Determination of Selected Heavy Metals (Zn, Ni, Cd, Cr, and Pb) in Leafy Vegetables (Ethiopian Cabbage) Cultivated around Dilla Town

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Abstract

In the Dilla area, vegetables like cabbage can be grown in a variety of methods and are eaten in large quantities. In recent years, it has become more and more popular for people to eat more green leafy vegetables (GLV). Of the several GLVs that are acceptable for eating by humans, some are only found in a certain area, while others are found all over the world. The latter category, which includes the Brassica family, includes cabbage. The aim of this study was to use the technique known as flame atomic absorption spectrometry to investigate the levels of specific harmful heavy metals (Cr, Zn, Ni, Cd, and Pb) that are detected in the vegetables. To extract the metals from the samples, the most effective wet digestion technique was used. Heavy metal buildup in the body can result in a number of health risks. Therefore, accurate identification of these pollutants is necessary to ensure safety. Zn and Ni were discovered for the kinds of Kale examined, according to the analytical results from this study. Zn (47 to 114 mg/kg), Ni (28.6 to 39.7 mg/kg), Cr, Pb, and Cd are below detection limit, and other metal concentrations (mg/kg) were discovered in the edible sections of cabbage. However, the highest concentration of heavy metals was found in vegetables grown in Andida. From the study human nutrition in the subject area is the foods that are analyzed for metal content. Accordingly, both low-risk (adults and adolescents) and high-risk (children and pregnant women) must reduce their cabbage intake in light of the findings. The metals may bio accumulate with repeated ingestion, raising the danger to human health.

Key word: Brassica family, Bioaccumulation, Cabbage, Wet digestion, Heavy metals

1. Introduction

Originating in the eastern Mediterranean and Anatolia, kale was farmed for food starting at least in 2000 BC. In the fourth century BC, flat-leaved and curly-leaved cabbage cultivars were already known in Greece. These varieties are thought to be the progenitors of contemporary kale; the Romans called them Sabellian kale [1]. This plant is referred to as Gomen domestically in Ethiopia. Omega 3 and 6 fatty acids are among the important fatty acids that kale is recognized as a great source of. These fatty acids are necessary for healthy development, growth, and survival [2]. Herbaceous plants that may be consumed as the primary meal or as a supporting one are collectively referred to as vegetables [3]. Vegetables are important parts of a person's diet since they provide proteins, carbs, vitamins, minerals, and trace elements [4]. Vegetables are an essential component of human nutrition whether they are cooked or raw since they are low in fat and carbohydrates and high in vitamins, minerals, and fiber [5]. Governments generally encourage the eating of vegetables, with a common recommendation of five or more servings per day. Eating veggies is a quick and inexpensive way to receive enough vitamins, minerals, and fiber [3]. When compared to starchy foods, leafy greens are not significant providers of carbohydrates. According to scientific research, eating plenty of vegetables helps prevent cancers of the esophagus, stomach, pancreas, bladder, and cervical regions. A diet rich in vegetables can also prevent 20% of all

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cancers. Consuming veggies on a daily basis might reduce the risk of numerous illnesses and help the body turn fats and carbs into energy. According to [6], The use of wastewater for irrigation has led to a rise in the amount of heavy metals contaminating the edible portions of vegetables, which is dangerous for human health. According to [7], perishable vegetables are frequently grown in urban locations, which are more likely to be contaminated with heavy metals due to a range of industrial and urban activities. Heavy metals are absorbed by plants through contaminated soil [8]. Toxic levels for a few heavy metals are frequently only above the background quantities that are normally present in the environment. In order to prevent excessive exposure, it is critical that we have to understand heavy metals [3].

2. Materials and Methods

2.1. Equipments and Apparatus

In this study, the samples were oven dried for 24 h at 105 °C. Dried vegetable samples were ground and homogenized according to type using a ceramic mortar and pestle. Ground sample powder was sieved using sieves, and the dried and sieved samples were packed in transparent plastic bottles. The materials were dried and weighed using a dry heat oven (Griffin and George Ltd, Britain) and a digital analytical balance (22 ADAM, PW254, 250g). Samples of leaves were broken down and digested using the Kjeldhal equipment.

2.2. Instrument

Atomic absorption spectrophotometry (model ZEEnit 700P, Germany) fitted with a deuterium background and a hollow cathode lamp for each metal was used to measure the chosen metals from the kale samples.

