Screening of zinc, copper and iron in lettuce and Chinese cabbage cultivated in Durban, South Africa, towards human health risk assessment

Human well-being and ecological reliability continue to face a major threat resulting from heavy metal pollution to soils caused by untreated discharge from metropolitan and industrial wastewater. The potential human health risks of zinc (Zn), copper (Cu) and iron (Fe) contamination to native inhabitants through the food chain were assessed in Pinetown, Durban, where their irrigation processes are from the Umgeni River passing through the highly industrialised Pinetown area. River water, vegetables (cabbage and lettuce) and soil were analysed for Zn, Cu and Fe; transfer factor, health risk index and the daily intake of metals were also calculated. The concentrations of heavy metals indicated the pattern trend as Fe > Zn > Cu for both cabbage and lettuce. The levels of transfer factors for heavy metals ranged from 0.02 mg/kg to 1.89 mg/kg. The health risk index (0.0002–0.01430) was found to be within the recommended range (<1), which poses no human health risk with respect to all heavy metals tested.

Significance:
The present study has generated data on heavy metal pollution in and around the area and associated risk assessment for consumers’ exposure to the heavy metals. These data can assist decision-makers in understanding the suitability status of vegetable consumption and irrigation by providing an understanding of the human health risk of the studied area. This database can be used as a tool to pinpoint the mechanisms and processes influencing public health implications of heavy metals in foods, soils and water.

Introduction
Heavy metals are elements that occur naturally and are known to have a high density – more than five times that of water – and high atomic weight. Their wide distribution in the environment arises from their numerous applications such as agricultural, industrial, technological, domestic, and medical which raises worries over their potential effects on the environment and human well-being. Copper (Cu), zinc (Zn), and iron (Fe) are some of the fundamental elements that are needed for different biological, chemical and physiological functions of plants. However, an insufficient supply of these elements in the environment causes syndromes, disorders, and deficiency diseases in human beings. Wastewater systems of businesses and municipalities discharge heavy metals into the environment. These inappropriate actions result in contamination of water and soil, which are further used for farming, bringing about increased accumulation of heavy metals in vegetable plants and thereby affecting food security all through the world. It has been documented that heavy metals can be taken up by vegetables and then accumulate in the edible parts of the plant, and, if the accumulation is sufficiently high, it could cause clinical issues for humans and other animals who consume these plants. Hence, heavy metal contamination of vegetables cannot be overlooked because vegetables are a significant part of the human diet and their intake may be hazardous to human health and lead to various long-term diseases. Heavy metal sources include weathering of metal-bearing rocks and volcanic eruptions, while anthropogenic sources include mining and various industrial and agricultural activities.

Copper is an essential nutrient involved in the creation of red blood cells, maintenance of the body defences and functioning of brain cells. Copper is found in soil and water within industrialised communities. Consumption of high levels of copper can cause nausea, vomiting, diarrhoea, gastric (stomach) complaints and headaches. Long-term exposure over many months and years can cause liver damage and death. Zn is considered a fundamental component for human existence; however, acute and chronic exposure to excessively high concentrations of Zn can cause nausea, vomiting, diarrhoea, fever and lethargy. Fe is a widely distributed mineral that is vital for human, plant and animal life – it is necessary for the production of haemoglobin, myoglobin and certain enzymes. However, excess Fe in the system can cause cirrhosis when deposited in the pancreas, liver cancer when deposited in the liver and cardiac arrhythmias when deposited in the heart.

An assessment of the risk to human health is essential as it informs the management stage of risks, including recommendations to ensure that human health is protected. The assessment, correction, regulation, monitoring, and protection of environmental attributes that can have a negative impact on human health, as well as the advancement of environmental consequences that can improve human health, are the focus of health and environmental systems. A number of studies have demonstrated that polluted environments with heavy metals may cause certain vegetables, including cabbage and lettuce, to accumulate high concentrations of these metals. Given the potential toxicity, recalcitrant nature, and cumulative behaviour of heavy metals, the frequency of vegetable consumption; and safety and health concerns, such research is essential. Primary objectives of this study were to:

1. investigate the current level of local heavy metal pollution in vegetated soils, plants and water,
2. assess potential health risks from heavy metals in the soil–vegetable and water systems, and
3. serve as a resource on heavy metal pollution prevention and treatment for policymakers and decision-makers.

