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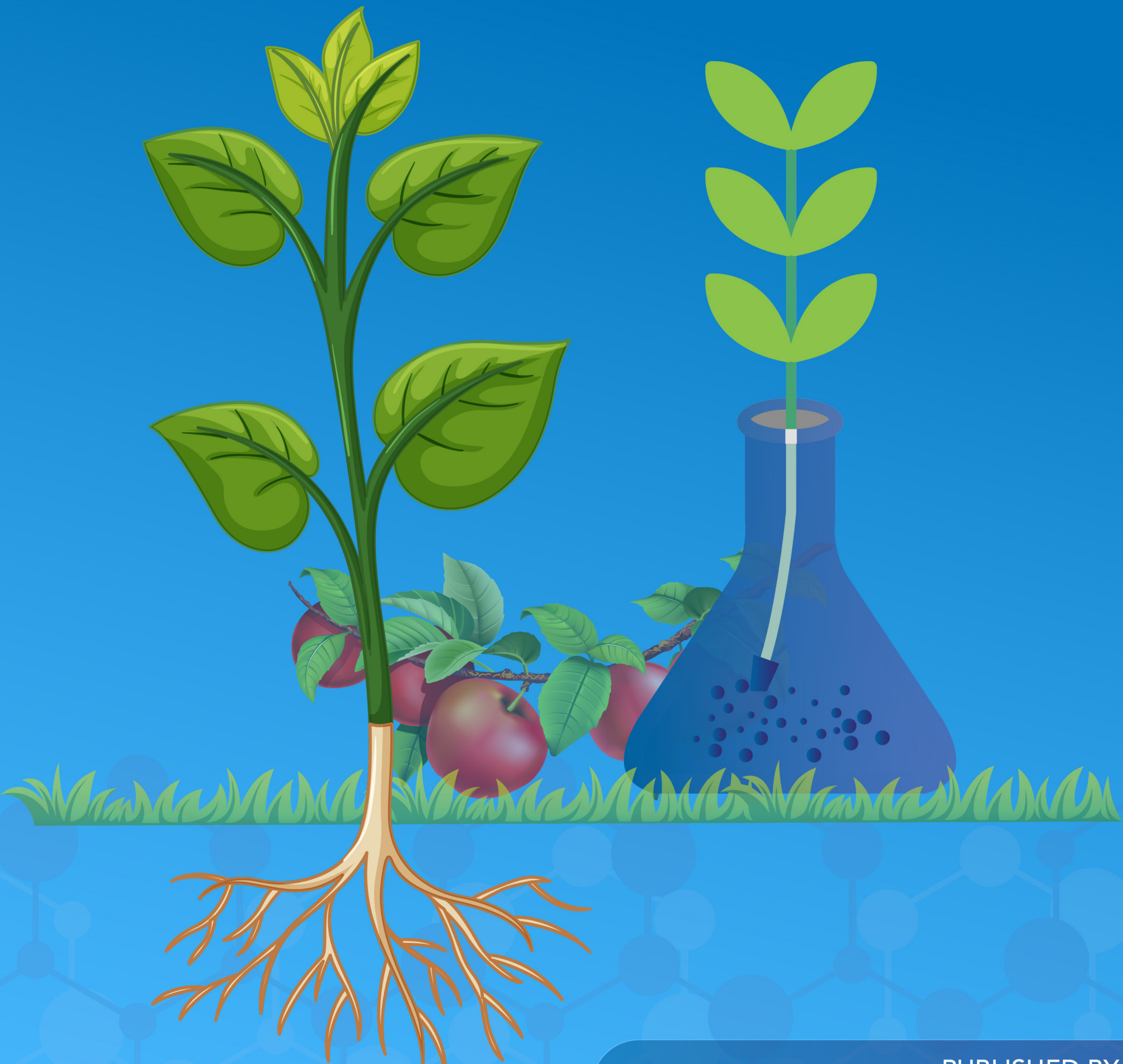

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The Safety of Leafy Vegetables in Oman