2.3. Reagents and Chemicals

Every chemical employed in this investigation was of the highest analytical purity. In order to digest the materials, for example, 69% HNO_3 and 70% $HClO_4$ were utilized. The fabrication of standard samples and experimental spiking tests both employed a 1000 mg/L stock standard solution of (Zn, Ni and Cd, Cr, Pb) metals. The equipment used for analysis was rinsed, a sample was diluted, and double-distilled water was utilized.

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2.4. Description of the Study Areas

The study locations were Guangua, Andida, Oddo Miqee and Darra which are found in the four corner part of Dilla City. They were chosen purposely, because the areas are known for producing large amount of vegetables.

2.5. Study Design and Sample Collections

From March 2023 to the month of October 2023, a laboratory-based survey was carried out to determine the concentration of heavy metals on a selection of leafy vegetables on the Ethiopian kale (Brassica Carinata) that is grown in the vicinity of Dilla. For the purpose of determining the levels of heavy metals, about 1 kilogram of the edible parts (leaves) of these plants were processed. The quantities of heavy metals such as zinc (Zn), nickel (Ni), cadmium (Cd), chromium (Cr), and lead (Pb) were measured in the vegetable samples using flame Atomic Absorption Spectrophotometry (FAAS). All samples were collected randomly from each sites in March 2023 and transported to Dilla University, Food Engineering Department laboratory for heavy metal analysis.

2.6. Preparation of Sample Vegetables

Every sample was gathered, sealed in plastic bags, and sent to the lab for processing. To get rid of adsorbed dust and other particulates, the obtained samples were first cleaned with tap water and again with distilled water. To help with drying, the leaves were broken off from the stem with bare hands and sliced into tiny pieces using scissors. To remove moisture and maintain consistent mass, the samples were first dried by air for five to six days and then dried again for 24 hours at 80 to 120 degrees Celsius in a hot air oven. To allow the sample to drop to room temperature, the oven was shut off at the conclusion of the drying process and left overnight. Using a mortar and pestle, the dry samples were crushed into a powder and then sieved through a 300 micron mesh sieve. The material that had been sieved was maintained in plastic bottles until the digesting process was complete.

2.7. Wet digestion

As part of wet digestion procedures for elemental analysis, sample matrices are broken down chemically in solution. This is usually done with a mix of acids to dissolve the samples more soluble. In order to reduce sample contamination from airborne contaminants, the surrounding environment, and vessel walls, the different acid and flux treatments are performed at high

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temperatures in specially made containers. Adsorption on to the vessel walls, volatilization, and co-extraction can all result in sample losses, although these can be minimized by changing the procedure. It may be possible to lessen contamination and sample loss by using closed systems, in which the digesting reaction is totally isolated from the environment [9].

2.8. Optimization of digestion procedures

Prior to doing any experiments, the best working method should be identified. Using the Kjeldhal apparatus and the wet acid digestion method, the optimization approach in this work aimed to find the smallest reagent volume, lowest temperature, and shortest time required to fully degrade the leaf samples. It is expected that organic components would break down into various gaseous forms, such as CO_2, NO_2, and H_2O. The measurement of the total breakdown involved the visualization of colorless and transparent liquids. Each leaf sample weighed 0.25 g of powdered material, which was then put to a 250 mL round-bottom flask. Various quantities of 69% nitric acid and 70% perchloric acid were added in the prescribed amounts, and the samples were then digested at various temperatures and times.

2.9. Digestion of Vegetable Samples

Vegetable samples that had been homogenized and powdered weighed 0.25 g were put into a 250 ml round-bottom flask together with 4 ml of HNO_3-$HClO_4$ (3:1, v/v). During 3:30 hours, the mixture was digested using a Kjeldhal device that was heated to 270°C. Following the end of the digestion process, the sample was allowed to cool at room temperature. Clear solutions were then filtered using Whatmann filter paper to remove any remaining material and put into a 50 mL volumetric flask. Following a double-distilled water washing, each digestive flask was fed to the volumetric flask until it reached the desired level of fill. To increase the accuracy of the outcome, each vegetable sample was broken down and examined three times. Three milliliters of HNO_3 and one milliliter of $HClO_4$ were combined, and the mixture was treated in the same way as the samples to create the blank solution. Following that, flame atomic absorption spectrophotometry was used to examine the heavy metals.

