00	
2	12.38	5.98	48.28	14.00	-	-	3.58	3.08	86.04	-	-	-	
3	11.30	0.42	3.75	13.00	-	-	1.20	0.00	0.00	12.88	1.58	12.30	
4	14.95	8.91	59.60	19.25	9.55	49.59		B.D.L		18.50	5.00	27.00	
5	8.68	0.32	3.66	12.00	0.00	0.00	9.78	13.83	141.42	12.50	1.63	13.00	
6	11.07	5.91	53.43	11.50	1.41	12.30	11.03	15.60	141.42	16.00	0.72	4.50	
7	14.45	5.54	38.31	17.00	7.78	45.75		B.D.L		15.50	2.48	16.00	
8	10.07	2.33	23.15	11.50	2.12	18.45	12.40	17.54	141.42	15.00	4.65	31.00	
9	14.20	6.13	43.15	21.25	10.25	48.25	8.20	0.00	0.00	15.50	4.34	28.00	
10	10.43	1.46	13.97	14.00	2.83	20.20		B.D.L		-	-	-	
Mean	12.14	4.17	32.10	15.03	4.82	27.75	7.62	8.29	88.87	14.36	2.82	19.47	
Median	11.84	5.10	35.99	14.00	3.71	23.82	8.20	8.00	111.79	15.25	2.32	20.00	
Range	8.0	58 – 14.	95	11.	50 - 21	.25		1.2 – 12	.4	9	.00 – 18.5	50	
95% Confidence level		0.800			0.760			0.898			0.087		

 Table 4.9: Descriptive Statistics of the Concentration of Copper in FUTMX7316SRM Obtained from Four Laboratories in Nigeria

There were no significant differences (P > 0.05) in the mean concentration of copper between the ten subsamples for each of LAB 1 LAB 2 and LAB 3. Those of LAB 4 showed some level of significant difference (p < 0.05). The mean separation using the Duncan Multiple Range Test (DMRT) of the data from LAB 4 revealed that there were some extreme values.

4.4.6 Cadmium

Table 4.10 shows the concentration of Cd in FUTMX7316SRM obtained from the different Laboratories in Nigeria. The mean \pm SD and median \pm MAD of the concentration of Cd in mg/kg of the ten subsamples of the FUTMX7316SRM were 3.13 \pm 0.43 and 2.96 \pm 0.23, for LAB1, 0.64 \pm 0.32 and 0.64 \pm 0.42 for LAB 2, 0.42 \pm 0.59 and 0.42 \pm 0.42 for LAB 3 and 0.21 \pm 0.06 and 0/20 \pm 0.06 for LAB4. The mean CVs of the obtained data were 22.81 %, 50.62 %, 141.42 %, and 30.46 % for LAB1, LAB2, LAB3, and LAB4 respectively. The CVs were high and revealed the presence of extreme values (Cezera *et al.*, 2009; Konieczka and Namiesnik, 2018; Hineman *et al.*, 2018; Tan *et al.*, 2018). There were no significant differences in the mean concentration of Cd between the ten subsamples obtained from each of the four laboratories.

Sample	LAB 1		LAB 2			LAB 3	LAB 4			
	Mean	SD	CV	Mean	SD	CV	Mean SD CV	Mean	SD C	CV
1	2.75	1.05	38.31	0.75	0.42	56.57	0.41 0.58 141.42	0.14	0.05 35	5.71
2	2.73	0.74	27.25	0.93	0.53	57.33	B.D.L.	0.22	0.06 25	5.42
3	3.90			0.43	0.11	24.96	B.D.L.	-	-	-
4	3.98	0.11	2.67	0.63	0.04	5.66	0.43 0.60 141.42	0.18	0.05 27	7.78
5	2.05	0.35	17.25	0.50	0.57	113.14	B.D.L.	0.25	0.10 37	7.52
6	3.40	0.00	0.00	0.55	0.42	77.14	B.D.L.	0.19	0.08 42	2.11
7	2.75	0.85	30.86	0.78	0.46	59.31	B.D.L.	0.15	0.06 37	7.55
8	3.80	-	-	0.65	-	-	B.D.L.	0.22	0.07 30	0.03
9	3.15	-	-	0.50	0.28	56.57	B.D.L.	0.24	0.02 6.	.45
10	2.77	1.20	43.31	0.73	0.04	4.88	B.D.L.	0.19	0.06 31	.58
Mean	3.13	0.61	22.81	0.64	0.32	50.62	0.42 0.59 141.42	0.20	0.06 30).46
Median	2.96	0.74	27.25	0.64	0.42	56.57	0.42 0.59 141.42	0.19	0.06 31	1.58
Range	2.05 - 3.98			0.43 - 0.93			0.41 - 0.43	0.14 - 0.25		
95% Confidence level	0.535		0.940			0.770	0.384			

