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Spectroscopic investigation of metal level in Aloe Vera plant, and the soil where the Aloe Vera grows: Arba Minch, southern Ethiopia

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Abstract: The main objective of this study was to assess the metals level in Aloe Vera and in the soil where the Aloe Vera plant has grown in three sites, namely; Konso, Welaita and Arba Minch in southern region of Ethiopia using flame atomic absorption spectrometry (FAAS). The results of this study reveal that Aloe Vera plant has the ability to accumulate relatively higher amounts of K and Mn among the determined macro-and micro-elements, respectively. The mean levels of metal in Aloe Vera among three geographical areas in this study could be put in the following order: K (51.880 mg/Kg>> Ca (27.440 mg/Kg) > Mg (7.890 mg/Kg) > Na (2.335 mg/Kg) > Mn (2.083 mg/Kg) > Fe (2.043 mg/Kg) > Ni (2.033 mg/Kg) > Cr (1.670 mg/Kg) > Co (0.947 mg/Kg) > Cu (0.189 mg/Kg). But the metals level in soil are decreased in the order of: Ca (25.110 mg/Kg) > Mg (7.600 mg/Kg) > K (2.764 mg/Kg) > Na (2.330 mg/Kg) > Ni (2.240 mg/Kg) > Fe (2.157 mg/Kg) > Cr (1.700 mg/Kg) > Co (1.650 mg/Kg) > Mn (1.510 mg/Kg) > Cu (0.189 mg/Kg). Hence, the metal level in Aloe Vera are more dominant since plants absorb metal ions from different sources and then hyper-accumulated.

Keywords: *Aloe Vera, FAAS* (Flame Atomic Absorption Spectrometry), Metal Level

1. Introduction

Aloe Vera is a medicinal plant belonging to Liliaceae family [1, 2]. It grows in warm regions, mainly in tropical and sub-tropical countries [3-5]. Various species (around 360 species) of Aloe Vera plant grow as ornate plant in hot regions [4, 5]. Among these species, *Aloe barbandensis miller* and *Aloe aborescens* are the most potent and the most popular one [6-8].

Aloe barbandensis Miller

Aloe aborescens

Fig. 1. *Aloe barbandensis Miller and Aloe arborescence along with their flowers.*

Aloe Vera is the most versatile and nutritional store house nature favor to it by giving enormous nutrients and bioactive compounds [2, 9]. It is a nutrient tower that contains around 75 nutrients and 200 active compounds including minerals, amino acids, enzymes and vitamins as summarized in Fig.2 [9-11]