2.10. Determination of metals in kale leaf samples

Using the atomic absorption spectroscopy standard, calibration metal standard solutions were Created for each metal using an intermediate standard solution containing 10 mg/L.The metal Concentrations (Zn, Ni, Cd, Cr, and Pb) in the 1000 mg/L stock solutions were determined using an FAAS equipped with a standard air acetylene flame and a deuterium background correction.

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As appropriate, a hollow cathode lamp served as the main source line. In tandem with the kale samples, the same methodology was used to determine the metal content of the digested blank solution while maintaining the same settings. The metal concentrations were all expressed in milligrams per kilogram of dry weight.

2.10.1. Limit of detection and quantification

The lowest amount recorded in a sample that may be detected with probability is known as the Limit of detection (LOD). It can be calculated in this way; LOD is equal to three times the standard deviation of the blank solutions or 3x SD blank. Lower LOD values suggest that the approach is capable of detecting traces of metals of interest in the sample. The lowest quantity of analyte that, under specified experimental circumstances, may be quantified with reasonable precision and accuracy is known as the limit of quantification, or LOQ. It is computed as follows: LOQ = 10 x SD blank, which is ten times the blank solution's standard deviation.

2.10.2. Method validation

The process of verifying that the analytical technique used for a particular test is appropriate for its intended use is known as method validation. Analytical data can be evaluated for quality, consistency, and dependability using the results of method validation. Using spiking experiments, the optimized procedure's validity was evaluated. This was accomplished by adding 140 µL of Zn and 355 µL of Ni to 0.25 g of kale samples. A standard solution of 1000 mg/L was utilized, and an intermediate standard solution containing 10 mg/L was created. The validated procedure was used to digest and analyze both the spiked and un-spiked samples under identical conditions.

2.10.3. Precision

Precision is the level of consistency between distinct outcomes. Separate identical samples should undergo the entire process several times. The findings from the mean values and standard deviation should be used to measure it. Precision is expressed as the relative standard deviation using the formula mentioned below.

$$RSD = \frac{Standard\ Deviation}{Mean}\ x100 \qquad (1)$$

2.10.4. Accuracy

Accuracy is the degree to which test results agree with the true value, or how closely the

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Procedure's results match the true value. It is measured by the recovery test of Zn and Ni spiked to the sample. Accuracy is calculated as percent recovery using the formula mentioned below.

$$R = [(Amount\ after\ spike - amount\ before\ spike)/Amount\ added]x100\% \qquad (2)$$

3. Results and Discussion

3.1. Comparision of heavy metals in kale samples collected from four different areas

The sensitivity of the heavy metal detection device, the possibility of sample contamination, the possibility of interference from other chemicals when determining the levels of specific heavy metals, the use of precise digestion methodology, and other influencing factors can all result in different values from the same sample that is tested in laboratories. Therefore, every effort was made to ensure that the data presented in this paper was reliable. A total of five metals were determined by using flame atomic absorption spectrophotometry, the results are expressed in terms of mg/L and were converted into mg/kg.

Table 1: Mean Concentration ± standard deviations in (mg/kg) of metals in kale samples

	Andida		Darra		Oddo Miqee		Guangua	
Metal	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ±SD	RSD (%)
Zn	114 ± 10.7	9.4	47 ± 4.0	8.5	49.6 ± 2.6	5.3	69.5 ± 3.7	5.3
Ni	35.4 ± 1.4	3.8	36.4 ± 2.3	6.4	39.7 ± 0.9	2.3	28.6 ± 1.1	3.7
Cd	0.05	-	0.06	-	0.03	-	0.01	-
Cr	0.02	-	0.02	-	0.05	-	0.04	-
Pb	0.07	-	0.05	-	0.09	-	0.07	-