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ABSTRACT

Leafy vegetables are potential carriers of foodborne diseases that threaten the community's well-being. Therefore, monitoring leafy vegetable's microbial and heavy metal contamination is crucial. This study evaluates the safety of leafy vegetables consumed in Oman by examining pathogenic bacteria such as Escherichia coli, Staphylococci, Salmonella, and Listeria, as well as the heavy metals concentration. Results indicate high levels of microbial contamination in all samples, exceeding the Gulf Standard Organization permissible levels for E. coli and Staphylococci. The highest levels of E. coli were 5.96, 6.08, 6.09, 6.25, and 6.01 log CFU/g in Arugula, Radish, Lettuce, Cabbage, and Spring onion, respectively. While for Staphylococcus the highest values were 5.59, 5.71, 4.33, 5.07, and 4.46 log CFU/g in Arugula, Radish, Lettuce, Cabbage, and Spring onion, respectively. No Salmonella or Listeria colonies were found in any samples even after several days of incubations. The Arsenic and Lead concentrations in all samples were significantly higher than the permissible levels set by FAO/WHO, while Chromium was high in some samples. The concentrations of Copper and Iron in most samples fell below their respective permissible levels, while Zinc was undetectable in all samples. These findings underscore inadequate hygiene, harvesting, storage, and agricultural practices, raising concerns about the health implications of consuming contaminated leafy vegetables.

INTRODUCTION

Vegetables are the vital constituents of the human eating regimen providing essential dietary components preserving prime health. Among diverse varieties, leafy vegetables stand out at a distinct position due to their nutrient balance and culinary diversity. The consumption of leafy vegetables continuously increases in Oman due to their promising nutrient profile. Leafy vegetables such as Arugula, Cabbage, Lettuce, Spring onions, and Radish are sources of certain vital minerals (iron, calcium, potassium, and magnesium), vitamins (K, C, E, and many types of B vitamins), and phytonutrients (beta-carotene, lutein, and zeaxanthin) (Gupta *et al.*, 2022) . Settaluri *et al.* (Settaluri *et al.*, 2015) reported the nutritional constituents of Omani leafy vegetables such as Lettuce, Cabbage, and Arugula, they found that all the leafy vegetables possessed high contents of starch, sucrose, and other biomolecules vital for biological functions. Saiwal *et al.* (Saiwal *et al.*, 2019) also found that leafy vegetables are excellent sources of antioxidants, omega-3 fats, selenium, and other healthy minerals. Ismail & Cheah (Ismail & Sook, 2003) proved in their study that Cabbage contains a high content of beta-carotene, riboflavin, vitamin C, and vitamin K per serving. However, irrespective of their exceptional dietary advantages, leafy vegetables are potential carriers of foodborne diseases that threaten dietary safety and community well-being.

Consuming contaminated foods can result in foodborne infections posing challenges to public health and the global economy. Contaminated foods have been associated with outbreaks of foodborne diseases, due to which contaminated leafy vegetables are feared to be the potential cause of foodborne outbreaks. According to the World Health Organization (WHO), as many as 600 million people, or 1 in every 10 people suffer from foodborne diseases every year and almost 420,000 people die (Li *et al.*, 2020). Another study in 2016 discovered that most of the leafy vegetables sold in Finland contained E.coli, Listeria, and Salmonella, highlighting the poor microbial safety of leafy vegetables in Finland (Nousiainen *et al.*, 2016). Among various pathogenic bacterial strains, Escherichia coli, staphylococcus, salmonella, and listeria are the agents responsible for the contamination of leafy vegetables and possible suspects of launching foodborne outbreaks (Marshall *et al.*, 2020). Similarly, Yafetto *et al.* (Yafetto *et al.*, 2019) also reported 8.29 and 8.09 log CFU/g bacterial counts on nutrient agar for cabbage and lettuce, respectively. The resilience and aggressiveness of these pathogenic culprits highlight the cruciality of microbiological analysis of leafy vegetables guaranteeing their safety and preventing the consumers from serious health consequences. A threshold of 2 and 3 Log CFU/g for E. coli and Staphylococcus respectively in leafy vegetables has been established by the Gulf Standard Organization (GSO 1016), which is a regulatory body within the Gulf Cooperation Council (GSO, 2019). This threshold is 0 for Salmonella and Listeria, which shows the zero-tolerance policy of GSO for Salmonella and Listeria in leafy vegetables.

Apart from the microbial hazards, leafy vegetables also host a variety of heavy metals sourced from soil, water, and agricultural products. Plants absorb heavy metals through

