ASSESSMENT OF HEALTH RISKS ASSOCIATED WITH CITRUS FRUITS EXPOSED TO HEAVY METAL TOXICITY IN DIVERSE IRRIGATION REGIMES

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Abstract

Heavy metals play a very important role in plant development, but exposure to these essential compounds at higher concentrations cause severe toxic effects in plants. Heavy metals are regarded as fundamental food supply pollutants due to their endurance, biomagnification, and non-biodegradability. Elevated trace metal levels in the diet imply a possible risk to human and environmental health. Despite that, these trace metals are an important part of our food. The present study was conducted at Telsil Sargodha (Site FW-I and SW-II), Punjab, Pakistan to analyze Copper (Cu), Cobalt (Co), Nickel (Ni) and Zinc (Zn) concentrations in citrus fruits. The samples were analyzed with the help of atomic absorption spectrophotometer. The assessment of the bio-concentration factor (BCF), daily intake of metal (DIM), pollution load index (PLI), enrichment factor (EF), and health risk index (HRI) were carried out in the present findings. The EFs of Co and PLIs of Copper were remarked as more than 1 while other indices were found less than 1 for Cu, Co, Ni and Zn, indicated that fruits cultivated in water rich soil were not harmful, therefore heavy metal analysis was necessary to assess the extent of environmental contamination.

Key words: Sargodha; HRI; DIM; Metal contamination; PLI.

Introduction

The Rutaceae family includes the genus Citrus, represented by flowering shrubs and trees. Plants in the genus Citrus produce citrus fruits like lemons, limes, oranges, pomelos, and grapefruits. Citrus is native to Australia, East Asia, Southeast Asia, South Asia, and Mediterranean region (Zech-Matterne, et al., 2018). In south Asia, citrus fruits account for about one-third of total fruit export value. The main developed citrus crops are mandarins, oranges, grapefruits, limes and, lemons which have been widely known for their nutritive worth (Satari & Karimi, 2018).

Heavy metals are considered essential contaminants in the food chains because of their persistence in the environment. Both humans and environment are potentially at risk due to elevated levels of these metals (Khan et al., 2019a, Raja et al., 2016). Assessment of risk is generally concerned with likelihood of any risk capable of connecting with exposure to pollutants. Human health risk assessment includes gathering, identifying, and integrating information about hazardous pollutant exposure and its negative health effects (Sobhanardakani, 2017a, 2017b).

The quantity of dangerous smoke and pollutants emitted into the atmosphere is strongly correlated with the number of heavy cars on the roads or highways. Some of these toxins are copper, chromium, cadmium, nickel, arsenic and lead which, although occasionally being helpful, pollute the environment (Chen et al., 2021, Suvarapu & Baek, 2017). Cu is one of these contaminants, and while it is occasionally useful, it also pollutes the environment (Chen et al., 2021).

In fact, the deficiency of copper, phosphorus, and manganese caused by zinc toxicity in plants is defined by the typical purple-red hue of foliage (Bhalakiya et al., 2019). In addition, zinc limits the development of soil microorganism by altering their shape and metabolic activity (Baran et al., 2018). Because of the toxicity, abundance, non-biodegradability and accumulative nature of heavy metals, their contamination and accumulation pose a severe risk to human community worldwide. As a result of rapid industrialization, global trade and several other anthropogenic activities, the variety of environmental contaminants has substantially increased (Khan et al., 2019a, Töth et al., 2016). Human activity in areas without household sewage or trash processing facilities may lead to discharge of metals into the environment. The fruits may become contaminated with trace and hazardous metals. As a result, human health is effected by this contamination (Ngo et al., 2021).

Wastewater may be more reliable for irrigation than rainfall or groundwater in terms of availability and nutrient supply (Chaganti et al., 2020, Martínez-Cortijo & Ruiz-Canales, 2018). Despite the availability of freshwater in some areas, farmers prefer wastewater to irrigate the fields because of high yield (Deh-Haghi et al., 2020).
Many landowners in Pakistan employ eccentric water resources (treated and untreated) for forestry and farming due to the state’s arid climate and lack of freshwater. City discharge, industrial effluent, wastewaters, and tainted canal water are important sources of irrigation water that several farmers have explored as an alternate supply of water. In practically every community across the country, this method is growing quickly. Wastewater has thus evolved into a practical method for lowering shortage of water (Hassan et al., 2013, Riaz et al., 2022, Ugulu et al., 2019a).