 Table 4.10: Descriptive Statistics of the Concentration of Cadmium in FUTMX7316SRM Obtained from Four Laboratories in Nigeria

B.D.L.: Below detectible limit

4.4.7 Chromium

The concentrations in mg/kg of Cr in the ten subsamples of FUTMX7316SRM obtained from two laboratories in Nigeria are shown in Table 4.11. The mean \pm SD and median \pm MAD of the obtained data were 127.65 \pm 39.95 and 133.25 \pm 33.94 for LAB2 and 88.73 \pm 125.49 and 95.36 \pm 134.86 for LAB 3. The percent CVs for the mean were 31.94 and 144.42 for LAB 2 and LAB 3 respectively. The ANOVA analysis showed that there were no significant differences (P > 0.05) in the concentration of Cr between the subsamples. The differences between the mean and median as well as the high CVs showed the presence of some extreme values within the data.

Sample		LAB 2		LAB 3			
	Mean	SD	CV	Mean	SD	CV	
1	176.50	90.51	51.28		B.D.L.		
2	47.50	12.73	26.80	36.09	51.03	141.42	
3	157.25	61.87	39.35		B.D.L.		
4	170.25	6.01	3.53	95.36	134.86	141.42	
5	116.50	19.80	16.99	56.70	80.19	141.42	
6	85.00	10.61	12.48	54.13	76.55	141.42	
7	170.00	37.48	22.05	113.40	160.37	141.42	
8	114.50	30.41	26.56	108.25	153.08	141.42	
9	150.00	56.57	37.71		B.D.L.		
10	89.00	73.54	82.63		B.D.L.		
Mean	127.65	39.95	31.94	77.32	109.35	141.42	
Median	133.25	33.94	26.68	76.03	107.52	141.42	
Range	47.	50 - 176.5	0	36.09 - 157.22			
95% Confidence level		0.227		0.876			

Table 4.11: Descriptive Statistics of the Concentration of Chromium inFUTMX7316SRM Obtained from Four Laboratories in Nigeria

B.D.L.: Below detectible limit
4.5 Inter-Laboratory Comparison

The inter-laboratory comparison of the mean concentration of some heavy metals is shown in Table 4.12 below. The data used for this comparison were those without the outliers and extreme values. There were significant differences (P<0.05) in the mean concentrations of all the heavy metals studied between the selected laboratories.

Metal (mgkg)	LAB 1	LAB 2	LAB 3	LAB 4	
Zn	$265.75\pm2.68^{\mathrm{a}}$	$45.15\pm6.00^{\circ}$	$25.14\pm0.87^{\circ}$	$192.25 \pm 15.70^{\rm b}$	
Fe	8810.77 ± 257.15^{a}	$9332.50 \pm 857.24^{\rm a}$	332.40 ± 47.75^{b}	-	
Mn	153.78 ± 1.77^{b}	338.63 ± 33.56^a	$12.74\pm0.50^{\circ}$	147.33 ± 5.25^{b}	
Pb	$13.42\pm0.61^{\circ}$	$19.55\pm1.92^{\rm a}$	$14.15\pm0.77^{\text{bc}}$	17.99 ± 1.78^{ab}	
Cu	13.57 ± 1.35^{ab}	$27.08 \pm 10.09^{\mathrm{a}}$	$1.23\pm0.80^{\text{b}}$	$20.50\pm4.12^{\rm a}$	
Cr	-	127.65 ± 13.91	B.D.L.	-	
Cd	$3.13\pm0.20^{\rm a}$	$0.73\pm0.10^{\rm b}$	B.D.L.	$0.21\pm0.02^{\rm c}$	

 Table 4.12: Average concentrations of some heavy metals in the prepared

 FUTMX7316SRM analyzed by four laboratories in Nigeria

It was expected that different laboratories analyzing the sample should get the same data but in this study, it was not so possible because the error in analytical processes differs from place to place influenced by an environmental factor, differences in the equipment used, and contaminations (Konieczka and Namiesnik, 2018)

The concentration of Zn in FUTMX7316SRM from LAB 1 was significantly higher than those from LAB 4, LAB 2, and LAB 3. LAB 4 was significantly higher than those of LAB 2 and LAB 3, which were both statistically comparable. The concentration of

Means on the same row with different superscripts are significantly different (p < 0.05) BDL: Below detectible limit

Fe in the FUTMX7316SRM obtained from LAB 1 and LAB 2 was not significantly different from each other but was significantly higher than that of LAB 3. The concentration of Mn in the FUTMX7316SRM obtained from LAB 2 was significantly higher than those of the other laboratories, LAB1, LAB 4, and LAB 3.