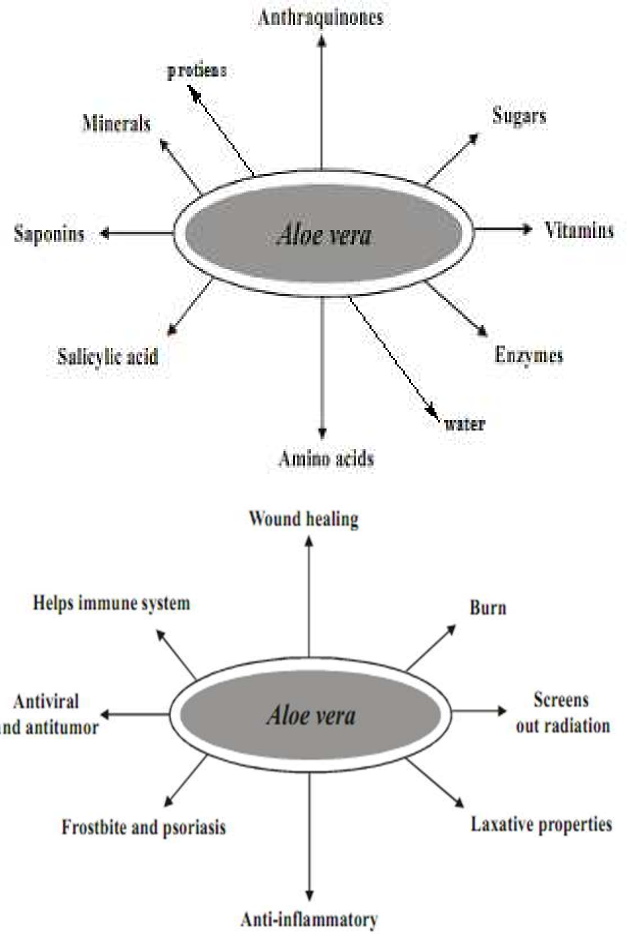


Fig. 2. *Medicinal uses and nutritional composition of Aloe Vera plants.*

Aloe Vera plant can be contaminated with heavy metals during cultivation, processing and from soils [6, 12]. Once these essential and heavy metals entered in the biological cycle of plants, vegetables and fruits through the roots and the leaves from different sources [2.13]; they can be accumulated excessively in different parts of the plant [7, 13]. This harms the enzymatic metabolism, human and animal nutrition [2, 3, 13]. The absorbed metals also bind to functionality of essential S-H groups in enzymes and proteins, and can substitute functional elements in prosthetic groups of enzymes resulting in an inactive catalysis [14, 15, 16]. They also enhance the generation of ROS, deoxyribonucleic acid (DNA) damage and depletion of protein sulfhydryl [15, 17].

Currently, there are many Aloe Vera products such as Aloe drinks, Aloe Vera gels, powders, capsules, creams that are used for medical and cosmetic purpose on the market [2, 13, 18]. But, the safety of these products can be concerned since they have the capability of accumulating excess metal ions by absorbing from soil, water, air and other sources [9, 12, 16]. Therefore, this study was designed to analysis the metal level in both Aloe Vera and soil samples where the Aloe Vera plant has grown from three sites namely Arba Minch, Konso and Welaita in the southern region of Ethiopia.

2. Experimental

2.1. Description of Study Area

The plant samples were collected from three different sites, namely; Konso, Arba Minch and Wolaita in southern Ethiopia along with the soil sample where the plant has grown. Arba Minch is located in the Gamo Gofa zone, Southern Nations, Nationalities and Peoples' Region (SNNPR) of Ethiopia. It is 505 Km south of Addis Ababa with longitude 6° $2^{'}$ $0^{''}$ N, 37° $33^{'}$ $0^{''}$E and altitude of 1300 m above sea level. The average temperature and the annual rainfall are 29°C and 900 mm respectively. Konso is located about 600 Km south of Addis Ababa with longitude 5° $15^{'}$ $0^{''}$ N, 37° $29^{'}$ $0^{''}$ E and altitude of 500 to 2,500 m above sea level. It's average rain fall is 570 mm and its temperature varies from 16^{o}C to 32°C. Wolaita is located about 360 Km south of Addis Ababa. The city is situated at longitude 6° $54^{'}$ $0^{''}$ N, 37° $45^{'}$ $0^{''}$ E and altitude of 2800 m above the sea level. It's average rain fall is 1,212 mm and mean monthly temperature is 20°C.

2.2. Equipment and Instruments

Atomic absorption spectrometer (*Buck Scientific Model 210VGP AAS, East Norwalk, USA*) was used for analysis of metals level in Aloe Vera and soil samples. Flame photometry (*Model: AnA-135, OSK, Japan*) was used for determination of Na and K. "Kjeldahl digestion apparatus (*Gallankamp, UK*), was used for digestion purpose.

2.3. Chemicals and Reagents

Reagents that were used in this study were all analytical grade. 69%HNO_3 (BDH, England), 30% H_2O_2, (Scharlau, European Union) and 37% HCl (Merk, Munich, Germany) were used for digestion of Aloe Vera samples. Stock standard solutions containing 1000 mg/Kg of the metals Ca, Mn and Cr (AnalaR, Hopkin and Williams LTD, Essex, England), and Co, K, Mg, Cu, Na, Fe and Ni (Buck Scientific Puro-Graphic, USA) were used for preparation of standards solutions. Aqua-regia (Riedel-deHaën) and 30% H_2O_2, (Scharlau European Union,) were used for digestion of soil sample.

2.4. Samples Collection

Plant Samples: Mature healthy and fresh leaves of Aloe Vera were collected from three different sites namely; Konso, Arba Minch and Welaita. To draw a representative sample from each sampling site, five subsamples were taken about 5-7 Km away from each other and put in clean polyethylene plastic bags by systematic name. They were brought to the laboratory for further pre-treatment.

