COMPARATIVE ANALYSIS OF Pb, Cr, and Cd PRESENT IN *Brassica Oleracea* L. var, acephala FROM MUNYAKA SLUMS AND KESSES MARKETS

MOI UNIVERSITY ISO 9001:2015 Certified Institution

PROJECT REPORT

BY

A Research Project submitted in partial fulfilment of the requirements for the award of Bachelor of Science Degree in Biochemistry

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ABBREVIATIONS

AAS – Atomic Absorption Spectrum.
Pb – Lead Metal
Fe – Iron Metal
Cd – Cadmium Metal
Cr – Chromium Metal
RBD – Randomized Block Design
LSD – Least Significant Difference

ABSTRACT

Kales are listed as one of the major groceries people utilize within the environment. The crop is rich in vitamins and other nutrients essential for the growth and development of the body. The plant's leaves are the major part that people consume within the environment. Kales originates from the Brassica oleracea L.var, acephala. This project is majorly focused on the phytoaccumulation of heavy metals and their ascertained levels in different samples of kales obtained from the Munyaka and Kesses regions. The project aims to determine the level of pollution in the plants cultivated in different environmental contaminants rates and create awareness on the impacts of pollution on food and human beings. Sources of contamination include natural sources, anthropogenic sources, mining, and mineral extraction. Most people practice urban agriculture, using untreated sewage water to irrigate their crops. Random sampling was used such that two samples from spread-apart places will be collected from Munyaka, Eldoret and two spread apart sites in Kesses. They have been subjected to analytical techniques carried out at the Moi University Laboratories and prepared through wet digestion. The products of the digestion process of all the samples provided were taken to the AAS section to analyze heavy metals (Pb, Cr, and Cd) and a readout containing the heavy metals concentration in the instances, which will be displayed in the computer and a printout obtained for data analysis. The data was analyzed using Microsoft excel to the mean and standard error, which was then subjected to the statistical test of significance using paired t-test at 95% confidence interval. The project results are meant to educate the general public on the importance of eating safe foods and help them indulge in agricultural practices that produce safe foods. It is also important for the government to enact laws that facilitate the production of safe foods.

CHAPTER 1
1.0. INTRODUCTION

1.1 Research Background

Kales (Brassica Oleracea) is a leaf vegetable consumed as a source of vitamins A, K, B6, and C, Calcium, potassium, copper, and manganese. The principal nutritional value of the forementioned crop is vitamins. It is a leaf cabbage mostly grown in East and Southern Africa. The harvest has been widely cultivated in Kenya, supporting a wide range of middle and lower classes. It is among the main food items for the people who exhibit lower living standards. The crop is grown in a wide range of environments within the country and mostly utilizes irrigation in limited water supply areas. Kales are cultivated in regions close to water supplies like rivers and streams (Samec et al., 2019). The hydroponics method has also been used to develop the crop, but it is not widely used. A larger percentage of the crop has been planted near river banks, and others are constantly irrigated. The proximity of the Brassica oleracea to water sources and diverse irrigation methods has exposed the crop to a wide range of pollution within the environment. Most of the water sources in the urban setting exhibit a wide range of pollution influx exposed to kales.

Water pollution is a major problem in society, and it exhibits a wide range of heavy metals that are dangerous to the health of human beings and domesticated animals (Joseph et al., 2019). Heavy metals absorption in plants, even in low concentrations, results in various health complications that might reduce the lifespan of human beings. The ecological distribution of the food chain exhibits the aspect of energy accumulation in the higher chain. The exposure of kales to heavy metals toxicity within the soil and water results in bioaccumulation of the toxic elements in the human beings who are the primary consumers of the crop. The larger portion of the Kenyan population depends on Kales for consumption due to their availability and affordability. When the plan has absorbed the heavy metals, it poses a huge risk and danger to the health of the larger population that depends on it for consumption.

Most of the non-degradable toxic elements within the soil emerging from a wide range of environments include Arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pb), and Zinc (Zn). These elements are the major pollutants to plants that the EPA has depicted (Lin et al., 2020). These elements are soluble in water and exist as cations that are accumulated in the soil through diverse pathways that may include: atmospheric deposition, sewage, stormwater, and leachate that carry the contaminants which emerge from diverse residential, industrialized, and cultivated areas.

The level of toxicity in terms of heavy metals varies depending on the nature of environmental activities within the designated region. Most of the urban agriculture that farmers have conducted in the metropolitan areas utilizes an irrigation method that uses untreated sewage water (Khalid et al., 2021). The dangers of these activities are about to be exposed by the research undertaken. The adversity knowledge of water sources, pollution/contamination, and kales consisting of heavy metals provide the general public the awareness of the most suitable foods they should buy, especially in regions with higher pollution. The research also provides the government with the ideology of developing policies that foster the growth and cultivation of suitable crops that are healthy for consumption.

1.2 Problem Statement

At such a time when environmental pollution and the quality of food products are a major concern to man, a better understanding of the safety of foods that we consume seems particularly significant. Certain trace elements are essential in plant nutrition (as micronutrients). Still, plants growing in polluted environments like sewage systems can accumulate trace elements and other heavy metals to high proportions, causing a serious risk to human health when consumed especially plant-based foods. Research indicates that heavy metal contaminations are only second to pesticides on the pollution proportion, and the trend may increase in the future (UNEP,1980). Thus, in the public interest, people should know and understand to what extent the foods they consume are polluted, especially those grown in urban areas.

1.3. Justification

Kales are some of the foods people consume in many places since they are nutritious and cheaper but most of them are not aware of where they are produced and have no information about the safety of these foods. Also, as a result of the high demand for food crops kales, farmers have gone a step ahead cultivating these crops in polluted land and selling them to residents. Therefore this research aims at enlightening people on the difference between taking a healthy diet and eating safe foods. Therefore, as presented earlier by (Sumiahadi & Acar, 2018) on the fatal effects of these heavy metals and unhealthy agricultural practices in the urban and suburban regions, this research aims at creating awareness on the dangerous effects of pollution on the human body and the sources of contamination.

