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Assessing the Occurrence of Heavy Metals in Edible Fruits Grown around Mine Tailings Dam in Kitwe

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Abstract

Mining activities in the Copperbelt province of Zambia have been of great environmental concern, despite recorded improvements in the socio-economic sector. Heavy metal contamination of soils resulting from depositing mine tailings and rock waste has posed possible health risks for communities nearer such facilities. The current study was conducted around residential homes surrounding Kitwe slimes (TD25). This work investigated levels of Co, Cu, Fe, Mn and Pb in the soils, and avocado and lemons fruits, growing near TD25. Heavy metals were analyzed using Atomic Absorption Spectrophotometer. The work further assessed the estimated daily intake (EDI) associated with potential health risks to humans. In the soil, concentration levels of heavy metals showed a trend in the order: Fe > Cu > Mn > Co > Pb. Heavy metal contents in avocado and lemon fruits at 200 and 400 m away from TD25, all had p-values < 0.05. The distances from the suspect source (TD25) to where the fruit trees were grown, had no influence on their concentration in the fruit samples. Fruit sample results showed that Fe had the highest EDI of 0.22 $mg \cdot kg^{-1}$ (avocado) and 0.14 $mg \cdot kg^{-1}$ (lemon), though both results were far below the FAO/WHO standard range (12.5 - 19.6 $mg \cdot kg^{-1}$). For both fruits, Co and Pb results were negligible. The concentration of the five heavy metals in avocado and lemon fruits grown around Kitwe slimes did not pose any health risks to the consumers.

Keywords

Avocado, Concentration, Lemon, Mining, Permissible Limits

1. Introduction

Heavy metals can be defined as those metals with a high specific weight of more than 5 g·cm^{-3} [1], and occur naturally as elements in the soil depending on the geological formation [2]. This is due to the various concentrations in the bedrock [3]. Heavy metals are mainly introduced into the environment by natural means (volcanic eruptions) and through anthropogenic activities, which include mining, industrial production, agriculture and transportation [4] [5]. Among the anthropogenic activities, mining produces large volumes of waste, mainly tailings, often stored in impoundments behind dams, with potential economic, environmental and human health impacts [4] [6]. Mine tailings are mainly a waste mixture of pulverized rock and water during mineral extraction with potential to contaminate soils with heavy metals [2] [5].

Naturally, most metals exist in the soil in various forms and as they become ionized plant roots absorb them as plant nutrients into their tissues [2] [7] [8]. Accumulation of heavy metals in crop plants is of great concern due to the probability of food contamination through the soil root interface, as plant roots take up nutrients [6] [7] [9]. Though the heavy metals like, cadmium (Cd), lead (Pb) and nickel (Ni) are not essential for plant growth; they may be readily taken up and accumulated by plants in toxic forms. Ingestion of vegetables or fruits irrigated or grown in soils contaminated with heavy metals poses a possible global risk to human health and wildlife [10]. Studies indicate that tailings increase the concentration levels of heavy metals in soil above human permissible limits, leading to serious environmental and health problems [4] [6] [11].

The human body needs some metal ions at low and acceptable concentrations, and yet the same metal ions can be toxic and carcinogenic at elevated concentrations [2] [3] [12]. As human beings feed on plants and fruits these metal ions end up in various body tissues [8]. Heavy metals, if ingested in quantities above permissible limits could induce chronic toxicity and negatively affect human health [2] [4]. For instance, lead one of the well-known toxic heavy metals can bio-accumulate in bones [13] and result in irreversible impairment to the nervous system, lead to hearing challenges and could affect the male reproductive system [14] [15].

One of the common tropical fruits recognized for its health benefits, rich in fat-soluble vitamins, with high levels of plant proteins, potassium and unsaturated fatty acids is avocado (*Persea americana*) [16] [17]. Additionally, avocado is associated with a balanced diet, especially in reducing cholesterol and preventing cardiovascular diseases [18]. A number of fruit trees, including avocados, have a fibrous root system located within 30 - 60 cm deep [19].

Lemon (*Citrus limon* (L.), a famous citrus fruit, is well-known because it is rich in vitamin C [20]. Lemon trees have shallow lateral and fibrous root systems, absorbing nutrients from the soil within a depth of 30 cm [21].

