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Study of Heavy Metals Accumulation in Leafy Vegetables of Ethiopia

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Abstract : *Energy dispersive X-ray Fluorescence (EDXRF) technique was applied for determination of heavy metals present in some selected varieties of leafy vegetables, mostly consumed in Ethiopia such as Ethiopian kale (Brassica carinata), Lettuce (Lactuca Sativa var.capitata), chard (Beta vulgaris), cauliflower (brassica oleracea.var.botrytis), Cabbage, Collard green (brassica oleracea), Siberian kale (Brassica napus var.pabularia) and Russian kale collected from Akaki, Kera and Debre Berhan. The concentration of 9 heavy metals (Zn, Cu, Ni, Co, Fe, Mn, Cr, As and Pb) were analyzed in the selected leafy vegetables. The foodstuffs examined for metal constituents are the basis of human nutrition in the study area except Siberian kale and Russian kale. The highest level of heavy metals were found in vegetables which are grown in Akaki and Kera, compared to samples of Debre Berhan , because Debre Berhan city is the place with no specific type of pollution. Metal levels observed in different sources were compared with WHO/FAO, and established permissible levels reported by different authors. Accumulation of most of the heavy metals in vegetables studied was higher than the recommended maximum tolerable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives (1999), with the exception of copper, iron, cobalt and nickel, which exhibited low-lying content. Among all selected leafy vegetables, Swiss chard accumulate much higher concentrations of metals and toxic metals like As and Pb was not detected in Abyssinia kale collected from Debre Berhan.*
Keywords: *EDXRF, leaf vegetable, Heavy metal, permissible limit*

I. Introduction

Vegetables constitute essential diet components by consisting carbohydrates, proteins, vitamins, iron, calcium and other nutrients that are highly beneficial for maintaining health and also preventing diseases. They also contain both essential and toxic elements over a wide range of concentrations. Until recently vegetables did not constitute as major part of the Ethiopian diet. In addition, there is an annual cycle of shortage of grains in some parts of Ethiopia, where families exhaust their grain supply before the next harvest; then they heavily supplement their food intake with leafy vegetables [1, 2]. However, during recent years their consumption is increasing gradually, particularly among the urban community. Now- a-days vegetables began to play a major role in the agriculture of Ethiopia by providing nutritional food and economic security.

Presently environmental pollution is a big concern. Heavy metals are present in all types of ecosystems. Their existence is mainly due to anthropogenic sources such as industrial and agricultural activities [3]. This causes an increase in the concentration of heavy metals content related to soil and water, thus contaminating them. The increased uptake of these metals by plants influence the natural contents of vegetables and thus poses serious health impacts. Heavy metals are potential environmental contaminants with the capability of causing human health problems such as cancer, mutations, or miscarriages; if present to excess in the food we take [4]. Plants and vegetables take up heavy metals by absorbing them from contaminated soils and waste water used for irrigating them as well as from deposits on different parts of the plants exposed to the air from polluted environment [5]. The major sources of elements in vegetable crop are their growth media, (soil, air, nutrient solutions) from which these elements are taken up by their roots or foliage.

The food chain contamination is the major pathway of heavy metal exposure for humans [6]. Industrial or municipal wastewater irrigation is a common reality in some parts of the cities in Addis Ababa. The vegetable farms located at Akaki and Kera areas are biggest among the farms that present in the capital city i.e. Addis Ababa, where a substantial amount of vegetables is being produced seasonally. These farms are irrigated with the waste water that obtained from the rivers of Akaki and Kera. Before several decades, the water of these rivers in the capital was clean. However, with the increase of urban population and industrialization, the water has now become contaminated with various pollutants, among which heavy metals are present. The Akaki river is used as open waste disposal site for the city of Addis Ababa. Apart from its unfortunate fact, the river is also still used for various purposes including irrigation and animal drink. Furthermore, vegetables consumed in the nearby cities are also produced using polluted waters from the river. The major industries from which effluents enter into Kera River are: Addis Ababa Tannery, Tikur Abay Shoe Factory, Gulele Soap Factory, Ethio Marble

Industry and Gulele Shirt Factory [7]. The daily domestic and industrial wastewater discharge from the above industries (excluding Gulele Shirt Factory) is 305, 200, 62, and 13 m^3, respectively. Wastewater from industries or other sources carries an appreciable amount of toxic heavy metals that create a problem for safe rational utilization of agricultural soil [8-10]. Long-term use of industrial or municipal wastewater in irrigation is known to have significant contribution to trace elements such as Cd, Cu, Zn, Cr, Ni, Pb, and Mn in surface soil [11]. Excessive accumulation of trace elements in agricultural soils through wastewater irrigation may not only result in soil contamination but also affect food quality and safety [12, 13].

There are number of studies that reported about the deposition of heavy metals in soil, crops, and vegetables grown in the vicinity of industrial areas. However, there are very few publications from Ethiopia related to the investigations on heavy metals contamination in soil; irrigation water and their transfer to vegetable crops grown in the vicinity of industrial areas. Vegetable farms of Akaki and Kera are important areas belong to Ethiopia in which research work related to heavy metals contamination has not been performed so far. The main objective of the present study is to focus on the status of heavy metals accumulation in some leafy vegetables those grown in Akaki, Kera areas and comparing the results with the concentration of heavy metals accumulated in the samples that collected from Debre Berhan, a location having far from industrial area. It is to observe the difference with the established safe limit of the content of heavy and toxic metals in various leafy vegetables.