1.4. Objectives

1.4.1. Overall Objective

To determine contaminants level of heavy metals in of Heavy metals in Brassica oleracea l. acephala

1.4.2. Specific Objectives

- To determine the amount of Pb, Cr, and Cd in Brassica Oleracea.1 acephala from Munyaka slums and Kesses region.
- To compare the heavy metals concentrations from the two regions; Munyaka and Kesses with required standards by the World Health Organization for healthy consumption.

1.5. Hypothesis

1.5.1 Null Hypothesis

- There is no significant difference in the concentrations of lead (Pb), chromium (Cr), and cadmium (Cd) present in *Brassica oleracea L. var. acephala* (kale) samples collected from Munyaka slums and Kesses markets, and the mean levels of these metals do not exceed the World Health Organization (WHO) permissible limits for heavy metals in vegetables.

1.5.2 Alternative hypothesis

- There is a significant difference in the concentrations of lead (Pb), chromium (Cr), and cadmium (Cd) present in *Brassica oleracea L. var. acephala* (kale) samples collected from Munyaka slums and Kesses markets, and the mean levels of these metals exceed the World Health Organization (WHO) permissible limits for heavy metals in vegetables.

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CHAPTER 2
2.0. LITERATURE REVIEW

2.1. Introduction to Heavy Metals

In order to produce cations, metals give up their electrons to conduct electricity, be malleable, and shine. In the earth's crust, they are found in varying amounts depending on where they are located. The characteristics of the metal in question, as well as different environmental conditions, determine the atmospheric dispersion of that metal. In general, heavy metals are defined as metals with a density more than 5g/cm3 that have a negative impact on the environment and living creatures. Low levels of these metals are crucial to the functioning of many biochemical and physiological processes in living beings. However, they become dangerous when concentrations rise over a certain level.

For ecological, evolutionary, nutritional, and environmental reasons, heavy metal poisoning is becoming a major issue. Wastewater contains a wide range of heavy metals such as arsenic and cadmium in addition to the more prevalent zinc and nickel. All of these metals pose a risk to human health (Rehman et al., 2021). Natural weathering, soil erosion, industrial effluents, mining, urban runoff, sewage discharge, pest or disease control applied to crops are only some of the sources of heavy metals that can be found in the environment. Heavy metals are initially accumulated in plants, and then passed up the food chain to higher trophic levels. They become accessible to the next tropic level, in this instance humans, when they accumulate in plants beyond the permitted limit and can no longer be scavenged by chelating chemicals. The chemical coordination and oxidation-reduction capabilities of these heavy metals have given them an extra advantage that allows them to escape numerous regulatory systems such as homeostasis, compartmentalization, transport and binding to necessary cell components. Displacement of other metals from their native binding sites causes cell dysfunction and eventually toxicity, since these metals wind up attaching to protein sites (such as enzymes) that were not designed for them.

Heavy metals were the primary focus of previous studies on DNA and nuclear protein degradation. There are several sources of these heavy metals in water, and we need to be aware of how they affect our health when eaten (Wang et al., 2018). Even though these heavy metals have biological functions in living cells, sometimes their chemical coordination and oxidation-reduction properties have given them an additional advantage that enables them to escape various control mechanisms such as homeostasis, compartmentalization, transport, and binding to required cell constituents. These metals end up binding to protein sites (for example enzymes) which were originally not meant for them by displacing original metals from their natural binding sites causing malfunctioning of cells and ultimately toxicity (Alengebawy et al., 2021). It therefore important for us to highlight various sources of these heavy metals in water and their ultimate effects on human beings when consumed.

2.2. Sources of Heavy Metal Contamination in Water

2.2.1 Natural sources

In nature excessive levels of trace metals may occur by geographical phenomena like volcanic eruptions, weathering of rocks, leaching into rivers, lakes, and oceans due to actions of water.

2.2.2. *Anthropogenic sources*

Small amounts of heavy metals are released while mining and uncontrolled smelting of large metal ores in open fires. Traces of heavy metals get into the environment through discharge of waste-both domestic, agricultural, and from auto exhausts (Zhou et al., 2020). The human activities through which heavy metals get into the environment include:

- Smelting or processing of ores of metals
- Mining
- Burning of fossil fuel
- Discharging agricultural waste
- Discharging industrial waste
- Discharge from domestic waste
- Discharge from auto exhausts

Using pesticides containing compounds of heavy metals (Siddiqui & Pandey, 2019).
Many industrial processes can generate heavy metal pollution and in a large number of ways. Some industries will become more likely to pollute than others (Boateng et al., 2019).

Figure 1: Industrial Discharge in a river.

Figure 2: Washing vehicles in the river

2.2.3. *Mining activities*

Heavy metals occur in the earth's geological structures, and can therefore enter water resources through natural processes. For example, heavy rains or flowing water can leach heavy metals out of geological formations. Such processes are exacerbated by economic activities such as mining. These processes expose the mined-out area to water and air and can lead to consequences such as acid mine drainage (Li et al., 2019). The low Ph conditions associated with acid mine drainage mobilize heavy metals, including radionuclides where these are present.

Figure 3: Mining Activity.

2.2.4. *Mineral extraction*

Mineral processing operations can also generate significant heavy metal pollution, both from direct extraction processes as well as through leaching from ore and tailing stockpiles.

2.2.5. *Fertilizer petroleum industry*

Cadmium is produced as an inevitable by-product of zinc (or occasionally lead) refining since these metals occur naturally within the raw ore. However, once collected the cadmium is easy to recycle since it has got many uses especially in corrosion resistance (Maurya et al., 2019). Cadmium is also present as an impurity in several products including phosphate fertilizers, detergents, and refined petroleum products.

2.2.6. Biological practices

The degassing of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water is the major source of mercury. World-wide mining of the metal leads to indirect discharges into the atmosphere (Zeng et al., 2020). Mercury is used in dentistry as an amalgam for fillings and by the pharmaceutical industry.