The objective of this study was to analyze the bioconcentration of cobalt, nickel, zinc and copper metal in soil, fruits and water and to evaluate the risks posed to humans. The metal flow in the food chain can be assessed by examining the metal profile in the collected samples. This research also aimed to assess the mode of transfer of these trace metals in water–soil–fruits continuum with hazard risk assessments.

Material and Methods

Area of research: Sargodha is the third largest division in Punjab province of Pakistan. Sargodha district spreads over 5,864 square kilometers. Sargodha has a latitude of 32.082 and a longitude of 72.669 (Fig. 1). The average temperature of the area fluctuates between 42-105 °F. Sargodha has mostly fertile, flat plains, with only a few small hills along the Sargodha-Faisalabad Road (Khan et al., 2019b, Khan et al., 2019c).

Sample collection: The soil, water and plant samples were gathered from two different sites arbitrarily. Samples were collected from Chak 75 N.B. (named as Site-FW-I irrigated with fresh water) and from Risala No. 5 (named as Site-SW-II irrigated with sewage water) (Table 1).

Table 1. Botanical and common names of analyzed fruits.

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>English name</th>
<th>Botanical names</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Sweet lime</td>
<td>Citrus limetta</td>
</tr>
<tr>
<td>2.</td>
<td>Sweet orange</td>
<td>Citrus sinensis</td>
</tr>
</tbody>
</table>

Water and soil sampling: For collection of water samples, at each site, 500 mL plastic containers were used. The soil samples were gathered from selected sites and saved in polythene bag. The containers were sealed tightly, put in an ice box (5°C), and then brought to the lab to be tested for EC (electrical conductivity) and other physicochemical characteristics. After that, the water samples were filtered and refrigerated before the analysis of metals.

Fruit samples: The samples of edible portion of fruits (C. limetta and C. sinensis) were collected from the selected sites. Each sample was chosen randomly, sealed in a particular brown envelope, labelled, and transferred to the research lab for further evaluation. Each sample included three replicates.

Soil and fruit sample preparation: Soil and fruit (edible portion) samples were left to dry in the air before being placed in an oven for 3 days at 72°C. Samples were air-dried until no moisture content remained and weighed using an electronic balance. The samples were crushed into a powder and kept in desiccators at room temperature. The samples were digested using the wet acid digestion method (Abbasi et al., 2016, Sun et al., 2017).

Wet acid digestion and analysis of samples: To ensure complete solubilization, three replicates of samples of soil, water, and fruits were digested in an acid mixture. Approximately, 1 ml water sample and 1.0 g of the powdered samples of soil and fruit were treated with 10 mL of concentrated HNO₃ (65%) and left unheated for overnight. Next day, the mixture was heated on electric burner for 70°C till evaporation took place (Abbasi et al., 2015, 2016).

The mixture was cooled to room temperature and 5 ml of undiluted HClO₄ (70%) was added. The mixture was then reheated at 70°C until thick, white fumes began to appear, signifying that the digestion was complete. Whatman paper # 42 was then used to filter the samples. The digested samples were put in a flask of 50 mL and filled on-to the proper level with 0.1 N HNO₃ (Parveen et al., 2020). The following metals were evaluated i.e., Cu, Co, Ni and Zn was explored with atomic absorption spectrophotometer (Añón & Calvelo, 1980).

Fig. 1. Map of study area.
Evaluation of metal profile by statistical analysis: The data of soil, fruits and water samples was statistically analyzed. SPSS 23 software and Graphed Prism Pad was used to determine variance and correlations.

Pollution load index: The pollution load index is established on the rate of the metal in the soil, seeks to provide an estimate of the pollution status. The following formula (1) was used in this study according to Liu et al., (2005).

\[
\text{PLI} = \frac{\text{Metal, content in tested soil}}{\text{Metal, reference value for soil}}
\]  (1)

Reference values for cobalt, nickel, copper and zinc were 9.1, 9.06, 8.39 mgkg\(^{-1}\) (Singh et al., 2010) and 44.19 mgkg\(^{-1}\) (Hassan et al., 2013) respectively.