Soil Sampling: For comparative analysis of metal levels in Aloe Vera and in soil where the Aloe Vera plant has grown, the soil samples were collected from the surface horizon about 20-30 cm in depth and one meter radical diameter. Finally, a representative bulk soil sample from stated areas was made ready for final analysis.

2.5. Sample Digestion

Digestion of Soil Sample: The dried and homogenized soil sample was ground into powder using mortal and pastel. The powdered materials were sieved with 1.5 mm and 2 mm sieves. The sieved soil was redried and homogenized to constant weight. 0.5 g of the dried soil sample was weighted and transferred into digestive flask. To this, 75 mL of aqua-regia was added followed by 15 mL of H_2O_2. The mixture was digested using 'Kjeldahl Method' till the clear solution was observed. The clear solution was filtered into 100 mL volumetric flasks through Whatman paper # 42. It was diluted with deionized water up to the mark and was stored for further analysis.

Digestion of Plant Sample: The plant sample was washed with distilled water and the leaves were cut in to small pieces. It was dried at 80-100°C in electric oven and ground using pestle and mortar. The powdered materials were stored in electric oven till they acquired constant weight. 05 g Aloe Vera powder was weighted into separate digestive flasks and was digested with 25 mL HNO_3. The flasks were heated in digestive apparatus around 80-100°C in hood. After heated for an hour, the content of flasks was digested by adding 10 mL HNO_3 followed by 2 mL of H_2O_2. Heating was continued until the volume of the content was reduced to semi dried mass via increment of H_2O_2. The contents of flasks were cooled by diluting around 5 mL HNO_3. It was filtered through Whatman # 42 paper into volumetric flasks and marked as stock sample solution for the determination of metals by FAAS.

2.6. Preparation of Standards

Standard Solution: Standard solutions were prepared for each of the metals from an intermediate standard solution containing 10 mg/Kg. The intermediate standard solution was prepared from standard stock solutions that contained 1000 mg/Kg. These secondary standards were prepared by diluting with deionized water to obtain four/five working standards for each metal of interest.

2.7. Determination of Elements

The concentration of Ca, Mg, Cu, Zn, Mn, Ni, Co, Fe and Cr were analyzed with FAAS. The parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Hallow cathode lamps for each metal operated at the manufacturers recommended conditions was used at its respective primary line source. The acetylene and air flow rates were managed to ensure suitable flame conditions. Finally, the absorbance of each standard was measured and then absorbance versus concentration curve was plotted. Once the FAAS was activated, the plant and the soil sample solution were aspirated into FAAS via sample introduction valve.

2.8. Recovery Test

In this work, the method validation was rechecked by spiking experiments [23]. The spiked samples of both Aloe Vera and soil were prepared by adding a known quantity of metal standard solutions.

For spiking Aloe Vera sample: 1000 µL of 1000 mg/Kg K, 600 µL of 1000 mg/Kg Mg, 800 µL of 1000 mg/Kg Ca, 400 µL of 1000 mg/Kg Na, 200 µL of 1000 mg/Kg Mn, 100 µL of 1000 mg/Kg Ni, 100 µL of 1000 mg/Kg Cr, 50 µL of 1000 mg/Kg Co and 40 µL of 1000 mg/Kg Cu standard solutions were added to digestive flask containing 0.5 g of Aloe Vera sample.

For soil sample spiking: 800 µL of 1000 mg/Kg Ca, 600 µL of 1000 mg/Kg Mg, 400 µL of 1000 mg/Kg K, 100 µL of 1000 mg/Kg Mn, 300 µL of 1000 mg/Kg Na, 200 µL of 1000 mg/Kg Ni, 150 µL of 1000 mg/Kg Cr, 50 µL of 1000 mg/Kg Co and 30 µL of 1000 mg/Kg Cu standard solutions were added to digestive flask containing 0.5 g of soil sample. The spiked samples were digested and measured in similar condition and procedure of the non-spiked samples. Then the percentage recovery of the analyte was calculated by [19]:

$$\%R = \frac{(C_M\,spike\,samples - C_M\,nonspike\,samples)}{C_M\,added\,solution} \times 100\% .$$

Where, C_M = concentration of metal.

2.9. Statistical Analysis

After the results were obtained and recorded, the mean, standard deviation, concentration, regression and correlation factors were performed using data analysis packages such as Microsoft Excel 2007, originlab 8.1. All measurements were done in triplicate and the results were reported as average values ± SD. Mahts type software also use to write mathematical equations.

