Detection of heavy metals concentration in vegetables and analyze the health risks

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ABSTRACT

Heavy metals are elements found in Earth's crust but introduced into soil and water bodies by human activities. They are not biodegradable, so they persist for a long time in the environment. Heavy metals are incorporated into to human body through the food chain, resulting in various health problems. Akaki Rivers, which are major water sources in Addis Ababa, are contaminated with various wastes, including heavy metals. This research aimed to detect heavy metal concentration in cabbage, potato, tomato, and beetroot irrigated with the Akaki Rivers and evaluate associated health risks. Following the vegetable sample collection, a laboratory-based study was used in sample processing, digestion, and heavy metal detection. Mean concentration (mg/kg dry weight) of Cd (26.11-26.34), Pb (17-33.84) in all samples, and Hg (0.124) in beetroot exceeded the permissible limits set by WHO/FAO. The HRI of Cd (28.3-140.96), Pd (10.9-27.35), both in adults and children, and Hg (1.727 for children) exceeded the safe limit (<1). The health of adults and children is at risk due to Cd, Pb, and Hg, with children facing approximately 2.5 times higher. Minimization of the release of wastewater into the Akaki Rivers, and dietary diversification should be encouraged, and the health of permanent consumers should be checked.

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1. INTRODUCTION

Heavy metals are a group of metallic elements with relatively high density and are considered among the most serious pollutants. They are naturally found in Earth's crust but incorporated into the environment due to various anthropogenic activities like industrialization [1]. Pollution of the environment with heavy metals is a significant global public health concern. They are not biodegradable, can persist for a long time in the environment, and are absorbed by plants [2]. Plants like vegetables are a main part of the human diet and are pathways in transferring toxic elements from the environment to humans [3]. Exposure to heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and mercury (Hg) leads to mental retardation, hypertension, blood pressure, neurological disorders, renal dysfunction, and cancers [4].

The Great and Little Akaki Rivers are major water sources for Addis Ababa City and are used for the irrigation of crops [5]. Rapid urbanization, industrialization, and inadequate waste management systems in the city lead to contamination of the rivers [6]. The city hosts more than 65% of the nation's total industrial sector, most of which are situated along the Akaki River banks and release their wastes directly into the river. About 90-96% of industries in the city lack any form of treatment facilities, allowing them to discharge wastes into the rivers [7]. Expansion of the city results in improper waste disposal and untreated sewage discharge to the rivers [8].

The mean concentration (mg/kg dry weight) of Cr (109.51), Cd (3.14), Pb (129.7), and Zn (148.28) in Akaki River sediment [9]. The median heavy metal concentration in mg/L of Akaki Rivers water was Pb (3.9), Cd (0.4), and Ni (1.22), surpassing the respective permissible limits 0.1, 0.1, and 0.2 set by FAO to use for irrigation purposes [10]. According to [9], the average concentration of Lead (Pb): 0.775 mg/L, Chromium (Cr): 1 mg/L, Cadmium (Cd): 0.075 mg/L, Nickel (Ni): 0.425 mg/L and Zinc (Zn): 5.75 mg/L in Akaki Rivers all of which were above respective FAO permissible limit for irrigation (0.1 mg/L, 0.1 mg/L, 0.01 mg/L, 0.2 mg/L, and 2 mg/L) [10].

However, there is limited research on the extent of heavy metal contamination of vegetables irrigated with the Akaki Rivers. This study aimed to (1) detect the concentration of Pb, Cd, Cr, As, and Hg in beetroot, cabbage, potato, and tomato samples irrigated by the Akaki Rivers and (2) evaluate the potential human health risks associated with consuming those vegetables.

2. METHOD

2.1. Study area

Figure 1 presents the map of the study area, covering the Great Akaki and Little Akaki Rivers in Addis Ababa, Ethiopia, along with the locations of the sampling sites. These sites were selected to represent environmental variations between upstream and downstream areas that are potentially exposed to heavy metal contamination from industrial and domestic activities. The map provides a clear spatial overview of the study area and supports the analysis of the relationship between sampling locations and the level of heavy metal contamination in vegetables irrigated by the rivers.