Bio concentration factor: It was calculated using the following formula by Akhtar et al., (2022).

\[
\text{BCF} = \frac{\text{MV (Fruits)}}{\text{MV soil}}
\]  (2)

where,
\(\text{M}_i (\text{Fruits}) = \text{Metal value. (mgkg}^{-1}\) in fruits; \(\text{M}_i (\text{Soil}) = \text{Metal value. (mgkg}^{-1}\) in soil

Enrichment factor: EF is calculated by following formula (Ahmad et al., 2016).

\[
\text{EF} = \frac{\text{MV in sampled fruit/MV in sampled soil}}{\text{MV \left( \frac{\text{Fruit}}{\text{Soil}} \right) Standard value}}
\]  (3)

| Table 2. Standard concentrations of metals. All values are according to FAO/WHO (2010). |
|-----------------|-----------------|
| **Heavy metals** | **Fruits**       |
| Cu              | 0.05-0.5        |
| Co              | 2               |
| Ni              | 2               |
| Zn              | 60              |

Daily intake of metal (DIM): There is a diversity of pathways for heavy metals to accumulate in human body, including through skin contact, inhaling, and eating contaminated forages (Table 2). The regular consumption of metals was calculated using the following formula (Shahid et al., 2015).

\[
\text{DIM} = \frac{\text{C x F x D food intake}}{\text{W}}
\]  (4)

C represents the concentration of metal in plants, F represents conversion factor, \(D_{\text{food intake}}\) represents fruits intake per day, and W is Average body weight of humans (Table 3).

Health Risk Index (HRI): Health index was utilized to assess the potential metal exposure that might happen if humans consumed the sampled citrus fruit samples (Citrus limetta and Citrus sinensis). The daily metal intake (DIM) in fruit products separated by the oral reference dosage is used to determine HRI (Khan et al., 2020b).

\[
\text{HRI} = \frac{\text{Daily intake of metals (DIM)}}{\text{Oral reference dose (RfD)}}
\]  (5)

RfD for Co, Ni, Zn and Cu was 0.043 (Campbell, 2004, Trumbo et al., 2001), 0.02, 0.37 and 0.04 mg/kg (USEPA, 2011) respectively.

Results

Metal concentration (mgL\(^{-1}\)) in irrigation water: The mean concentration of Cu, Co, Ni and Zn in water varied from 0.062 to 0.37 mg/L, 0.08 to 0.14 mg/L, 1.54 to 2.24 mg/L and 0.56 to 0.761 mg/L respectively. The means of Cu, Co and Zn concentration was found higher in water at SW-II compared to FW-I, while highest level of Ni was found in water samples at FW-I (Tables 4, 5).

Metal concentrations in soil samples: The results from analysis of the variance of data revealed that the concentration of Cu was non-significant while Ni and Zn concentrations were highly significant \(p<0.001\) and the concentrations for Cu, Co, Ni and Zn ranged from 0.24 to 0.39 mg/kg, 0.10 to 0.36 mgkg\(^{-1}\), 0.062 to 0.37 mgkg\(^{-1}\) and 0.23 to 1.52 mgkg\(^{-1}\) respectively. The concentrations of Cu and Co were highly significant \(p<0.001\) but Ni and Zn were non-significant (\(p>0.05\)) (Tables 8, 9).

Pollution load index for Cu, Co, Ni and Zn: The values of PLI fluctuated from 0.76 to 1.24 for Cu, 0.02 to 0.047 for Co, 0.032 to 0.56 and 0.019 to 0.054 for zinc (Table 10). Soil of C. limetta at SW-II confirmed elevated PLI value (1.24) for Cu compared to others. While, least value detected was 0.021 in the soil of C. sinensis at FW-I for Co.

Bio concentration factor for copper, cobalt, nickel and zinc: The BCF values for Cu, Co, Ni and Zn varied from, 0.025 to 0.062 mgkg\(^{-1}\), 0.53 to 0.98 mgkg\(^{-1}\) for Cu, 0.10 to 0.75 and 0.16 to 0.66 respectively (Table 11). BCF content of Zn (0.66 mg/kg) in C. limetta was higher at SW-II compared to other samples.

Enrichment Factor for copper, cobalt, nickel and zinc: The values for EF fluctuated among 0.42 to 1.047 for Cu, 2.43 to 4.46 for Co, 0.34 to 3.39 for Ni and 0.12 to 0.49 for Zn (Table 12). Cu metal showed the highest Enrichment Factor (1.047) at site SW-II in C. sinensis.