In Zambia, avocado and lemon trees are usually planted around residential areas. Some residential areas are located close to old mine tailings sites, where fruit trees do grow. Possible presence of heavy metals in soils found in areas with high anthropogenic activities such as mining, has greatly contributed to environmental pollution [2] [3] [5]. Therefore, fruits grown in such residential areas may bio-accumulate heavy metals in their edible parts during their growth period [22] [23].

Old copper mine tailings dams in Zambia, such as TD25, are slowly being surrounded by human settlements due to rapid urbanization. Some of these old mine tailings dams could be possible sources of heavy metal contamination in the surrounding communities. There is limited information on possibilities of heavy metal contamination in some of the consumed fruits on the Copperbelt in Zambia, particularly those fruits grown around Kitwe slimes (TD25). Public awareness of the levels of heavy metals in edible fruits (avocado and lemon) grown around TD25 is therefore essential. This study investigated the levels of heavy metals in fruit samples (lemon and avocado) collected from residential areas bordering TD25. Additionally, the study assessed the estimated daily intake of each heavy metal in the sample fruits, in accordance with Food and Agriculture Organization/World Health Organization (FAO/WHO) permissible limits.

2. Materials and Methods

In order to assess the levels of heavy metals in avocado and lemon fruit samples grown at 200 and 400 m away from Kitwe slimes (TD25), fruit and soil samples were collected around the suspected point source (**Figure 1**). Soil and fruit samples

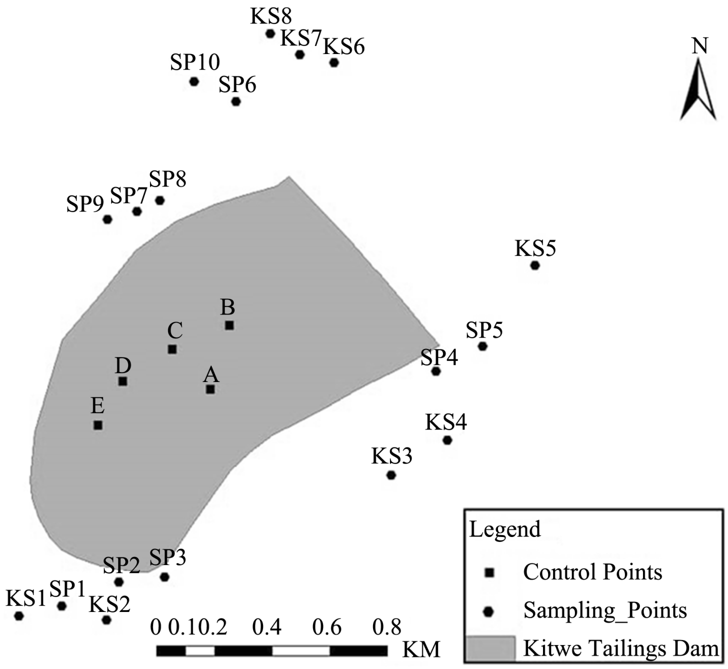

Figure 1. Map of study site showing the location of the Kitwe slimes (TD25) and the sampling points. (Note: SP1 - SP10 represents sampling points within 200 m, while KS1 – KS8 are sampling points within 400 m away from TD25).

were collected within Nkana East and Parklands residential areas of Kitwe. Soil and ripe fruit samples (avocado and lemon) were collected during the ripening season in May 2021. Both samples were taken to the laboratory for analysis.

2.1. Soil Sample Collection

Representative soil samples were collected at three different locations around each fruit tree (avocado and lemon). A total of 18 sampling sites for soil samples were considered, 10 sites within 200 m and 8 sites within 400 m away from TD25. This was achieved by digging into the ground at depths ranging from 30 to 40 cm and the collected soil put into sampling bags. Avocado root systems may extend from 30 to 60 cm, while lemon fibrous root system is concentrated within 30 to 90 cm [19] [21]. The sampling bags were labelled according to their sampling points for easier identification. Two main sets of samples were collected, those around 200 and 400 m away from TD25. Soil samples were also collected on the suspected point source (TD25) at 5 different points (A, B, C, D and E) to act as the control (**Figure 1**).