The present study was conducted on a local farm, the Fair Food Company & Edamame Development Programme in Pinetown, Durban, South Africa; with the aim of assessing Zn, Cu, and Fe concentrations in irrigation water, soil,
Chinese cabbage (Brassica rapa pekinensis), and leafy lettuce (Lactuca sativa) from two different sites on the farm, which uses river water for irrigation of agricultural land on a long-term basis. The concentrations of these heavy metals in the soil, vegetables, and water were also compared to recommended limits for human safety.

Materials and methods

Study area

The study area was located in Marianhill, Pinetown in KwaZulu-Natal, South Africa. Two sampling sites were chosen and were located at The Fair Food Company & Edamame Development Programme (Site 1: 29°50′27.9″S, 30°49′21.2″E; Site 2: 29°50′40.9″S, 30°49′25.9″S). River water from the Umgeni River was used for irrigation at both sites; the River passes through a highly popularised industrial area in Pinetown.

Water sampling

Samples of irrigation water were collected from a single location in the Umgeni River. Samples were collected into polyethylene bottles that were cleaned with metal-free soap, soaked in 2% nitric acid and then washed with demineralised water.\(^\text{14}\) Samples were brought to the Analytical Services Laboratory, Technology Station in Chemicals, Mangosuthu University of Technology, Durban, and kept at 5 °C.

Soil sampling

Soil samples from the farm were collected by digging approximately 500 g of soil (0–30 cm) from four different areas (125 g) of each site using a plastic scoop. The samples were completely blended to form a uniform soil sample. Foreign particles such as grass and stones were removed from the samples and the soils were dried in the oven. Samples were filtered through a sieve (2 mm) and preserved in a labelled polythene bag.\(^\text{15}\)

Vegetable sampling

The study area contained a range of vegetables; cabbage and lettuce were chosen for the study because they are sometimes eaten raw through salads. For each zone, 3–6 replicates of the entire vegetable parts (leaves, roots, and stems) were collected. Samples were placed in a properly marked sampling bag and taken to the laboratory. They were thoroughly washed under running tap water to eliminate aerial contaminants and soils and rinsed with distilled water. Roots, stems and leaves were separated and the samples were oven dried (55–60 °C), blended and sieved (40 mesh).\(^\text{16}\)

Digestion of samples and analysis

Half a gram of each vegetable and soil sample was weighed into a Teflon vessel and digested by 12 ml of aqua regia, i.e. a mixture of nitric acid and hydrochloric acid in a 3:1 ratio. The vessels were closed and placed on the microwave digestion system (UniClever BM—1z, Plazmatronika, Poland). At the end of the digestion, a 20-min airing process (no microwave power) was undertaken in order to cool the vessels so as to reduce the pressure to within the ambient values. The samples were poured into 100-mL volumetric flasks and filled with distilled water. Whatman filter papers (No. 42) were used to filter the samples; a filtrate of 5 L per sample was stored at 5 °C. Heavy metal content was determined by a PerkinElmer 2380 Atomic Absorption Spectrophotometer fitted with a lamp specifically designed for specific heavy metals. The remainder of the parameters were the same as in the method described by Welz et al.\(^\text{17}\)

Determination of soil and water pH

Soil pH was measured using a pH meter (soil solution ratio 1:2 in water); water pH was determined in situ using a portable pH meter (201T ATC) from Laboratory Equipment suppliers.

Determination of transfer factor

The bioaccumulation of metals in the environment is a highly dynamic process which relies upon explicit combinations of synthetic, natural and ecological conditions.\(^\text{18}\) Heavy metals are taken up by plants from the soil through the roots, and also from the environment through overground vegetative organs.\(^\text{18}\) Plants’ requirements for micronutrients play a controlling role in metals availability for plants as well as their ability to assimilate and kill harmful elements. This accessibility is unique, and depends on the plant species and their adaptation to the climatic conditions. The soil-to-plant tissue transmission is investigated using the transfer factor (TF) index (Equation 1). TF is calculated as a ratio of the concentration of a specific metal in plant tissue (C\text{plant}) to the concentration of the same metal in soil (C\text{soil}) both represented in the same unit (mg/kg fresh weight).\(^\text{20}\) Higher values of TF (≥1) translate to increased absorption of metals from soil to plants; meaning the phytoextraction and phyto-extraction will have higher suitability for the plant. A TF value lower than one (<1) indicates poor metal absorption by the plant and therefore no obvious risk will be observed.\(^\text{20}\)