Daily intake of copper, cobalt, nickel and zinc: DIM of Cu, Co, Ni and Zn had variation among all locations. The values of daily intake in this study for Cu varied from 0.0041 to 0.014 mgkg\(^{-1}\)day\(^{-1}\), 0.0090 to 0.015 mgkg\(^{-1}\)day\(^{-1}\) for Co, 0.002 to 0.014 mgkg\(^{-1}\)day\(^{-1}\) for Ni and for Zinc 0.0091 -0.041 mgkg\(^{-1}\)day\(^{-1}\). Highest values of daily intake were detected for zinc (0.059 mgkg\(^{-1}\)day\(^{-1}\)) at SW-II in C. limetta, while lower values were noticed for Ni (0.002 mgkg\(^{-1}\)day\(^{-1}\)) at site FW-I in C. limetta (Table 13).
Table 3 Values for the daily food intake, conversion factor and average body weight.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>D&lt;sub&gt;food intake&lt;/sub&gt;</th>
<th>Conversion factor</th>
<th>Average body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>31.5 g/person/day*</td>
<td>0.085**</td>
<td>70 kg</td>
</tr>
</tbody>
</table>

* WHO (2003), **Jan et al., (2010)

Table 4. Analysis of variance for Cu, Co, Ni and Zinc in water.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>Cu means</th>
<th>Co means</th>
<th>Ni means</th>
<th>Zn means</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites</td>
<td>1</td>
<td>0.117**</td>
<td>0.001***</td>
<td>0.063**</td>
<td>0.061**</td>
</tr>
<tr>
<td>Error</td>
<td>4</td>
<td>0.003</td>
<td>0.000</td>
<td>0.157</td>
<td>0.008</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Mean and SE (standard error) for Cu, Co, Ni and Zinc in water (mgL<sup>-1</sup>).

<table>
<thead>
<tr>
<th>Sites</th>
<th>DF</th>
<th>Mean squares of Cu</th>
<th>Mean squares of Co</th>
<th>Mean squares of Ni</th>
<th>Mean squares of Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW-I</td>
<td>1</td>
<td>1.217***</td>
<td>0.082568*</td>
<td>37.1680***</td>
<td>4.10600***</td>
</tr>
<tr>
<td>SW-II</td>
<td>1</td>
<td>22.325**</td>
<td>0.012224*</td>
<td>2.8036**</td>
<td>0.38636**</td>
</tr>
<tr>
<td>Plants</td>
<td>1</td>
<td>8.098*</td>
<td>0.034382ns</td>
<td>4.5425**</td>
<td>0.21403**</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>1.349</td>
<td>0.014954</td>
<td>0.2406</td>
<td>0.04816</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** Indicates highly significant data at level 0.001

Table 6. Analysis of variance data for Cu, Co, Ni and Zinc in soil (mgkg<sup>-1</sup>).

<table>
<thead>
<tr>
<th>Sites</th>
<th>DF</th>
<th>Mean squares of Cu</th>
<th>Mean squares of Co</th>
<th>Mean squares of Ni</th>
<th>Mean squares of Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW-I</td>
<td>1</td>
<td>0.051798***</td>
<td>0.180811***</td>
<td>0.02708***</td>
<td>3.21782**</td>
</tr>
<tr>
<td>SW-II</td>
<td>1</td>
<td>0.002380**</td>
<td>0.000000ns</td>
<td>0.05165**</td>
<td>0.19799**</td>
</tr>
<tr>
<td>Plants</td>
<td>1</td>
<td>0.000002**</td>
<td>0.0000095ns</td>
<td>0.14734**</td>
<td>0.12706**</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.001189</td>
<td>0.000672</td>
<td>0.02755</td>
<td>0.02042</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>0.051798***</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** Significant at when p<0.001 level; ns= Non-significant at p>0.05

Table 7. Cu, Co, Ni and Zn concentration (mean and standard error, SE) in soil mgkg<sup>-1</sup>.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cu</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW-I</td>
<td>9.37</td>
<td>1.09</td>
<td>0.29</td>
<td>0.88</td>
</tr>
<tr>
<td>SW-II</td>
<td>10.38</td>
<td>1.36</td>
<td>0.50</td>
<td>1.26</td>
</tr>
<tr>
<td>Plants</td>
<td>8.29</td>
<td>0.36</td>
<td>0.55</td>
<td>0.91</td>
</tr>
<tr>
<td>Sites*Plants</td>
<td>6.01</td>
<td>0.42</td>
<td>2.84</td>
<td>2.39</td>
</tr>
</tbody>
</table>

Table 8. Analysis of variance data for Cu, Co, Ni and Zinc in fruits (mgkg<sup>-1</sup>).