Earlier X-ray fluorescence analysis (XRF) was used in quantitative elemental analysis of a wide range of organic and inorganic samples [14-16]. The basis of the technique is that all elements emit secondary ('fluorescent') X-rays of characteristic energy when exposed to X-rays of appropriate higher energy. Energy and intensity of emitted X-rays are used to determine elemental composition. In general, heavier the element being analyzed, higher the energy of X-rays required to elicit fluorescence, higher the energy of fluorescence, then it is easier to detect fluorescence. The lightest elements exist in biological samples (e.g. H, B, C, N, O) are not generally detectable by XRF, while the elements such as Na, Mg, P, S, Cl, K, Ca are detectable only at higher concentrations or under highly specialized conditions, and heavier elements namely Mn, Fe, Cu and Zn (trace metals) or toxic heavy metals are readily analyzed, even at trace levels. Major advantages of XRF over other analytical methods are that analyses are nondestructive, simultaneous multi-element analysis, use no noxious chemicals and produce no toxic wastes, losses that encounter in chemical methods during dry ashing and acid extractions are avoided and can be made on solid samples. Since XRF signal is obtained from transitions among inner shell electrons, not bonding electrons, XRF also has the advantage that signals are independent of chemical form. Of the various types of X-ray spectrometry available, laboratory 'bench-top' Energy-Dispersive X-ray Fluorescence (EDXRF) is the foremost commonly used for routine analysis of large number of samples. In EDXRF, detectors are used that can discriminate X-rays based on energy enabling the simultaneous detection of multiple elements. Energy dispersive X-ray fluorescence (EDXRF) technique has been employed for the analysis of heavy metal accumulation of leaf vegetables from the study areas.

II. Material and methods

SAMPLE COLLECTION AND PREPARATION

During September 2015 to January 2016 samples of the present studies were collected in and around Addis Ababa, Ethiopia, which has a large industrial area comprised of a large number of small and big industries like fabric printing; dyeing, food processing, metal workshops, garages, tanneries, textiles, electric cables, pharmaceutical, chemical etc., located in the capital city (Addis Ababa). Most of the industries discharge their effluents through open drain without any prior treatment contaminating water, soil, and vegetables of the adjacent areas. All samples of leaf vegetables such as Ethiopian kale (Brassica carinata), Russian kale, Collard, Siberian kale, Lettuce (Lactuca Sativa var.capitata), chard (Beta vulgaris), cauliflower (brassicaoleracea.var.botrytis) and Cabbage with their corresponding soil samples were collected from different fields of Addis Ababa particularly from the location of Akaki river area. Akaki farm is located in the south western part of Addis Ababa near Lake Aba Samuel in Sakelo village (at latitude of 9.01^0, longitude of 38.71^0). Being in the downstream of the Little Akaki river, this farm receives no direct industrial discharges and Kera farm (at latitude of 8.98^0, longitude of 38.7^0 and altitude of 2253.11 m above ellipsoid) is nearer to Kera abattoir and is irrigated with the Little Akaki river (also called Kera river) as well as agricultural research center of Debre Berhan university (located at latitude of 9.6^0, longitude of 39.5^0 and altitude of 2769.68 m above ellipsoid, at a distance of 130 km from capital Addis Ababa).

For each plant sample, leaves of vegetable were collected at random from the fields of the sampling sites by hand using gloves, carefully packed into polyethylene bags, and brought to the laboratory. The samples were washed with double distilled water to remove dust particles and non-edible parts were removed from them. After washing and cutting, the vegetables leaves were dried in an oven at temperature of 65^0C to remove moisture in them. After drying, the samples were gently grinded with clean agate mortar and pestle to make fine powder and stored in clean dried plastic containers. The homogenized samples were prepared by weighing 150mg and Pellets

(1mm thick and 13mm diameter) were prepared using a tabletop pelletize machine at a pressure of 100− 110 kg/cm2. A minimum of 3 pellets of each sample was made to reduce the error in the analysis.

Experimental studies and Method of Validation

Experimental studies of the samples have been carried out at UGC-DAE Consortium for Scientific Research, Kolkata Centre using a Xenemetrix (erstwhile Jordan Valley) EX 3600 EDXRF spectrometer, which consists of an oil-cooled Rh anode X-ray tube (maximum voltage 50 kV, current 1 mA). The measurements were performed in vacuum using different filters (between the source and sample) for optimum detection of elements. A 0.05-mm-thick Ti filter was used in front of the source for Cr, Mn, Fe, Co, Ni, Cu and Zn with an applied voltage of 14 kV and a current 900 mA. For higher Z elements such as Pb, Bi, Ag and As, a Fe filter of 0.05 mm thickness was used at a voltage of 37 kV and 45 mA current. All the spectra of the samples were collected for duration of 1400 seconds as shown in table 1. The X-rays were detected using a liquid nitrogen-cooled 12.5 mm^2 Si (Li) semiconductor detector (resolution 150 eV at 5.9 KeV). The X-ray fluorescence spectra were quantitatively analyzed by the '**nEXt**', system software runs under the **Windows NT™** operating system integrated with the system. The precision and trueness of the EDXRF method was checked by analyzing certified reference materials made from leaves (NIST 1515 'Apple leaves' and Oriental Tobacco leaves (CTA-OTL-1) from National Institute of Standards and Technology), therefore expected to have a similar matrix composition of leafy vegetable samples and the recovery percentages of the elements were not significantly different from them as shown in table 2.

Acquiring spectra is the first and one of the most important steps performed in both qualitative and quantitative analysis. Acquisition parameters are used in the spectrum capture process to determine the spectrum profile and parameters. They are chosen to enhance the number of counts obtained for the elements of interest. As tabulated in below table 1 for the present study the following parameters were used.

Table 1: Operating condition of EX 3600 EDXRF spectrometer e1 (spectrum1), e2 (spectrum2), e3 (spectrum3)

Parameters	*e1*	*e2*	*e3*
Filter	None	3-Ti	4-Fe
Emission current(µA)	240	900	45
High voltage (Kv)	6	14	37
Preset time(S)	200	900	300
Atmosphere	vacuum	vacuum	vacuum
Energy Range (KeV)	10	10	40
Throughput	low	low	low

Table 2: Concentrations of elements obtained from NIST (SRM 1515) Apple leaves and Tobacco leaves (CTA-OTL-1) with our experimental set-up. (*Italic (percent), normal (ppm))

	NIST (SRM~ 1515$)		(CTA-OTL-1)	
Elements	Certified value	Present work	Certified value	Present work
Ca	15260.00	15581.52	3.17±0.12 *	3.038 *
K	16100.00	15878.04	1.56±0.05*	1.36 *
S	18000.00	18780.64	0.732±0.081*	0.684 *
P	15900.00	15278.01	2892±134	4723.83
Zn	12.5	14.5	49.9±2.4	46.69
Cu	5.64	7.08	14.1±0.5	12.57
Ni	0.91	0.73	6.32±0.65	6.68
Fe	83.00	71.70	989	1000.34
Mn	54.00	47.98	412±14	421.12
Cr	0.30	1.15	2.59±0.32	2.13
Ba	49.00	67.97	84.2±11.5	82.26
Sr	25.00	29.29	201±20	205.27
Rb	10.20	9.85	9.79±1.27	10.61
Br	1.80	4.26	9.28±1.06	11.78
Se	0.05	0.11	0.153±0.018	0.11