LAB 1 and LAB 4 had statistically comparable Mn concentrations, which were significantly higher than that of LAB 3. The concentration of Pb in the FUTMX7316SRM obtained from LAB 2 was significantly higher than those of LAB 3 and LAB 1 respectively. LAB 4 had the concentration of Pb in the FUTMX7316SRM statistically comparable to those of LAB 2 and LAB 3 while that of LAB 3 was statistically comparable to LAB 1.

The concentrations of Cu in the FUTMX7316SRM from LAB 2 and LAB 4 were not significantly different from each other but were significantly higher than that of LAB 3. LAB 1 had Cu concentration in the FUTMX7316SRM statistically comparable to those of the other laboratories, LAB 2, LAB 3, and LAB4. The concentration of Cr in the FUTMX7316SRM was only detected by the determination from LAB 2. LAB 3 had a concentration below the detectible limit. The concentration of Cd in the FUTMX7316SRM obtained from LAB 1 was significantly higher than those of LAB 2 and LAB 4. LAB 2 was significantly higher than LAB 4 while the concentrations of Cd in the sub-samples from LAB 3 were below the detectible limit.

The variation in the agreement of data from the four laboratories was similar to that of Naseem *et al.* (2005) that found that the data obtained from an Inter-laboratory study on the determination of lead and copper in wastewater from the printed circuit board industry showed much variation between the laboratories and less than 50 % of the laboratories showed good performance concerning the quality control measure used.

4.6 Repeatability of the Measurement Method

Repeatability is the agreement between results from the same method on the same sample either from the same laboratory (within laboratory repeatability) or different laboratory (between laboratory repeatability) which is a measure of the standard deviation or coefficient of variance (CV) of the results (Tan *et al*, 2018). The between-laboratory repeatability for this study is shown in Tables 4.13 and 4.14 below.

Metal (mg/kg)	LAB 1	LAB 2	LAB 3	LAB 4	Mean	Standard deviation	% CV
Zn	265.75	45.15	25.14	192.25	132.07	116.16	87.95
Fe	8810.77	9332.5	332.4	-	6158.56	5052.34	82.04
Mn	153.78	338.63	12.74	147.33	163.12	133.86	82.06
Pb	13.42	19.55	14.15	17.99	16.28	2.96	18.20
Cu	13.57	27.08	1.23	20.5	15.60	11.05	70.87
Cr	-	127.65	B.D.L.	-	127.65	-	-
Cd	3.13	0.73	B.D.L.	0.21	1.36	1.56	114.81

 Table 4.13: Between Laboratory Repeatability of the Determination of Heavy Metals in the Prepared FUTMX7316SRM

B.D.L.: Below detectible limit

The between laboratory standard deviation and percent coefficient of variance for all the metals in the FUTMX7316SRM except that of Pb were very high; they were higher than the recommended value of < 20% recommended as the acceptable range by Hineman *et al.* (2018). The between-laboratory standard deviation of Pb was below 20% which revealed that the precision of its determination was high and acceptable.

Metal (mg/kg)	LAB 1	LAB 2	LAB 3	LAB 4	Mean	Standard deviation	%CV
Zn	337.15	109.5	84.5	465	249.04	183.44	73.66
Fe	27780	18267	200	-	15415.67	14009.34	90.88
Mn	775	1000	37.8	3202	1253.70	1362.33	108.66
Pb	185.5	310.5	31.9	265.5	198.35	122.42	61.72
Cu	135.5	143	23.2	142.5	111.05	58.67	52.83
Cr	-	148	B.D.L.	-	148.00	-	-
Cd	8.4	10.55	B.D.L.	7.25	8.73	1.68	19.18

 Table 4.14: Between Laboratory Repeatability of the Determination of Heavy Metals in the CRM - 989

BDL: Below detectible limit

Though the between laboratory standard deviation for the FUTMX7316SRM was below 20 %. It was found that it was not so for the CRM – 989. The reason for this instability in the precision for Pb determination was not known. The between laboratory standard deviation for all the metals were high, above 20 % acceptable maximum limit recommended by Hineman *et al.* (2018) in the determination of the metals in the CRM – 989 except for Cd with a deviation lower than 20 %.

4.7 Property value of FUTMX7316SRM

The property value of FUTMX7316SRM traceable to the certified reference material (CRM 989) is shown in Table 4.14. The total nitrogen and total organic carbon values from the homogeneity test after bottling was included in the property values because Shukla (2015) reported that property values are assigned to materials that are sufficiently homogeneous and well established. The property value reveal that the prepared reference material contained 0.41 ± 0.02 % total organic carbon, 0.16 ± 0.04 %

total nitrogen, 236.21 ± 41.78 mg/kg Zn, 213.25 ± 108.63 mg/kg Mn, 17.46 ± 3.50 mg/kg Pb, 18.38 ± 7.55 mg/kg Cu, 1.35 ± 1.56 mg/kg Cd, 9071.64 ± 368.92 mg/kg Fe and 127.65 ± 39.95 mg/kg Cr. FUTMX7316SRM may be fit for use in the calibration AAS, assessment of measurement methods in heavy metal determination, purity test, and for assigning values to materials (APLAC, 2018; Shukla, 2015); in a general term, it can be used for quality control in the determination of the concentration of Zn, Mn, Pb, Cu, Cd, Fe, and Cr as well as in the determination of total organic carbon and total nitrogen.