2.2. Treatment of Soil Samples

The collected soil samples were sieved through standard soil sieves up to 250 µm in order to obtain a homogenous sample. Two grams of each soil sample was put in beakers into which 3 - 4 drops of hydrochloric acid (HCl), [35% purity supplied by LABCHEM] added in order to break-down the silicate in the soil. For decomposition to take place, 30 ml of nitric acid (HNO_3) [purity 69% - 70% supplied by Himedia] was added to each sample, and then heated on a hot plate for 15 minutes. The solution was then diluted with distilled water up to 100 ml using 100 ml volumetric flasks, shaken thoroughly and filtered using Whatman filter paper (grade 91 qualitative filter paper wet-strengthened, 100 circles, 12.5 cm).

The treated soil samples were analyzed for copper (Cu), cobalt (Co), iron (Fe), manganese (Mn) and lead (Pb) using the PerkinElmer Atomic absorption spectrophotometer (AAS) PinAAcle 500. Triplicate tests were conducted for each soil sample.

2.3. Fruit Sample Collection and Treatment

Fruit samples were randomly sampled from each of the avocado and lemon trees. Three ripe fruits were plucked from the opposite ends of each fruit tree. The fruit samples were put in polythene bags and labelled. Similarly, 18 sampling stations as outlined in Section 2.1 were considered for avocado and lemon fruits.

In the laboratory, the fruit samples collected, were washed with distilled water to remove any possible contamination. The samples were cut into slices using a sharp clean knife and then oven-dried at 70˚C for 72 hours. Thereafter, the dried samples were weighed and ground into powder using a pestle and mortar [24].

The powdered samples were then stored in new sampling bags in readiness for laboratory analysis.

Wet digestion method was used to analyze the pulverized fruit samples. 2.5 g of the powdered sample was obtained and put into a beaker. Thereafter, 25 ml of nitric acid was added and the mixture was left for 24 hours for digestion to take place. Thereafter, 15 ml of perchloric acid was added to each mixture earlier prepared and heated until white fumes of perchloric acid appeared. 50 ml of distilled water was then added to each beaker, heated for at least 20 minutes and left to cool. The solution was mixed thoroughly and diluted with distilled water up to 100 ml per sample using a 100 ml volumetric flask and then filtered through Whatman filter paper (grade 91 qualitative filter paper wet-strengthened, 100 circles, 12.5 cm).

The analysis of heavy metal content was determined using the PerkinElmer Atomic absorption spectrophotometer (AAS) PinAAcle 500. Triplicate tests were conducted for each sample.

2.4. Estimated Daily Intake

The estimated daily intake of heavy metals depends on the concentration of heavy metals in the fruit (avocado and lemon) being consumed and the average body weight of the population [25]. The average body weight of 60 kg for adults and 15 kg for children are assumed for most populations globally, for dietary exposure purposes. However, the average body weight for adults may differ for other regions significantly from 60 kg. Estimations of food consumption can be determined through individual and household surveys or approximated through statistical national food balance sheets [26].

The daily intake of heavy metals through the consumption of fruits was calculated using Equation (1) by [25];

$$\text{EDI} = \frac{C_{\text{metal}} \times W_{\text{food}}}{B_{\text{weight}}} \tag{1}$$

where,

C_{metal} is the heavy metal concentration in the food sample (mg·g^{-1}),

W_{food} is the daily average weight of the consumed food sample (g·day^{-1}), and

B_{weight} is the average body weight (kg) with an assumption that the food samples are being consumed by both adults and children.

2.5. Data Analysis

Multivariate analysis of variance (MANOVA) was used to analyze the data on the heavy metal content (Co, Cu, Fe, Mn, and Pb) amongst multiple groups. This method takes into account the intercorrelations among the dependent variables in the analysis, to provide a better protection against the inflation of the probability of type I error and has a greater sensitivity for detecting differences [27].