2.3. Mechanism of Heavy Metal Uptake by Plants

Various researchers have explored the many mechanisms used by plants to absorb heavy metals from the soil. The main aim of this uptake is to improve the performance of the plant. It has been found by some researchers that plants could be "accumulators" or "excluders" where accumulators survive regardless of the contaminant concentration inside their tissues. They bio-degrade or bio-transform the contaminants into inert forms inside their tissues. Contaminant uptake into their biomass is limited by the excluders

Even when present at low ppm levels, plants have evolved extremely precise and efficient methods to acquire necessary micronutrients from the environment. Plant roots can solubilize and take up micronutrients from very low quantities in the soil, even from virtually insoluble precipitates, thanks to plant-produced chelating agents and plant-induced pH shifts and redox processes (Sumiahadi & Acar, 2018). Micronutrient translocation and storage mechanisms have emerged in plants as well. These same systems are engaged in the intake, transport, and storage of hazardous elements, which have chemical characteristics that are similar to essential elements. Evaporating water from plant leaves acts as a pump, allowing nutrients and other soil components to be absorbed into plant roots. This mechanism, known as evapotranspiration, is also responsible for transporting contaminants into plant shoots.

Contamination is eliminated while the original soil is left untouched because contamination is translocated from roots to shoots, which are collected. "Hyperaccumulators" are plants that are employed in phytoextraction methods. They are plants with a greater than one shoot-to-root metal concentration ratio. The shoot-to-root ratio of non-accumulating plants is often less than one. Hyperaccumulators should, in theory, thrive in poisonous environments (Emamverdian et al., 2018). Heavy metals such as Cr, Cd, Zn, Co, Mn, Ni, and Pb can be concentrated up to 100 or 1000 times higher in metal accumulating plant species than in non-accumulator (excluder) plants. In most circumstances, bacteria and fungi dwelling in the rhizosphere around plants can help mobilize metal ions, increasing the bioavailable percentage. Their role in removing organic contaminants is even more important than it is in removing inorganic contaminants.

2.4. Selected Metals and Their Toxicity

2.4.1. Lead (Pb)

Lead is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the globe. Lead is a bright silvery metal, slightly bluish in a dry atmosphere. It begins to tarnish on contact with air, thereby forming a complex mixture of compounds, depending on the given conditions. The sources of lead exposure include mainly industrial processes, food and smoking, drinking water, and domestic sources. The sources of lead were gasoline and house paint, which has been extended to lead bullets, plumbing pipes, pewter pitches, storage batteries, toys, and faucets.

Some of these lead produced are taken up by plants, fixation to the soil, and flow into water bodies, hence human exposure to lead in the general population is either due to food or drinking water. Lead is an extremely toxic metal that disturbs various plant physiological processes and unlike other metals, such as zinc, copper, and manganese, it does not play any biological functions (Naranjo et al., 2020). A plant with a high lead concentration fastens the production of reactive oxygen species (ROS), causing lipid membrane damage that ultimately leads to damage of chlorophyll and photosynthetic processes and suppresses the overall growth of the plant.

2.4.2. Chromium (Cr)

It exists in different oxidation states in the environment ranging from Cr^{3+} to Cr^{6+}. The most common forms are the trivalent and the hexavalent forms. Anthropogenically, chromium is released into the environment through sewage and fertilizers. The discharge of industrial wastes and groundwater contamination has drastically increased the concentration of chromium in the soil. Modern agriculture has led to an increase of chromium in the ecosystem resulting in soil pollution affecting the soil-plant system and thus affecting their yield. Its accumulation beyond the permissible limits is harmful to the plant and the whole food chain at large. It is characterized by reduction of root growth, leaf chlorosis, inhibition of seed germination, and depressed biomass-phytotoxicity. Enzymes that are affected by this metal include catalase, cytochrome oxidase, and peroxidase. Chromium (III) requires simple diffusion to enter the cell as the other oxides can easily pass through the cell membrane.

2.4.3. Cadmium (Cd)

It is classified as the seventh most toxic heavy metal as per the Agency for Toxic Substances and Disease Registry (ATSDR) ranking. It is a byproduct of zinc production. It is known to influence enzymatic systems and yet has no biological advantage. This happens by inducing ROS production and nutritional deficiency in plants.

2.5. Theory and Basic Principle of the Atomic Absorption Spectrum

Atomic absorption spectroscopy (AAS) is one of the commonest instrumental methods for analyzing metals and some metalloids. It is a viable analytical technique for determining trace elements, because its highly specific and relatively highly sensitive when compared to other metals. AAS is a Spectro analytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state. Atomic absorption spectroscopy is based on the absorption of light by free metallic ions (Wang et al., 2019). The main advantage of AAS is that as an analytical technique the absorption by atoms takes place within narrow spectral regions and only those involving the ground state are normally absorbed, yielding extremely simple spectra, and there is very little possibility of coincidence of spectral lines and spectral interference.

The most important components of a typical AAS are:

a) The spectral source which emits the spectrum of the element of interest,
b) The atom cell, usually a flame furnace or graphite furnace, in which the atoms of
c) the sample is formed by thermal dissociation,
d) A monochromator for the spectral dispersion of the source radiation and an exit
e) slit for selection of the wavelength of the analyte resonance line,
f) A detector, normally a photomultiplier tube, to permit measurement of the radiation
g) intensity at the resonance line,
h) An amplifier and display units for the recording of the absorption values.

2.5.1. Principle

When an atom is heated (in atomizers), it gets excited which results in energy transitions between orbitals that are close by and the following relaxation produces a light of a unique wavelength. The wavelength uniqueness is specific to an atom of analysis (Frati et al., 2020). The specificity brings about the qualitative analysis and the intensity of the emitted light forms the quantitative analysis of the test sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration.

Figure 4: Atomic Absorption Spectrophotometer.

CHAPTER 3
3.0. RESEARCH METHODOLOGY

3.1. Description of the Study Area

The research project is based on the quantitative analysis of the samples from various rural areas of Kesses, namely: Mabatini and Stage. From around Eldoret in areas like Munyaka in two spread areas.

3.2. Apparatus and Materials

- Conical flasks (100 cm3) and digestion flask (Pyrex)
- Brassica oleracea l. acephala
- Beakers (100 cm3) and measuring cylinders
- Collecting/polypropylene bags
- 6 weighing boats
- Analytical balance
- Face masks
- Deionized water
- Concentrated Nitric acid (HNO3)
- Concentrated Hydrochloric acid (HCl)
- ANALYTICAL INSTRUMENTS
- The AAS (PG-990)
- The Class I hood

3.3. Sample Preparation

Brassica oleracea l. acephala, commonly consumed leaves, was the sample for analysis. Preventive measures were taken to avoid contamination of the model. Once obtained from the stipulated areas, the samples were washed thoroughly to remove mud and dust particles which would have been possible contaminants. After washing with deionized water, they were sliced into smaller pieces then left to dry. After which, a powder will be obtained by crushing the sliced kales. Each sample was labeled concerning the area from which they were collected.