Metal	FUTMX7316SRM			
Total organic carbon (%)	0.41 ± 0.02			
Total Nitrogen (%)	0.16 ± 0.04			
Zn (mg/kg)	236.21 ± 41.78			
Mn (mg/kg)	213.25 ± 108.63			
Pb(mg/kg)	17.46 ± 3.50			
$C_{\rm u}$ (mg/kg)	18 38 + 7 55			
Cd(mg/kg)	125 ± 1.55			
	1.35 ± 1.30			
Fe (mg/Kg)	9071.64 ± 368.92			
Cr (mg/kg)	127.65 ± 39.95			
Soil class: Loamy sand				

 Table 4.15: Average property values of the prepared In-House Soil Reference

 Material (FUTMX7316SRM)

CHAPTER FIVE

5.0 CONCLUSION AND RECCOMMENDATIONS

5.1 Conclusion

One hundred bottles, each containing 500 g, of the prepared homogenized composite soil (FUTMX7316SRM) were obtained. No significant differences were observed in between-bottle and within-bottle homogeneity tests, showing that the material was homogeneous for the determination of Fe, Zn, Mn, Cr, Cu, Pb, and Cd as well as the homogeneity indicators, total nitrogen, and total organic carbon.

The result of the quality control check using CRM-989 revealed that the recoveries of Fe, Cu and Cd for LAB 1, 78.25, 88.56, and 102.19 % respectively, were within the specified range of 75 - 125 % but those of Pb and Mn to the nearest whole number was 70 % and that of Zn was 33.05 %. The recoveries of Mn, Pb, Cu and Cr for LAB 2, 92.24, 110.11, 93.46 and 83.15 respectively, were within the range of 75 - 125 % but that of Cd was slightly above 125 % (128.35 %); Fe had a recovery of 51.46 % while that of Zn was also very low (10.74 %). The recoveries of Cu, Pb and Cd, 93.14 %, 94.15 % and 88.20 % respectively, obtained from LAB 4 were within the range of 75 - 125 %. The recoveries of all the metal from LAB 3 were very low; they were all less than 16 %; Cr and Cd were below detectible limit.

The property values of FUTMX7316SRM were 0.41 ± 0.02 % total organic carbon, 0.16 ± 0.04 % total nitrogen, 236.21 ± 41.78 mg/kg Zn, 213.25 ± 108.63 mg/kg Mn, 17.46 ± 3.50 mg/kg Pb, 18.38 ± 7.55 mg/kg Cu, 1.35 ± 1.56 mg/kg Cd, 9071.64 ± 368.92 mg/kg Fe and 127.65 ± 39.95 mg/kg Cr traceable to CRM-989. It can therefore

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be used for the inter-laboratory campaign and other quality control purposes, thereby fulfilling the objectives of this study.

5.2 Recommendations

- The use of in-house reference material like the prepared FUTMX7316SRM should be incorporated as an important part of quality control measures in the determination of heavy metals.
 - ii. Further research work is also recommended on FUTMX7316SRM to obtain other heavy metals which can be included in the property value. This can be made more reliable through the use of more laboratories within and outside Nigeria.