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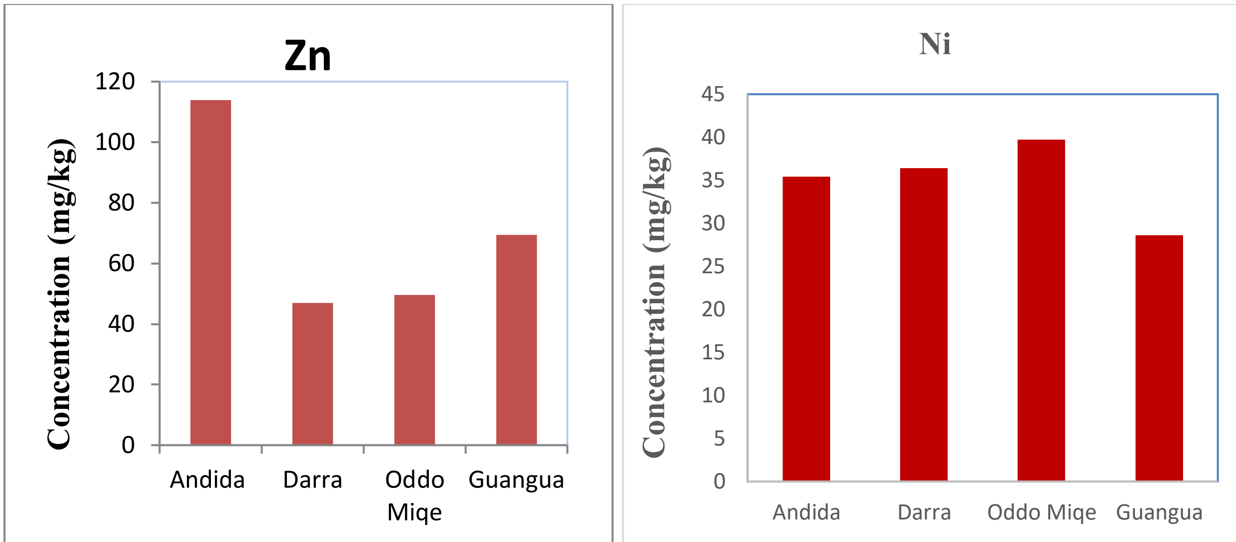

Figure 1: Bar graphs of mean concentration of metals (mg/kg) in four different sample sites

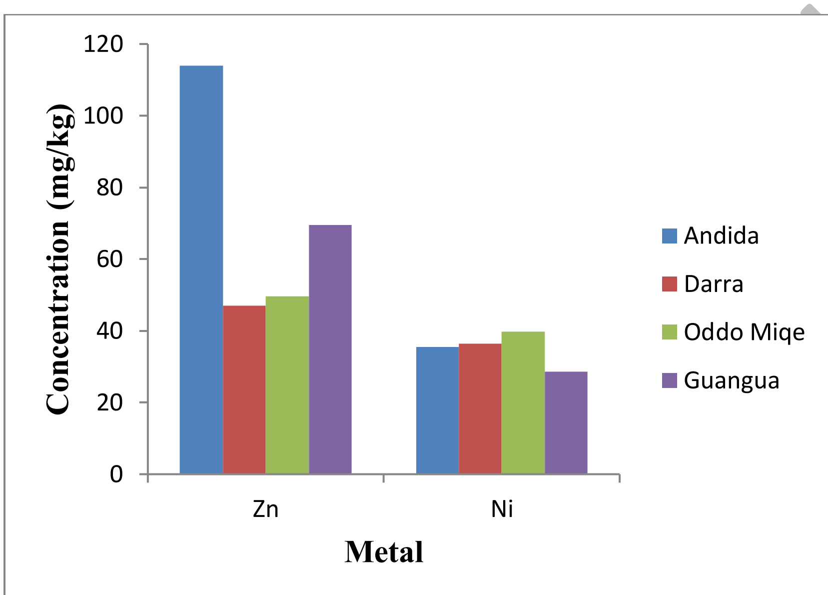

Figure 2: Comparison of Zinc and Nickel content in kale samples from four different areas

Zinc

All living organisms accumulate considerable amount of Zn in their system without any damaging effect. 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. Iron deficiency causes comparable symptoms to those of zinc deficiency.Zinc deficiency results in immunological problems, growth retardation, and appetite l oss.According to recent studies, men require more zinc than women do.Therefore; it makes sense that the RDA for zinc is sex-specific. Exposure to zinc beyond physiological requirements can represent a hazard for human health.

Zinc pollutes the environment and can be detected in a variety of foods, soil, and pharmaceutical samples. Significant concentrations of zinc may reduce the soil microbial activity and also is a common contaminant in agricultural and food wastes [10]. Both the requirement and toxicity of trace metals make them significant.

By binding to substrates and favoring different reactions, such as the mediation of redox reaction , through reversible changes in the oxidation state of the metal ions, zinc, an essential functional and structural element in biological systems, frequently catalyzes reactions. World Health Organization has set permissible amounts for a sufficient dietary consumption for zinc, which is a micronutrient. According to WHO, adults intake should be comprised between 5.0 and 22.0 mg. At high concentrations, Zn causes nephritis, anuria and extensive lesions in the kidneys [11]. In the present investigation, the values of Zn range from 47 to 114 mg/kg in various kale samples. The maximum concentration (114 mg/kg) of zinc was recorded in samples collected from Andida, while minimum concentration (47 mg/kg) was obtained from Darra.