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different trails such as contaminated water, irrigation, agrochemicals, and atmospheric depositions (Liu *et al.*, 2005, Hardaway *et al.*, 2016). Additionally, sewage sludge and the application of fertilizers, pesticides, and manure may also influence plants' intake of trace elements. It was found that the uptake of metals by crops is directly linked to the heavy metal concentration in the soil (McBride, 2003). It is vital to ensure that the concentrations of heavy metals in vegetables are less than the permissible limits set by FAO/WHO (WHO, 1989). The permissible concentrations for Arsenic (As), copper (Cu), zinc (Zn), iron (Fe), lead (Pb), and chromium (Cr) are 0.2 mg/kg, 73 mg/kg, 99 mg/kg, 425 mg/kg, 0.3 mg/kg, and 2.3 mg/kg, respectively. Several studies have reported higher concentrations of heavy metals in vegetables than those allowed by FAO/WHO. For instance, Mensah *et al.* (Mensah *et al.*, 2009) reported in their study that all heavy metals except nickel have surpassed the FAO/WHO standardized heavy metals concentrations. Similarly, much higher concentrations of heavy metals were reported in Palestine vegetables by Bawwab *et al.* (Bawwab *et al.*, 2022) compared to the FAO/WHO limits. Excessive intake of certain heavy metals including cadmium, lead, copper, and chromium can cause serious adverse effects including bone, neurological and cardiovascular disorders, renal impairment, and gastrointestinal disturbances. Depending on the exposure time, the toxicity due to heavy metal exposure may be acute or chronic (Jean-Lou Dorne *et al.*, 2010).

To ensure food safety, it is extremely important to monitor the microbial and heavy metal contamination in leafy vegetables along with their associated health risks for the consumers. Therefore, this study aimed to evaluate the safety of leafy vegetables consumed in Oman. For this purpose, we determined the microbiological quality of leafy vegetables by determining the number of colonies of pathogenic bacterial strains that are Escherichia coli, Staphylococci, Salmonella, and Listeria in leafy vegetables. Additionally, we have also determined the heavy metals concentration in leafy vegetables.

MATERIALS AND METHODS

Samples Collection

Twenty-five samples (approximately 500 g each) of edible portions of five leafy vegetables including Lettuce (Lactuca sativa), Cabbage (Brassica oleracea), Arugula, (Eruca sativa), Radish (Raphanus sativus), and Spring onion (Allium fistulosum) were collected in sterile sample bags from different Hypermarkets in Nizwa, Oman. The samples were stored at 5 ◦C until analysis. The microbiological analysis was conducted within 6 hours of the collection. To keep the sources of the samples anonymous, they were represented by numbers i.e.; 1, 2, 3, 4, and 5 along with the first letter of the vegetable's common name.

Microbiological Analysis

The samples were cut down into small pieces using a sterile knife. A sample of 10g was then added to 90 ml of sterile Buffered Peptone Water (BPW), which was made from10 g of peptone powder, 5g of sodium chloride, 3.5 g of disodium phosphate, and 1.5 g of mono-potassium phosphate were added to 1000 ml of water and mixed well using hot-plate stirrer for 5 minutes. For Salmonella and Listeria, the sample was incubated in BPW overnight. However, no overnight incubation was given for E.coli and Staphylococcus as it can affect the threshold colony count of these strains. Different selective mediums were used for all four types of microorganisms used in this study.

Escherichia Coli Count

MacConkey agar (15.45 g in 300 mL H2O) was used for the isolation of E. coli. The media was heated using a hot plate stirrer until boiling followed by sterilization at 121 oC for 15 minutes using an autoclave. The media was poured into sterile petri plates and let solidify at room temperature. Then, three different volumes (0.1 mL, 0.2 mL, and 0.3 mL) of the 1:10 diluted sample were spread on the agar plates using a sterile spreader. After culturing all the samples, the Petri plates were covered with parafilm to prevent cross-contamination during incubation. All the plates were then incubated overnight at 44 ◦C. After 24 hours of incubation, the number of colonies on all the plates was counted (Degaga *et al.*, 2022).

Staphylococci Count

To isolate Staphylococci, Mannitol salt agar was prepared (33.3 g in 300mL H2O). Similar to E. coli isolation, the media was heated and autoclaved. The same volumes and dilutions were added to the plates and incubated at 37 ◦C for 24 hours before counting the colonies (Degaga *et al.*, 2022).

Salmonella Count

Xylose-lysine-deoxycholate (XLD) was prepared (17.004 g of XLD was added to 300 mL dH2O) for the selective isolation of Salmonella. The medium was heated at a very low temperature and then transferred to a water bath for a few minutes at 45-50 oC before use. The same volumes and dilutions were added to the plates and incubated at 37 ◦C for 24 hours before counting the colonies (Degaga *et al.*, 2022, Sant'Ana *et al.*, 2011).

Listeria Count

For Listeria isolation, 20.66 g of PALCAM agar was dissolved in H2O. The medium was heated till boiling, then sterilized at 121oC for 15 minutes. After sterilization, 0.6 mL of selective supplement (Polymyxin B, acriflavine, and ceftazidime combined) was also added to PALCAM agar for the selective isolation of Listeria. The same volumes and dilutions were added to the plates and incubated at 37 ◦C for 24 hours before counting the colonies (Degaga *et al.*, 2022, Abatcha *et al.*, 2020).

