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Determination of Trace Metals (Mn, Cu &Ni) Content in *Moringa Oliefera* using Atomic Absorption Spectroscopy

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Abstract
The levels of trace metals were determined in Moringa Oleifera. The leave sample of Moringa Oleifera was analyzed for trace metals using atomic absorption spectrometry after dry ashing the sample at 350^{o}C in furnace for 1hr. Generally, the plant accumulate metals in order Ni >Cu>Mn. The result revealed the accumulated amount of trace metals in leave sample Cu, Mn and Ni 2.866$^{0 \pm 0 \pm}$0.0436, 1.1050$\pm 0.0019 \pm 0.0019$ and 3.2002$\pm 0.0241 \pm 0.0241$ mg/kg respectively.
Keywords: Flame Atomic Absorption spectrometry, Moringa Oleifera, Trace Metals

1. INTRODUCTION

Minerals are necessary for health add as such are part of all aspect of cellular function [1].They are involved in structural components. Example calcium and Phosphorous are structural components in bone and acts as cofactors for enzyme activities.Minerals also forman integral part of enzyme or protein structure. Minerals are essential for growth, development and maintenance of tissues and are also linked to the expression of genetic information, the effectiveness of immune system, the prevention of cell damage. In general, minerals increase resistance to many chronic and some infectious diseases. Minerals are nutritionally important components in food. They could be classified as essential or non essential elements. Essential elements are not synthesized internally and must be consumed from its environment. These include the major elements and minor elements. [2] Major elements are those elements that are required in large quantities. The body requires more than hundred (100mg/day) of each major elements. The major elements are components of cells and body fluids and are structural components of cells [2]. Examples of major elements include calcium, chloride, magnesium, phosphorous (present as phosphates), potassium, sodium.

Trace elements essential for life generally occur in the body in micrograms per gram of tissue and are usually required by humans in milligrams per day. Thus the body requires less than twenty (20mg/day) of each trace elements [2]. These trace elements include copper, iron, manganese, zinc, selenium, and iodine. The newer trace elements are ones that are possibly essential. Chromium, manganese nickel, tin, vanadium, arsenic, lithium, strontium, cesium, and silicon are regarded as new trace elements in the sense that they have only recently been considered essentialin human diets. Non-essential components of foods can still have significant impact on health and can either be beneficial or toxic. These non essential elements are however frequently consumed and accumulated in living organisms, though are not required.

Among the roles played by essential elements are growth and production of bones, teeth, hair, blood, nerves, skin, vitamins, enzymes and hormones. Essential elements also play a major role in nerve transmission, blood circulation, cellular integrity, energy production and muscle contraction. It is now well recognized that several trace elements are essential constituents of enzymes and play vital role in human metabolism. All the nutrient elements are primarily supplied throughdiet. The amount neededdepends on age, sex, and health status, geographical and climatic conditions [3].

There exists a range of intake over which the supply of essential elements adequate for the body. However, above and below this rang, toxic and deficiency effects are observed respectively [4]. As a result of this, it is essential to determine elemental contents of food items and to estimate their daily dietary intake. Essential elements can be systemic toxins with specific neurotoxin, nephrotoxic, fetotoxic, and tetratogenic effects. Essential elements can influence behavior by impairing immune, mental and neurological function, influencing neurotransmitter production and utilization and altering other metabolic process singin the body [5]. Uptake of elements from both the atmosphere, leaf surfaces and from soil through roots may account for the elevated levels of elements in plants

A major source of human exposure to trace elements (as well as heavy metals) from environment is from blood [6]. Elements can also find their way into humans by direct absorption via air or drinking water. Other elements find their way into food either naturally or through anthropogenic activities such as agricultural practices, industrial emission and exhaust fumes. Moringa like most plants is a cheap source of essential trace metals. However, little attention has been given to their exact concentrations presentin different preparations of the plants. Studies have shown that high intake of elements can lead to metal poisoning whereas low intake

levels can lead to deficiency effects. This study was therefore conducted to investigate the essential metals in leaf powder of Moringa oleifera.