<table>
<thead>
<tr>
<th>Source of variation (S.O.V)</th>
<th>DF</th>
<th>Mean squares of Cu</th>
<th>Mean squares of Co</th>
<th>Mean squares of Ni</th>
<th>Mean squares of Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sites</td>
<td>1</td>
<td>0.051798***</td>
<td>0.180811***</td>
<td>0.02708***</td>
<td>3.21782**</td>
</tr>
<tr>
<td>Plants</td>
<td>1</td>
<td>0.002380**</td>
<td>0.000000ns</td>
<td>0.05165**</td>
<td>0.19799**</td>
</tr>
<tr>
<td>Sites*Plants</td>
<td>1</td>
<td>0.000002**</td>
<td>0.0000095ns</td>
<td>0.14734**</td>
<td>0.12706**</td>
</tr>
<tr>
<td>Error</td>
<td>8</td>
<td>0.001189</td>
<td>0.000672</td>
<td>0.02755</td>
<td>0.02042</td>
</tr>
<tr>
<td>Total</td>
<td>11</td>
<td>0.051798***</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*** Significant at when p<0.001 level; ns= Non-significant at p>0.05

Table 9. Cu, Co, Ni and Zinc concentration in fruits.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cu</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW-I</td>
<td>0.24</td>
<td>0.10</td>
<td>0.062</td>
<td>0.28</td>
</tr>
<tr>
<td>SW-II</td>
<td>0.36</td>
<td>0.35</td>
<td>0.37</td>
<td>1.52</td>
</tr>
<tr>
<td>Plants</td>
<td>0.26</td>
<td>0.35</td>
<td>0.37</td>
<td>1.52</td>
</tr>
<tr>
<td>Sites*Plants</td>
<td>0.39</td>
<td>0.36</td>
<td>0.28</td>
<td>1.06</td>
</tr>
</tbody>
</table>

Table 10. Pollution load index for copper, cobalt, nickel and zinc.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cu</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>FW-I</td>
<td>1.12</td>
<td>0.99</td>
<td>0.032</td>
<td>0.019</td>
</tr>
<tr>
<td>SW-II</td>
<td>1.24</td>
<td>0.76</td>
<td>0.040</td>
<td>0.052</td>
</tr>
</tbody>
</table>
Health risk index of copper, cobalt, nickel and zinc: The values of HRI for Cu, Co, Ni and Zinc varied from 0.23 to 0.38 mgkg⁻¹day⁻¹ and 0.64 to 2.43 mgkg⁻¹day⁻¹, respectively (Table 14). Site FW-I in C. sinensis showed maximum concentration of HRI for Ni, while least was examined at FW-I in C. limetta for Zn.

Discussion

Heavy metal contamination is a worldwide issue, especially in the food commodities, causing environmental challenges in the developing world. This study was particularly carried out to assess the level of contamination in citrus grown fields of district Sargodha and have an insight on risks posed to consumers of sweet orange and sweet lime. Sargodha region is well known for its citrus production and amid shortage of freshwater sources, farmers have now shifted to alternate sources of irrigation like wastewater (Khan et al., 2023).

Cu concentrations in water samples ranged from 0.062 to 0.37 mg/L. In comparison to the value of 0.10 mg/L stated by Chaoua et al., (2019), both irrigation waters had significantly higher Cu concentrations at SW-II. A study carried out on wastewater and groundwater, however, yielded higher mean levels of Cu (1.29, 1.03 mg/L) in comparison to the current study (Khan et al., 2017). Some samples had Cu concentrations above the values determined by. Higher copper levels (1.842 mg L⁻¹) were also found as reported by Kumar & Chopra (2015).

In the current study, the mean concentration of cobalt in water ranged from 0.079 mg/L to 0.140 mg/L that was higher than the value of 0.0030 mg/L (Khashkhoussy et al., 2013). According to Chiroma et al., (2014) and USEPA (2011), the threshold limit of Co accumulation in water is 0.05 mg/L and the value of cobalt in irrigation water at both sites (FW-I and SW-II) exceeded this threshold value. In comparison to other studies, the current Co concentrations were below the values documented by Yaqub et al., (2021); Ugulu et al., (2021a) and Ugula et al., (2022).

All water samples had Ni concentrations that ranged between 1.54 and 2.24 mg/L. In waste water, Hassan et al., (2013) and Ugulu et al., (2020) recorded a lower Ni concentration of 0.05 mg/L compared to our results. According to Ahmad & Goni (2010) and Khan et al., (2020a) the concentration of Ni in recent study was higher than their findings. The present value of Ni in water was found to be higher than the findings of Pescod (1992) which was 0.2mg/L. Farmers utilize industrial and municipal waste excessively and untreated, which contaminates the water. This is a significant source of Ni contamination in water storage reservoirs.