Heavy Metal Analysis

The collected leafy vegetables were chopped into small pieces, dried in an oven at 60 °C until constant weight, and crushed to powder form using a regular blender. For the accurate analysis of heavy metals, complete digestion is essential. For this purpose, 0.5 g of sample was weighed and 5mL of nitric acid (HNO3) was added. Then the sample was introduced to a microwave digestion apparatus (model: ULTRAWAVE, SN:16040687, Milestone srl, Sorisole, Italy). After digestion in the microwave combustion system, the solution was filtered using Whatman filter paper. Then the filtrate was analyzed with Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES, OPTIMA 8000, PerkinElmer, Massachusetts, America). Five calibration standards were taken for each sample. The calibration concentration ranged from 0.07-3 for As, 1-20 for Cu, 1-100 for Zn, 1-150 for Fe, 1-20 for Pb, and 1-20 mg/L for Cr. The amounts of As, Cu, Zn, Fe, Pb, and Cr, were determined in mg/kg. All the samples were experimented on twice and the resulting values are the average of the duplicates (Bingöl *et al.*, 2010).

Statistical Analysis

The data were evaluated statistically using Microsoft Excel for the mean value and standard deviation (SD). Analysis of variance (ANOVA) was used to determine the level of significance ($P < 0.05$).

RESULTS AND DISCUSSION

Microbiological Analysis

In this study, five different leafy vegetables i.e., Arugula, cabbage, onion, lettuce, and radish from five different hypermarkets, were evaluated for their microbiological qualities. Table 1 represents the log values ± standard deviation of the calculated colonies per gram compared to the standard values outlined by GSO 1016 for each bacterium (GSO, 2019). The results showed that all the samples had high microbial contamination levels, and their log CFU/g values were much higher than the standard values for E. coli and Staphylococci. This highlights the poor hygiene, harvesting, storage, and agricultural practices. However, the log CFU/g values varied among the varieties of leafy vegetables and the hypermarkets from where the samples were collected.

The E. coli counts in all samples were significantly higher than the permissible limits set by GSO 1016 (2.0 Log CFU/g) (GSO, 2019). The E. coli count ranged between 5.21-5.96 in Arugula, 5.46-6.08 in Radish, 5.32-6.25 in Lettuce, 3.77-6.09 in Cabbage and 5.30-6.01 in Spring onion. Even the lowest Log CFU/g values in this study are much higher than those previously reported by Khalil (Khalil, 2016) for arugula which was 4.51 for E. coli. Our results are also in line with those of Hashemi *et al.* (Hashemi *et al.*, 2019) who reported 4.2 Log CFU/g for E. coli in radish. These values are higher than the average Log CFU/g values of 3.34 for E. coli in lettuce reported by Choi *et al.* (Choi *et al.*, 2016). Among the Spring onions of all the brands, O2 and O5 were the most contaminated with 6.01 Log CFU/g each for E.coli. O1 exhibited the next highest Log CFU/g for E.coli, leaving O3 to be the least contaminated among all the samples for E. coli. In a previous study reported by Xu *et al.* (Xu *et al.*, 2013), the initial E.coli contamination in onion leaves was 5.2 log CFU/g, which is comparatively less than the one found in our study.

Also, the Staphylococci counts in all samples were significantly higher than the permissible limits set by GSO 1016 (3.0 Log CFU/g) (GSO, 2019). The Staphylococci counts ranged between 4.59-5.59 in Arugula, 4.62-5.71 in Radish, 3.10-5.07 in Lettuce, 3.27-4.33 in Cabbage and 3.48-4.46 in Spring onion. Our results showed higher log CFU/g values than those of Hashemi *et al.* (Hashemi *et al.*, 2019) who reported 3.3 Log CFU/g for Staphylococci in radish. Even though the values for Staphylococci in the cabbage sample are surprisingly much less than the rest of the sample, they are almost similar to the log CFU/g values for both E.coli and Staphylococci (4.44) reported by Kothe *et al.* (Kothe *et al.*, 2019) when stored for 5 days at 30°C. They also reported Log CFU/g values for E. coli much less than the values that resulted in our study. However, their values increased significantly when incubated for a whole week. This indicates that improper storage of vegetables for extended periods provides microorganisms optimal conditions for growth resulting in the microbial contamination and spoilage of vegetables. These values are higher than the average Log CFU/g values of 4.62 for Staphylococci in lettuce reported by Choi *et al.* (Choi *et al.*, 2016). Sample O5 resulted in being the most contaminated with 4.46 Log CFU/g for Staphylococci among the onion samples. The sample O2 exhibited the next highest Log CFU/g in Staphylococci, leaving O4 to be the least contaminated among all the samples for Staphylococci.