3. Result and Discussion

3.1. Recovery Test

Recovery tests use to check whether the proposed method was valid or not using non-spiked and spiked samples for both Aloe Vera and soil samples [19], and each sample was determined in triplicate. The concentration of both spike and non-spike samples were presented in Table 1 for both Aloe Vera and soil samples.

Table 1. *The recovery tests for the optimized procedure of Aloe Vera and soil samples.*

	Metals	Mg	Ca	Cr	Mn	Fe	Co	Ni	Cu
Aloe Vera	Non-spiked value(mg/Kg	7.89	27.40	1.70	2.08	2.04	0.95	2.03	0.19
	Spiked value (mg/Kg)	14.70	34.20	2.60	3.82	4.86	1.35	2.91	0.51
	Added value (mg/Kg	6.00	8.00	1.00	2.00	3.00	0.50	1.00	0.40
	%Recovery	85	84	91	87	94	80	83	79
Soil	Non-spiked value(mg/Kg	7.600	25.11	1.70	1.51	2.16	1.65	2.24	0.19
	Spiked value (mg/Kg)	12.82	32.31	3.09	2.32	4.77	2.06	4.00	0.41
	Added value (mg/Kg	6.00	8.00	1.50	1.00	3.00	0.50	2.00	0.30
	%Recovery	87	90	93	81	87	82	88	74

As shown in Table 1, the result of percentage recoveries for the studied metal in Aloe Vera and soil samples lies between 74 to 94% which is within the acceptable range [19]. This verified the validity of the proposed method for analysis of both Aloe Vera and soil sample. Hence, an efficient digestion procedure for Aloe Vera and soil sample was validated and a good percentage recovery was obtained.

3.2. Determination of Metal Level in Aloe Vera and Soil Samples

The concentration of ten metals (Ca, Mg, Cu, Co, Cr, Mn, Na, Fe, K and Ni) in the digested samples of Aloe Vera and soil were determined by FAAS and flame photometry. The average concentration (mean ± SD) of metals in Aloe Vera samples from three sites (Arba Minch, Wolaita and Konso) is shown (Table 2).

The result reveals that, Aloe Vera sample contains the highest amount of K (35.220-77.080 mg/Kg), Ca (25.100-29.840 mg/Kg) and Mg (7.820-8.440 mg/Kg) among others. The highest levels of K, Ca and Mg in the Aloe Vera are probably due to the fact that elements such as N, P, K, Mg and Ca are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue [20, 21]. The other probable reason for higher concentration of K, Mg and Ca is due to the present of fertilized soil with manure and organic residues which is rich in K, Ca and Mg [21, 22]. As the result, the Aloe Vera plant has higher amount of K, Mg and Ca metals.

Table 2. *The distribution pattern of metals in Aloe Vera samples in three geographical areas (mg/Kg).*

Metals	AMPS	WPS	KPS	Mean
	mean± SD	**mean± SD**	**mean± SD**	**mean± SD**
Na	3.000±0.600	1.000±0.004	3.000±0.003	2.335±0.003
K	35.220±0.166	43.360±0.071	77.080±0.185	51.88±0.174
Mg	7.820±0.011	8. 044±0.012	7.820±0.012	7.890±0.012
Ca	25.100±0.0165	27.670±0.018	29.840±0.02	27.440±0.018
Cr	1.000±0.003	1.000±0.003	3.000±0.003	1.670±0.003
Mn	1.831±0.055	2.142±0.054	2.275±0.054	2.083±0.054
Fe	1.890±0.016	2.090±0.003	2.150±0.003	2.043±0.007
Co	0.368±0.014	0.159±0.013	2.320±0.016	0.947±0.014
Ni	2.177±0.016	1.742±0.016	2.177±0.016	2.033±0.016
Cu	0.246±0.064	0.085±0.064	0.235±0.066	0.189±0.064

AMPS=Arba Minch plant sample, WPS=Wolaita plant sample, KPS=Konso plant sample

In general, the concentrations of the examined metals in Aloe Vera sample are decreased in the order of: (51.880 mg/Kg) >> Ca (27.440 mg/Kg) > Mg (7.890 mg/Kg) > Na (2.335 mg/Kg) >Mn (2.083 mg/Kg) > Fe (2.043 mg/Kg) > Ni (2.033 mg/Kg) > Cr (1.670 mg/Kg) > Co (0.947 mg/Kg) > Cu (0.189 mg/Kg).