Nickel

The nickel content was highest in Oddo Miqee (39.7 mg/kg), while it was found lowest in Guangua (28.6 mg/kg) which was found to be lower than the permissible value recommended by FAO/WHO [12]. When compared the present results with the data reported by [13] and [10] is found to be higher, this difference might be due to the level of contamination of the studied areas.

3.2. Comparison of metal concentration in kale samples with other literature values

Comparison of metal concentration in kale samples with other literature values is summarized in table 2.

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Table 2: Comparison of metal concentration in kale samples with other literature values

Sample	Mean Concentration (mg/kg)					Method	Country	Reference
	Zn	Ni	Cd	Cr	Pb			
Ethiopian kale	52.8	1.0	NR	2.7	2.8	EDXRF	India	[10]
Ethiopian kale	99.8	NR	0.2	0.3	0.7	GFAAS	Ethiopia	[14]
Ethiopian kale	88.3	11.1	6.3	13.3	7.2	FAAS	Ethiopia	[15]
Ethiopian kale	63.7	4.6	0.1	0.9	0.5	FAAS	Ethiopia	[13]
Kale from Andida	114	35.4	0.05	0.02	0.07	FAAS	Ethiopia	This study
Kale from Darra	47	36.4	0.06	0.02	0.05	FAAS	Ethiopia	This study
Kale from Oddo Miqee	49.6	39.7	0.03	0.05	0.09	FAAS	Ethiopia	This study
Kale from Guangua	69.5	28.6	0.01	0.04	0.07	FAAS	Ethiopia	This study

Table 2 indicates mean concentrations of this study compared with research done by [10], [14], [13] *and* [15]. From this data the variation between mean concentration of metals in this study and literature values is highlighted.

The mean concentration of Zn in the samples from the different sites in this study 114, 47, 49.6, 69.5 mg/kg for kale samples is somewhat in a good agreement with that reported by the values reported [13] and [10]. The study done by [13] indicates 63.7 and 35.08 mg/kg of kale from Kera and Akaki farms and a study done by [10] indicates 52.8 and 10.9 mg/kg of kale from Akaki and Debre Birhan.

For Nickel table 2 indicates concentration of 35.4, 36.4, 39.7, 28.6 mg/kg for kale samples and it is in a good agreement with that reported by the researchers [15] and [13].

In Table 2 Cd, Cr and Pb are found below detection limit. But according to the study done by [15], [13], [10], Cd, Cr, and Pb were detected and reported.

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3.3. Comparisons for metal Concentration of kale with other leafy vegetables.

Table 3 summarizes the levels of metal concentration in the vegetables (Ethiopian Kale) and compares them to other edible plants reported worldwide. The comparison shows that the current study's findings are consistent with the majority of the published values.

Table 3: Comparison of metal concentrations in kale with other leafy vegetables reported in the literature from Ethiopia.

Sample	Metal Concentration (mg/kg)					Reference
	Zn	Ni	Cd	Cr	Pb	
Cabbage	27.8	7.4	1.6	10	3.8	[15]
	43.2	1.0	NR	2.2	12.3	[10]
Swiss chard	106.1	NR	0.37	0.45	1.06	[14]
	48.6	51.8	1.4	11.6	9.5	[15]
Lettuce	111.2	NR	0.39	0.45	1.43	[14]
	64.5	48.14	0.23	6.6	2.38	[15]
Kale from Andida	114	35.4	0.05	0.02	0.07	This study
Kale from Darra	47	36.4	0.06	0.02	0.05	This study
Kale from Oddo Miqee	49.6	39.7	0.03	0.05	0.09	This study
Kale from Guangua	69.5	28.6	0.01	0.04	0.07	This study

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3.4. Comparison of results of the present study with WHO/FAO joint CODEX maximum permissible levels

Table 4: Comparison of results of the present study with WHO/FAO Values

Parameters		Metals (mg/kg)				
		Zn	Ni	Cd	Cr	Pb
WHO/FAO Values		99.4	66.9	0.2	2.3	0.3
Kale samples	Andida	114	35.4	0.05	0.02	0.07
	Darra	47	36.4	0.06	0.02	0.05
	Oddo Miqee	49.6	39.7	0.03	0.05	0.09
	Guangua	69.5	28.6	0.01	0.04	0.07

Table 4 presents the comparisons between the results in the present study with WHO/FAO reported in 2012. The level of Zn in kale from Andida farm was found to be higher than the permissible value recommended by FAO/WHO and the level of nickel is lower than the permissible value recommended by FAO/WHO [12]. The daily average consumption per person per day of leafy vegetables is 50g.