The overall results showed that leafy vegetables from hypermarkets 2 and 5 were the most contaminated with E. coli and Staphylococci when compared to the samples from the rest of the Omani hypermarkets. However, all the samples showed significantly higher microbial contamination than the GSO 1016 standards (GSO, 2019). This raises a critical concern regarding the microbial quality of leafy vegetables from all the hypermarkets. Our study highlights the questionable transportation, distribution, storage, and handling of leafy vegetables in hypermarkets. Encouragingly, not a single colony of Salmonella and Listeria was observed in any of the samples from all the hypermarkets even after several days of incubation. Both strains are very pathogenic and have the potential to cause severe health complications in consumers. Unlike the previous studies of Aytaç *et al.* (Aytac *et al.*, 2010), Abadias *et al.* (Abadias *et al.*, 2008), and Fröder *et al.* (Fröder *et al.*, 2007), who reported the high prevalence of Salmonella and Listeria in leafy vegetables, our results suggest that Omani leafy vegetables are free of Salmonella and Listeria contamination. The reason behind this might be due to competition between Salmonella and Listeria, and the background bacteria which in this case are E. coli and Staphylococcus. This hypothesis was also stated by Abadias *et al.* (Abadias *et al.*, 2012), who suggested that different bacterial species on a single food sample can compete for physical space as well as nutrient availability. Similarly, Babic *et al.* (Babic *et al.*, 1997) also reported in their study that the background microbiota such as E.coli inhibited the growth of Listeria in spinach.

This indicates that leafy vegetables with elevated levels of one bacterium can cause growth inhibition of other pathogenic bacteria. The absence of these strains highlights the appreciable food safety of Omani hypermarkets. However, the presence of E. coli and staphylococcus at such a significantly high number cannot be neglected. The high prevalence of E.coli and Staphylococcus in leafy vegetables raises concerns regarding safety and public health. Both these bacteria are pathogenic, making these leafy vegetables the potential source of food-borne outbreaks. These leafy vegetables contaminated with these bacterial strains can cause severe food-borne communicable illnesses, especially the local consumers unaware of the consequences of contaminated food. Protective measures should be taken by the food safety authorities to address the raised concerns and ensure public health.

Table 1: The average microbial count of selected leafy vegetables collected from markets in Nizwa, Oman

Samples	**E. coli Log CFU/g**	**Staphylococci Log CFU/g**	**Salmonella Log CFU/g**	**Listeria Log CFU/g**
GSO 1016	2.0^{a}	3.0^{a}	0	0
Arugula:				
A1	5.73 ± 0.11^{b}	5.33 ± 0.24^{b}	ND	ND
A2	5.21 ± 0.25^{c}	5.59 ± 0.15^{b}	ND	ND
A3	5.75 ± 0.23^{b}	4.90 ± 0.13^{c}	ND	ND
A4	5.27 ± 0.04^{c}	4.59 ± 0.12^{d}	ND	ND
A5	5.96 ± 0.14^{b}	5.43 ± 0.16^{b}	ND	ND
Radish:				
R1	5.46 ± 0.21^{b}	5.65 ± 0.16^{b}	ND	ND
R2	5.63 ± 0.11^{b}	5.08 ± 0.16^{c}	ND	ND
R3	5.62 ± 0.03^{b}	4.62 ± 0.08^{d}	ND	ND
R4	5.98 ± 0.19^{b}	5.30 ± 0.11^{b}	ND	ND
R5	6.08 ± 0.13^{b}	5.71 ± 0.14^{b}	ND	ND
Lettuce:				
L1	5.71 ± 0.15^{b}	3.72 ± 0.16^{e}	ND	ND
L2	6.25 ± 0.20^{b}	4.51 ± 0.12^{d}	ND	ND
L3	5.32 ± 0.13^{c}	4.84 ± 0.06^{c}	ND	ND
L4	5.80 ± 0.09^{b}	3.10 ± 0.17^{a}	ND	ND
L5	5.74 ± 0.21^{b}	5.07 ± 0.08^{c}	ND	ND
Cabbage:				
C1	3.77 ± 0.15^{d}	3.43±0.13f	ND	ND
C2	5.85 ± 0.15^{b}	4.33 ± 0.11^{d}	ND	ND
C3	5.65 ± 0.11^{b}	3.27 ± 0.14^{a}	ND	ND
C4	6.09 ± 0.20^{b}	4.07 ± 0.10^{j}	ND	ND
C5	5.63 ± 0.17^{b}	3.69 ± 0.13^{e}	ND	ND
Spring Onion:				
O1	5.84 ± 0.14^{b}	4.23 ± 0.12^{d}	ND	ND
O2	6.01 ± 0.14^{b}	4.11 ± 0.12^{j}	ND	ND
O3	5.49 ± 0.11^{b}	3.48±0.16e	ND	ND
O4	5.30 ± 0.03^{c}	3.91±0.06j	ND	ND
O5	6.01 ± 0.19^{b}	4.46 ± 0.09^{d}	ND	ND