\[
TF = \frac{C_{\text{plant}}}{C_{\text{soil}}}
\]  
Equation 1

Determination of daily intake of metals

Tolerable daily intake is an indication of the actual amount in nutrition or potable water that can be ingested on a body mass basis, commonly mg/kg body weight every day for a long period by people who do not face a significant health risk. The daily intake of metals (DIM) was determined from the edible part of the plants using Equation 2:

\[
\text{DIM} = M \times K \times \frac{I}{W}
\]  
Equation 2

where M is the concentration of metals in plants, K is the conversion factor, I is the daily intake of vegetables and W is average body mass. Average body mass for an adult and child were projected to be 59.9 kg and 32.7 kg, respectively.\(^\text{20}\) Vegetable daily intake for adults and children was projected to be 0.345 and 0.232 kg/person/day, respectively. The fresh weight of vegetables was converted to the dry weight using a conversion factor of 0.085.

The health risk index (HRI) for ingestion of Cu, Fe, and Zn through polluted vegetables was calculated using Equation 3:\(^\text{23}\)

\[
\text{HRI} = \frac{\text{DIM}}{\text{Rfd}}
\]  
Equation 3

where the reference oral dose is represented by Rfd. Rfd values for Fe, Cu, and Zn are 0.70, 0.04, and 0.30 mg/kg bw/day, respectively.\(^\text{4}\)

Statistical analysis

Microsoft Excel was used to determine statistical data and the results are presented as the mean ± standard deviation.

Results and discussion

Hydrogen potential

Water and soil samples were also analysed for hydrogen potential (pH) as it has a significant impact on determining the suitability of water for irrigating vegetables. Optimal soil pH plays a major part in growing the best plants and vegetables. Adjusting soil pH or matching plants or vegetables to the soil pH is important; most plants grow between a pH of 4.5 to 8.0. Soil pH is important because acidity or alkalinity of the soil determines what plant supplements are available to plant roots. The best pH for cabbage and lettuce is 6.5–6.8 and 6.0–6.5, respectively, for ideal growth and to debilitate club root infections. The irrigation water was found to have a pH of 6.93, which is almost neutral, and soil samples from Sites 1 and 2 showed a pH of 5.55 and 6.35, respectively.

Concentrations of heavy metals in water and soil samples

Heavy metal concentrations in water and soil samples from Sites 1 and 2 are illustrated in Table 1. The content levels of Cu and Fe in water were measured to be 0.075 mg/kg and 0.731 mg/kg, respectively, which exceeds the WHO/FAO standard parameters of 0.017 mg/kg and 0.50 mg/kg, respectively. Zn levels in water were measured to be 0.131 mg/kg, which is in the range of the permissible limit of 0.20 mg/kg. All soil samples from Sites 1 and 2 were in the range of the permissible limits of the WHO/FAO: 0.285 mg/kg and 0.266 mg/kg for Cu, 89.87 mg/kg and 86.35 mg/kg for Fe, and 1.405 mg/kg and 1.361 mg/kg for Zn, respectively.
Heavy metal concentrations in vegetables

The concentrations in mg/kg were assessed in roots, stem and leaves, as presented in Table 2 for both cabbage and lettuce grown at two different sites irrigated with river water. The mean concentrations of heavy metals in vegetables appeared to have a wide range: 0.061–0.339 mg/kg for Cu, 1.650–10.24 mg/kg for Fe and 0.387–2.566 mg/kg for Zn. The highest levels of Cu, Fe and Zn were recorded in lettuce leaves, cabbage roots and lettuce roots, respectively, from Site 2 for all. The lowest levels of Cu, Fe, and Zn were all recorded in cabbage leaves from Site 2. It is understood that the differences and variations in concentrations could be attributed to the plant’s abilities in terms of absorbing and accumulating heavy metals, and differences in growth phase and rate of growth.