MANOVA tests were then followed by a series of separate ANOVAs, using the Bonferroni-adjusted significance level of $\alpha' = \alpha/g$ (where g was the number of heavy metals considered in the analysis), to identify whether group differences were present for that variable. Post hoc Tukey tests were conducted as follow-up procedures after significant univariate ANOVA tests with an adjusted significance level of $\alpha' = \alpha/g$. This procedure is recommended because it provides a strict control of experiment-wise type I error rate for all possible pairwise comparisons [27].

When assessing whether heavy metal contents in fruit samples were within the recommended permissible safe limits by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), Hotelling's T^2 test [28] was used. Similarly, the same technique was used for testing the multivariate null hypothesis that the population mean vector of heavy metal content was equal to the mean vector of FAO/WHO permissible limits. Follow-up analyses consisted of constructing 95% simultaneous Bonferroni confidence intervals [28] for all heavy metals.

Preliminary analyses were conducted to assess the validity of the MANOVA and ANOVA assumptions to check for any univariate or multivariate outlier. Univariate outliers were identified as observations with z-scores greater than 3 or less than −3, while multivariate outliers were recognized by their large Mahalanobis values, that is, multivariate outliers' distances exceeding x^2 critical value with g (number of dependent variables) degrees of freedom at the 0.001 significance level [27]. In order to check if the use of MANOVA was justified with the data at hand, bivariate correlations were examined and the Bartlett's test of sphericity was conducted.

For all analyses conducted, neither univariate nor multivariate outliers were detected as all z-scores in absolute values were less than 3, and all Mahalanobis distances were less than the x^2 critical value with g (number of dependent variables) degrees of freedom at the 0.001 significance level. In addition, the Bartlett's test of sphericity was significant at the 5% significance level (all p-values < 0.05), suggesting that there were sufficient correlations between dependent variables to justify the use of MANOVA.

All analyses were conducted using the Statistical Package for Social Sciences (SPSS) version 20 with a significance level of $\alpha = 0.05$.

3. Results and Discussion

3.1. Heavy Metal Content in the TD25 Soil, Lemon and Avocado Soil Samples

The analyses in this section were to investigate the heavy metal contents in TD25 and fruit tree soil samples and to check whether the distance from TD25 had any effect on the concentrations of heavy metals in soils. The results of the analysis of the TD25 soil samples at the five sampling points suggest that the concentration levels of heavy metals were significantly different (MANOVA and ANOVA p-values < 0.05; Tukey p-values < 0.01), while the comparison of the heavy metal content for avocado and lemon soil samples at both 200 and 400 m away from TD25 showed that group differences were not statistically significant (all p-values > 0.05).

Concerning the comparison of the soil samples at 200 and 400 m, the results show that the concentration levels of heavy metals in (lemon and avocado) soil samples within 200 and 400 m were not the same (MANOVA p-values = 0.00). A further investigation using the t-tests and the 95% t-confidence intervals (not reported) found that the mean concentrations of Co and Pb in lemon soil samples at 200 m were less than those at 400 m. For avocado soil samples, the mean concentration of Pb at 200 m was lower than that at 400 m, while Mn concentration was higher at 200 m than at 400 m. [24] obtained similar results for soil and mango fruit samples around a mine tailings dam, though the influence of distance on heavy metals from suspected point source was not investigated.

3.2. Comparison of Heavy Metal Content in Soil and Fruit Samples

A comparison of heavy metal content in soil samples with that in fruit samples was made to ascertain the level of abstraction of the heavy metals from the soil through the roots of the trees up to the fruits. At both distances of 200 and 400 m away from TD25, significant group differences of the concentration of heavy metals in soil and fruit samples were found (all p-values < 0.05). Following significant MANOVA tests, post hoc analyses, based on the 95% confidence intervals (CI) of the mean differences of heavy metal content in soil and fruit samples were done (see **Table 1**). In **Table 1**, the mean differences, and lower and upper bounds of the confidence intervals were all positive indicating that heavy metal contents in (lemon and avocado) soil samples were very high as compared to those in (lemon and avocado) fruits at both locations. [29] investigated Cd, As, Cr and Pb in mangoes and found all the four heavy metals below the acceptable limits. [9] found that levels of concentration for Cd, Ni and Pb in plant samples

Table 1. Confidence intervals (CI) of the mean differences of heavy metal content in soil and fruit samples at 200 and 400 m away from Kitwe slimes (TD25).