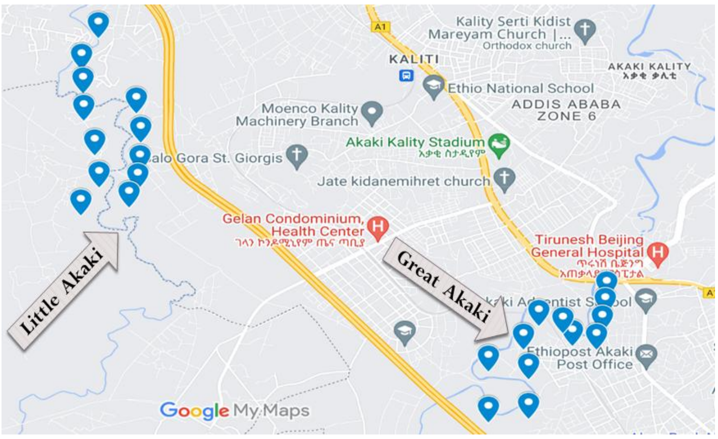

Figure 1. Map of the Akaki rivers with sampling sites

2.2. Instrument and analytical reagent

Atomic absorption spectrometer (AAS) novAA 800 was used to measure the concentration of Cd, Cr, Pb, As and Hg in vegetable samples. AAS is a quantitative analytical instrument used to identify approximately 70 elements and used to measure the concentration of an element by passing light of a specific wavelength, emitted from a radiation source corresponding to that element, through a cloud of atoms derived from the sample. The atoms absorb light from a hollow cathode lamp (HCL) energy source, and the reduction in light intensity detected is proportional to the concentration of the element in the sample [11]. All chemicals used were analytical reagent grade. HNO3 (65%), H2O2 (30%), and HClO4 (70%) were used for digestion. Stock standard solutions of 1000 ppm for Cd, Cr, Pb, As, and Hg were used to prepare all calibration curves by diluting to known concentrations [12]. Distilled water was used for sample preparation and dilution purposes throughout the study.

2.3. Sample collection and preparation

Edible parts of cabbage (Brassica juncea), beetroot (Beta vulgaris), potato (Solanum tuberosum), and tomato (Lycopersicon esculentum Miller) samples were collected from farmlands irrigated by the Akaki Rivers in dry months (January and February). A total of twenty-four samples, twelve from each of the Great and Little Akkaki River sites, and six samples for each vegetable type were selected and collected by a stratified random sampling method. Sub-samples as a representative fraction of the vegetables were used to prepare each of the samples in a composite sample form [5].

Samples were washed with tap water to remove soil and other dirt and rinsed with distilled water to remove adsorbed elements. The samples were cut into small pieces and dried using the dry oven (Memmert UF 260 plus 230 V Sunon) at a temperature of 105 °C for 24 hrs to remove moisture. The dried samples were powdered with a mortar and pest, and sieved with a 2.5 micrometer (µm) sieve size, and placed into polythene bags until digestion began [12]. One gram of powdered sample was digested in a borosilicate flask with a mixture of HNO_3 (65%), $HClO_4$ (70%), and $H2O_2$ (30%) in a ratio of 5:2:2 at 240 °C for 15 minutes. A clear and colorless solution was diluted with distilled water to raise the volume up to 50 mL and filtered through Whatman filter paper no 42, [12].

2.4. Quality control and assurance

To ensure the quality of data, the samples were collected in dry months (January and February) when vegetables were grown entirely with irrigation waters from the Akaki Rivers. They were picked with gloved hands and collected in polythene bags to prevent cross-contamination and stored at 4 °C until used. The blank solution was prepared by mixing HNO_3 (65%), $HClO_4$ (70%), and H_2O_2 (30%) in a volume ratio of 5:2:2 and then diluted the mixture to 50ml with distilled water. The method detection limit was calculated as three times the standard deviation of multiple blank measurements and divided by the slope of the calibration curve. In addition, the method was evaluated by a spike recovery test, and the mean recovery rate was 90.5% [13]. Stock standard solution of each metal was used to prepare calibration standards for AAS. All analytical reagents were within their expiration date. Each vegetable sample was analyzed in hexaplicate (n = 6), and the mean concentration was calculated to minimize bias. The analysis was performed using novAA 800 AAS. The concentrations of Cr, Cd, and Pb were determined using the flame technique, and those of As and Hg were analyzed using the hydride generation technique [14].