Mean concentration of Zn (mgL⁻¹) in water ranged from 0.55 to 0.761. When the present research was compared with other studies, it was observed that the values of Zn in the present water samples were below the concentrations reported by Aurangzeb et al., (2014). In comparison to the results of the current investigation, Mousavi & Shahsavari (2014) recorded low values for Zn (0.010-0.021 mgL⁻¹) in ground water. Zn content in water was reported to be higher than the findings by Hassan et al., (2013). High Zn concentrations were recorded in Lahore canal water by Kashif et al., (2009).

For the soil samples, Cu concentration in this study varied from 6.01 to 10.38 mg/kg. Elevated Cu level (32.71 mg/kg) in soil treated with sewage water was reported by Alghobair & Suresha (2016). In contrast to this experiment, Wang et al., (2015) evaluated the low copper range (1.09-1.55 mg/kg). Compared to the level (1.09, 1.55 mg/kg) provided by Khan et al., (2017) our estimated value for Cu was greater. Ahmad et al., (2016) and Wajid et al., (2020) found that Cu concentrations ranged from 2.79-4.13 mg/kg which were below the concentrations of Cu in present study.

### Table 11. Bio concentration factor for Cu, Co, Ni and Zn.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cu</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. limetta</td>
<td>C. sinensis</td>
<td>C. limetta</td>
<td>C. sinensis</td>
</tr>
<tr>
<td>FW-I</td>
<td>0.025</td>
<td>0.032</td>
<td>0.53</td>
<td>0.57</td>
</tr>
<tr>
<td>SW-II</td>
<td>0.035</td>
<td>0.062</td>
<td>0.98</td>
<td>0.82</td>
</tr>
</tbody>
</table>

### Table 12 Enrichment factor for copper, cobalt, nickel and zinc.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cu</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. limetta</td>
<td>C. sinensis</td>
<td>C. limetta</td>
<td>C. sinensis</td>
</tr>
<tr>
<td>FW-I</td>
<td>0.42</td>
<td>0.53</td>
<td>2.43</td>
<td>2.61</td>
</tr>
<tr>
<td>SW-II</td>
<td>0.59</td>
<td>1.047</td>
<td>4.46</td>
<td>3.73</td>
</tr>
</tbody>
</table>

### Table 13. Daily intake of Cu, Co, Ni and Zn (mgkg⁻¹day⁻¹).

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cu</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. limetta</td>
<td>C. sinensis</td>
<td>C. limetta</td>
<td>C. sinensis</td>
</tr>
<tr>
<td>FW-I</td>
<td>0.0041</td>
<td>0.0043</td>
<td>0.0090</td>
<td>0.011</td>
</tr>
<tr>
<td>SW-II</td>
<td>0.014</td>
<td>0.013</td>
<td>0.014</td>
<td>0.015</td>
</tr>
</tbody>
</table>

### Table 14. Health risk index for copper, cobalt, nickel and zinc.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Cu</th>
<th>Co</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. limetta</td>
<td>C. sinensis</td>
<td>C. limetta</td>
<td>C. sinensis</td>
</tr>
<tr>
<td>FW-I</td>
<td>0.23</td>
<td>0.25</td>
<td>0.094</td>
<td>0.099</td>
</tr>
<tr>
<td>SW-II</td>
<td>0.35</td>
<td>0.38</td>
<td>0.32</td>
<td>0.31</td>
</tr>
</tbody>
</table>
Co was found in soil at various concentrations. The mean value of Co in soil varied from 0.195 mg/kg to 0.428 mg/kg. Overall, Co accumulation in soil was within the WHO (2003) guidelines. Heavy metal contamination of soil raises the risk of metal uptake by plants and deposition in various edible parts (Ali & Al-Qahtani, 2012). USEPA (1997) reported that the permissible maximum limit of Co accumulation in soil was 750 mg/kg.

In both soils at FW-I and SW-II, the observed mean levels of Ni ranged from 0.19 to 0.42 mg/kg. Nickel buildup may be influenced by variables such soil pH, plant characteristics and kind. Nickel (Ni) accumulation is also influenced by anthropogenic soil contamination. According to Khan et al., (2017), Ni concentration in soil irrigated with control (1.36 mg/kg) and sewage water (1.61 mg/kg) varied a little, however, it was still higher than the results of the current study. In comparison to the current findings, Shah et al., (2009) found greater Ni level (36.0 mg/kg) in soil. Ni concentration in soil was lower than the value of 1.90-3.74 mg/kg reported by Ahmad et al., (2016). The concentration 13.29 to 13.99mg/kg of Ni in soil was observed by Leogrande et al., (2019) which was greater than the present study. The current values of Ni in soil were lower than the values recorded by Addis & Abebaw (2017).

