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## **Research Article**

## Copper levels in three commonly edible fruits: Are consumers at risk?

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## Abstract

This study aimed to investigate levels of copper in three commonly edible fruits, namely, Lycopersicon esculenta (tomato), Citrullus lanatus (watermelon) and Cucumis sativus (cucumber) traded in Dar es Salaam city. Also, the study scrutinized risk associated with heavy metal intake in the fruits. The fruits were collected from Buguruni Market, one of the biggest markets in the city, and thereafter, were processed, and analyzed to determine copper levels. The average daily intake and human health risk indices were calculated based on the obtained copper levels. The findings revealed that the mean levels and average daily intake in *L. esculenta* (0.3267 mg kg<sup>-1</sup>, 0.0279 mg kg<sup>-1</sup> person<sup>-1</sup>) and *C. lanatus* (0.2523 mg kg<sup>-1</sup>, 0.0216 mg kg<sup>-1</sup> person<sup>-1</sup> <sup>1</sup>) did not differ considerably (p = 0.05) from each other while the two fruits had significantly higher values than *C. sativus* (0.1610 mg kg<sup>-1</sup>, 0.0137 mg kg<sup>-1</sup> person<sup>-1</sup>). The copper levels and average daily intake values were below WHO/FAO and Tanzania Bureau of Standards permissible limits. The human health index (HRI) was in the order: L. es*culenta* > *C. lanatus* > *C. sativus* and all the values were less than unit, suggesting that there is no health risk from consuming the fruits. Concerning copper levels, the study concludes that the fruits are safe for human consumption.

## Introduction

Copper (Cu) is an indispensable micronutrient for humans and animals (Babaali et al., 2020; Bost et al., 2016; Chen et al., 2020). The metal is required for numerous physiological and biochemical processes and functions. For instance, Cu plays a role as a biocatalyst (Escobar et al., 2017; Murugappan and Sreeram, 2021), which is vital for body pigmentation, upholding central nervous system and preventing anemia (Angelova et al., 2011). It plays a vital role in human metabolism by allowing many critical enzymes to function properly (Bost et al., 2016). Cu is essential for maintaining the proper functioning of the thyroid gland and production of melanin, myelin and hemoglobin. Also, it maintains strength of arteries, veins and capillaries, skin, synovial and epithelial membranes (Bhattacharya et al., 2016). Cu acts

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as both anti-oxidant and pro-oxidant in human body (Rahal et al., 2014). It scavenges or neutralizes free radicals and helps by reducing or preventing any damage that can be caused by radicals. As pro-oxidant, the metal sometimes promotes damage of free radicals, hence causing Alzheimer's disease (Bagheri et al., 2017; Rembach et al., 2013). When Cu surpasses permissible safe limits in foods and average daily intake level, it is likely to cause hypertension, sporadic fever, coma, stomach and intestine irritation as well as liver and kidney damage in humans (Bermudez et al., 2011). On the other hand, low Cu levels in humans have detrimental effects too due to the nature of the roles it plays (Jaishankar et al., 2014).

Various studies conducted in Dar es Salaam (Othman, 2001; Sahu and Kacholi, 2016) have shown that poor agricultural practices are the main cause of contaminating the vegetables and fruits with heavy metals. On the other side, the use of chemical fertilizers, pesticides, herbicides, using the waste water from industries flowing as streams in the nearby urban farms are responsible factors for the increasing levels of heavy metals in soil, water and ultimately get transferred to the agricultural produces. Additionally, in urban areas, heavy traffic movement in the highways contaminate soil with heavy metals (Kacholi & Sahu, 2018) and sometimes fruits and vegetables are cultivated along dumpsites and irrigated with water polluted with leachates run-offs (Kihampa et al., 2011), a practice that endangers urban population who consume fruits and vegetables.

The increasing anthropogenic activities associated with metal dispensation and the hitor-miss disposal of domestic wastes, calls for a research on the heavy metal pollution in fruits marketed in Dar es Salaam because some of the fruits sold at markets in Dar es Salaam are grown near metal polluted areas (Kihampa et al., 2011). Due to difficulties in establishing levels of Cu from each area of Dar es Salaam, commonly consumed fruits were collected from Buguruni Market. The market is among the biggest and highly dependable markets of the city that sells fresh vegetables and fruits from various parts of the city. Therefore, this study was undertaken to investigate the levels of Cu in the three commonly eaten fruits, namely; tomato (*Lycopersicon esculenta*), watermelon (*Citrullus lanatus*) and cucumber (*Cucumis sativus*) sold at Buguruni Market. Moreover, average daily intake and human health risk index was calculated based on the fruit consumption.