Means ± SD followed by the same letter, within a column, are not significantly different (P > 0.05). Samples 1-5: samples from 5 distinct locations, ND: Not detected.

Heavy Metal Analysis

Table 2 represents the concentrations of As, Cu, Zn, Fe, Pb, and Cr per Kg of leafy vegetables from five different hypermarkets in Oman. The concentrations of As, and Pb, in all the leafy vegetable samples from all the hypermarkets were significantly higher than the permissible limits set by FAO/WHO (WHO, 1989) while some samples had higher concentrations of Cr. The concentrations of Cu and most of the Fe samples fell below their respective permissible levels, while Zn was non-detective in all the samples. However, the concentrations of heavy metals varied between the samples.

The results showed that the concentrations of As range from 1.67 to 2.14 mg/kg in arugula, 1.89 to 2.19 mg/kg in radish, 1.98 to 2.19 mg/kg in lettuce, 1.95 to 2.26 mg/kg in cabbage, and 1.65 to 1.92 mg/kg in spring onion. All these values are far higher than the 0.2 mg/kg permissible concentration set by FAO/WHO (WHO, 1989). Our study completely contradicts previous studies reporting As concentrations in leafy vegetables. For instance, Ma *et al.* (Ma *et al.*, 2007) reported 0.09 and 0.14 mg/kg of As in lettuce and spring onion. However, Gezahegn *et al.* (Gezahegn *et al.*, 2017) also previously reported 1.4 mg/kg of As in cabbage. such elevated concentrations of As are associated with cardiovascular problems in humans such as hypertension, atherosclerosis, and peripheral cardiovascular diseases (Balarastaghi *et al.*, 2022). The consumption of these vegetables contaminated with As can lead to serious health issues.

Cu concentrations in all the samples are significantly lower than the permissible limit of 73 mg/kg for leafy vegetables. The Cu concentration ranged from 13.8 to 19.4 mg/kg in arugula, 13.9 to 23.7 mg/kg in radish, 12.7 to 18.4 in lettuce, 9.1 to 13.2 mg/kg in cabbage, and 12.6 to 18.7 mg/kg in spring onion. Our results align with those of Kananke et al (Kananke *et al.*, 2014), who reported that the concentrations of Cu fell below the permissible limits. Even though Cu is important for several body functions such as central nervous system maintenance and anemia prevention, its elevated levels can be toxic to the human body such as liver damage, gastrointestinal symptoms, and kidney damage (Kananke *et al.*, 2014). Fortunately, our study reassures the Cu levels below the threshold associated with toxicity.

Even though Fe has a very high threshold concentration of 425 mg/kg in leafy vegetables, most of the arugula samples still crossed that limit. The samples in which the Fe concentrations were higher than the FAO/WHO limit are A1 (665 mg/kg), A2 (699 mg/kg), A4 (744 mg/kg), and A5 (633 mg/kg) in arugula, R1 (479 mg/kg) in radish, and O1 (524 mg/kg) in spring onion. Fe concentrations in cabbage were non-detectable. All the remaining samples have permissible levels of Fe. Interestingly, lettuce samples from all the hypermarkets have Fe concentrations below the threshold levels. These results agree with the previous studies (Uriu-Adams & Keen, 2005, Hanif *et al.*, 2006, Zwolak *et al.*, 2019), which reported that Fe levels in lettuce fall within the permissible limits. Fe is important for many biological functions in the body such as oxygen transport, energy production, and immune functions (Demi-Rezen & Aksoy, 2006). However, some of the tested samples, especially arugula possess elevated levels of Fe that can potentially cause negative effects such as increased oxidative stress, pancreas damage, and cardiomyopathy (Lieu *et al.*, 2001, Sampaio *et al.*, 2014)