Average concentration of zinc in soil samples collected from all sites fluctuated between 0.86 to 2.39 mg/kg. Eissa & Almaroai (2019) recorded the Zn level in the soil (600 mg/kg) to be much higher compared to current study. Zn levels in the soil of the current region were significantly lower than those of 3.205-6.910 mg/kg as reported by Orisakwe et al., (2017). The concentration of 1.09-1.55 mg/kg was provided by Khan et al., (2017) whereas the current reported range for Zn was lower. When compared to recent research values, Özayzici (2013) obtained the highest value for zinc, i.e., 83.3-58.8 mg/kg. Similarly, Yu et al., (2016) established that Zn had a higher value than attained in current work. Current Zn values in soil were also lower than calculated values of Ran et al., (2016).

The copper level in the analysis of fruits ranged from 0.24 to 0.39 mg/kg. The buildup of Cu is influenced by the types of plants and their needs. Small amounts of copper are necessary for plants to develop normally (5-20 mg/kg). However, a concentration of more than 20 mg/kg can make copper hazardous. In comparison to this study, Kumar (2016) reported the highest Cu content (5.5-18.7 mg/kg) in the green leafy vegetables. According to Chaoa et al., (2019) in the edible fraction, copper buildup was lower (6.57-6.86 mg/kg). The Cu content (0.52 mg/kg) suggested by Auranzeg et al., (2014) was discovered to be lower than the present range.

For fruits, the mean concentration of cobalt varied from 0.106-0.357 mg/kg. The observed values were according to the permissible limits of FAO/WHO (2010). Co in fruits were below the global average and appeared to be less than the allowable limit (Al-Sayegh & Al-Yazichi, 2001). Plants require cobalt for functioning of certain enzymes which is why cobalt affects metabolism in plants. Co also interacts with other elements and forms complexes which may be phytotoxic depending on the type of complex.

Nickel was found in abundance in citrus fruits harvested from the FW-I irrigated site. The current results were thought to be above those determined by Ihesinachi & Eresiya (2014). Ni was found in high concentrations (0.08 mg/kg) in oranges sold in Owerri, Nigeria (Orisakwe et al., 2012). However, in another study, Ni mean level in orange was 0.129 mg/kg (Sobukola et al., 2010). According to our findings, the Ni concentration in citrus fruit was 0.415 mg/kg. These values were found to be above the allowable level of 0.14 mg/kg set by FAO/WHO (2010).

The zinc concentration in fruits was lower than the acceptable limit (100 mg/kg) established by Chiroma et al., (2014), which ultimately showed a deficiency of Zn in fruits in the current investigated area. In the study, Zn average content was varied from 0.23 to 1.52 mg/kg. Zinc is a vital nutrient required for plant growth and several metabolic processes. Zn is easily available to plants in solution forms and its accumulation depends on amount of zinc in nutrient solution and soil. Chaoua et al., (2019) reported maximum Zn values in plants (60.515, 86.35 mg/kg) than the current findings. Singla & Dhawan (2017) observed a zinc content ranging from 26.0 to 31.30 mg/kg, which was found to be significantly higher than the currently detected range. The concentrations of Zn from this study were found to be about ten times lower in all the samples compared to the permissible level of 99.40 mg/kg by (WHO, 2003).

The pollution load index values, which stayed within the range of 1, indicated that the soil in the current research region was not contaminated with copper and the fruits grown there were safe for human health. This study's PLI value was lower than the Cu reference values (8.39) as suggested by Singh et al., (2010). The value of PLI for Co in soil ranged from 0.047 to 0.0214 which was found to be lower compared to the value reported by Khan et al., (2015).

In these soil samples, PLI values ranged between 0.032 and 0.31. The PLI value for Ni found in this study was lower than earlier report (Proshad et al., 2017). Recent findings indicated that the soil in the studied site was safe for growing fruit. The pollutant load index value for Ni in the current analysis fell below the reference values of 9.06 and 2.89-3.67 as reported by Singh et al., (2010) and Ahmad et al., (2016) respectively when compared to the current study. It was determined that the Ni value of 1.62 proposed by Ahmad et al., (2014) fell within the parameters noted in this study.

The soil of the researched region is not polluted, as shown by the current data, and the soil is safe for fruit cultivation. The Zn PLI level was also less than 1. PLI values for Zn were lower than the report of Singh et al., (2010) reference value (44.19). Ahmad et al., (2016) confirmed a higher PLI value for Zn (1.47) in contrast to the current study. It was found that the Zn PLI level (0.05) recommended by Ezemokwe et al., (2017), was below the current concentration.