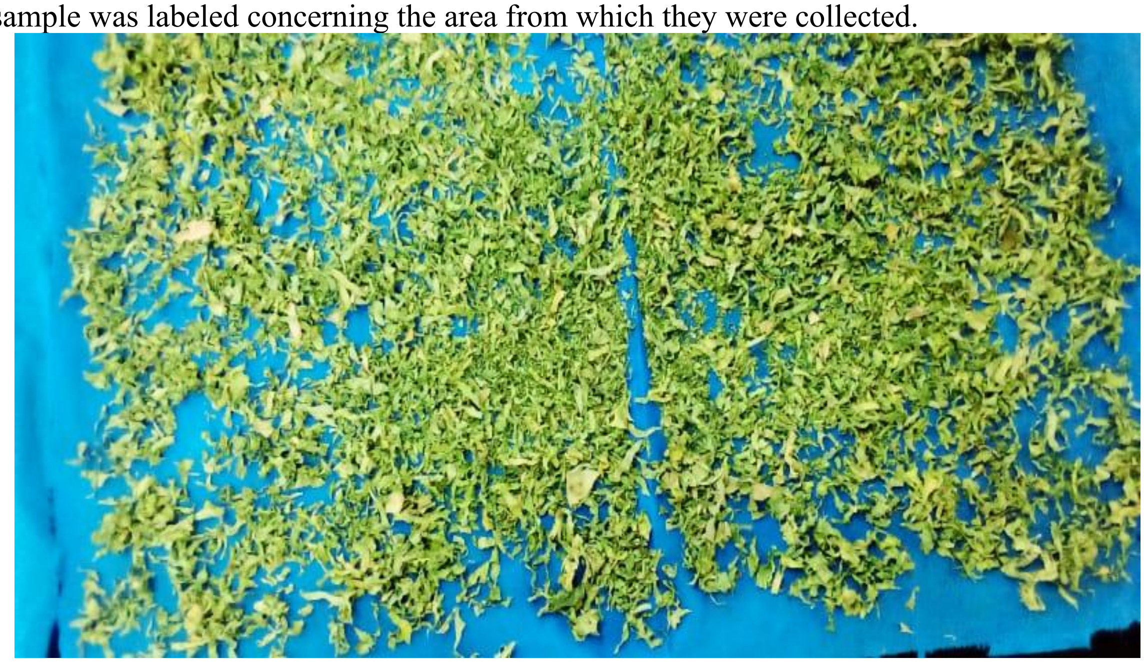


Figure 5: Brassica Oleracea.1 acephala under drying conditions.

3.4. Study Area

This research was conducted be done in the Moi University chemistry laboratory with the supervision of the assigned lab technician; Madam Florence and Mr. Samson Lutta. Samples was collected from various places in Munyaka Slums and Kesses region: Stage and Mabatini, and the two areas are believed to have exactly opposite heavy metals (Pb, Cr, Cd and Fe) phytoaccumulation and concentration.

Figure 6:Brassica Oleracea.1 acephala from Chebaiywo habitat

3.5. Sampling Method

We used a random sampling method. Whereby samples were randomly collected from two stipulated points in Munyaka slums and two spread-apart places in Kesses.

3.6. Wet Digestion and Ashing of the Kales

Ashing in analytical chemistry is a technique defined as heating a substance to leave incombustible ash that can be analyzed for its elemental properties. Digestion is the process of burning (combusting) volatile materials in the sample. The volatile materials, in this case, are usually organic materials such as carbohydrates, fats and oil, protein, and other materials. After the digestion, what remains is the inorganic materials mostly the trace metal elements (Ghorpade et al., 2021). The acid method will be used to digest the sample because this method has the advantage of reducing the loss of volatile compounds from the sample, also it takes short time to digest the sample compared to the dry Ashing/digestion. All acid procedure makes utilizes oxidizing agents to disintegrate the organic matters. The method has some merit when compared with dry Ashing as there is no loss of volatile compounds that occurs. The nutrient elements can be analyzed in one digest solution.

Figure 7: Ashed Brassica Oleracea.1 acephala.

Various chemicals such as Nitric acid, Hydrochloric acid, and Sulphuric acid can be used in the analysis of Lead, Copper, Arsenic, Iron, and other heavy metals. Hydrochloric acid will be used in this project because digestion is very fast though it is dangerous in the sense that an explosion may occur if care is not taken. Hence care will be taken to avoid such cases occurring. The solution after digestion will then be made up to the mark in the volumetric flask.

3.6.1. ***Wet Digestion Method (Aqua Regia)***

0.5 grams of Kale sample were taken in a 250ml conical flask, added 15 ml of concentrated HCl followed by slow addition of 5 ml of concentrated HNO3 with the help of a 25ml measuring cylinder through the inner wall of the conical flask. The contents were heated on hot plate to the boiling point until the content reduced to 2-3ml. 50ml of DDW was added to the contents and mixed the content thoroughly (Mickova et al., 2018). The mixture was filtered through Whatman No.42 filter paper in a 100ml volumetric flask with 2 to 3 times of washing the conical flask with DDW. It was made up the volume to the mark. Stored the extracts for the estimation of total microelements by atomic absorption spectrophotometer.

3.7. Data Collection and Tools

The heavy metals presence was done on the AAS and the printout of the results was obtained for data analysis.

3.8. Data Analysis

The heavy metals were analyzed using Microsoft Excel to find the mean and standard error of the mean which was then be subjected to the statistical test of significance ANOVA at a confidence interval of 95 % ($p<0.05$).

CHAPTER 4
4.0. RESULTS AND DISCUSSION

Three elements were analyzed from the samples, namely Lead, Chromium and Cadmium (Pb, Cr, and Cd). The results below were obtained as a printout from the Atomic Absorption Spectrophotometer analysis. The mean for each metal was calculated using Microsoft excel and presented as shown below.