REFERENCES

- Aba, A., & Ismaeel, A. (2013). Preparation of in-house calibration source for the use in radioactivity analysis of the environmental samples: consideration of homogeneity. *Journal of Radioanalytical and Nuclear Chemistry*, 295(1), 31-38.
- Akinyele, I. O., & Shokunbi, O. S. (2015). Comparative analysis of dry ashing and wet digestion methods for the determination of trace and heavy metals in food samples. *Food Chemistry*, 173, 682-684.
- Almasoud, F. I., Usman, A. R., & Al-Farraj, A. S. (2015). Heavy metals in the soils of the Arabian Gulf coast affected by industrial activities: analysis and assessment using enrichment factor and multivariate analysis. *Arabian Journal of Geosciences*, 8(3), 1691-1703.
- American Industrial Hygiene Association (AIHA) Laboratory accreditation programs LLC (2014). Guidance on traceability of measurement. Quality system guidance. Fairview Park Dr, Suite 777, Falls Church, VA 22042: American Industrial Hygiene Association, 1 – 15.
- Analytical Methods Committee Technical Briefs (A. M. C. T. B.) & Analytical Methods Committee. (2013). What causes most errors in chemical analysis?. *Analytical Methods*, 5(12), 2914-2915.
- Arif, N., Yadav, V., Singh, S., Singh, S., Ahmad, P., Mishra, R. K., ... & Chauhan, D. K. (2016). Influence of high and low levels of plant-beneficial heavy metal ions on plant growth and development. *Frontiers in environmental science*, *4*, 69.
- Asia Pacific Laboratory Accreditation Corporation (APLAC) (2018). Guidance on reference material use and production. North Melbourne, Australia: Asia Pacific Laboratory Accreditation Corporation, APLAC TC 008, 6, 1 18.
- Atkins P. (2013). *Certified reference materials*: Beyond the basics. SPEX CertiPrep.P Inorganic and organic reference materials; <u>https://www.spexcertiprep.com/getattachment/4bca99ad-8e6a-49fb-848f-</u> <u>a74c53fe3f38/WebinarSlides_CRMsBeyondTheBasics.pdf?lang=en-</u> <u>US&ext=.pdf</u>.
- Bonsucesso, J. S., Gloaguen, T. V., do Nascimento, A. S., de Carvalho, C. A. L., & Dias, F. D. S. (2018). Metals in geopropolis from the beehive of Melipona scutellaris in urban environments. *Science of the Total Environment*, 634, 687-694.
- Cezara, V., Dehelean, A. & Pamula, A. (2009). Method validation for determination of heavymetals in wine and slightly alcoholic beverages by ICP-MS. *Journal of Physics Conference Series*, 182, 1–6.
- Chan, M., & Ma, L. Q. (2001). Comparison of three aqua regia digestion methods for twenty Florida soils. *Soil science society of America Journal*, 65(2), 491-499.
- Chauhan, A., & Mittu, B. (2014). Various Types of Interferences during Sample Analysis in Atomic Absorption Spectrometry. *Journal of Textile Science & Engineering*, 4(6), 1.

- Chen, H., Teng, Y., Lu, S., Wang, Y., & Wang, J. (2015). Contamination features and health risk of soil heavy metals in China. *Science of the total environment*, *512*, 143-153.
- Das, S., & Ting, Y. P. (2017). Evaluation of wet digestion methods for quantification of metal content in electronic scrap material. *Resources*, 6(4), 64.
- Dudzik, D., Barbas-Bernardos, C., García, A., & Barbas, C. (2018). Quality assurance procedures for mass spectrometry untargeted metabolomics. a review. *Journal* of pharmaceutical and biomedical analysis, 147, 149-173.
- Dumčius, A., Paliulis, D., & Kozlovska-Kędziora, J. (2011). Selection of investigation methods for heavy metal pollution on soil and sediments of water basins and river bottoms: a review. *Ekologija*, 57(1).
- Emons, H., Linsinger, T. P. J., & Gawlik, B. M. (2004). Reference materials: terminology and use. Can't one see the forest for the trees?. *TrAC Trends in Analytical Chemistry*, 23(6), 442-449.
- Evans, J. R., & Lindsay, W. M. (2002). *The management and control of quality* (Vol. 5, pp. 115-128). Cincinnati, OH: South-western.
- Gallhofer, D., & Lottermoser, B. G. (2018). The influence of spectral interferences on critical element determination with portable X-ray fluorescence (pXRF). *Minerals*, 8(8), 320.
- Gopalakrishnan, R. (2005). Preparation and certification of international rock standards. *Journal of Geological Society of India (Online archive from Vol 1 to Vol 78)*, 65(5), 658-662.
- Helaluddin, A. B. M., Khalid, R. S., Alaama, M., & Abbas, S. A. (2016). Main analytical techniques used for elemental analysis in various matrices. *Tropical Journal of Pharmaceutical Research*, 15(2), 427-434.
- Helmeczi, W., Helmeczi, E., Baker, L. A., Wang, Y., & Brindle, I. D. (2018).
 Development of a general acid method for the digestion of gold ore samples together with a comparison of extraction solvents for gold and determination by microwave-induced plasma-atomic emission spectrometry (MIP-AES). *Journal of Analytical Atomic Spectrometry*, 33(8), 1336-1344.
- Hineman, A., Purcell-Joiner, R.& Astill T. (2018). Digestion, testing, and validation of heavy metals in cannabis. *The Emerald Test, Perkin-Elmer, Canada*, 1-5.
- Hou, S., Zheng, N., Tang, L., Ji, X., Li, Y., & Hua, X. (2019). Pollution characteristics, sources, and health risk assessment of human exposure to Cu, Zn, Cd, and Pb pollution in urban street dust across China between 2009 and 2018. *Environment international*, 128, 430-437.
- Idris, A. M. (2019). Between-bottle homogeneity test of new certified reference materials employing wavelength dispersive X-ray fluorescence spectrometry. *BMC chemistry*, *13*(1), 1-11.
- International Organization of Standard (ISO) 9000 (2000). *Quality management systems requirements*. Geneva: International Organization for Standardization.