2.5. Human health risk assessment

In this study, the human health risks associated with Cd, Cr, Pb, As and Hg consumed by beetroot, cabbage, potato, and tomato were evaluated by estimating health risk indicators such as Daily Intake of Heavy Metals and Health Risk Index [13].

2.5.1. Daily intake of heavy metals (DIHM)

The DIHM, which is measured in (mg/kg/d) of heavy metals consumed with vegetables, was calculated as;

$$DIHM = \frac{DIV x\ C}{BW} \quad (1)$$

Where, C represents the concentration of heavy metal in the edible part of the vegetable in mg/kg, DIV is a daily intake of the vegetable, which is 0.1 and 0.05 kg for Ethiopian adults and children, respectively and BW is the body weight in kg, which has been 60 for adults and 12 kg for children in Ethiopia [15].

2.5.2. Health risk index (HRI)

The HRI was used to determine human health risk because of the consumption of contaminated vegetables with heavy metals and it is the ratio of the daily intake of metals and the reference oral dose,

$$HRI = \frac{DIHM}{RFD} \quad (2)$$

Where, RFD is reference oral dose values which are 1×10^{-3}, 4×10^{-3}, 1.5, 5×10^{-4}, and 3×10^{-4} (mg/kg/d) for Cd, Pb, Cr, Hg, and As, respectively [16].

2.6. Data analysis

The data was analyzed using SPSS version 25, and descriptive analysis was used to calculate the mean and standard deviation of concentration of heavy metals in the samples at a 95% confidence interval, and One-way-ANOVA was used to analyze the significant difference of heavy metals concentration within and across vegetables at $p<0.05$.

3. RESULT AND DISCUSSION

3.1. Heavy metal contents in vegetable samples

The mean concentration with SD of Cd, Cr, Pb, As, and Hg in hexaplicate (n = 6) samples of beetroot, cabbage, potato, and tomato were analyzed and compared with the WHO/FAO permissible limit, as shown in Table 1. The mean concentration of Cd ranged from (17 to 33.84 mg/kg), which was higher than the recommended limit (0.2 mg/kg) [17]. This is likely due to cadmium accumulation in plants through contaminated soil with phosphate fertilizers and industrial waste [18]. The level of Cd was higher in cabbage than in other vegetable samples, which might be due to leafy vegetables having a higher potential to accumulate Cd [19]. The concentration of Cd in all vegetable samples was greater than (6.03 and 6.43 mg/kg) in tomato and cabbage samples, respectively, collected from Koka Ejersa, and it might be because of Akaki Rivers are located upstream of Koka Ejersa [15].

All vegetable samples had around 26 mg/kg of lead concentration, which was by far above the WHO/FAO stipulated limit of 0.3 mg/kg [17]. The sources of lead for this contamination might be vehicle emissions, industrial pollution, and lead-based pesticides [17]. These results were greater than lead concentration (2.02, 3.63, and 7.56 mg/kg) in potato, tomato, and cabbage samples, respectively studied at Koka areas [15]. The reason for higher contamination might be that Akaki Rivers are located upstream of Koka areas. The mean concentration of Cd and Pb was also significantly higher than the concentration of Cd: (1.06 mg/kg) and Pd: (5.6 mg/kg) in vegetables collected from the Mojo area, which is likely due to discharges from lead-acid manufacturing, recycling, and electroplating in Addis Ababa [20].

The concentration (mg/kg) of Hg in beetroot (0.124), potato (0.025) and tomato (0.047) samples were a little bit exceeded the stipulate limit, 0.02 mg/kg [21]. It was accumulated more in beetroot, which might be due to its high capacity for metal translocation and storage in its edible taproot [22]. The values were less than the concentration of Hg (3.430 and 4.230 mg/kg) in tomato and cabbage samples collected from Koka areas respectively investigated by [15]. Chromium (Cr) concentration was below the detection limit (<0,001 mg/kg) and within safe limit (2.3 mg/kg) set by WHO/FAO [23]. Chromium can be found in Cr (III) and Cr (VI) forms. Cr (III) is less toxic and exists in the soil, and is preferred by plants but less to be translocated into edible parts. Cr (VI) is highly toxic and mostly found in industrial wastes and not preferred by plants [24]. The concentration of Arsenic (As) in tomato (0.035), beetroot (0.012), potato (0.012) and cabbage (0.005) was within stipulate limit (0.1 mg/kg) [23], because the rivers might not be contaminated with arsenic which likely due to absence of mining around sampling sites [25]. The concentration of Cr, As, and Hg was very low compare to respective concentrations (3.06 mg/kg, 3.83 mg/kg and 3.83 mg/kg) in vegetables collected from Mojo area, which might be due to less tannery industries and metal-based pesticides and herbicides in agricultural activities in Addis Ababa [20].