The concentration patterns of ten metals in soil sample were also determined as comparative study with Aloe Vera sample and the result verified that the highest concentration of magnesium and calcium are found. The results as shown in Table 3 are decreased in the order of: Ca > Mg > K > Na > Ni > Fe > Cr > Co >Mn> Cu.

Table 3. *Concentration of metals in soil samples where the Aloe Vera plant has grown (mg/Kg).*

Metals	**AMSS**	**WSS**	**KSS**	**Mean**
	Mean± SD	**Mean ±SD**	**Mean± SD**	**Mean± SD**
Na	3.000±0.003	1.000±0.0033	3.000±0.003	2.330±0.003
K	0.830±0.168	3.1501±0.171	4.314±0.173	2.764±0.171
Mg	7.378±0.011	7.600±0.012	7.822±0.012	7.600±0.012
Ca	46.158±0.032	4.579±0.006	24.579±0.016	25.110±0.018
Cr	1.000±0.0026	1.100±0.003	3.000±0.003	1.700±0.003
Mn	0.747±0.083	2.940±0.066	0.840±0.083	1.510±0.077
Fe	1.850±0.093	2.640±0.054	1.980±0.056	2.157±0.067
Co	0.368±0.014	2.211±0.016	2.368±0.016	1.650±0.015
Ni	2.900±0.015	1.967±0.016	1.840±0.016	2.240±0.016
Cu	0.110±0.064	0.0214±0.065	0.353±0.064	0.189±0.064

AMSS=Arba Minch soil sample, WSS=Wolaita soil sample, KSS=Konso soil sample

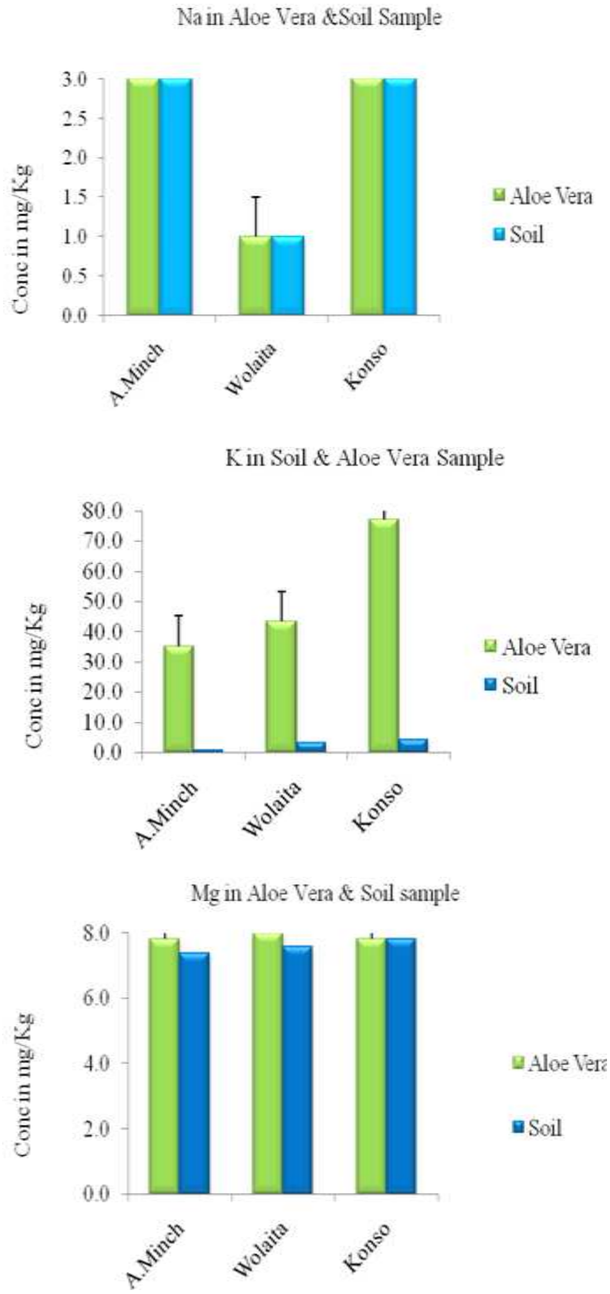
3.3. Comparisons of Metal Levels between Aloe Vera and Soil Sample