- International Organization of Standard (ISO) Guide 80 (2014). *Guidance for the In-House preparation of quality control materials (GCM. International organization for standardization* (ISO). Geneva: International Organization of Standards.
- International Atomic Energy Agency (IAEA) (2003). *Development and use of reference materials and quality control materials*. Vienna, Austria: Industrial applications and chemistry section, IAEA TEC DOC – 1350.
- Jain, P., Jain, A., Singhai, R., & Jain, S. (2017). Effect of bio-degradation and nondegradable substances in the environment. *International Journal of Life Sciences*, 1(1), 58-64.
- Jaiswal, A., Verma, A., & Jaiswal, P. (2018). Detrimental effects of heavy metals in soil, plants, and aquatic ecosystems, and humans. *Journal of Environmental Pathology, Toxicology and Oncology*, 37(3).
- Kane, J. S. (2001). The use of reference materials: A tutorial. *Geostandards Newsletter*, 25(1), 7-22.
- Khan, A., Khan, S., Khan, M. A., Qamar, Z., & Waqas, M. (2015). The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. *Environmental Science and Pollution Research*, 22(18), 13772-13799.
- Kim, J., Anawati, J., & Azimi, G. (2018). Matrix complexity effect on platinum group metals analysis using inductively coupled plasma optical emission spectrometry. *Journal of Analytical Atomic Spectrometry*, 33(8), 1310-1321.
- Konieczka, P., & Namiesnik, J. (2018). *Quality assurance and quality control in the analytical chemical laboratory: a practical approach.* CRC Press.
- Lawler, J. E., Sneden, C., Den Hartog, E. A., & Cowan, J. J. (2019). Quantitative atomic spectroscopy, a review of progress in the optical-UV region and future opportunities. *Proceedings of the International Astronomical Union*. *International Astronomical Union*, 350, 73.
- Leśniewska, B., Kisielewska, K., Wiater, J., & Godlewska-Żyłkiewicz, B. (2016). Fast and simple procedure for fractionation of zinc in soil using an ultrasound probe and FAAS detection. Validation of the analytical method and evaluation of the uncertainty budget. *Environmental monitoring and assessment*, 188(1), 29.
- Liatu, T. Y., Auta, I. K., Tanko, K., Gajere, E. N. & Joshua, M. (2017). Study on heavy metals concentration in irrigated soil samples of some selected locations along River Kaduna, Nigeria. *IJRDO-Journal of Biological Science*, 3(7), 79 – 90.
- Lima, E. S. A., Amaral Sobrinho, N. M. B. D., Pérez, D. V., & Coutinho, I. B. (2016). Comparing methods for extracting heavy metals from Histosols for establishing quality reference values. *Revista Brasileira de Ciência do Solo*, 40.
- Llauradó, M., Torres, J. M., Tent, J., Sahuquillo, A., Muntau, H., & Rauret, G. (2001). Preparation of a soil reference material for the determination of radionuclides. *Analytica chimica acta*, 445(1), 99-106.