The mean concentration of Cd was significantly different across vegetable samples in which cabbage with maximum concentration at $p<0.05$ (One-way-ANOVA). This is likely due to their distinct physiology and the function of their edible parts. Leafy vegetables like cabbage have higher transpiration rates that pull Cd into their leaves [21]. The mean concentration of Pd was not significantly different across vegetable samples. This might be due to all vegetables had equivalent absorption and accumulation rate for Pb [17]. The mean concentration of Cd and Pb were statically significantly different with in vegetable samples (One-way-ANOVA). This might be due to Cd and Pb are not equally absorbed and translocated by all vegetables [19]. Figure 2 as presented Cd and Pb concentration variation with in and across vegetables.

Table 1. Heavy metals concentration in vegetable samples

Vegetables	Concentration; Mean ± SD (mg/kg), n = 6				
	Cd	Cr	Pb	As	Hg
Beetroot	21.92 ± 1.05	<0.001	26.13 ± .10	0.012 ± .57	0.124 ± .76
Cabbage	33.84 ± 1.1	<0.001	26.11 ± .04	0.005 ± .56	0.018 ± 1.16
Potato	17 ± 1.27	<0.001	26.26 ± .13	0.012 ± .74	0.025 ± 3.02
Tomato	20.27 ± 2.05	<0.001	26.34 ± .21	0.035 ± .86	0.047 ± .48
WHO/FAO stipulate limit	0.2	2.3	0.3	0.1	0.02

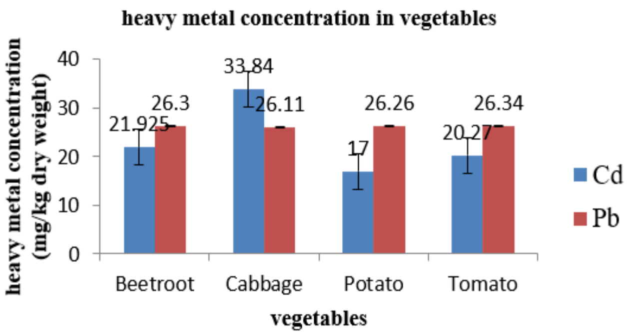

Figure 2. Cd and Pb concentration variation with in and across vegetables

3.2. Human health risk assessment
3.2.1. Daily intake of heavy metals (DIHM)

Health risks of heavy metal intake with investigated vegetables were assessed by estimating the average DIHM. The DIHM values obtained via (question 1) vary between adults and children. The highest intakes were Cd and Pb by children with all studied vegetables as showed in Table 2. This is due to their smaller body size and higher consumption rates [15].

3.3. Health risk index (HRI)

The HRI is mainly dependent on a DIHM and studied heavy metals' recommended reference oral doses (RFD) (question 2). If HRI<1, means that it is unlikely to going to pose a population health risk but HRI ≥ 1 means that the consumers are going to in pose to health risk [26]. In this study, the estimated HRI for adults and children who consumed beetroot, cabbage, potato, and tomato which accumulated Cd and Pb, and children who consumed beetroot with Hg were above safe limit as shown in Table 2.

Children faced higher health risk due to cadmium than adults Table 2. Consumption of cabbage by children posed the highest risk (140.96), likely because leafy vegetables accumulate more cadmium [27]. Chronic exposure to cadmium consumption can cause kidney damage, bone loss and cancer [28]. The health risk of children was again higher than that of adults due to lead consumption. Lead is a neurotoxin metal in nature and children are at risk of developmental delays, learning disabilities, and behavioral issues, whereas, adults may face cardiovascular and kidney problems [29]. Mercury showed elevated risk in beetroot especially in children than adults, which may potentially affect the nervous system and kidneys [30]. Consumption of arsenic (As) with studied vegetables can cause low risks but prolonged exposure may contribute to skin lesions or cancer. Overall, children faced approximately 2.5 times higher risk of cadmium, lead and mercury than adults due to lower body weight and higher intake relative to their body size [27]. These findings agreed with [31], who assessed the potential health risk of heavy metals via consumption of rice and vegetables grown in the industrial areas of Bangladesh. The findings also agreed with that of [15], who reported that the children were more exposed to health risks due to intake of toxic metals through consumption of vegetables irrigated with wastewater.