III. Results and discussion

From the same sample that tested at various laboratories yield different values due to the influencing factors such as used precise digestion methodology, the sensitivity of the heavy metal detection instrument, the potential for contamination of samples and the potential for interference from other chemicals when assessing the levels of certain heavy metals etc. Hence, great care was taken to assure the reliability of data being presented in the present work. Internationally certified plant standards reference material (SRM) is an important tool for the quality control process. This material has known concentrations of the heavy metals that are concerned in the present investigation; therefore it is used to test the reliability of findings that obtained in the measurements. As tabulated in table 2 the standard deviation of the measured values are mostly with in ±5-10%, which may represent a good agreement between the measured and certified values.

Table 3 shows the mean concentrations of heavy metals those presented in the investigated leafy vegetables that commonly consumed in Debre Berhan; Addis Ababa of Ethiopia. The results are means of three replicates and values are given as mean ± SD. The determined concentration of heavy metals is based on plants dry weight. A wide variation in the heavy metal concentrations of different leafy vegetable samples is studied. Vegetable leaves in the investigated samples; and the corresponding soil samples, Energy dispersive x-ray fluorescence technique analysis, which allowed for determination of 9 heavy metals such as zinc, copper, nickel, cobalt, iron, manganese, chromium, arsenic and lead are presented.

Table 3: Concentration of Heavy metal (mean ± Standard deviation, mg/Kg) in samples of leaf vegetables collected from Akaki, Debre Berhan university Agriculture research centre and Kera

Sample name	Place	Zn	Cu	Ni	Co	Fe	Mn	Cr	As	Pb
Abyssinia kale (dark green leaf)	Akaki	35.1 ±0.9	2.6 ±0.8	1.4 ±0.7	0.47	184.9±73.8	28.1±3.2	8.8 ±6.5	0.05	0.5
Abyssinia Kale (bright green leaf)	Akaki	52.85 ±2.3	6.8 ±1.2	1.0 ±0.3	0.42±0.02	151.5 ±1.8	14.2±1.6	2.7 ±0.3	0.3±0.2	2.8
cabbage	Akaki	43.2 ±12.7	2.5 ±0.2	1.0 ±0.4	0.4 ±0.1	82.9±3.4	18.3±2.2	2.2 ±0.2	1.4±0.3	12.3±3.1
Abyssinia kale (dark green leaf)	Kera	49.3 ±1.4	6.1 ±0.4	1.5 ±0.5	0.47	92.7±2.6	11.9±0.7	3.9 ±0.4	0.11	1.06
Cauliflower leaf	Kera	17.9 ±1.9	1.5	0.7	0.39±0.14	67.5±7.9	0.26	2.0 ±0.4	0.05	0.56
Swiss Chard	Kera	219.3 ±4.9	11.6±1.4	11.6±1.4	0.35±0.1	158.5 ±1.6	868.5±13.9	2.2 ±1.1	0.09	0.9±0.6
Cabbage	Kera	40.1 ±5.1	10.4±5.7	1.5 ±0.9	0.3 ± 0.2	100.7 ±1.9	22.6±1.0	6.2 ±0.9	0.7±0.3	6.9±2.9
Lettuce	Kera	48.9 ±0.8	7.0 ± 1.0	1.2 ±0.2	0.46	132.5 ±1.4	23.7±1.3	6.1 ±0.2	0.1	1.07
Abyssinia Kale (dark green leaf)	DB	16.1 ±0.7	4.1 ± 0.8	1.47±0.5	0.3 ± 0.2	59.4±2.9	76.9±1.0	1.8 ±1.1	ND	ND
Abyssinia kale (bright green leaf)	DB	10.9 ±1.3	2.1 ± 1.0	1.1 ±0.4	0.47	73.5±1.7	94.4±4.7	2.5 ±0.5	0.04	0.5
Swiss Chard	DB	60.1 ±0.3	9.4 ± 1.7	1.2 ±0.3	0.47	193.6 ±4.7	49.6±2.3	2.15±1.1	0.05	0.5
Collard	DB	48.78 ±1.8	2.4 ± 0.5	7.5 ±0.2	0.47	40.8±1.9	53.9±1.4	1.7 ±0.5	0.16	1.5
Cabbage	DB	20.2 ±0.6	1.9 ± 0.3	3.1 ±1.7	0.39±0.1	44.3 ±10.5	65.4±1.5	3.5 ±1.2	ND	1.05
Lettuce	DB	84.9 ±1.7	9.9 ± 0.8	5.3 ±0.9	0.47	115.3±11.4	299.5 ±3.9	2.6 ±0.2	0.04	0.46
Russian kale	DB	50.5 ±0.8	8.5 ± 2.4	2.8 ±1.4	0.47	92.3±5.2	42.1±0.9	4.3 ±0.2	0.08	0.9
Siberian kale	DB	78.9 ±1.9	8.7 ± 1.3	7.7 ±1.4	0.4	85.8 ±17.4	106.8 ±1.1	3.0 ±0.6	0.16	1.5
Min		10.9 ±1.3	1.5	0.7	0.3 ±0.2	40.8±1.9	0.26	1.7 ±0.5	0.09	0.5
Max		219.3 ±4.9	11.6±1.4	11.6±1.4	0.47	193.6 ±4.7	868.5±13.9	8.8 ±6.5	1.4±0.3	12.3±3.1

ND =Not detected

Table 4: Maximum permissible concentrations (mg/Kg) of some heavy metals in vegetables

Elements	Cu	Mn	Pb	Fe	Zn	Cr	Ni	As	Co
FAO/WHO	73.30_a	500.00_a	0.30_a	425.50_a	99.4_a	2.3_a	66.9_a	0.03_a	50_a
SEPA	20_b		9_b	-	100_b	0.5_b	10_b	-	-
Indian safe limit	30_c	-	2.5		50_c	20_c	1.5_c		
WAV	$5\text{-}30_d$	$30\text{-}300_d$			$27\text{-}150_d$				$0.02\text{-}1_d$

[a]source: FAO/WHO (2001) Joint Codex Alimentarius Commission [17]
[c]source: Awashthi (2000) [18]
[b]source: SEPA (2005) [19] and
[d]source: WAV, World average value(pendias 2000).