4.1. Lead (Pb) Concentration in (ppm)

Table 1: Lead Concentration in Brassica Oleracea.1 acephala.

Munyaka Estate Block B	Munyaka Estate Block A	Stage	Chebaiywo
2.8814	2.2201	1.3103	0.8986
2.8812	2.2200	1.3106	0.8978
2.8712	2.2108	1.3106	0.8976

4.2. Chromium Concentration in (ppm)

Table 2: Chromium Concentration of Brassica Oleracea.1 acephala.

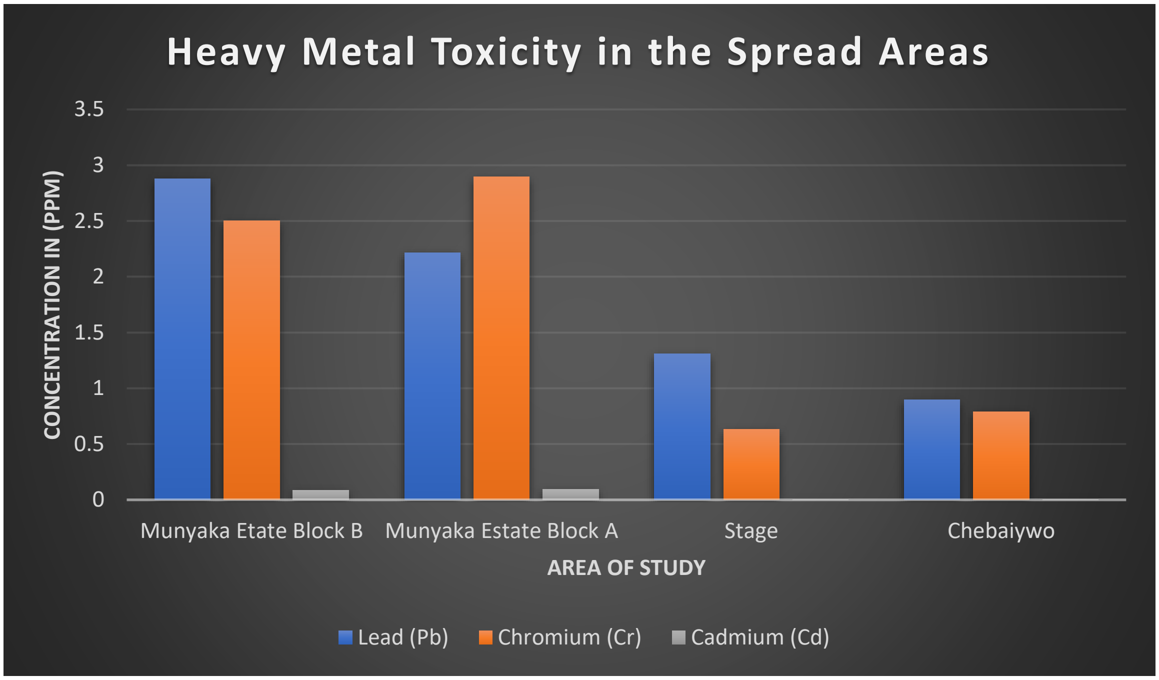
Munyaka Estate Block B	Munyaka Estate Block A	Stage	Chebaiywo
2.5368	2.8963	0.6321	0.7899
2.5366	2.8897	0.6318	0.7897
2.4365	2.8964	0.6320	0.78998

4.3. Cadmium Concentration in (ppm)

Table 3: Concentration of Cadmium in Brassica Oleracea.1 acephala.

Munyaka Estate Block B	Munyaka Estate Block A	Stage	Chebaiywo
0.087	0.0967	0.00161	0.00186
0.0842	0.0954	0.00145	0.00117
0.0876	0.0938	0.00163	0.00185

Table 4: Heavy Metal Toxicity in the Spread Areas.



4.4. Data Analysis

To analyze for the difference in means of the concentration level of the metal, a randomized block design was used. RBD experiments are effective and provide higher accuracy since it groups relatively homogeneous groups together there by decreasing the error rate. This test is carried out at 5% significance level and uses Place as blocks and Metals as Treatments. The least Significant Difference (LSD) was used for post hoc analyses. ###

Hypothesis:

1. Place (Block) Null: No difference between the 4 different locations Alternative: At least one of the locations is different
2. Metal (Treatment) Null: Lead, Chromium and Cadmium have same concentration rates. Alternative: At least one is different.

Decision Rule If p-value is less than alpha (0.05) we reject the null hypothesis.

4.4.1. ANOVA

Table 5: One factor ANOVA table for lead concentration.

Anova: Single Factor **Lead Metal Analysis**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Munyaka Estate Block B	3	8.6338	2.877933	3.4E-05
Munyaka Estate Block A	3	6.6509	2.216967	2.85E-05
Stage	3	3.9315	1.3105	3E-08
Chebaiywo	3	2.694	0.898	2.8E-07

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	7.159028	3	2.386343	151883.5	2.34E-19	4.066181
Within Groups	0.000126	8	1.57E-05			
Total	7.159154	11				

Table 6: One factor ANOVA table for chromium concentration

Anova: Single Factor

Chromium Analysis

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Munyaka Estate Block B	3	7.5099	2.5033	0.003347
Munyaka Estate Block A	3	8.6824	2.894133	1.47E-05
Stage	3	1.8959	0.631967	2.33E-08
Chebaiywo	3	2.36958	0.78986	2.08E-08

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12.12061	3	4.040203	4807.651	2.32E-13	4.066181
Within Groups	0.006723	8	0.00084			
Total	12.12733	11				

***Post hoc* analysis**

p-values for pairwise t-tests

		KESSES 0.7109	MUNYEKA 2.6987
KESSES	0.7109		
MUNYAKA	2.6987	2.32E-13	

Tukey simultaneous comparison t-values (d.f. = 10)

		KESSES	MUNYEKA
		0.7109	2.6987
KESSES	0.7109		
MUNYEKA	2.6987	0.8524	

critical values for experimentwise error rate:

0.05	2.23
0.01	3.17

Table 7: One factor ANOVA table for cadmium concentration

Anova: Single Factor **Cadmium Analysis**

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Munyaka Estate Block B	3	0.2588	0.086267	3.29E-06
Munyaka Estate Block A	3	0.2859	0.0953	2.11E-06
Stage	3	0.00469	0.001563	9.73E-09
Chebaiywo	3	0.00488	0.001627	1.56E-07