- Loken, E., & Gelman, A. (2017). Measurement error and the replication crisis. *Science*, *355*(6325), 584-585.
- Lymperopoulou, T., Tsakanika, L. A., & Ochsenkuohu-Petropoulou, M. (2014). Method validation for the determination of agua regia extractable trace elements in soils. *Eon Metsob Nonvtexneion*, 1.
- Machado, R. C., Silva, A. B. S., Amaral, C. D., Virgilio, A., & Nogueira, A. R. A. (2020). Internal standardization as a strategy to overcome non-spectral interferences in the determination of As, Cd, and Pb in mineral fertilizers by synchronous vertical dual view (SVDV) ICP OES. *Analytical Methods*, 12(1), 39-45.
- Mackey, E. A., Christopher, S. J., Lindstrom, R. M., Long, S. E., Marlow, A. F., Murphy, K. E., & Nebelsick, J. (2010). Certification of three NIST renewal soil standard reference materials for element content: SRM 2709a San Joaquin Soil, SRM 2710a Montana Soil I, and SRM 2711a Montana Soil II. *NIST Special Publication*, 260(172), 1-39.
- Mádlíková, M., Krausová, I., Mizera, J., Táborský, J., Faměra, O., & Chvátil, D. (2018). Nitrogen assay in winter wheat by short-time instrumental photon activation analysis and its comparison with the Kjeldahl method. *Journal of Radioanalytical and Nuclear Chemistry*, 317(1), 479-486.
- Magharbeh, M., Fayyad, M., & Bartarseh, M. (2012). Preparation of natural standard reference material (SRM) for El-lajjun oil shale/Jordan. *Jordan Journal of Earth and Environmental Sciences*, 4(2), 15-22.
- McAlinden, C., Khadka, J., & Pesudovs, K. (2015). Precision (repeatability and reproducibility) studies and sample-size calculation. *Journal of Cataract & Refractive Surgery*, *41*(12), 2598-2604.
- Mead, M. (2019). Quality Assurance and Documentation, Handbook for Citizen Science. USA: United States Environmental Protection Agency (EPA), 1, 1 – 31.
- Miller, C. M., Fadel, J. G., Heguy, J. M., Karle, B. M., Price, P. L., & Meyer, D. (2018). Optimizing accuracy of protocols for measuring dry matter and nutrient yield of forage crops. *Science of the total environment*, 624, 180-188.
- Moat, S. J., Schulenburg-Brand, D., Lemonde, H., Bonham, J. R., Weykamp, C. W., Mei, J. V., & Carling, R. S. (2020). Performance of laboratory tests used to measure blood phenylalanine for the monitoring of patients with phenylketonuria. *Journal of inherited metabolic disease*, 43(2), 179-188.
- Mohamed, R., Zainudin, B. H., & Yaakob, A. S. (2020). Method validation and determination of heavy metals in cocoa beans and cocoa products by microwave-assisted digestion technique with inductively coupled plasma mass spectrometry. *Food Chemistry*, *303*, 125392.

- Mutwalli, H., Braian, M., Mahmood, D., & Larsson, C. (2018). Trueness and precision of three-dimensional digitizing intraoral devices. *International journal of dentistry*, 2018.
- Naseem, R., Tahir, S. S., Haq, A. & Saeed, K., (2005). An inter-laboratory comparison study for the determination of copper and lead from the wastewater of printed circuit board manufacturing industry in Pakistan. *Accreditation and quality assurance*, 10(7), 362-368.
- Oliveira, A. F., Gonzalez, M. H., & Nogueira, A. R. A. (2018). Use of multiple lines for improving accuracy, minimizing systematic errors from spectral interferences, and reducing matrix effects in MIP OES measurements. *Microchemical Journal*, 143, 326-330.
- Palma, P., Ledo, L., & Alvarenga, P. (2015). Assessment of trace element pollution and its environmental risk to freshwater sediments influenced by anthropogenic contributions: the case study of Alqueva reservoir (Guadiana Basin). *Catena*, *128*, 174-184.
- Parvizi, S., Behbahani, M., & Esrafili, A. (2018). Preconcentration and ultra-trace determination of hexavalent chromium ions using tailor-made polymer nanoparticles coupled with graphite furnace atomic absorption spectrometry: ultrasonic assisted-dispersive solid-phase extraction. *New Journal of Chemistry*, 42(12), 10357-10365.
- Prenesti, E., & Gosmaro, F. (2015). Trueness, precision, and accuracy: a critical overview of the concepts as well as proposals for revision. *Accreditation and Quality Assurance*, 20(1), 33-40.
- Ramamoorthi, V., & Meena, S. (2018). Quantification of Soil Organic Carbon-Comparison of Wet Oxidation and Dry Combustion Methods. *International Journal of Current Microbiology and Applied Sciences*, 7, 146-154.
- Rawar, E., & Rohman, A. (2016). Validation of atomic absorption spectrophotometry and mercury analyzer for analysis of cadmium, lead, and mercury in red chili pepper. *Asian J Agric Res*, 10(1), 65-71.
- Rodríguez-Eugenio, N., McLaughlin, M., & Pennock, D. (2018). Soil pollution: a hidden reality. FAO.
- Samin, S., & Sunanti, S. T. Analysis Methods for Development of Standard Reference Material (SRM) Zircon Minerals Synthesis. *Indonesian Journal of Chemistry*, 18(3), 448-456.
- Santoro, A., Held, A., Linsinger, T. P., Perez, A., & Ricci, M. (2017). Comparison of total and aqua regia extractability of heavy metals in sewage sludge: The case study of certified reference material. *TrAC Trends in Analytical Chemistry*, 89, 34-40.
- Sarwar, N., Imran, M., Shaheen, M. R., Ishaque, W., Kamran, M. A., Matloob, A., ... & Hussain, S. (2017). Phytoremediation strategies for soils contaminated with

heavy metals: modifications and future perspectives. *Chemosphere*, *171*, 710-721.

