Cactus is a member of the Cactaceae family, which was originated in Mexico and grows in many other parts of the world, such as Africa, Australia and the Mediterranean basin. Cactus is generally known as a plant has great economical value, which occurs in the dry zone of north Ethiopia, mostly in south tigray and east tigray. It is a popular fruit all over tigray. Heavy metals are natural components of the earth’s crust and they can enter the water and food cycles through a variety of chemical and geochemical processes. Living organisms require trace amounts of some heavy metals, including Co, Cu, Fe, Mn, Mo, Ni and Zn. Excessive levels of these metals, however, can be detrimental to living organisms. This paper was focus on level of the metals and to investigate the effect of digestion temperature and time on the concentration metals. The method was developed by adjusting reagent volume and by varying the time and temperature, then the digested sample was run into atomic absorption spectrophotometer (AAS). Sample leaf collected from Kidane Mhiret (KM), the concentration of metals with respect to time was found in the order of Cu>Mn>Ni but, with respect to temperature Ni>Mn>Cu. Therefore, in the leaf of cactus in KM, Mn abundantly existed as the micronutrients. At the MK the concentration of metals with respect to time was found in the order of Ni>Cu>Mn but, with respect to temperature Mn>Cu>Ni. Therefore, in the leaf of cactus in MK, Mn abundantly existed as the micronutrients.

Keywords: Cactus; Metals; Flame atomic absorption spectrophotometer; Digestion

1. INTRODUCTION

Cactus opuntia is an important feed for ruminants in drought regions especially during the dry season due to forage shortage. The high productivity of cactus under harsh conditions is mainly due to its CO₂ fixation, which enables it to covert water to dry matter more efficiently than most to ranges. Unlike other forages, cactus contains high level of water. Cactus is a member of the Cactaceae family, which is generally known as a plant has great economical value, which occurs in the dry zone of north Ethiopia, mostly in south tigray and east tigray. It is a popular fruit all over Tigray. The fruit is a fleshy berry, varying in shape, size, and colour and has a consistent number of hard seeds. The high sugar content and low acidity of the fruit make it very sweet and delicious like other fruits. The plant produces luxuriant edible ripe fruits which are rich lipids, protein, minerals, and fiber, do not differ significantly from other fruits. The mineral pattern is characterized by high amounts of calcium and magnesium while other minerals are in the normal range for fruits.

In many tropical countries, rural people traditionally harvest wide range of leafy vegetables, roots, tubers, fruits from wild because of its taste, cultural uses, as food supplements or to tide over food shortage. Among alternatives available, to meet the food demands cultivable and wild vegetables are cheap source of food for the marginal communities. Wild plants have been recognized to have potential to meet household food and income security. To apprehend the situation, interests have been centralized on the exploitation, quantification and
Utilization of food plants, especially the vegetables. Vegetable being the rich source of carbohydrates, fats and proteins, which form the major portion of the human diet, are the cheaper source of energy. Various scientists have recorded the importance of this biochemical. Besides these biochemicals, the moisture, fiber, and ash contents and the energy values of individual vegetable and plant species have also been regarded important to the human health. The wild plant species have antibacterial, hepatoprotective and ant carcinogenic properties, and therefore having medicinal values. Trace element and heavy metals have certain risk thus it is important to determine the levels of these compounds in common, popular, and widely used herbal plants. In general, information on edibility and therapeutic properties of wild plants is scanty but data on their nutritional composition and mineral content is negligible. For these essential metals there is a range of intake over which their supply is adequate to the body (Fe 8-18 mg/day, Mn 1.8-2.3 mg/day, Cu 0.9 mg/day, Zn 8-11 mg/day, Ni 0.5 mg/day). However, beyond this ranges, deficiency and toxic effects are observed. According to reports, the recommended limits of dietary consumption of copper is up to 10ppm and average daily requirement of cop 1-3mg, 3-7mg and 2-5mg per day for Copper, nickel and manganese respectively. Heavy metals constitute a very heterogeneous group of elements widely varied in their chemical properties and biological functions. The term heavy metals defined as commonly held for those metals that have specific weights more than 5g/cm³. Heavy metals are kept under environmental pollution category due to their toxic effects in plants, human and food. Some of the heavy metals i.e arsenic (AS), cadmium (Cd), lead (Pb), mercury (Hg) are cumulative poison. These heavy metals are persistence, accumulate and not metabolized in other intermediate compounds and do not easily break down in environment.