Table 2. Daily intake of heavy metals through vegetables and Estimation of health risk index

Vegetables	Person		DIHM (1) with HRI (2) of heavy metals			
			Cd	Pb	As	Hg
Beetroot	Adults	1	0.0365	0.000	0.0436	0.00001
		2	36.5	10.9	0.039	0.69
	Children	1	0.1826	0.000	0.2178	0.00001
		2	91.33	27.22	0.001	1.727
Cabbage	Adults	1	0.0563	0.000	0.0435	0.00001
		2	56.3	10.88	0.015	0.098
	Children	1	0.2819	0.000	0.2177	0.00001
		2	140.96	27.21	0.039	0.246
Potato	Adults	1	0.0283	0.000	0.0437	0.00001
		2	28.3	10.93	0.042	0.138
	Children	1	0.1416	0.000	0.2188	0.00001
		2	70.83	27.35	0.104	0.344
Tomato	Adults	1	0.0337	0.000	0.0437	0.0001
		2	33.7	10.93	0.115	0.26
	Children	1	0.1689	0.000	0.2188	0.00002
		2	84.46	27.35	0.289	0.651

4. CONCLUSION

The study obtained that beetroot, cabbage, potato and tomato irrigated with the Akaki Rivers water had cadmium (Cd), lead (Pb), and mercury (Hg) with concentration that exceeded the safe limits set by WHO/FAO. The significant surpass safe limit (HRI<1) of cadmium and lead in all vegetables for both adults and children and mercury in beetroot for children, shows a serious health risk in consuming these vegetables. Children were found to be approximately 2.5 times higher risk of cadmium, lead and mercury than adults due to lower body weight and higher intake. The study concludes that if the release of heavy metal is not prevented and controlled, the health of population who regularly consume the vegetables from the study area is at risk.

Based on the research finding, it can be recommended that; Administrative measures should be taken to prevent the release of waste water in to Akaki Rivers system prior to treatment, dietary diversification should be encouraged for permanent consumers of vegetables from study sites to limit exposure, methods of prevention and control of heavy metal pollutions like phytoremediation and biochar on study areas should be implemented, organic farming should be encouraged and the health status of permanent consumers of the vegetables from the research sites should be analyzed.

The study of concentration of Cd, Cr, Pb, As, and Hg in beetroot, cabbage, potato, and tomato was subjected to various limitations which could be considered in result interpretation. First, even though tries were made to diversify the sample representativeness, the obtained concentrations might not cover sample variability caused due to geographical origin, vegetable types and seasonal differences. Second, although quality control procedures were followed, the complex organic nature of vegetable samples posed risks of spectral interference during analysis which can be partially eliminated through validation techniques. This limitation is particularly a problem in the reporting of concentration of chromium and arsenic in which their toxicity is dependent on their chemical speciation like Cr (III) verses Cr (VI) and organic verses inorganic As, thus the reported values might not accurately show the real risk. Lastly, the HRI was assessed based on estimated daily intake model, which depends on entire population consumption data but does not consider individual dietary habit, body weight or bioavailability of metals in individuals. So, the health risk assessment provides preliminary screening level evaluation but not definitive measure of individual health risk. To overcome such limitations, future studies should have to include large scale sampling, chemical speciation analysis for Cr and As, and more nuanced exposure assessment model.

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AUTHOR CONTRIBUTIONS STATEMENT

This journal uses the Contributor Roles Taxonomy (CRediT) to recognize individual author contributions, reduce authorship disputes, and facilitate collaboration.

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C : **C**onceptualization
M : **M**ethodology
So : **So**ftware
Va : **Va**lidation
Fo : **Fo**rmal analysis
I : **I**nvestigation
R : **R**esources
D : **D**ata Curation
O : Writing - **O**riginal Draft
E : Writing - Review & **E**diting
Vi : **Vi**sualization
Su : **Su**pervision
P : **P**roject administration
Fu : **Fu**nding acquisition

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest with respect to the publication of this manuscript.

DATA AVAILABILITY

Derived data supporting the findings of this study are available from the corresponding author, [SG], on request.

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