Heavy metal	Distance at 200 m from TD25			Distance at 400 m from TD25		
	Mean difference	95% CI		Mean difference	95% CI	
		Lower	Upper		Lower	Upper
Fe (lemon)	4.09	3.27	4.68	5.03	4.38	5.73
Fe (avocado)	4.32	3.60	5.03	4.48	3.62	5.38
Cu (lemon)	3.64	2.51	4.76	4.20	3.50	4.89
Cu (avocado)	3.63	3.35	3.91	3.54	3.31	3.78
Mn (lemon)	2.47	1.47	3.46	2.75	2.09	3.40
Mn (avocado)	2.62	1.70	3.55	2.35	1.27	3.44

were higher than in soil samples. Similarly, [10] found that out of the 7 considered heavy metals in the soil, only Zn was below the WHO permissible limits. The results in the current study could suggest that the levels of abstraction of heavy metals from the soil up to the fruits by avocado and lemon trees were very low (see **Table 4** and **Table 5**).

3.3. Heavy Metal Content in Fruit Samples

In this section, heavy metal contents in lemon and avocado fruits were investigated. The MANOVA tests for comparing heavy metals in avocado with those in lemon fruits at both 200 and 400 m were significant (all p-values < 0.05).

Post hoc analyses based on the t-confidence intervals with 95% confidence level (see **Table 2**) indicated that the concentration levels of Fe, Cu, and Mn in lemon fruits were lower than those in avocado fruits at 200 m away from TD25 as suggested by negative mean differences, lower and upper bounds of the confidence intervals at 200 m. For fruits at 400 m, the mean concentration of Fe in lemon was higher than that in avocado, while the concentration of Cu was lower in lemon as compared with that in avocado. No significant difference was found between the concentration of Mn in lemon and in avocado. Variations in heavy metal ion uptake or translocation from plant root system upwards to the fruits have been reported by [24] (soil and mango); [22] (4 varieties of mangoes); [23] (fruits and vegetables).

Confidence intervals for the mean differences of heavy metal contents for fruits at 200 m and those at 400 m (not reported) suggested that, apart from Fe for both lemon and avocado fruits, the distance from TD25 did not have any effect on the concentration levels for other heavy metals.

3.4. Comparison of Heavy Metal Contents in Soil and Fruit Samples Using the FAO/WHO Limits

The Hotelling's T^2 test was significant for all analyses (all p-values = 0.000), indicating that the mean vectors of concentration levels in soil and fruits at both locations were highly statistically different from the WHO/FAO permissible limits. In addition, the 95% Bonferroni simultaneous confidence intervals in **Table 3** had lower and upper bounds less than the WHO/FAO limits for all heavy

Table 2. Confidence intervals (CI) of the mean differences of heavy metal contents in lemon and avocado fruit samples at 200 and 400 m away from Kitwe slimes (TD25).

Heavy metal	Distance at 200 m from TD25			Distance at 400 m from TD25		
	Mean difference	95% CI		Mean difference	95% CI	
		Lower	Upper		Lower	Upper
Fe	−0.09	−0.16	−0.02	0.06	0.01	0.11
Cu	−0.11	−0.12	−0.09	−0.04	−0.12	−0.01
Mn	−0.09	−0.17	−0.01	0.02	−0.02	0.05

metals, suggesting that the concentration levels of heavy metals in soil and fruit samples at 200 m (and equally at 400 m, results not shown) were well below the permissible WHO/FAO limits.

3.5. Estimated Daily Intake (EDI)

The amount of consumer exposure to heavy metals and other health related risks may be expressed in terms of estimated daily intake (EDI). Each heavy metal concentration for both fruits was computed at 200 and 400 m away from TD25 (Table 4 and Table 5) using Equation (1).

The highest concentration of heavy metals in avocado fruits (Table 4 was that for Fe at 200 m (0.53 mg·kg^{-1}), with an estimated daily intake of 0.22 mg·kg^{-1}, compared with a standard limit range of 12.50 - 19.60 mg·kg^{-1}. This result (EDI

Table 3. 95% Bonferroni simultaneous confidence intervals for heavy metals at 200 m away from Kitwe slimes (TD25).