## Materials and methods Collection and preparation of samples

The three studied fruits, namely Tomatoes, watermelon and cucumber were obtained from Buguruni Market between June 2018 and August 2018. The market is located in Buguruni ward in Ilala district. The market is among the most popular markets in Dar es Salaam city. It attends a total population of 67,028 of the Buguruni ward and those from nearby areas. The collected fruit samples were kept in plastic bags and transported to the laboratory for processing and analysis. In the laboratory, the fruits were thoroughly washed using purified water to remove specks of dusts, dirty and some surface pollutants. The fruits were peeled off, edible part sliced-of and dried at room temperature on sheets of paper for two hours to remove moisture. Before drying the samples at 80 °C for 72 hours to a constant weight, they were weighed. Each dry samples were then powdered and sieved using a 2 mm mesh sieve. Thereafter, the samples were kept at room temperature in clean, dry stoppered glass containers ready for further analysis.

## Sample digestion - dry ashing

The dry ashing method was used to destruct all organic matter present in samples. 1 g of each powdered samples were accurately measured in acid washed clean porcelain crucibles and ashed for 12 hours at 500°C in a muffle furnace. Thereafter, ashes samples cooled at room temperature. The ashes were then digested in 5 mL of 20 % (v/v) hydrochloric acid. The solution was warmed slowly in a steam bath for about 15 minutes to dissolve any residues. The solution was filtered through an acidwashed Whatman filter paper No 42 into a 50 mL volumetric flask. The filter paper was washed with distilled water and washings collected in the volumetric flask. The resulting solution was diluted to the 50 mL mark with distilled water, well mixed and used for determination of Cu in fruits samples.

#### Atomic absorption spectrophotometer (AAS)

Determination of copper levels was done using the Atomic Absorption Spectrophotometer, Perkin-Elmer 2380 model. Used analytical technique is the same as described in Kacholi & Sahu, (2018) and Sahu and Kacholi, (2016).

#### Quality assurance analysis

Quality control measures in studying procedure was undertaken to endorse the accuracy of the data. The standard solutions were prepared and used as per AAS instruction manual. In all valuations, a standard air-acetylene flame was used. A wavelength of 324.8 nm for Cu, the corresponding slit widths of 45  $\mu$ m and 10 mA lamp current were used.

#### Average daily intake (ADI)

For the purpose of evaluating risk of any contaminant, it is categorically compulsory to estimate a level of exposure. The evaluation of an average daily intake (ADI) is a very crucial aspect of such estimation. The ADI is calculated as a product of average daily intake per person, percentage of fruit dry weight and average heavy metal level per dry weight fruit (Equation 1).

$$ADI = AV_{consumption} \ge \% DW_{fruit} \ge C_{heavy metal} \dots (1)$$

Whereby: ADI is an average daily intake of heavy metal per person per day (mg person<sup>-1</sup> day<sup>-1</sup>), Av<sub>consumption</sub> is average daily consumption of fruit per person per day (g day<sup>-1</sup>); % DW<sub>fruit</sub> is percentage dry weight of fruit (% DW = [100-% Moisture)/100]) and C <sub>heavy metal</sub> is average heavy metal concentration of dry weight fruit (mg kg<sup>-1</sup>). The average daily consumption of fruits in human diet of 400 g per person and an average weight of person of 60 kg were considered in this study (World Health Organization, Food and Agriculture Organization, 2011).

#### Human health risk index (HRI)

The human health risk index (HRI) is calculated as a fraction of an average daily intake (ADI) to the reference dose (Equation 2). This HRI is used to determine human health risk associated with consumption of metal-contaminated fruits. When the HRI is less than one

Where; ADI is average fruit intake per day (mg kg<sup>-1</sup> day<sup>-1</sup>), and  $R_fD$  is the oral reference dose of the metal (mg kg<sup>-1</sup> day<sup>-1</sup>). The  $R_fD$  for Cu is 0.04 mg kg<sup>-1</sup> day<sup>-1</sup> [14].

#### Statistical analysis

The one-way ANOVA test followed by the multiple comparison Tukey *post hoc* test were used to compare the levels of copper between fruits, at 5% significance level. All the data were processed using the QED statistics and Microsoft Excel software.