- Sciacchitano, A., & Wieneke, B. (2016). PIV uncertainty propagation. *Measurement Science and Technology*, 27(8), 084006.
- Société Générale de Surveillance (SGS) (2012). *Certified reference materials*. Van Drunen RoadSouth Holland, Illinois 60473 USA: SGS minerals service division Laboratory Quality Services International (LQSI) 16130, 1-3
- Shukla, T. (2015). Certified reference materials (CRM): *Quality control tool for chemical pollutants SPEX Europe*. Retrieved from <u>www.spexeurope.com</u>.
- Silva, C. S., de Souza, G. B., & Nogueira, A. R. A. (2018). Preparation and characterization of phosphate rock as quality control material. *Accreditation and Quality Assurance*, 23(1), 39-45.
- Simone C. (2016). *Calibration*. CIRP Encyclopedia of production engineering, DOI: 10-1007/978 3-642-35950-7_16754-1.
- Stefanie, T., Botha, A., Linsinger, T. P., Mac Curtain, S., & Emons, H. (2017). The new International Standard ISO 17034: general requirements for the competence of reference material producers. *Accreditation and Quality Assurance*, 22(6), 381-387.
- Taghipour, H., Mosaferi, M., Armanfar, F., & Gaemmagami, S. J. (2013). Heavy metals pollution in the soils of suburban areas in big cities: a case study. *International Journal of Environmental Science and Technology*, 10(2), 243-250.
- Tan, M., Sudjadi, A., Astuti, R., & Rohman, A. (2018). Validation and quantitative analysis of cadmium, chromium, copper, nickel, and lead in snake fruit by inductively coupled plasma-atomic emission spectroscopy. *Journal of Applied Pharmaceutical Sciences*, 8 (2), 44-48.
- Tasker, T. L., Burgos, W. D., Ajemigbitse, M. A., Lauer, N. E., Gusa, A. V., Kuatbek, M., ... & Warner, N. R. (2019). Accuracy of methods for reporting inorganic element concentrations and radioactivity in oil and gas wastewaters from the Appalachian Basin, US based on an inter-laboratory comparison. *Environmental Science: Processes & Impacts*, 21(2), 224-241.
- Tchounwou, P. B., Yedjou, C. G., Patlolla, A. K., & Sutton, D. J. (2012). Environmental toxicology. *Molecular, clinical and environmental toxicology, Experientia Supplementum, 3*, 133-164.
- Turek, A., Wieczorek, K., & Wolf, W. M. (2019). Digestion procedure and determination of heavy metals in sewage sludge—An analytical problem. *Sustainability*, 11(6), 1753.
- Turkyilmaz, A., Sevik, H., Cetin, M., & Ahmaida Saleh, E. A. (2018). Changes in Heavy Metal Accumulation Depending on Traffic Density in Some Landscape Plants. *Polish Journal of Environmental Studies*, 27(5).

- Varela, B., & Pacheco, G. (2018). A comprehensive evaluation of the internal and external quality control to redefine analytical quality goals. *Biochemia Medica*, 28(2), 222-232.
- Velychko, O., & Gordiyenko, T. (2019). Metrological Traceability at Different Measurement Levels. In *Standards, Methods, and Solutions of Metrology*. IntechOpen.
- Vodyanitskii, Y. N. (2016). Standards for the contents of heavy metals in soils of some states. *Annals of agrarian science*, 14(3), 257-263.
- Wang, H., Ni, Y., Zheng, J., Huang, Z., Xiao, D., & Aono, T. (2019). Low-temperature zEfusion using NH4HSO4 and NH4HF2 for rapid determination of Pu in soil and sediment samples. *Analytica chimica acta*, 1050, 71-79.
- Wielgosz, R. I. (2004). CCQM workshop on comparability and traceability in food analysis, 18–19 November 2003, BIPM, Sèvres. Accreditation and Quality Assurance, 9(9), 523-529.
- Williams C. C. & Stephen G. C. (2014). *Elemental analysis manual for food and related products*. U.S. Food and Drug Administration (US FDA), 1 10.
- Zheng, H., Hong, J., Luo, X., Li, S., Wang, M., Yang, B., & Wang, M. (2019). Combination of sequential cloud point extraction and hydride generation atomic fluorescence spectrometry for preconcentration and determination of inorganic and methyl mercury in water samples. *Microchemical Journal*, 145, 806-812.
- Zhang, S., Liu, Y., Yang, Y., Ni, X., Arif, M., Charles, W., & Li, C. (2020). Trace elements in soils of a typical industrial district in Ningxia, northwest China: Pollution, source, and risk evaluation. *Sustainability*, 12(5), 1868.
- Zhao, X., Joo, J. C., Lee, J. K., & Kim, J. Y. (2019). Mathematical estimation of heavy metal accumulations in Helianthus annuus L. with a sigmoid heavy metal uptake model. *Chemosphere*, 220, 965-973.