This study was designed with the general objective of comparing determine the extent of accumulation of essential metals in Moringa oleiferal production in Wukro. With this general objective, the specific objectives of this study were:

- To determine selected nutrient composition both essential (Mn) and non essential (Ni, Cu) by using AAS
- ✓ To comparethe level of metals in Ethiopia Moringa oleifera with WHO Guide lines

1.1. TRACE METALS

Trace metals are metals in extremely small quantities that are presented in animal, plant cells and tissue [3]. Trace metals include iron, magnesium, lithium, zinc, copper, chromium and nickel, cobalt, vanadium, arsenic, molybdenum, manganese, selenium and other level of metals of in the Moringa oleifera. Sample is generally bellowing the WHO and FAO Maximum permissive limits [6].

The environmental metal contains a wide range of heavy metals with varying concentration depending on the surrounding geological environmental and natural activities occurring or that has once occurred. These have Fe, Zn, Cd, Mn, Hg, etc. However heavy metals like Pd, Zn, Cd, Hg, and there are great concern because of their potential effects on human health, agricultural and environmental.

1.2. MORINGA OLEIFERA

Moringa oleifera, family moringaceac is a native tree in arid and semi-arid regions in the southern Rift valley of ETHIOPIA. The local farmers use the species as one of the major arable tree inter-crop in multi-storey system especially by the konso people in gamogofa. Morinaoleifera has awide range of adaptation from arid to humid climates with a prospect of to be grown in a wide range of land use classes. The potential growth area fall in a rainfall rangefrom 300-1400mm per year with soil reaction of 6-7 Mayer [11]. It does not require fertile soils in Sudan as reported in Mayer [7].

Moringa oleifera, a smooth barked deciduous tropical plant, is a traditional medicinal and nutritional pilot in Ethiopia [11].It is widely distributed in south western part of Ethiopia at an altitude range of about 1100 to 1600 Meters. The major growing areas are Arbamich, Negelle and Wellayta sodo. Moringa oleifera is commonly called shiferaw in Amharic [8]. Moringaoleiferacommenlyusedin folk medicines as anti malarial, anti hypertensive, against stomach pain, anti diabetic, anti cholesterol, anti spasmodic and to expel retained placecentae during birth [9].

2. MATERIALS AND METHODS

This chapter starts by presenting and discussing about the study area, experimental site and sampling procedure. It also goes through the detailed methodology followed in the experiment such as experimental procedure, materials and reagents used and method of data analysis. Finally, it winds up by specifying the analytical method, and software used.

2.1. Experimental Site

Sample Moringa Oleifera Leaves were collected from Wukro Agricultural College which is located in northern part of Ethiopia in Tigray National Regional state, Eastern Tigray Zone, wukro wereda at a distance 826 km from Addis Ababa, 46 km from Mekelle. Its astronomical location is 39^0 37' Northern latitude and 39^o 29' EastLongitude. The sample Moringa Oleifera leaves was randomly collected. The leaves Moringa were set in white plastic. The representative sample was air dried in the laboratory, and then place in an oven to complete the drying at a temperature of about 105^oc.The dried sample were crushed in to powdered form and sieved to obtain finest powder.

2.2. Apparatus

The following apparatus were used in the study: sieve of 0.5 mm mesh sizes; crucible; analytical balance (OHAUS, made in Switzerland); oven (Genlab, UK); type 1500 furnace, desiccators, and Atomic Absorption Spectrophotometer. All glassware used were rinsed and soaked in 10% (v/v) HNO_3 overnight. They were rinsed with de-ionized water and dried before use.

2.3. Reagents and Chemicals

Reagent and chemicals used during the laboratory were all analytical grade; 99.6% $Ni(NO_3)_2.6H_2O$, 99% $CuSO_4.5H_2O$ and 99.4% $MnCl_2.4H_2O$. HNO_3 (65%) was used for Digestion of the sample.

a. Preparation of Standard solutions

Determination the metal concentration in the experimental solution was based on calibration curve. In plotting the calibration curves Nickel, Manganese, copper stock solutions of 1000 ppm were prepared by dissolving 1.6g Ni $(NO_3)_2.6H_2O$, 2.74g $MnCl_2.4H_20$ and 2.83g $CuSO_4.5H_2O$ in de-ionized water respectively. Blank solution were prepared for the methods and for the standard working solution, to prepare 100 ppm, 10 ml of the standard Ni $(NO_3)_2.6H_2o$, $MnCl_2.4H_2O$ and $Cuso_4.5H_2O$ stock solution were pitted and added in to 100 ml calibrated

flasks finally diluted with de-ionized water and the solution was mixed thoroughly. Net toprepare50ppm standard solution of each metal, 50 ml each of 100 ppm stock solution was pipette in to 100 ml volumetric flasks and diluted with de-ionized water. Finally to prepare 0.5, 1.0, 2.0, 4.0, 6.0, ppm aliquot ion of this standard working solution 2.5, 5, 10, 20, 30 ml was pipette flasks from 50 ppm standard solution in to 50 ml calibrated flasks and made up to volume with De-ionized water (AOAC, 1995).