Pb concentrations ranged from 2.86 to 15.89 mg/kg in arugula, 2.8 to 6.01 mg/kg in radish, 3.55 to 4.85 mg/kg in lettuce, 4.07 to 5.46 mg/kg in cabbage, and 2.97 to 3.32 mg/kg in spring onion. These concentrations are significantly higher than the maximum permitted by FAO/WHO for leafy vegetables. However, these concentrations are far lower than the 12.3 mg/kg of Pb in cabbage reported by Gezahegn *et al.* (Gezahegn *et al.*, 2017). These elevated levels of Pb in vegetables can result either from the ink-contaminated water absorbed by the soil or from the water used for watering the plants directly, especially the water leaked from industries as Gezahegn *et al.* (Gezahegn *et al.*, 2017) suggested that the high levels of Pb in cabbages can be attributed to the ink leakage from industries to the water. Such elevated levels of Pb can have negative impacts on the human body such as kidney dysfunction, and brain damage as it is toxic to the central and peripheral nervous system (Murphy & Oudit, 2010).

Most of the tested samples possess elevated Cr concentrations exceeding the permissible limit of 2.3 mg/kg for vegetables. A2 exhibited the highest concentration of 11.65 mg/kg, while the lowest Cr concentration of 1.13 mg/kg was exhibited by C5. Arugula, was found to be the most contaminated with Cr with an average Cr concentration of 6.7 mg/kg, followed by radish, lettuce, and Spring Onion with average concentrations of 3.73 mg/kg, 3.51 mg/kg and 3.27 mg/kg respectively. Kananke *et al.* (Kananke *et al.*, 2014) reported a much lower average Cr concentration of 1.79 mg/kg in leafy vegetables that falls within the permissible level. Such high levels of Cr can be attributed to the using waste for the irrigation of leafy vegetables (Nava-Ruíz & Méndez-Armenta, 2013). Due to the persistence and high toxicity of Cr, it is considered to be very toxic for human biological processes (Soumi *et al.*, 2016).

Some of the heavy metals such as Cu, Zn, and Fe are vital for the biological functions of the human body. However, the elevated levels of these metals can exhibit adverse effects and can be toxic to the human body. Therefore, it is crucial to ensure the concentrations of heavy metals are not above the FAO/WHO recommended levels in foods (leafy vegetables in this study). There could be multiple reasons for the elevated contents of such toxic heavy metals in vegetables but fertilizers and irrigation water are the most convincing reasons. Bawwab *et al.* (Bawwab *et al.*, 2022) suggested that sometimes compost contaminated with industrial waste is utilized as a fertilizer containing high contents of heavy metals.

Mensah *et al.* (Mensah *et al.*, 2009) argued that apart from fertilizers, the use of contaminated water can also increase the heavy metals absorption in plants. When roots absorb Pb, it cannot go through the root's endoderms. However, the results from the study of Domergue & Vedy (Domergue & Védy, 1992) showed that leafy vegetables not only accumulate Pb from the soil but also use leaves to accumulate Pb from the atmosphere. Other factors such as the plant species, the type of soil, growth phase, and the surrounding environment greatly affect the accumulation of heavy metals in plants. Even the atmospheric deposition and marketing can be the potential reasons for the elevated heavy metals contents. The elevated samples are directly associated with the health consequences of consumers. As we discussed above, intake of vegetables with such prominent levels of heavy metals can lead to many serious health issues such as hypertension and cardiovascular diseases due to As, gastrointestinal, kidney, and liver damage due to copper, cardiomyopathy, and pancreas damage due to Fe, compromised central and peripheral nervous system due to Pb, and kidney dysfunction due to Cr.

Table 2: The heavy metal concentration of selected leafy vegetables collected from markets in Nizwa, Oman