Zinc (Zn): All living organisms accumulate considerable amount of Zn in their system without any damaging effect [20]. It is essential to carbohydrate metabolism; protein synthesis and inter nodal elongation (stem growth). Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs with zinc mal-absorption occurs [21]. Zinc deficiency leads to iron deficiency causing similar symptoms. Deficiency of zinc causes loss of appetite, growth retardation and immunological abnormalities. The RDA of Zn is 15 milligrams per day for men and 12 milligrams per day for women. Recent research suggests that men have a higher need of zinc relative to women. Thus, it is appropriate that the RDA is sex-specific for zinc. Zinc can be toxic when exposures exceed physiological needs. Zinc is present in many foods, soil and is also found in a number of pharmaceutical samples, causing environmental pollution. Significant concentrations of zinc may reduce the soil microbial activity and also is a common contaminant in agricultural and food wastes.

The highest concentration of Zinc (219.3 ± 4.9 mgKg^{-1}) is detected in Swiss chard that belong to Kera farm followed by Lettuce (84.9 ± 1.7 mg/Kg) and Siberian kale (78.9 ± 1.9 mgKg^{-1}), which were collected from Debre Berhan locality. The lowest content of Zn (10.9 ± 1.3 mg/kg) is detected in Abyssinia kale (bright green leaf) that collected from Debre Berhan. The observed high content of zinc in Swiss, which belongs to kera might be due to presence of higher zinc content in its soil when compared with soil of the other locations. Four years ago a study [22] was conducted in the same site by which, results of Zn concentration in Swiss chard, Abyssinia kale and Lettuce 17.38±0.28, 11.40±0.25 and 3.07± 0.12 mgKg^{-1} respectively were reported. The obtained present results showed the relative increase of Zn in this site indicating its increase from time to time. A study [23**]** performed in Romania was reported the Zn concentration in Lettuce and spinach as 45.5±3.6 and 82.4±15.2 mgkg^{-1} respectively; this data agree well with the values obtained in the present investigation. Zn low level was also observed in the Study carried out in Ghana [24] and Burkina Faso **[**25**].** The concentration of accumulated Zn in cabbage and lettuce those irrigated with waste water related to the Nagodi mining site are found to be 1.641 and 1.853 while it is 0.82±0.06 in cabbage and 0.40±0.04 in lettuce belong to selected farms of Burkina Faso. On the other hand high level of zinc was found in India too in the earlier study [26] 'Heavy metal accumulation in the vegetables grown in a long term west water-irrigated agricultural land of tropical India' having Zn level in cauliflower (90.87±4.33) and Spinach (148.04±4.04). The result of investigations carried out [27] relating to industrial area of Dhaka, Bangladesh and Pakistan [28**]** agree well with the results of the present study. Investigations of the study [29], was also indicated that Swiss chard accumulates much amount of Zn among other leafy vegetables. Zn level of Swiss chard from Kera farm was found to be higher than the permissible value recommended by FAO/W HO [17].

Copper (Cu): Copper works with many enzymes such as those involved in protein metabolism and hormone synthesis **[**30, 34**].** Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting; nervous system disorder **[**30**]** and10 mg of copper can have a toxic effect **[**25]**.**

A very narrow range of copper concentration **(**8.5 ± 2.4 - 11.6 ± 1.4 mg/Kg) is found in the present samples Russian kale (DB), Siberian kale (DB), Swiss chard (DB), lettuce (DB), cabbage (Kera) and chard (Kera). The highest content of Copper (11.6 ± 1.4 mg/Kg) is detected in chard and cabbage (10.4 ± 5.7) mg/Kg those collected from Kera farm followed by lettuce (9.9 ± 0.8 mg/Kg), Swiss chard (9.4 ± 1.7 mg/Kg), Siberian kale (8.7 ± 1.3 mg/Kg) and Russian kale (8.5 ± 2.4 mg/Kg) belong to Debre Berhan research center, while the lowest content (1.5 mg/Kg) is detected in cauliflower leaf collected from Kera farm. Four years ago a study was conducted at Kera and peacock farm**s [**22**],** which reported that Cu level was found to be (2.86±0.04) mg/Kg in Swiss chard, (3.77±0.02) mg/Kg in Abyssinia kale and (1.63±0.05) mg/Kg in Lettuce indicating the level of heavy metal is relatively increasing. An interesting observation that obtained in the present work is Russian kale and Siberian kale is not known in the diet of Ethiopian, but they have a good source of essential elements as well as they accumulate low amount of heavy metals. The concentrations of Cu in the present study are found to be lower than the permissible value recommended by earlier investigators **[**17, 18-19**].** The present study agrees well with the study conducted in Pakistan [28] and in industrial area of Dhaka, Bangladesh **[**27**]**. On the other hand high level of Cu was reported from the study conducted in Romania [23], Cu concentration values in Lettuce and spinach are 25.5±1.7 and 17.4±4.6 mgkg^{-1} respectively. Similarly the study conducted in India by Gupta reported the level of Cu in some vegetables such as cauliflower (15.26±0.80) and Spinach (32.11±2.08). The result reported by [29] is in a close agreement with the present work. The observed variation may be due to change of vegetables variety, growing area, type of soil, climate, used fertilizer, water and agricultural practice.

Nickel (Ni): Nickel and nickel compounds have many industrial and commercial uses, and the progress of industrialization has led to increased emission of pollutants into ecosystems. Nickel is considered to be a normal constituent of the diet and its compounds are generally recognized as safe when used as a direct ingredient in human food. Little is known about the actual chemical forms of nickel in various foods or whether dietary nickel has distinct "organic" forms with enhanced bioavailability analogous to those of iron and chromium. Nickel levels in foodstuffs generally range from less than 0.1 mg/kg to 0.5 mg/kg [31]. The maximum level of Nickel is observed in Swiss chard that collected from Kera irrigated farm with the concentration of 11.6 ± 1.4 mg/Kg while the minimum content (0.7 mg/Kg) is detected in cauliflower leaf of the same farm.