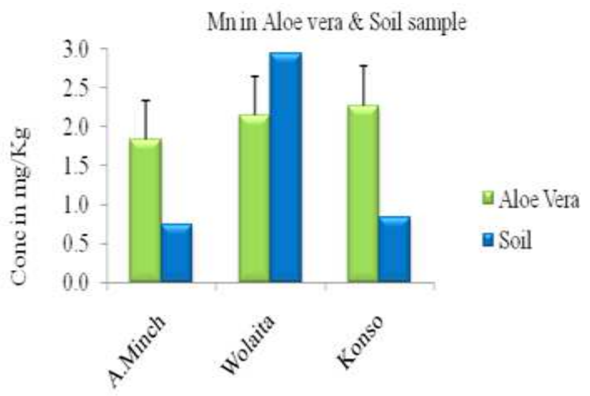
In this work, comparative study has been done to correlate the metal level of Aloe Vera with the soil where the plant grows. As it has seen from (Fig3, Table 2 and 3), the level of metals in Aloe Vera sample are found in higher amount than soil samples.

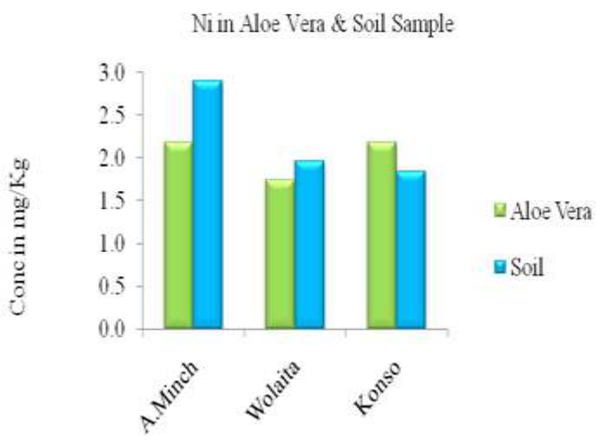
The data obtained in this study reveals that the concentration of Mn, K and Mg were considerably higher in Aloe Vera samples than soil samples. This indicates that the plant is capable to remove the metals from the soil matrix, and the removed metals are trans-located from the root to the shoots and to the leaves, then hyper-accumulated in fatty leaves of Aloe Vera plant [20, 21]. Moreover, plants extracted and concentrated several elements from their environmental fonts such as water, air, industrial waste, mining activities, atmospheric deposition, agricultural chemicals and other sources [13, 20] which greatly influence the metal level in plants.

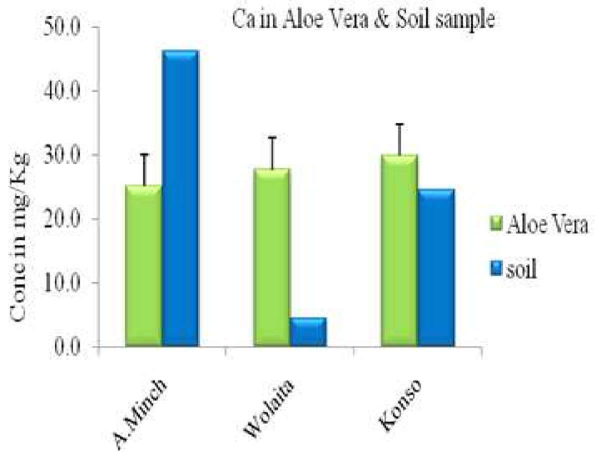
In many ways, the results reveal that the level of metals in Aloe Vera is a function of the metal level in the soil where the plant has grown. This is the fact that the composition and the bioavailability of metals are dependent on the nature of the soil such as sand, silt, clay, organic and inorganic matters [21].