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.023986	3	0.007995	5742.247	1.14E-13	4.066181
Within Groups	1.11E-05	8	1.39E-06			
Total	0.023997	11				

Post hoc analysis

p-values for pairwise t-tests

		KESSES	MUNYEKA
		0.001595	0.0908
KESSES	0.001595		
MUNYEKA	0.0908	.0021	

Tukey simultaneous comparison t-values (d.f. = 10)

KESSES	MUNYEKA
0.001595	0.0908

KESSES	0.001595		
MUNYEKA	0.0908	4.12	

critical values for experimentwise error rate:

0.05	2.23
0.01	3.17

The test reported a p-value of (2.34E-19) which is less than alpha (0.05) *Decision* Reject the null hypothesis *Conclusion* With 95% confidence, there exist sufficient statistical evidence to support the claim that the average sample concentrations rates are different for the different locations. Post hoc analysis reported Munyaka having the most significant difference with mean (2.54745). There was no statistically significant difference at 5% between the concentration levels in Munyaka Block B, Chebaiywo and Stage.

The test reported a p-value of (2.32E-13) which is less than alpha (0.05) *Decision* Reject the null hypothesis *Conclusion* With 95% confidence, there exist sufficient statistical evidence to support the claim that the average sample absorbance rates are different for the three metals. Post hoc analysis reported Cadmium having the most significant difference with mean (0.0908). There was no statistically significant difference at 5% between the absorbance levels in Lead and Chromium.

4.5. Discussion

From the observations made from the results above, it can be depicted that in Munyaka, there is much more heavy metal concentration in the Kales compared to the ones in Kesses. This could be attributed to the *Jua kali* activities that are so many in Munyaka. Car washing along river banks is among the source of pollutants from petroleum found in oils used by motor vehicles and the ones present in gasoline additives (Li et al., 2019). Spraying of vehicles with paints rich in heavy metals contributes to pollution in this urban center, this is through runoffs that later sweep away the remains of paint into lands that are used for cultivation.

Figure 8: Jua Kali activities in Langas region.

Kesses is a rural area with limited chances of pollution from sewage, industries, and petroleum products. Additionally, it could be attributed to the nature of farming practiced in the area where the crop is grown around the natural ecosystem and supplied with rain water which is less polluted than Munyaka region where most of the crops are grown around sewage trenches. However, slight pollution in the area gave positive results that these Kales are slightly polluted, this could be attributed to human activities like poor waste disposal systems and poorly structured and located sanitation facilities and septic tanks which allow leaking of wastes and their leaching into soil (Emamverdian et al., 2018). These weaknesses or faults are quite rampant in Kesses region and they pose a silent threat towards heavy metal concentration. Cadmium was found to be in little quantities which could be assumed that the samples had not accumulated the metal or due to its scarcity.

Various parameters contribute to high heavy metal concentration in Munyaka. Based on (Joseph et al., 2019), first, runoff from roofs with metal features (roofing material, gutters), particularly Lead, had the highest quantities of heavy metals. Chromium and lead concentrations are occasionally found in runoff from streets and parking lots, which is likely due to metals being leaked from vehicles, elements, and metallic constructions of streets and parking lots. Large disparities in heavy metal concentrations are detected in runoffs released into urban waterways from different systems. Concentrations are low in residential catchments but high in industrial catchments and places with a high proportion of traffic.

Therefore, high pollution in Munyaka due to open garages whose wastewater is swept to the rivers, petroleum products, runoffs from roofs, and sewages bursts are among the causes of heavy metal pollution in the area. Kales of Kesses are “safer” than those grown in Munyaka as they are less polluted.

CHAPTER 5
5.0. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

Brassica Oleracea samples obtained from Munyaka region exhibited high levels of heavy metal contamination compared to those obtained from Kesses region. This is attributed by the waste water sewage in Munyaka region which contain high levels of heavy metal concentration. The toxicity of these metals within the stipulated areas can also be contributed by Jua kali activities within the region which promote the ease of heavy metal contamination like lead and cadmium which scrap off from equipment used. Car washing and painting is also found to contribute to the contamination through runoffs containing petroleum and paint materials which contain heavy metals of lead, cadmium and chromium. In Kesses region, the levels of heavy metal concentrations differed greatly from those in Munyaka region. These levels were lower, explained by the fact that the Brassica Oleracea samples were grown in areas without sewage waste exposure, car wash garages, lack of excessive use of car paints and also lack of Jua kali enterprises.

In spite of these factors, the Brassica Oleracea samples from Kesses region still exhibited levels of heavy metal concentration and these we attributed to factors of human negligence and fault. They include improper disposal of wastes and poor waste disposal systems that allow for the build-up of heavy metals from substances like dry cells which contain lead, sanitary towels which being made from cotton treated with pesticides and insecticides which are toxic and carcinogenic (Wang et al., 2019). There is also the poor structuring and locations of sanitary facilities in Kesses region that promote soil contamination through leaking of waste materials and their seeping into soil e.g., from septic tanks that are poorly constructed. The low levels of heavy metal concentration in the Brassica Oleracea samples obtained from Kesses regions especially Chebaiywo was explained by the fact that these were grown in soils covered and mixed with charcoal which acts as a biochar that works to remove heavy metals from soil through sequestration (Wang et al., 2019). The required standards by the Food and Drug Administration (FDA) and the World Health Organization (WHO) include the following;

Table 8: WHO Permissible Limits for Heavy Metals in Brassica Oleracea.

Metal	**Pb**	**Cr**	**Cd**
Standard Concentration in Plants	2 ppm	1.30 ppm	0.02 ppm
Sample Concentration in Brassica Oleracea (Munyaka)	2.54745 ppm	2.6987 ppm	0.0908
Sample Concentration in Brassica Oleracea (Kesses)	1.10425 ppm	0.7109 ppm	0.001595 ppm

From the table above, it is conclusive proof that Brassica Oleracea samples from Munyaka region exhibit high levels of heavy metals concentration compared to the permissible limits as set by WHO. The samples from the Kesses region exhibit a lower heavy metal concertation compared to the World Health Organization standards thus they are for consumption. These observations imply that the samples from either region are unsafe for human consumption.