Results of the present study is found to be higher than the data reported by earlier studies [22**]** and has good agreement with the study conducted in Pakistan [43], in which the level of Ni in cabbage ranges between 0.96-14.67 mgKg^{-1} and in cauliflower it ranges between 0.76-8.91 mgKg^{-1}. When compared the present results with the data reported by [27] is found to be higher, this difference might be due to the level of contamination of the studied areas. The result of the present study is found to be higher than the permissible value recommended by **[**18-**19],** but it s lower than the permissible level set by FAO/WHO [16]. Nickel is one of many carcinogenic metals known to be an environmental and occupational pollutant. Chronic exposure has been connected with increased risk of lung cancer, cardiovascular disease, neurological deficits, and developmental deficits in childhood, and high blood pressure. Nickel exposure introduces free radicals, which lead to oxidative damage and may also affect the kidneys and liver.

Cobalt (Co): Cobalt deficiency symptoms include a loss of appetite, emaciation, weakness, anemia, and decreased production. RDA value for cobalt is 0.3mg/day **[**32**].** Inorganic cobalt has no nutritional value but sometimes added to beer as anti-foaming agent. To be biologically useful, cobalt must be obtained from foods.

The highest concentration of Cobalt (0.47 mg/Kg) is detected in Abyssinia kale (dark green leaf) belong to both Kera and Akaki locations besides lettuce, Russian kale, collard, chard of Debre Berhan area. But lowest level of cobalt is observed in Abyssinia kale (dark green leaf) of Debre Berhan area having concentration 0.3 ± 0.2 mg/Kg. The present results have good agreement with the result reported by earlier study [22**].** Co concentrations in different samples are; Swiss chard (0.32±0.05), Lettuce (0.36± 0.03), cabbage (0.057±0.03) those collected from Akaki farm and Swiss chard (0.407±0.01), Abyssinia kale (0.417±0.02), Lettuce (0.267±0.01) obtained from Kera farm. On the other hand higher level of Co was reported by earlier investigators also [28]. The level of cobalt obtained in the present study is found to be lower than the permissible limit set by **[**17-19**].**

Iron (Fe): The highest content of Fe (193.6 ± 4.7 mg/Kg) is noticed in Swiss chard collected from Kera farm followed by Abyssinia kale particularly dark leaf collected from irrigated farm of Akaki with the concentration of 184.9 ± 73.8 mg/Kg, Abyssinia kale of Akaki (151.5 ± 1.8 mg/Kg), Swiss chard (158.5 ± 1.6 mg/Kg) and lettuce (132.5 ± 1.4 mg/Kg) related to Kera farm. The lowest content of Fe (40.8 ± 1.9 mg/Kg) is detected in collard of Debre Berhan. The obtained content of iron in the present study areas ranges from 40.8 ± 1.9 to 193.6 ± 4.7 mg/Kg. The concentration of Fe in leafy vegetables samples collected from the study areas is found to be in the recommended level of FAO/WHO and it is safe to the consumer.

An excess amount of iron could not be taken and if excess of iron is stored in the body's tissues; it adversely affect the body's immune function, cell growth and heart health [**32, 33, 39**]. Iron absorption can be influenced by calcium, magnesium, manganese, zinc, anti-acids and tetracycline (a common antibiotic). Deficiency of iron deprives body tissues of oxygen and results in anemia, which is recognized by its symptom such as low blood iron level, small red blood cells and low blood hemoglobin values, fatigue, paleness, dizziness, sensitivity to cold, irritability, poor concentration and heart palpitation[34]. RDA of iron depending on age level and health condition is 10 to 30 mg. Recommended daily intake is 15 mg. Low level of Fe was reported in vegetables from some African country, such as Ghana [24] and Burkina Faso [25]. Results reported by [29] and [27] are in good agreement with the present work.

Manganese (Mn): A deficiency of manganese may affect brain health, glucose tolerance, normal reproduction, skeletal and cartilage formation. Grains and cereal products are the best food sources of manganese, while animal products are the poorest. The RDA levels and the recommended "not to exceed" daily maximums (tolerable upper intake levels) for manganese is 1.2 and 11 mg respectively [35]. Exposure to high level of Mn can cause both mental and emotional disturbance along with increased slowness and clumsiness of the body movements [35, 36]. In the present study it is found that the distribution of Mn in all the samples varies in a wide gap.

The maximum content of manganese (868.5±13.9 mg/Kg) is found in Swiss chard sample while the lowest (0.26 mg/Kg) is detected in cauliflower leaf those collected from Kera. The former is much higher when compared to the values reported by [29] and [22]. The content of Mn in the remaining vegetable samples is found to be in a good agreement with the values reported by [23]. Except Swiss chard of Kera farm, Mn accumulation in leafy vegetables of the present study areas are lower than the recommended maximum tolerable levels proposed by the Joint FAO/WHO Expert Committee on Food Additives (1999).

Chromium (Cr): The highest concentration of chromium (8.8±6.5 mg/Kg) is detected in Abyssinia kale (dark green leaf) of Akaki, followed by green cabbage (round) 6.2 ± 0.9 mg/Kg and lettuce(6.1 ± 0.2 mg/Kg) of Kera while the lowest content (1.7 ± 0.5 mg/Kg) is found in collard belong to Debre Berhan. In the present study the content of Cr in leafy vegetables those collected from Kera and Akaki farms is found to be higher than the recommended limit that set by FAO/WHO and Indian safe limit [18] but it is lower than the value set by [19]. Chromium is a mineral. It is called an "essential trace element" because very small amounts of chromium are necessary for human health. There are two forms of chromium: trivalent chromium and hexavalent chromium. The first is found in foods and supplements and is safe for humans. The second is a known toxic that can cause skin problems and lung cancer.

Chromium (Cr) is considered an essential nutrient and also cause hazard to health. Medical warning is need to be issued if inhalation of dust containing Cr in high oxidation states (IV) and (VI) is associated with malignant growth in the respiratory tract and painless perforation in nasal septum among trivalent and hexavalent states, which being the most stable and common in terrestrial environments. Hexavalent Chromium form is considered to be the greatest threat because of its high solubility, its ability to penetrate cell membranes and its strong oxidizing ability. Hence, Cr (VI) is more toxic than Cr (III) because of its high rate of absorption on living surface [37].