3.5. Statistical analysis

In this study, a sample of kale leaves was collected randomly from four different places in Ethiopia. Several random errors may be introduced in each replicate measurement during this process. As a result, depending on the type and nature of the results, the statistical method is used to determine whether or not these random errors contribute to the difference in analysis results. If there is a difference in the statistical analysis, it indicates whether the differences are significant or not at a specified confidence level.

To determine if there are significant differences between the means of each plant sample, the statistical analysis is conducted using one-way analysis of variance (ANOVA) with the kale samples as an independent variable and the metal content as an independent variable. An ANOVA is used to determine whether or not the means of three or more independent groups are equal. If this p-value is less than ($p<0.05$), we reject the null hypothesis of the ANOVA and conclude that there is a statistically significant difference between the means of each groups.

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Otherwise, if the p-value is not less than ($p>0.05$), then we fail to reject the null hypothesis and conclude that we do not have sufficient evidence to say that there is a statistically significant difference between the means of the each groups. Microsoft Excel was used to compare the statistical parameters, and the result of the analysis is indicated in Table 5.

Table 5: ANOVA between and within kale samples at 95% confidence level

Metals	F_{cal}	F_{crit}	P-value	Remark
Zn	3.8365	5.0503	0.2063	No significance difference between sample means
Ni	3.1798	5.0503	0.1171	No significance difference between sample means

F_{cal} = Fcalculated, F_{crit} = Fcritical

The values of the analysis of variance (ANOVA) shown on Table 5 indicated that there was no significance difference ($p > 0.05$) in the mean values of Zn and Ni between Andida, Darra, Oddo Miqee, and Guangua kale samples. The maximum mean concentration of Zn was found in Andida (114mg/kg) and the minimum mean concentration of Zn was found in Darra (47mg/kg). The maximum mean concentration of Ni was found in Oddo Miqee (39.7mg/kg) and the minimum mean concentration of Ni was found in Guangua (28.6mg/kg). The level of Zn and Ni metal are not significantly different in vegetables sampling sites (at 0.05 levels). The mean concentration of Cd, Cr and Pb are below detection limit. On the study the four data set gives different value and the p-value is almost >0.05. So there is no significance difference in each group.

3.6. The correlation coefficient of metals

The highest correlation coefficient ($r = 0.76$) was found in the sample correlation coefficient matrices for the samples Ni and Zinc.

4. Conclusion

In this study the levels of selected heavy metals in kale leaves collected from four different areas around Dilla city were analyzed for their contents of Zn, Ni, Cd, Cr and Pb using flame atomic absorption spectrometry. By adding known concentration standards to the solid sample, the recovery experiment assessed the best wet digestion technique for kale samples, yielding a good percentage recovery (92.3% for Zn and 110% for Ni). The edible portions of the kale contained the following metal values (mg/kg): Ni (28.6 mg/kg to 39.7 mg/kg) and Zn (47 to 114 mg/kg). Cr, Pb, and Cd are below the limit of detection. Vegetables grown in Andida had the greatest concentration of heavy metals. The study indicated that while Ni is not above the maximum

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allowable levels, the concentrations of Zn in the veggies were found to be above the safe limits established by WHO/FAO for eating and to pose a major health concern to humans. The mean Zn and Ni values for the Guangua, Andida, Darra, and Oddo Miqee kale samples did not differ significantly ($p > 0.05$), according to statistical analysis performed using one way analysis of variance (ANOVA). The samples in Ni and Zinc exhibited the highest correlation coefficient ($r = 0.76$) in the study's sample correlation coefficient matrices. Thus, it is crucial to regularly check vegetables in order to avoid an excessive accumulation of harmful heavy metals in meals. As a result, both the degree of heavy metal contamination and the health risk can be decreased.

Authorship contribution statement

Ayalew Mekuria: Visualization, Investigation, Formal analysis, Writing – original draft, Project administration, Funding acquisition. **Elias Ture:** Methodology, Supervision, Writing – review & editing, Validation, Resources. **Samuel Gemeda:** Data collection, Writing – review & editing, Validation. **Bilise Getachew:** Conceptualization, Data collection, Methodology, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests.

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