5.2. Recommendations

- Kales should not be cultivated for food in polluted areas such as polluted springs, swamps, wetlands, or in polluted soils where they would absorb poisonous heavy metals.
- Kales should also not be cultivated along busy motorways where they would be exposed to lead emissions from vehicular exhaust fumes.

- Application of inorganic fertilizers on Kale's plantations should be avoided to minimize the chances of the plant absorbing cadmium.
- The Water and Sewerage Company and the Ministry of Water and Irrigation should regularly monitor the heavy metal pollution in the piped water which has been drawn by respective communities from Munyaka and Kesses before the respective consumers use it.
- National Environmental Management Agency and the Ministry of Public Health should enact legislation and guidelines to prevent pollution of rivers and proper maintenance of busy road reserves for the cultivation of the Kales plant.
- Further research on the interaction of heavy metals with the Kales plant and the practice of safe urban agriculture is highly recommended.
- Farmers should be encouraged and educated on safe cultivation techniques e.g., the use of biofertilizers.

6.0. REFERENCES

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8.0. APENDIX

Table 9: Standard Calibration curve for Lead Standard

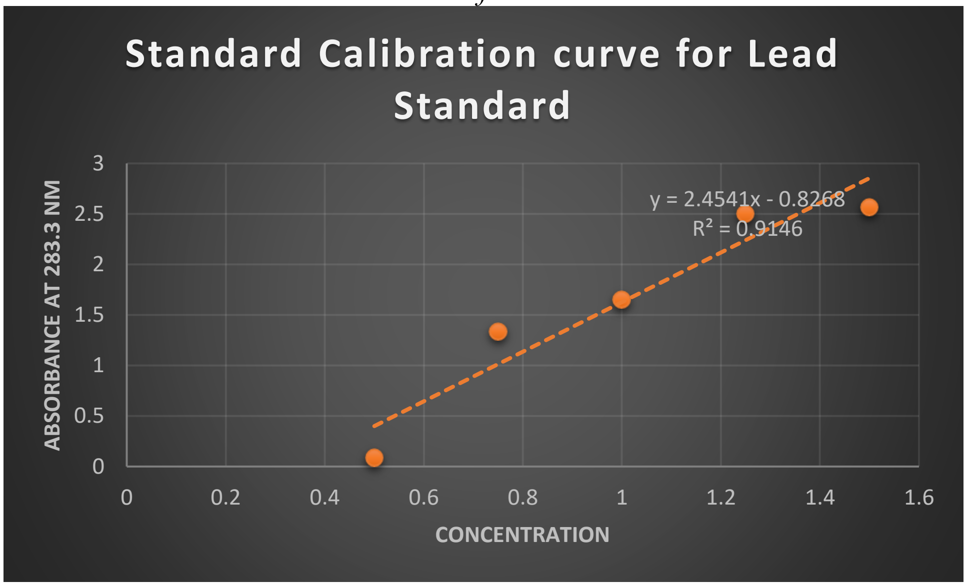

Table 10: Standard Calibration curve for Cadmium

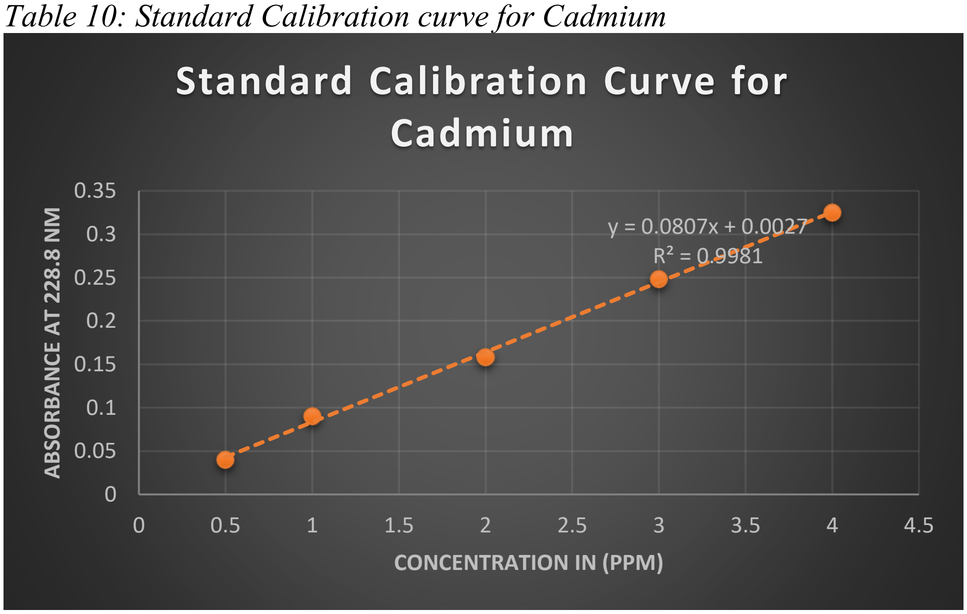

Table 11: Standard Calibration Curve for Chromium

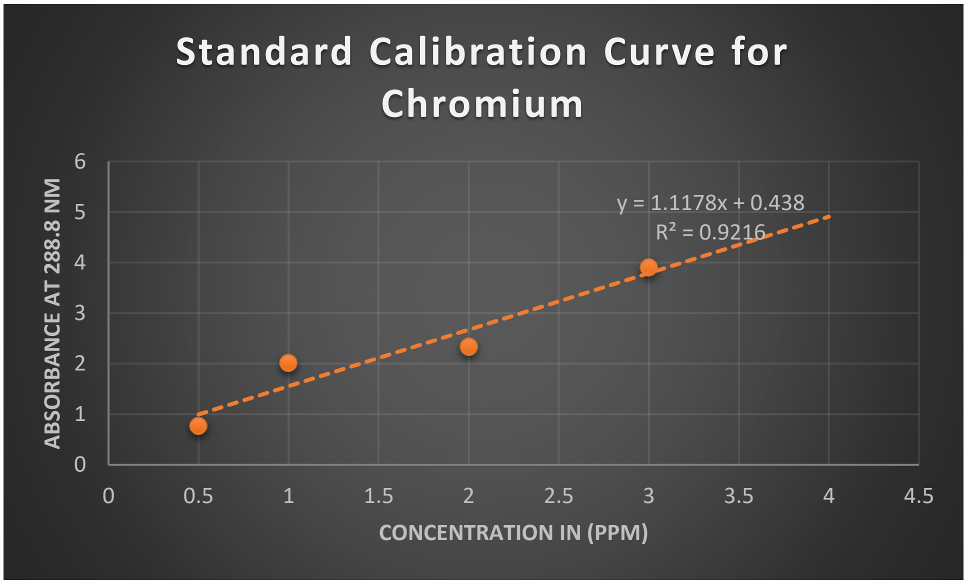

Figure 9: Brassica Oleracea.1 aephala in Munyaka region.