Lead (Pb): Excessive lead accumulation in plant tissue impairs various morphological, physiological, and biochemical functions in plants either directly or indirectly, and induces a range of deleterious effects. Leafy vegetables, potatoes and beans are likely to absorb more lead than fruiting crops like tomatoes etc. Lead obstructs the utilization of oxygen and glucose for the life sustaining energy production. The interference with normal metabolic functions starts when the blood-lead level reaches 0.3 ppm. When the blood-lead level reaches about 0.8 ppm, symptoms of anemia will be observed due to deficiency of hemoglobin. Higher levels of lead in the blood can cause kidney dysfunction and brain damage as it is a toxic to the central and peripheral nervous system.

As shown in table 3, the highest level of lead (12.3 ± 3.1 mg/Kg) is found in cabbage obtained from Akaki, (6.9 ± 2.9 mg/Kg) followed by a Kera farms and Ethiopian kale (2.8 mg/Kg) of Akaki while minimum level of lead (0.46 mg/Kg) is detected in lettuce of Debre Berhan. In the present study the level of Pb in all samples are found to be much higher than the permissible level (0.3 mg/Kg) set by FAO/WHO. The accumulation of elevated level of Pb in leafy vegetables of Akaki and Kera farms might be attributed to the leakage of ink effluent from the industry to the water and the gas emission from vehicles because those sites are not far from road. Higher level of Pb was reported by earlier investigators [27] related to study place in Dhaka, Bangladesh. The value of Pb obtained in the present study has good agreement with data reported by [27] and [38].

Arsenic (As): Arsenic is an extremely toxic element. Its pollution occurs due to its release into air from smelting of As-containing ores, burning of coal and use of arsenic compounds in various applications such as fungicides, insecticides, herbicides, pesticides and preservatives. Arsenic released from natural agencies such as weathering processes on a global scale is estimated to be about 8×104 metric tons per year while man-made activities account for about 24 × 104 metric tons per year. Arsenic is a general protoplasmic poison and it affects all systems in the body. It is a cumulative poison. The concentration of arsenic exceeding the maximum permissible limit (0.03mg/Kg) in food stuff cause short term (nausea, vomiting, diarrhea, weakness, loss of appetite, cough and headache) and long term (cardiovascular disease, diabetes and vascular diseases) health effects. Arsenic poisoning also affects bone marrow and cellular elements of blood. Arsenicals are known to be carcinogenic to lungs. They may also lead to skin cancer through the initial skin lesions.

In the present study the maximum level of Arsenic (1.4 ± 0.3 mg/Kg) is detected in cabbage sample of Akaki while the lowest level (0.04 mg/Kg) is found in lettuce and dark green leaf of Abyssinia kale samples collected from Debre Berhan. Investigators [25] studied samples of Burkina Faso to assess presence of heavy metals in irrigation water; vegetables in selected farms and reported the level of As in Cabbage (0.120±0.001), lettuce (0<0.04. 'Metals in leafy vegetables grown in Addis Ababa and their toxicological implications' reported [29] that the content of Arsenic was found to be (0.13) in Cabbage, Lettuce (1.04) and Swiss chard (1.21) those collected from Kera.

Table 5: Heavy metals concentration (mean ± Standard deviation, mg/Kg) in the soil of Akaki, Debre Berhan University Agriculture research centre and Kera farm

Elements	Akaki	Debre Berhan	Kera	Safe limit (Source: Awashthi (2000)) [18]
Zn	175.3 ± 6.7	162.4 ± 5.8	386.2 ± 12.5	300-600
Cu	49.5 ± 4.5	61.7 ± 1.1	87.6 ± 2.7	135-270
Ni	51.2 ± 8.9	44.6 ± 2.2	48.2 ± 2.6	75-150
Fe	91347.9±1279.1	86378.2±1531.1	94734.8 ±2684.2	-
Mn	2546.7	2555.3 ± 156.7	3988.0 ± 101.6	-
Cr	195.2 ± 12.0	170.7 ± 8.3	204.6 ± 20.3	-
As	7.0 ± 0.01	6.98 ± 0.01	7.4 ± 0.03	-
Cd	2.3	1.5 ± 1.1	2.32	3-6
Pb	19.1 ± 7.3	18.1 ± 4.6	208.9 ± 27.5	250-500

Heavy metal concentration in soil:

Chemical composition of soil plays important role in composition of plant materials. Overall toxic metals availability in soil contributes to contents of metals in vegetables. Anthropogenic activity such as application of fertilizers, manures also affects soil metal content.

The concentration of heavy metals (mg kg^{-1} dry soil) in agricultural soils of the study areas (Table 5) ranged from 162.4 ± 5.8 to 386.2 ± 12.5 for Zn, 49.5 ± 4.5 to 87.6 ± 2.7 for Cu, 44.6 ± 2.2 to 51.2 ± 8.9 for Ni, 86378.2±1531.1 to 94734.8 ±2684.2 for Fe, 2546.7 to 3988.0 ± 101.6 for Mn, 170.7 ± 8.3 to 204.6 ± 20.3, 6.98 ± 0.01 to 7.4 ± 0.03 for As, 1.5 ± 1.1to 2.32 for Cd and 18.1 ± 4.6 to 208.9 ± 27.5 for Pb. Besides Fe, the mean highest concentrations recorded in soil are Mn followed by Zn, Cr, Pb, Cu, Ni, and As. The minimum concentration is observed for Cd, which is lower than the safe limit [18]. Highest deposition of Fe in soil might be due to its long-term use in the production of machine tools, paints, pigments, and alloying in various industries of the study areas that may contaminate soil changing the soil structure and thus make it less fertile for cultivation. The extent of metals observed in agricultural soil of the industrial areas in the present investigation is found to be higher than the samples of Debre Berhan.

There are various known mineral interrelationship in which an additional dietary quantity of one mineral element will influence absorption or utilization of another mineral element. As tabulated in table 6, in the leafy vegetables those collected from Akaki farm, the concentration of Zinc is in strong positive and significant correlation with Cu(r=0.880) ,Ni(r=0.839), Mn(r=0.960) and strong negative correlation with Cr(r=0.800) at level of significance 0.05.