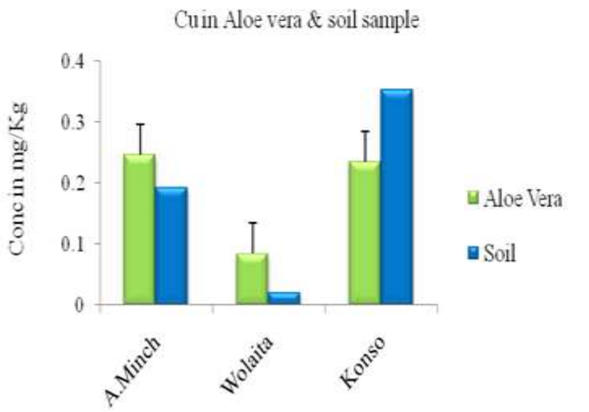
This result also reveals that the metal levels greatly rely on their sample areas; thus, the highest metal concentration is obtained from Konso sample site (Fig4a). This might be due to the fact that Konso people are well known in conservation of soils from being eroded, and they used natural and synthetic fertilizers during cultivation of different crops. Hence, the soil is enriched with these metals, and Aloe Vera has the ability to absorb these deposit minerals from soil and trans-locates to the leave and accumulated [13, 20, 21].

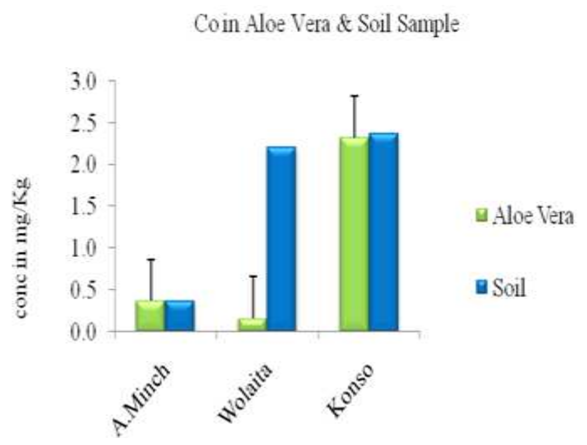












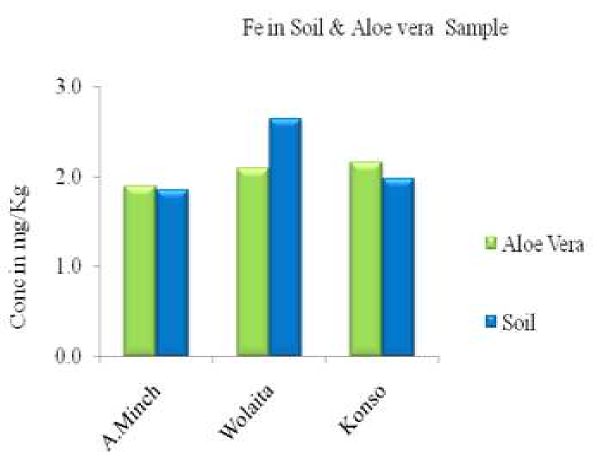


Fig. 3. *Comparison of metal content in soil and Aloe Vera samples.*

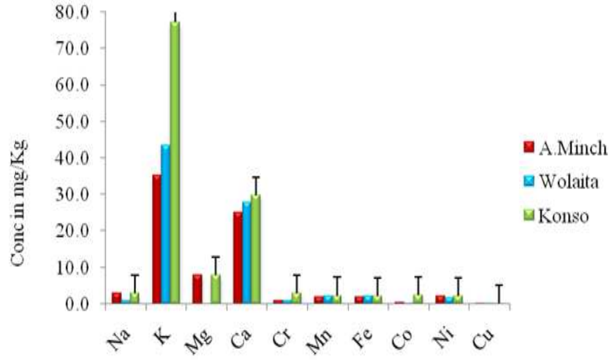


Fig. 4a. *the level of metals in Aloe Vera.*

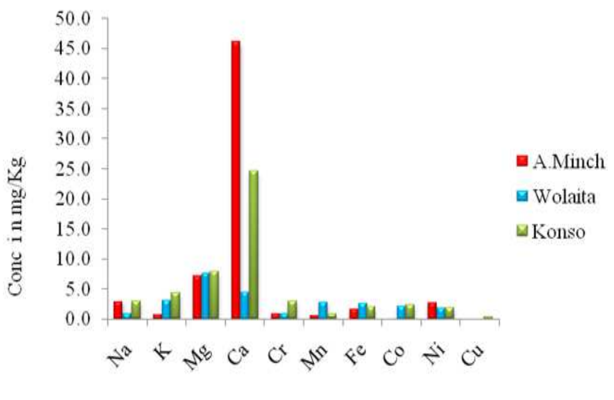


Fig. 4b. *The level of metals in soil.*

3.4. Comparison of Metal Content with Previous Works

The metal level in this study was compared with previous literature values as presented in Table4. This comparison helps to identify the difference in composition if there exist a deviation from a certain guide lines.