## **Results and discussion** *Copper levels in the fruits*

There was significant differences in the mean copper levels between the fruits (F =14.42, df<sub>1</sub> = 2, df<sub>2</sub> = 24, p < 0.05) and average daily intake (F = 14.49,  $df_1 = 2$ ,  $df_2 = 24$ , p < 0.05). The mean levels and ADI in L. esculenta and *C. lanatus* were significantly higher than that of C. sativus. The L. esculenta and C. lanatus did not differ considerably in terms of mean copper levels and ADI (Table 1). The observed Cu levels in the present study are lower than the WHO/FAO and TBS permissible levels in food stuffs (40 mg kg-1 dry weight) and fruit juices (2.0 mg kg<sup>-1</sup>) (Kihampa et al., 2011). Other studies (Kirmani et al., 2011; Lugwisha and Othman, 2016) conducted elsewhere have recorded copper levels below permissible limits in studied fruits like L. esculenta. Variation in the levels of the copper among the studied fruits may possibly be due to the disparity in the metal soil-plant transfer coefficients. The transfer coefficient is actually influenced by factors like soil pH (Gupta et al., 2013; Tasrina et al., 2015), metal bioavailability, quantity of metal in soil, plant absorption capacity and plant growth rate, and the interaction of other heavy metals, for instance selenium, in growing areas (Mubofu, 2012) as well as ability of plant roots to penetrate where the heavy metals are found (Osredkar & Sustar, 2011). Moreover, the variation could be due to agronomic practices such as application of fertilizers and water managements on growing the fruits (Angelova et al., 2011; Mubofu, 2012). Low copper values were also reported in other various studies done elsewhere (Kacholi and Sahu, 2018; Lugwisha and Othman, 2016). During the investigation of Cu in the present study, other heavy metals like Lead (Pb) and Cadmiun (Cd) were analyzed but found to be below detection limit of 0.01 mg kg<sup>-1</sup>.

#### Average daily intake (ADI)

Determining heavy metal exposure level is crucial in assessing human health risk (Yu et al., 2021). The exposure is well expressed by the ADI. From the findings, the ADI values (Table 1) show that Cu is consumed below the permitted maximum tolerable daily intake (PMTDI) of 2.0 mg person<sup>-1</sup> day<sup>-1</sup> recommended by WHO/FAO. The order of contribution for the Cu intake is in the order of *L. esculenta* > *C. lanatus* > *C. sativus*. This is to say that consumers of the fruits are not at health risk.

Table 1. Mean copper levels (mg kg<sup>-1</sup>) and average daily intake (ADI, mg person<sup>-1</sup> day<sup>-1</sup>)

Common Name	Scientific Name	Mean ± SD	ADI ± SD
Tomato	Lycopersicon esculenta Mill	$0.3267 \pm 0.1073^{a}$	$0.0279 \pm 0.0091^{a}$
Watermelon	Citrullus lanatus (Thunb.) Matsum. & Nakai	$0.2523 \pm 0.0358^{a}$	$0.0216 \pm 0.0030^{a}$
Cucumber	Cucumis sativus L.	$0.1610 \pm 0.0087^{b}$	$0.0137 \pm 0.0080^{b}$

## Health risk index (HRI)

The HRI-based risk estimation method offers an early signal of the risk level due to exposure to contaminants (Hossain et al., 2015; Karimi et al., 2020; Mahmood and Malik, 2014). The findings of this study show that the calculated values of HRI were in the order of *L. esculenta* > *C. lanatus* > *C. sativus* (Figure 1). In all the three fruits, the HRI's were less than unit (< 1) signifying that no health hazard to the consumers for consuming the studied fruits. Normally, when HRI surpasses one, indicates presence of potential health risk/effects from consuming the fruits (Chen et al., 2014; Doležalová Weissmannová et al., 2019; Yu et al., 2021). Though no observed health risk linked with consumption of the metal in the studied fruits, the potential risks can multiply when other heavy metals are taken into account or when the fruits are consumed over time. Thus, it should be noted that, the HRI method used in this study reflected only exposure to Cu through consumption of the fruits and does not take into account any other exposure pathways.



Figure 1. Hazard quotients of copper per fruit

## Conclusion

The findings reported in this study show that Cu levels in the three common edible fruits were below the WHO/FAO and TBS permissible limits. Also, the results showed the significant variation in the Cu levels. The mean Cu levels in *L. esculenta* and *C. lanatus* did not differ significantly but the two fruits had significantly higher Cu levels than C. sativus. The calculated average daily intake for the three fruits revealed the values to be lower than the WHO/FAO recommended levels. The average daily intake of the metal among the three fruits differed significantly whereby the values for *L*. esculenta and C. lanatus did not differ appreciably but the two fruits revealed considerably high values than *C. sativus*. The human health indices (HRI) for each fruit were less than unit and were in the order of *L. esculenta* > *C. lanatus* > *C. sativus*. By being lower than unity (< 1), the findings suggest absence of health risk from consuming the three fruits. Therefore, as far as copper levels is concern, the studied fruits are safe for human consumption. The findings of this study are imperative to both consumers and policy makers.

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#### Author's declaration and contribution

Authors declare that they have no conflict of interest. M. S (Senior Lecturer and Researcher) and D. S. K (Senior Lecturer and Researcher) conceived the presented idea and both conducted field work. M. S did the laboratory analysis of the collected samples while D. S. K performed the computations, analyzed the data statistically and composed this article. Both authors read and approved the manuscript for publication consideration.

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