2.5. Sample Preparation

Moringa Oleifera leaves sample were washed thoroughly with tap water followed by de-ionized water and dried in the oven at a temperature of 105^{o}C for 24 hr was dried in oven and dried samples were ground using mortal and pestle to obtain fine particles that pass through a 0.5 mm mesh and kept dry in a polyethylene bag in desiccators until analysis.

2.6. Digestion Methods

2.6.1. Dry Ashing

One gram of each spice sample was placed into porcelain crucible. The furnace temperature was slowly increased from room temperature to 350˚C. The sample was ashed for about 3 hr until a white or grey ash residue was obtained. The residue was dissolved in 5 ml of HNO_3 (25%, v/v). The solution was transferred to a 10 mL volumetric flask and made up to volume [17].

2.7. Determination of Detection Limits

Detection limit is the lowest concentration level that can be determined at 95% confidence level or the minimum concentration that can be detected by the analytical method with a given certainty. For a measurement, detection limit can be properly estimated from the standard deviation of several blank determinations [18]. There are numerous ways of determining detection limits of a given measurement. A general accepted definition of detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal. In this study the detection limit of each element was calculated as three times the standard deviation of the blank (3δ blank, n = 5), as summarized in Table 2.

Table 1. Working Conditions of Atomic Absorption Spectroscopy

Element	Wavelength nm	Slit Width nm	Lamp Current mA	IDL mg/L	MDL mg/L	Flame type
Cu	324.8	1 .0	4-10	0.008	0.003	Rich/yellow
Ni	235	1.0	4-10	0.04	0.003	Lean/blue
Mn	279.5	1.0	4-10	0.01	0.003	Lean/blue

IDL =instrument detection limit MDL =method detection limit

3. RESULT AND DISCUSION

3.1. Total Metal Concentrations in the sample

Moringa sample were analysed for three heavy metals. Three of the metals namely manganese (Mn), copper (Cu) and Nickel (Ni) are essential micronutrients.

Table 2, Mean concentration (mg/kg) of trace metals in the various part of Moringa oleifera

Heavy metal (mg/kg)			
Moring oleifera	Cu	Ni	Mn
Current result	2.866^{0} ±0 ±0.0436	3.2002 ±0.0241 ±0.0241	1.1050 ±0.0019 ±0.0019
Previous results	2.906 ±± 0.025	2.9802±±0.012	2.2043±±0.003
Permissible levels in food as per WHO & FAO	4.0	3.5	2.5

The calibration curve were plots as a function of Absorbance Vs concentration of the standardsolutions in this study three calibration curves were plotted for the metals. Table 2 shows the mean concentrations of trace metals in mg/kg in the leave sample was Ni >Cu>Mn. And all the metal concentrations are below the WHO guide line.

4. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

In this study the metal content of some freshly prepared and commercially available
Ethiopian Moringa oleifera has been investigated.

The level of metals obtained showed a comparable result with other reported values in some cases. The concentration of Ni investigated in this study was higher than the values reported by different authors cited in

this paper. The concentration of the metals Cu, Ni and Mn is $2.866^{0 \pm 0 \pm}_{0.0436}$, $3.2002^{\pm 0.0241}_{\pm 0.0241}$, and $1.1050^{\pm 0.0019 \pm 0.0019}$ µg/kg respectively. This concentration is below the WHO acceptable level (maximum 4.0 mg/kg).

4.2. Recommendations

The following recommendations are made as a result of the outcome of this study

- This study might be repeated with ICP-OES to compare the metal contents of Moringa
- The concentrations of heavy metals in Moringa should be assessed.
- If it is possible sample would be directly sampled from the areas of cultivation
- Monitoring of the levels of heavy metals in Moringa should be encouraged.

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