Table 6: Linear correlation of elements in vegetables from Akaki farm

Properties	Zn	Cu	Ni	Co	Fe	Mn	Cr	As	Pb
Zn	1	0.880*	0.839*	-0.656	0.273	0.960**	-0.800*	0.124	0.134
Cu		1	-0.482	-0.220	0.215	-0.713	-0.421	-0.360	-0.351
Ni			1	0.960**	0.751	0.957**	0.997**	-0.643	-0.650
Co				1	0.905*	0.840	0.977	-0.830*	-0.835
Fe					1	0.530	0.794	-0.988**	-0.989**
Mn						1	0.936**	-0.396	-0.405
Cr							1	-0.693	-0.693
As								1	0.999**
Pb									1

***, ** Level of significance p<0.05, p<0.01**

Table 7 shows that the linear correlation of heavy metals in vegetables collected from Kera farm, as one can see from the table, Zn has a positive and significant correlation with copper, nickel, iron and manganese.

The highest positive and significant correlation is determined between content of Zn-Ni (r=0.994) and Zn-Mn (r=0.990) at level of significance 0.01 and Zn-Fe has significant and positive correlation with the significant level of 0.01. They do not block the action of one on the other. Copper has a positive and significant correlation with Nickel(r=0.648), Iron(r=0.778) and Manganese (r=0.619). Ni was found to have significant and positive correlation with Fe(r=0.777) with the significant level of 0.05; strong and positive correlation with Mn (0.998) at the significant level of 0.01. Negative and significant correlation is observed between the content of Co- As(r= 0.691) and Co-Pb(r= 0.692) at the significant level of 0.05.

Table 7: Linear correlation of elements in vegetables of Kera farm

Properties	Zn	Cu	Ni	Co	Fe	Mn	Cr	As	Pb
Zn	1	0.679*	0.994**	-0.276	0.828*	0.990**	-0.414	-0.221	-0.226
Cu		1	0.648	0.494	0.778*	0.619	0.314	0.476	0.471
Ni			1	-0.342	0.777*	0.998**	-0.474	-0.205	-0.210
Co				1	-0.089	-0.342	0.019	-0.691*	-0.692*
Fe					1	0.771	0.090	-0.110	-0.111
Mn						1	-0.496	-0.230	-0.235
Cr							1	0.624	0.628
As								1	0.999**
Pb									1

***, ** Level of significance p<0.05, p<0.01**

Table 8: Linear correlation of elements found in vegetables belong to Debre Berhan University Agriculture research centre

Properties	Zn	Cu	Ni	Co	Fe	Mn	Cr	As	Pb
Zn	1	0.835**	0.625*	0.398	0.496	0.519	0.091	0.538	0.340
Cu		1	0.139	0.270	0.755*	0.406	0.243	0.184	-0.079
Ni			1	0.142	-0.301	0.271	-0.045	0.803**	0.784
Co				1	0.395	0.156	0.170	0.359	0.288
Fe					1	0.147	-0.044	-0.083	-0.319
Mn						1	-0.081	-0.131	-0.237
Cr							1	-0.051	0.257
As								1	0.805**
Pb									1

***, ** Level of significance p<0.05, p<0.01**

As shown in table 8, for the vegetable those collected from Debre Berhan University Agriculture research centre, the highest positive and significant correlation has been found between the content of Zn-Cu(r=0.835) and moderate correlation found between the content of Zn and Ni (r=0.625) at the significant level of 0.05.

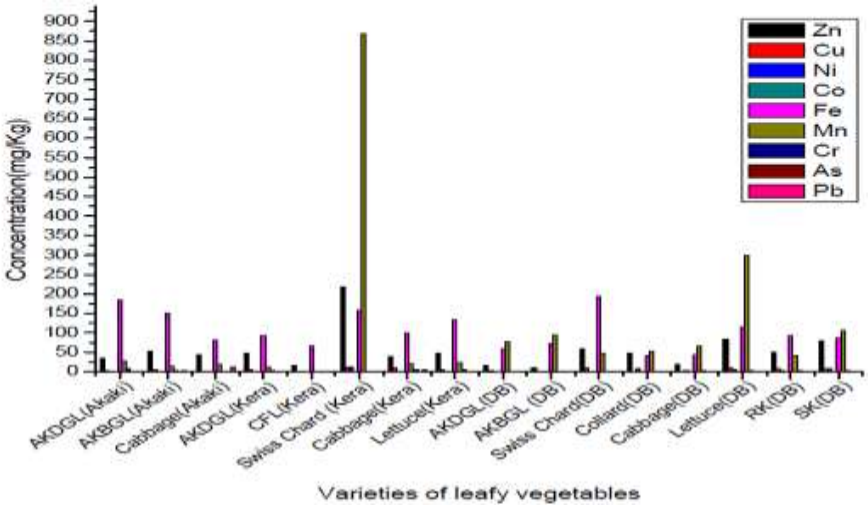

Fig 1: Heavy metals concentration in all leafy vegetables collected from Akaki farm, Kera farm and Debre Berhan University Agriculture research center

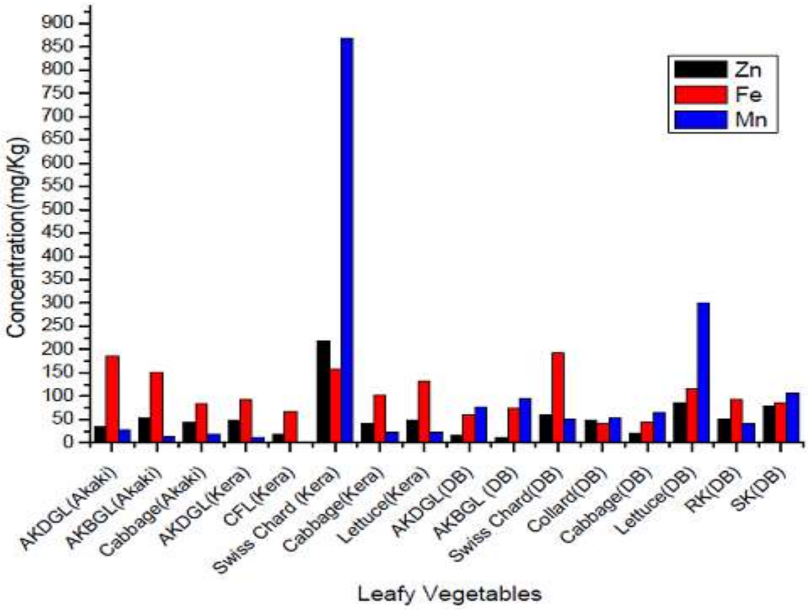

Fig 2: Level of Zn, Fe and Mn in leafy vegetables collected from Akaki farm, Kera farm and Debre Berhan University Agriculture research center