The contents of heavy metals studied were also compared to the permitted WHO/FAO maximum intake levels. Cu, Fe, and Zn concentrations in various parts of both vegetables were significantly lower than the maximum concentrations, meaning that the vegetables were safe for human consumption. The cabbage samples showed a trend for concentration to be in the order roots>stem>leaves, which may be due to the fact that roots transport nutrients, water content and heavy metals from the soil to the stem and then to the leaves. The results for cabbage followed a more or less similar trend to those of the study conducted by Meerkotter, in Cape Town, South Africa, in which the Cu concentrations in cabbage leaves were lower when compared to stems and roots; and the roots seemed to have a considerably higher concentration of Cu. In the same study, a similar trend was observed where cabbage roots showed greater concentrations of Fe and Zn than in either stems or leaves. The heavy metal concentrations for lettuce seemed to be portioned differently as there was no particular trend followed for Cu, Fe and Zn.

Transfer of heavy metals from soil to vegetables

The TF of trace elements from soil to plants is defined as the ratio of trace element contents in the plant (dry weight) to the total trace element contents in soil (dry weight). A plant with the TF value greater or equal to one (≥1) is deemed more suitable for phytoextraction and phytoremediation, which is known to be due to the higher soil to plant metal absorption. Lower numbers, on the other hand, are an indication that those plants have a poor response to metal absorption and that the plant may be consumable by humans. Table 3 summarises the heavy metal transfer factor in the vegetables; The TF range for cabbage is 0.28–0.77 for Zn, 0.25–0.89 for Cu and 0.02–0.11 for Fe; for lettuce is 0.42–1.87 for Zn, 0.22–1.27 for Cu and 0.03–0.07 for Fe. The highest (1.27) and lowest (0.22) TF for Cu were found in lettuce leaves from both sites. The highest TF for Zn (1.87) was observed in lettuce roots and the lowest (0.28) in cabbage leaves, both from Site 2. As for Fe, the highest TF (0.11) was observed in cabbage roots from Site 1 and the lowest (0.02) was observed in cabbage leaves from Site 2. The TF values are comparable to the findings published by Jan et al.

Table 1: Soil and water (mg/kg) heavy metal concentrations

| Heavy metal | Soil 1 | Soil 2 | Water 1 | Water 2 | WHO/FAO
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Cu</td>
<td>0.285 ± 0.020</td>
<td>0.266 ± 0.040</td>
<td>0.075 ± 0.001</td>
<td>0.017</td>
<td>100</td>
</tr>
<tr>
<td>Zn</td>
<td>1.405 ± 0.007</td>
<td>1.361 ± 0.017</td>
<td>0.131 ± 0.016</td>
<td>0.200</td>
<td>300</td>
</tr>
<tr>
<td>Fe</td>
<td>89.87 ± 0.460</td>
<td>86.35 ± 1.540</td>
<td>0.731 ± 0.050</td>
<td>0.500</td>
<td>50 000</td>
</tr>
</tbody>
</table>

Table 2: Heavy metal concentrations (mg/kg) in different parts of the vegetables

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Plant part</th>
<th>Mean concentration of heavy metals in vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>Roots</td>
<td>0.211 ± 0.032</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.129 ± 0.011</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.129 ± 0.042</td>
</tr>
<tr>
<td>Fe</td>
<td>Roots</td>
<td>10.24 ± 0.077</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>6.038 ± 0.311</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>3.788 ± 0.062</td>
</tr>
<tr>
<td>Zn</td>
<td>Roots</td>
<td>1.077 ± 0.279</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.905 ± 0.040</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.874 ± 0.022</td>
</tr>
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</table>

Table 3: Heavy metal transfer factors

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Plant part</th>
<th>Transfer factor plant/soil in vegetables</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu</td>
<td>Roots</td>
<td>0.74 ± 0.071</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.45 ± 0.013</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.45 ± 0.012</td>
</tr>
<tr>
<td>Fe</td>
<td>Roots</td>
<td>0.11 ± 0.020</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.07 ± 0.155</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.04 ± 0.314</td>
</tr>
<tr>
<td>Zn</td>
<td>Roots</td>
<td>0.77 ± 0.057</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>0.64 ± 0.023</td>
</tr>
<tr>
<td></td>
<td>Leaves</td>
<td>0.62 ± 0.033</td>
</tr>
</tbody>
</table>