Heavy metal	WHO/FAO limit (mg·kg^{-1})	Lemon		Avocado	
		Lower	Upper	Lower	Upper
Fe	50	3.15	5.41	3.61	5.58
Cu	75	2.13	5.24	3.40	4.17
Co	50	0.12	0.31	0.08	0.29
Mn	20	1.15	3.88	1.49	4.03
Fe	0.5	0.16	0.22	0.21	0.35
Cu	0.8	0.04	0.06	0.14	0.17
Mn	2.0	0.05	0.05	0.04	0.24

Table 4. Estimated daily intake (EDI) of heavy metals in avocado fruit samples grown around 200 and 400 m away from Kitwe slimes (TD25).

Heavy metal	Concentration (mg·kg^{-1})	Estimated daily intake (mg·kg^{-1})	Standard estimated daily intake for fruits (mg·kg^{-1})
Co (200 m)	0.00	0.00	0.05 - 1.00
Co (400 m)	0.00	0.00	0.05 - 1.00
Cu (200 m)	0.19	0.08	3.00
Cu (400 m)	0.31	0.13	3.00
Fe (200 m)	0.53	0.22	12.50 - 19.60
Fe (400 m)	0.32	0.13	12.50 - 19.60
Mn (200 m)	0.26	0.11	2.00 - 5.00
Mn (400 m)	0.06	0.03	2.00 - 5.00
Pb (200 m)	0.00	0.00	0.21
Pb (200 m)	0.00	0.00	0.21

Table 5. Estimated daily intake (EDI) of heavy metals in lemon fruit samples grown around 200 and 400 m away from Kitwe slimes (TD25).

Heavy metal	Concentration ($mg·kg^{-1}$)	Estimated daily intake ($mg·kg^{-1}$)	Standard estimated daily intake for fruits ($mg·kg^{-1}$)
Co (200 m)	0.00	0.00	0.05 - 1.00
Co (400 m)	0.00	0.00	0.05 - 1.00
Cu (200 m)	0.06	0.03	3.00
Cu (400 m)	0.08	0.03	3.00
Fe (200 m)	0.29	0.12	12.50 - 19.60
Fe (400 m)	0.33	0.14	12.50 - 19.60
Mn (200 m)	0.06	0.03	2.00 - 5.00
Mn (400 m)	0.11	0.05	2.00 - 5.00
Pb (200 m)	0.00	0.00	0.21
Pb (200 m)	0.00	0.00	0.21

of 0.22 $mg·kg^{-1}$ for Fe) was well below the allowable limit, an indication that there were no related health risks due to possible heavy metal intake from fruits grown around TD25. A similar study on assessment of EDI for 4 different heavy metals found that Cd and Pb in rice and vegetables, exceeded permissible limits [25]; while [5] reported health risks among local children due to contamination from Cd, As and Pb. In the current study, all EDI results for avocado fruits grown around 200 and 400 m away from TD25 were below the acceptable limit.

Similarly, the highest concentration of heavy metals in lemon fruits (see **Table 5**) was for Fe with an estimated daily intake of 0.14 $mg·kg^{-1}$, a result far below the permissible limit. The results for the EDI of heavy metals in avocado and lemon fruits grown at 200 and 400 m away from the suspect point source (TD25) were all within the permissible levels according to FAO/WHO guidelines.

4. Conclusion

This study showed that avocado and lemon fruits grown within 500 m away from Kitwe slimes (suspected point source) were found to be safe from possible heavy metal contamination and therefore fit for human consumption. The investigated heavy metals were Fe, Co, Cu, Mn and Pb in the soil as well as in fruits. Distance from the suspected point source had no influence on the concentration of the considered heavy metals in the soil around the fruit tree samples. Generally, the concentration levels of the heavy metals followed a trend in the order: Kitwe slimes soil > Soil samples around fruit trees > Fruit samples. Understanding the concentration levels of heavy metals in soils and plants around copper mine tailings (Kitwe slimes) is key for environmental management. This knowledge is also important in ensuring food safety and minimizing possible risks to human health.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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