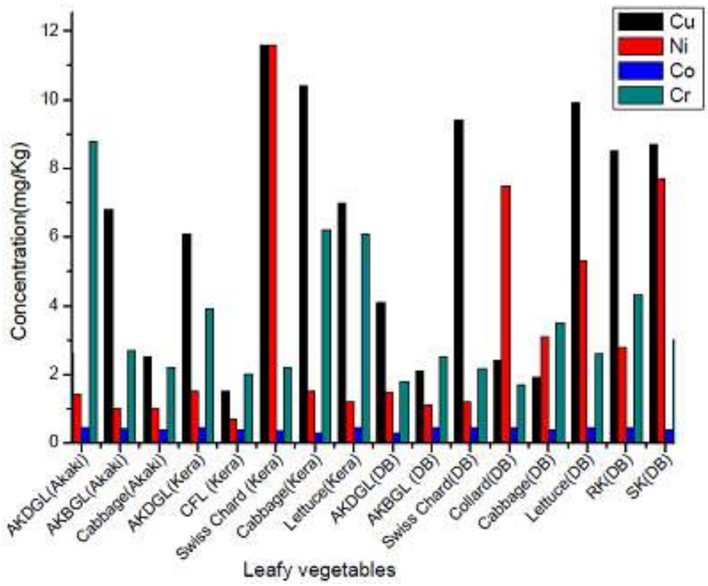

Fig 3: Level of Cu, Ni, Co and Cr in leafy vegetables collected from Akaki farm, Kera farm and Debre Berhan University Agriculture research center

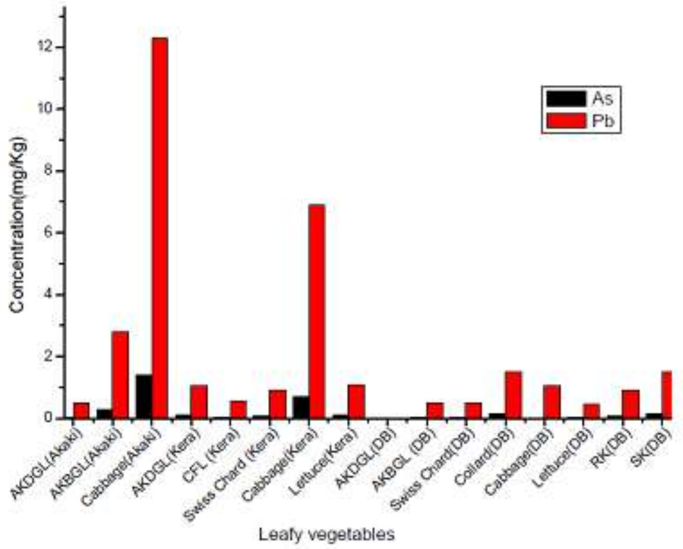

Fig 4: Level of toxic metals, As and Pb in leafy vegetables collected from Akaki farm, Kera farm and Debre Berhan University Agriculture research center

IV. Conclusion

a) In the present study nine heavy metals such as Zn, Cu, Ni, Co, Fe, Mn, Cr, As and Pb are analyzed in leafy vegetables those collected from industrially contaminated irrigated farms i.e. (Akaki farm and Kera farm) and Debre Berhan university research center using Energy Dispersive X-ray Fluorescence spectrometry. The order of metal contents is found to be Mn > Zn >Fe > Pb> Cu> Ni>Cr>As> Co. Heavy metal depositions of agricultural soils are associated with a wide range of sources such as small scale industries (including metal products, metal smelting and cable coating industries), vehicular emissions, re-suspended road dust and diesel generator sets. These can all be important contributors to the contamination found in vegetables. Akaki and Kera farms are the two main irrigated farms in industrial city Addis Ababa; these farms are irrigated with the waste water coming from Rivers Akaki and Kera respectively.

b) The result of the present investigation shows that irrigation of agricultural land with waste water is resulting to the accumulation of heavy metals in vegetables. Variation in the heavy metals content in the investigated leafy vegetables shows the difference in uptake capabilities and in the ability of crops to take up heavy metals through their roots and transport them to the edible portion of the plant. This depends not only on the type of heavy metal but also the species and cultivations of vegetable being grown; the prevailing soil and other growing conditions. Higher accumulations of heavy metals are noticed in Swiss chard belong to Akaki and Kera farms, which is irrigated with industrial effluent and urban pollution associated with sewage sludge, municipal waste water.

c) From the present study it can be concluded that Zn, Mn, Cr and Pb and As concentrations in some leafy vegetables obtained from the selected location are above the permissible limits set by FAO/WHO for human consumption. However, the levels of Cu, Fe, Co and Ni in all the leafy vegetables collected are found to be below the maximum permissible limit. Long term consumption of heavy metals contaminated leafy vegetables may possibly cause numerous health hazards in human. Therefore, regular monitoring of heavy metals in leafy vegetable is crucial to avoid excessive buildup of these metals in the human food chain.

d) Concentration of metals in the studied vegetables may not sufficient for determining health implications because this depends on the dietary pattern of the consumers also. The average amount of vegetables consumed per day by a person in Addis Ababa is 5g in contrast to the daily average of international consumption (50g) for leafy vegetables. It is because of this, that the intake of metals from the studied vegetables constitutes much less than the theoretical maximum daily intake (TMDI) or the provisional tolerable weekly intake (PWTI), which are used to express the exposure of consumers and associated health risk.

In the present study two new leafy vegetables are introduced belonging to Debre Berhan university research center such as Russian kale and Siberian kale, which are not familiar in the diet of Ethiopian. This study revealed that both vegetables have sufficient concentration of nutrients and low accumulation of toxic heavy metals.

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