These metals are accumulating in food chain through up take at primary producer’s level and than through consumption at consumer level. Metals are entering the human body either through in halation or injection. Heavy metals such as Cd, Ni, As, Pb cause a number of hazards to human. These metals are also forceful carcinogenic and mutagenic. Copper and Zinc serve either as cofactor as a activate bio chemical reactions and enzymatic for information of enzyme subset rate metal complex. The high concentration in take of cadmium cause itai disease and mercury intake leads to minamita disease and other heavy metals cause poisoning due to drinking water contamination. Heavy metals have large availability in soil and a aquatic ecosystem and to relatively smaller proportion in atmosphere at a particular vapors metal toxic city to plants varies with plant species, specific metals, concentration, chemical from soil composition, PH and many metals considered to for plants growth. Heavy metals are natural components of the earth’s crust and they can enter the water and food cycles through a variety of chemical and geochemical processes. Living organisms require trace amounts of some heavy metals, including Co, Cu, Fe, Mn, Mo, Ni and Zn. Excessive levels of these metals, however, can be detrimental to living organisms. Other heavy metals such as Cd, Hg and Pb have no known beneficial effect on organisms and their accumulations over time in the bodies of mammals can cause serious illness. Heavy metal pollutants are of significant ecological/environmental concern because they are not biodegradable and have long half-lives in the soil, thus predicating far-reaching effects on biological systems including soil microorganisms and soil biota. These metals also are accumulated when plants and crops cultivated along major roads are consumed by man and animals especially livestock, either directly or indirectly. The aim this study was the level of metal (Mn, Co and Ni) in cactus leaf by optimizing at different time and temperature parameters during digestion.

2. MATERIALS AND METHODS
2.1 Apparatus and Reagents and chemicals
A drying oven (DHG-9030A), grinder (JESO-100), electronic balance, round bottom flasks (100mL), reflux condensers, borosilicate volumetric flask, pipettes, micropipettes, and flame atomic absorption spectrophotometers (varian-AA240) equipped with deuterium arc back ground correctors and hollow cathode lamps with air-acetylene flame was used in this study.
The reagents and chemicals used in this study was HNO$_3$, H$_2$O$_2$ and H$_2$SO$_4$, stock standard solutions containing 1000 mg/L in distill water of the metals Mn, Ni and Cu.

### 2.2 Sample collection and transportation
Sampling was done in a zigzag manner in an area coverage surrounding Adigrat university approximate distance between two plants from which leaf samples was taken roughly 100 meters. Fresh leaf samples of *cactus*. The sample was collected in a three days interval. A very young, very old and yellow leaf was not sampled. The collected sample was placed and label in polyethylene plastic bags.

### 2.3 Sample preparation for elemental analysis
The leaf sample was further sun dried; leaves for three days. The dried and cleaned stem sample was crushes into small pieces grinder. Both leaf samples was further dried in air and then in an oven at 100 °C for 2hr hours. The dried leaf sample was milled using crusher and sieved through 1 mm sieve. The powdered sample was stored in plastic bags until digestion and analysis.

### 2.4 Optimization of the digestion procedure for cactus leaf samples
The wet sample digestion was carried out using a reflux condenser for both leaf samples. Four parameters: temperature, time, reagent volume and reagent ratio was optimized by varying one parameter and keeping the other parameters constant. Sample was pre-digested for 30 min to minimize the vigorous reaction that may occur during digestion in the hot plate. The optimum conditions for leaf digestion was: temperature, 100°C; time, 1 h and volume, 2.5+1+0.5 mL (HNO$_3$+H$_2$O$_2$+H$_2$SO$_4$). Applying the optimized condition, 2 g of the dried and homogenized leaf samples was added to a 100 mL round bottom flask. Then 4 mL mixture of HNO$_3$ (69-72%), H$_2$O$_2$ (30%) and (98% H$_2$SO$_4$) to leaf sample and the mixtures was pre-digested for 30 min at room temperature. After 30 min the mixtures was digested on a reflex digestion apparatus at the optimized condition. The digestion was carrying out in triplicate for each bulk sample. Digestion of a reagent blank was also performed with the same procedure in parallel with the digestion of the samples keeping all digestion parameters the same. The digest was allowed cooling to room temperature for 30 min. The solutions were store in the refrigerator until analysis.