Daily intake estimates and the health risk index of heavy metals for vegetables

The values in Table 4 represent data for DIM and HRI in adults and children. These values were calculated from the leaves as they are...
the most edible parts of the vegetables. The estimated dietary intakes for metals were below the tolerable limits. The results indicate that the highest intake in adults was 0.0057 kg Cu/person/day in cabbage from Site 1; and the highest intake in children was 0.0429 kg Fe/person/day in lettuce from Site 1. RID for heavy metals is known to be 0.04 mg/kg/day for Cu, 0.7 mg/kg/day for Fe and 0.3 mg/kg/day for Zn.30 The RID is seen as an estimate of day-to-day accessibility to the general public that is unlikely to present a tremendous risk of detrimental consequences over the course of a lifetime.30 The HRI results were obtained by dividing daily intake of heavy metals by their reference measurements to evaluate the health risk that comes with these heavy metals (Table 4). An HRI less than 1 demonstrates that the assessment is unlikely to pose a significant health risk. However, a HRI greater than 1 does not imply that a serious adverse health impact will emerge, it just indicates a strong probability of a health risk. The HRI of the study area suggests that cabbage and lettuce grown at both sites of The Fair Food Company & Edamame Development Programme were totally free from any risk and safe for consumption. Despite the fact that all of the heavy metals in the samples examined were within the global limit for agricultural use, long-term reuse of irrigated water causes an excessive build-up of those hazardous metals in soil and crops. Hence, proper waste management and environmental practices in the surrounding areas are critical.

### Conclusion

The concentration of various elements in plants is determined by the approximate degree of plant interaction with polluted soil as well as hazardous element deposition in air pollutants. Human health risk assessment helps experts to assess the overall situation and determine what advice or actions, if any, should be taken to ensure that human health is protected. Past, current or future exposures to heavy metals in air, soil, water, food, consumer products or other materials can be assessed.

Although the river water for irrigation passes through a highly industrial urban area, our results reveal that the vegetables were least contaminated by Cu, Fe and Zn, which indicates less discharge of these metals into the natural ecosystem by nearby industries. It is recommended that, to keep the environment less affected by heavy metals, proactive health agencies, trash disposal knowledge, and best practices should be maintained. Because the two farming sites share a single source of irrigation water, the overall differences in heavy metal concentrations were minimal and insignificant. It is noteworthy to mention that soil concentrations from the two selected sites were also not significantly different.

This study reports that the human assessment risk for selected metals is low, hence vegetables may be considered safe for consumption. However, some data in this study are lacking as the first screening focus was mainly on three heavy metals. A more detailed study is required to investigate a wider range of heavy metals in the area and surrounding areas. The outcome of this investigation could be used as a tool for farmers and decision-makers to adopt and implement action-oriented, sustainable strategies to prevent risk to the population from heavy metals in vegetables.

### Acknowledgements

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### Competing interests

We have no competing interests to declare.

### Authors’ contributions

S.M.N: Conceptualisation, methodology, sample analysis, drafted the first manuscript, project leadership. N.M.M: Sample analysis, data collection, manuscript revision.

### References


<table>
<thead>
<tr>
<th>Sites</th>
<th>Vegetables</th>
<th>Daily intake of metals</th>
<th>Health risk index</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cu</td>
<td>Fe</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(mg/kg/day)</td>
<td>(mg/kg/day)</td>
</tr>
<tr>
<td>Site 1</td>
<td>Cabbage</td>
<td>0.0057±0.014</td>
<td>0.0018±0.035</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>0.0003±0.010</td>
<td>0.0029±0.051</td>
</tr>
<tr>
<td>Site 2</td>
<td>Cabbage</td>
<td>0.0003±0.022</td>
<td>0.0008±0.023</td>
</tr>
<tr>
<td></td>
<td>Lettuce</td>
<td>0.0002±0.011</td>
<td>0.0014±0.021</td>
</tr>
</tbody>
</table>

**Table 4: Daily intake of metals (kg/person/day) and health risk index in adults and children**


27. Meerkotter M. Sources of heavy metals in vegetables in Cape Town, and possible methods of remediation (PhD thesis). Cape Town: University of the Western Cape; 2012.