Figure 10: Brassica Oleracea.1 acephala from Chebaiywo.

Figure 11: Brassica Oleracea.1 acephala drying.

Figure 12: Brassica Oleracea.1 acephala drying.

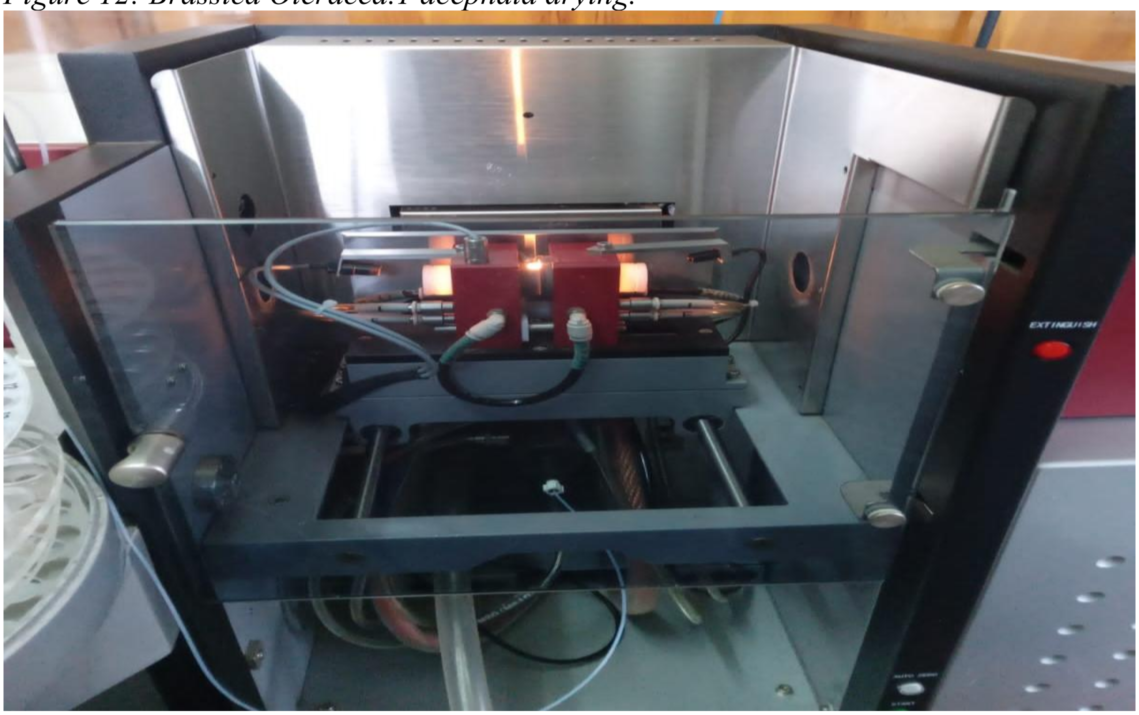

Figure 13: AAS under operation.

Figure 14: Digested Brassica Oleracea.1 acephala being filtered

Figure 15: Brassica Oleracea.1 acephala under wet digestion.

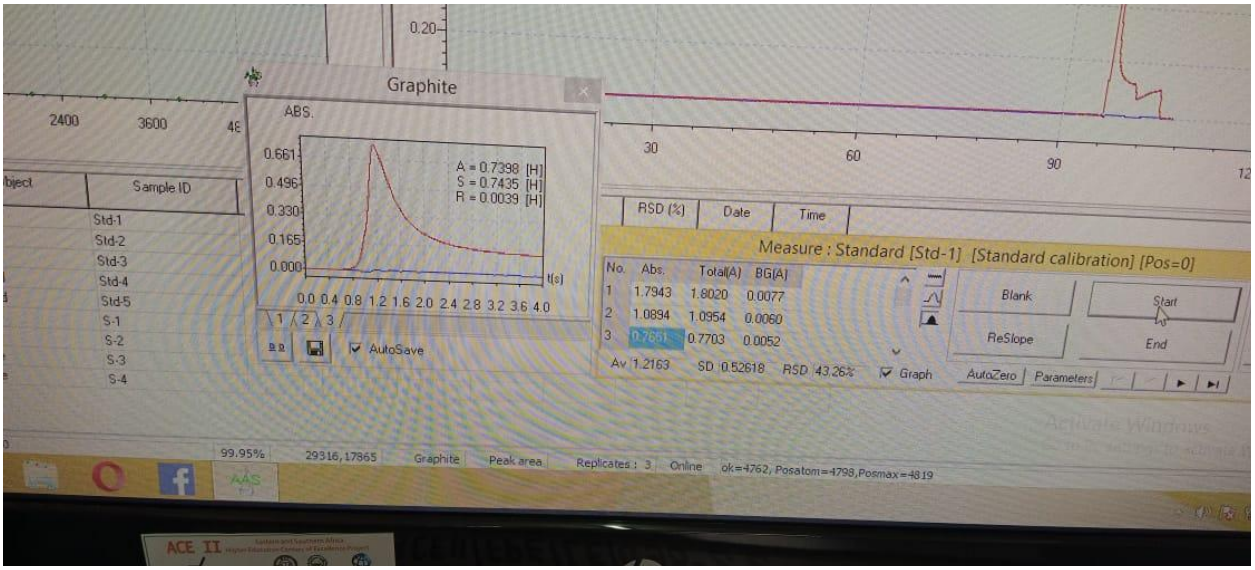

Figure 16: Standard Absorbance read from AAS.