Table 4. *Comparison of metals level in the Aloe Vera samples with previous reported value.*

Metals	Concentration in mg/Kg	Country	Reference
Na	13.9800-41.2300	Pakistan	[18]
	1.0000-3.0000	Ethiopia	this study
K	12.8700-21.2800	Pakistan	[18]
	35.2200-77.0800	Ethiopia	this study
Fe	1.8900-2.1500	Ethiopia	this study
	0.2292	India	[8]
Ca	2.4500-3.2700	Pakistan	[18]
	25.1100-29.8400	Ethiopia	this study
Mg	7.8220-8.0440	Ethiopia	this study
	0.6300-1.1600	Pakistan	[18]
Cr	0.0137-0.0275	Pakistan	[22]
	1.0000-3.0000	Ethiopia	this study
Mn	1.8310-2.2760	Ethiopia	this study
	0.0930-0.1240	Pakistan	[22]
Ni	1.742-2.177	Ethiopia	this study
	0.0042-0.0074	Pakistan	[22]
Co	0.1590-2.3200	Ethiopia	this study
	0.0161-0.0237	Pakistan	[22]
Cu	1.2822	India	[8]
	0.0031-0.0062	Pakistan	[22]
	0.0855-0.2460	Ethiopia	this study

As shown in Table 4, the level of both micro and macro elements in Aloe Vera plant in this study are far exceed from the literatures values. Concentration of K, Ca, Mn, Co, Ni and Mg in Aloe Vera plants are beyond previous values, which leads a great impact to public safety and health. Excessive intakes of Mg is harmful to people with impaired renal function resulting hypermagnesemia include nausea, vomiting and hypotension [2, 13]. High level of Ni causes allergic, carcinogen which adversely affect lungs and nasal cavities [21, 22]. Excessive intakes of Cr causes skin rash, nose irritations, bleeding, upset stomach and kidney [8, 10]. High intakes of Ca lead to urinary stone formation as well it inhibits the intestinal absorption of iron, zinc and other essential minerals [21, 22]. The problem is rather more worst in Africa particularly in Ethiopia, because medicinal plants which form the raw materials for the finished products are neither controlled nor properly regulated by quality control groups. Therefore, this paper gives a hint to the society regarding to the effect metals levels in human health and other organisms when they are presenting in excess in quantity.

4. Conclusion

The mean value of metals level in Aloe Vera samples among three sampling site in this study could be put in the following order of: K (35.220-77.080 mg/Kg) > Ca (25.100-29.840 mg/Kg) > Mg (7.820-8.044 mg/Kg) > Na (1.000-3.000 mg/Kg) ≥ Ni (1.000-3 .000 mg/Kg) > Mn (1.831-2.275 mg/Kg) > Fe (1.890-2.150 mg/Kg) > Co (0.159-2.320 mg/Kg) > Cu (0.0855-0.246 mg/Kg). The metal levels in the soil sample where the Aloe Vera plant has grown also decrease in the order of: Ca (25.110 mg/Kg) > Mg (7.600 mg/Kg) > K (2.764 mg/Kg) > Na (2.330 mg/Kg) > Ni (2.240 mg/Kg) > Fe (2.157 mg/Kg) > Cr (1.700 mg/Kg) > Co(1.650 mg/Kg) > Mn (1.510 mg/Kg) > Cu (0.189 mg/Kg). On the basis of the above results, the metal levels in Aloe Vera samples are more dominant than the corresponding soil samples. This is due to the fact that Aloe Vera plant has the potential to accumulate metal ions in it fatty leaf. The data obtained from this study may be helpful for researches that may be conducted on agronomy and physiology of the plant, nutrient requirement and soil-plant nutrient balance of Aloe Vera plant. It is also helpful for fertilizer applications, nutritional and toxicological studies in regard to human health as well as with respect to quality of Aloe Vera plant and its products.

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