### 2.5 Analysis of Cactus leaf samples for metal levels
Metal standard solutions for calibration was prepared from an intermediate standard solution Containing 10 mg/l, which was prepared from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L for each of the metals. All the metals included in this study were analyzed with AAS after the instrument was calibrated using four series of working standards. Three replicate determinations were carried out for each metal and the same analytical procedure was employed for the determination of the elements in blank solutions.

### 3. RESULTS AND DISCUSSION
#### 3.1 Analysis of metals (Mn, Cu, Ni) in cactus leaf
The analytical linear range, correlation coefficients and correlation equations of the calibration curves for the determination of metals in the samples by FAAS are given in Table 6.1 and 6.2. The correlation coefficients of all the calibration curves were > 0.999 and these correlation coefficients showed that there was very good correlation (relationship) between concentration and absorbance.

The average concentrations of the metals (mean ± SD) are given Tables 6.1 and 6.2. In both samples, Cu and Ni were found as the micronutrients abundantly with respect to time and temperature effect respectively. At the KM the concentration of metals with respect to time was found in the order of Cu>Mn>Ni but, with respect to temperature Ni>Mn>Cu. Therefore, in the leaf of cactus in KM, Mn abundantly existed as the micronutrients. At the MK the concentration of metals with respect to time was found in the order of Cu>Mn>Ni but, with respect to temperature Mn>Cu>Ni. Therefore, in the leaf of cactus in MK, Mn abundantly existed as the micronutrients.

When we were comparing the levels of metals (Mn, Cu and Ni) in the two sites (KMandMK), with respect of time except Nickel, the level of Mn(2.08mg/l) and Cu(3.9mg/l) at the KM were higher than MK. In addition to the above, with respect of temperature except copper, the level of Mn (2.75mg/l) and Ni (4.56mg/l) at the KM were higher than MK. Manganese was
abundantly available micronutrient than in both KM and MK sites than nickel and copper. Those metals are exist below the daily requirement intake per day as it was reported by [5] and [6].

Table 3.1: Average concentration of metal at KM

<table>
<thead>
<tr>
<th>Metals</th>
<th>Correlation coefficient ( R^2 )</th>
<th>Concentration (mg/l)</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>0.999</td>
<td>2.08±0.005</td>
<td>2.75±0.005</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.999</td>
<td>3.9±0.007</td>
<td>1.48±0.007</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.989</td>
<td>1.56±0.009</td>
<td>4.5±0.009</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.2: Average concentration of metal at MK

<table>
<thead>
<tr>
<th>Metals</th>
<th>Correlation coefficient ( R^2 )</th>
<th>Concentration (mg/l)</th>
<th>Time</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>0.999</td>
<td>0.0153 ±0.005</td>
<td>2.64 ±0.005</td>
<td></td>
</tr>
<tr>
<td>Cu</td>
<td>0.999</td>
<td>2.203 ±0.007</td>
<td>2.64 ±0.007</td>
<td></td>
</tr>
<tr>
<td>Ni</td>
<td>0.989</td>
<td>2.44 ±0.009</td>
<td>1.7 ±0.009</td>
<td></td>
</tr>
</tbody>
</table>

3.2 Effect of Digestion Time

As it indicated below in the fig.1 the effect of time on the concentration of Manganese during digestion of sample was investigated by varying the time (30, min, 60,90min) by keeping volume of the reagent (4ml) and temperature (100°C) constants. As the time of digestion increases the concentration of the metal manganese increase for the sample KM. However, as the time increases the concentration of manganese at MK was increases until the time of the digestion reached at time 60 minutes. Therefore the optimum digestion time of the manganese would be around 60 minutes.