It was found that the bio-concentration for Cu (4.38-5.05) provided by Ahmad et al., (2016) was greater than the present results. The highest concentrations observed in BCF for Co was 0.035. The highest concentration of nickel observed for BCF was 0.74. Compared to this experiment,
Ahmad et al., (2016) found greater BCF values for nickel (2.948-4.149). According to Khan et al., (2017) the nickel BCF value was found to be greater than the current study. The DIM for Cu (0.004 mgkg⁻¹·day⁻¹) determined by Chaoua et al., (2019) was found to be consistent with the findings of this study. Ismail et al., (2015) observed that the suggested DIM for Cu (0.0002 mgkg⁻¹·day⁻¹) was lower in comparison to the present study. The present Cu DIM was lower than the recommended highest acceptable level of 3 mgkg⁻¹·day⁻¹.

The investigation showed that the Ni DIM ranged from 0.002 to 0.016 mgkg⁻¹·day⁻¹. Khan et al., (2019d) investigated lower Ni DIM levels (0.006, 0.008, mgkg⁻¹·day⁻¹). Likewise, Ismail et al., (2015) suggested a related DIM value of 0.002 mgkg⁻¹·day⁻¹ for nickel as tested in this research. Ni consumption per day was lower in all samples than the expected daily permissible intake (1.4 mgkg⁻¹·day⁻¹) (USEPA, 2011).

Research revealed that the Zn DIM ranged between 0.0091-0.059 mgkg⁻¹·day⁻¹. There was no health risk for humans consuming polluted fruits in the study region because the DIM values for Zn were less than 1. Compared to the current study’s DIM levels (0.0091 - 0.059 mgkg⁻¹·day⁻¹), Nadeem et al., (2020) and Ugulu et al., (2022a), recorded a wider range of DIM values (0.039-0.769 mgkg⁻¹·day⁻¹).

Cu health risk index values for both youth and adolescents in the study are below 1. The HRI<1 indicates, in accordance with USEPA (2011), that consumers don’t face any immediate health risks (Liang et al., 2019d). Likewise, Ismail et al., (2020) and Ugulu et al., (2019b). According to our findings, HRI occurred between 0.22 and 0.37. Therefore, it can be said that Cu in citrus fruit samples does not pose a risk to consumers’ health. Similar results were also reported by Ugulu et al., (2021b, 2021c).

The mean concentration of HRI for Co ranged from 0.094 to 0.32. When the findings of HRI from this report’s analysis were compared with those from Bibi et al., (2014) and Khan et al., (2019d) study, the HRI value was found to be slightly higher.

In the current study, HRI for Ni was less than 1, demonstrating that Ni had no harmful effects on the health of the humans who would consume these fruits grown on two different irrigation waters. The outcomes of the research showed that the HRI for Ni observed in this study ranged between 0.11-0.79. As a result, it is possible to conclude that Ni in citrus fruit samples posed no health risks to consumers. The current Ni HRI values were higher than that of the 0.39 described by Singh et al., (2010).

Ahmad et al., (2016) found a higher HRI for Zn concentration than what was found in the current study (0.0165-0.257). In contrast to the present investigation, Khan et al., (2017) discovered greater HRI Zn values (0.537-0.609). A higher HRI Zn value (0.040-0.021) was also recorded by Lawal et al., (2017).

Conclusion

The Cu, Co, Ni and Zn translocation from the soil and fruits to the human being food chain revealed that Cu, Co, Ni and Zn concentration in soil and fruit samples was within the acceptable USEPA and WHO limits. It was found that there were variations in concentration of these metals across the soil-fruit-human continuum at various locations. This study analyzed soil and citrus fruits contamination with Cu, Co, Ni and Zn as well as potential future health risks, was found a step towards pollution by these metals, however, it did not represent a concern to human health because the HRI and DIM values were less than 1.

Recommendations and Implications

Ecotoxicological assays are useful tools for analyzing Cu, Co, Ni, and Zn contamination levels chemically. The evaluation of the toxicity in soil and fruits should not simply utilize the pollution indices. Biological markers are another crucial tool for understanding the impact of pollutants on the environment. By examining dangerous metals, we can monitor how poisonous the environment is to people. It is important to be aware of the many poisons, metal pollutants, and significant plant stages during which HMs might infiltrate the environment and food chain, as well as the illnesses caused by them, in order to safeguard the public’s health. In order to aid in the development of regulations, the government should regularly assess the quantity of HMs in biological and ecological aspects.

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ASSESSMENT OF METALS IN CITRUS FRUITS


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