As it shown in the fig.2 below the effect of time on the concentration of Copper during digestion of sample was examined by varying the time (30, mini,60,90min)by keeping volume of the reagent (4ml) and temperature (100°C) constants. As the time of digestion increases the concentration of the metal copper decrease for the sample KM. However, as the time increases the concentration of copper at MK was slightly increases until the time of the digestion reached at time 60 minutes and then decreases. Therefore the optimum digestion point of time of the copper would be around 60 minutes.

As it shown in the fig.3 below the effect of time on the concentration of Nickel during digestion of sample was examined by varying the time (30,mini,60,90min)by keeping volume of the reagent (4ml) and temperature (100°C) constants. As the time of digestion increases the concentration of the metal Nickel increase for the sample MK. However, as the time increases the concentration of Nickel at KM was slightly increases until the time of the digestion reached at time 60 minutes and then slowly decreases. Therefore, the optimum digestion point of time of the Nickel would be around 60 minutes.
3.3 Effect of digestion temperature

As it indicated below in the fig: 4 the effect of temperature on the concentration of Mn during digestion of sample was investigated by varying the temperature (100 \(^\circ\)C, 150 \(^\circ\)C, and 200 \(^\circ\)C) by keeping volume of the reagent (4ml) and time (60min) constants. As the temperature of digestion increases, the concentration of the metal manganese was constants for the sample MK. However, as the temperature increases the concentration of manganese at KM was increases until the temperature of the digestion reached at 150 \(^\circ\)C. Therefore the optimum digestion temperature of the manganese would be around 150 \(^\circ\)C.

![Fig. 4: Shows that the effect of temperature during digestion of on the concentration of Mn by keeping others constants](image)

As it indicated below in the fig: 5 the effect of temperature on the concentration of copper during digestion of sample was investigated by varying the temperature (100 \(^\circ\)C, 150 \(^\circ\)C, 200 \(^\circ\)C) by keeping volume of the reagents (4ml) and time (60min) constants. As the temperature of digestion increases, the concentration of the metal Copper was decreases for the sample KM. However, as the temperature increases the concentration of Copper at KM was decreases slightly until the temperature of the digestion reached at 150 \(^\circ\)C, then increases. Therefore the optimum digestion temperature of the Copper would be around 100 \(^\circ\)C.

![Fig. 5: Shows that the effect of temperature during digestion of on the concentration of Cu by keeping others constants](image)

As it point toward below in the fig:6 the effect of temperature on the concentration of Nickel during digestion of sample was investigated by varying the temperature (100\(^\circ\)C, 150\(^\circ\)C, 200\(^\circ\)C) by keeping volume of the reagents (4ml) and time (60min) constants. As the temperature of digestion increases the concentration of the metal Nickel was increases for the sample KM. However, as the temperature increases the concentration of Nickel at MK was goes constant slightly until the temperature of the digestion reached at 150 \(^\circ\)C, then decrease. Therefore the optimum digestion temperature of the Nickel would be around 150 \(^\circ\)C for MK and 200 \(^\circ\)C KM.

![Fig. 6: Shows that the effect of temperature during digestion of on the concentration of Mn by keeping others constants](image)

4. CONCLUSION

The cactus leaf is the main food sources during the drought dry season in the community around eastern and south Tigray. The main objective of this study is focused on the level of heavy
metals (Cu,Mn,Ni) in cactus leaf. Then the correlation coefficient of these heavy metals of all the calibration curves were >0.99 and this coloration coefficient showed that there was very good relationship between concentration and observance. The concentration of metals that were at the KM with the respect to time both found in the above of cu>Mn>Ni but with respect temperature Ni>Mn>Cu. There for in the leaf of cactus in Km, Mn is abundantly existed as the micronutrients. The concentration of heavy metals that were at the Mk with respect to time was found in the order of Ni>Cu>Mn but with respect to temperature Mn>Cu>Ni. Therefore, in the leaf of cactus in Mk, Mn is abundantly existed at the micronutrient. Those metals(Mn>Cu>Ni) are exist below the daily requirement intake per day as it was reported.

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