Samples	Arsenic mg/kg	Copper mg/kg	Zinc mg/kg	Iron mg/kg	Lead mg/kg	Chromium mg/kg
WHO/FAO limits	0.2[a]	73[a]	99	425[a]	0.3[a]	2.3[a]
Arugula						
A1	ND	19.4±0.4[b]	ND	665±67[b]	4.36±0.39[b]	10.40±0.89[b]
A2	1.84±0.19[b]	18.3±0.7[c]	ND	699±95[b]	15.89±1.62[c]	11.65±1.48[c]
A3	1.90±0.34[b]	16.8±1.5[d]	ND	149±11[c]	4.16±0.51[b]	2.17±0.11[d]
A4	2.14±0.15[c]	14.8±0.7[e]	ND	744±17[d]	2.86±0.12[d]	4.86±0.11[e]
A5	1.67±0.01[d]	13.8±0.1[f]	ND	633±38[b]	3.43±0.0.39[e]	4.43±0.04[f]
Radish						
R1	1.89±0.29[b]	15.2±0.8[g]	ND	479±62[e]	2.8±0.28[d]	6.8±1.1[g]
R2	1.92±0.10[b]	14.3±0.3[e]	ND	263±23[f]	3.34±0.22[e]	3.54±0.18[h]
R3	2.19±0.10[c]	23.7±1.8[h]	ND	271±10[f]	6.01±0.91[f]	3.12±0.42[i]
R4	2.06±0.03[c]	13.9±0.3[f]	ND	343±25[g]	3.26±0.79[e]	2.13±0.54[d]
R5	1.92±0.02[b]	15.3±0.6[g]	ND	106±8[h]	4.34±0.69[b]	3.07±0.17[i]
Lettuce						
L1	2.04±0.14[c]	12.7±0.1[i]	ND	207±13[i]	4.49±0.32[b]	2.90±0.38[i]
L2	2.05±0.01[c]	18.4±0.1[c]	ND	16± [j]	4.85±0.78[g]	1.49±0.08 [j]
L3	2.11±0.15[c]	13.5±1.1[f]	ND	52±5[k]	3.55±0.15[e]	4.29±0.21[f]
L4	1.98±0.22[b]	12.9±0.2[i]	ND	106±15[h]	ND	6.55±0.01[g]
L5	2.19±0.20[c]	13.5±1.1[f]	ND	40±8l	4.44±0.72[b]	2.34±0.40[k]
Cabbage						
C1	1.95±0.24[b]	13.2±0.5[f]	ND	ND	4.44±0.41[b]	3.42±0.77[h]
C2	2.19±0.21[c]	12.7±2.3[i]	ND	ND	4.07±0.68[h]	1.72±0.15[l]
C3	1.96±0.11[b]	9.1±1.5[j]	ND	ND	4.77±0.73[g]	1.21±0.05[m]
C4	2.26±0.09[c]	11.8±1.2[k]	ND	ND	5.46±0.21[i]	1.59±0.02[j]
C5	2.05±0.07[c]	11.1±1.4[k]	ND	ND	4.73±0.60[g]	1.13±0.10[m]
Spring Onion						
O1	1.91±0.27[b]	14.2±2.9[e]	ND	524±4[k]	3.28±0.68[e]	7.71±0.12[n]
O2	1.65±0.01[d]	12.6±0.2[i]	ND	ND	3.07±0.08[e]	1.44±0.07[j]
O3	1.92±0.02[b]	18.7±0.6[b]	ND	62±2[m]	3.32±0.49[e]	2.47±0.40[k]
O4	1.91±0.04[b]	15.6±0.7[g]	ND	233±12[n]	2.97±0.14[d]	2.76±0.03[o]
O5	1.9±0.03[b]	18.5±0.3[b]	ND	121±5[o]	2.97±0.04[d]	1.99±0.32[q]

Means ± SD followed by the same letter, within a column, are not significantly different (P > 0.05). Samples 1-5: samples from 5 distinct locations, ND: Not detected.

CONCLUSION

It can be concluded from the present study that leafy vegetables have high microbiological and heavy metals contamination. Most of the samples used in this study were contaminated with E. coli and Staphylococci as well as some toxic heavy metals such as As, Pb, and Cr. This study highlights the questionable production, irrigation, fertilization, harvesting, packaging, transportation, handling, and storage of leafy vegetables in Oman. The consumption of vegetables with such microbiological and heavy metals content can lead to concerning health issues among the consumers. Further work needs to be conducted to ensure microbiological safety, assessing more food samples for even more pathogenic bacterial strains. Additionally, the heavy metals accumulation by other vegetables and fruits also needs to be explored.

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REFERENCES

Abadias, M., Alegre, I., Oliveira, M., Altisent, R., & Viñas, I. (2012). Growth potential of Escherichia coli O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. *Food Control, 27*(1), 37-44. https://doi.org/10.1016/j.foodcont.2012.02.032

Aytac, S. A., Ben, U., Cengiz, C., & Taban, B. M. (2010). Evaluation of Salmonella and Listeria monocytogenes contamination on leafy green vegetables. *Journal of Food Agriculture & Environment, 8*(2), 275-279. https://doi.org/10.5072/zenodo.36512

Abadias, M., Usall, J., Anguera, M., Solsona, C., & Viñas, I. (2008). Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. *International Journal of Food Microbiology, 123*(1-2), 121-129. https://doi.org/10.1016/j.ijfoodmicro.2007.12.013

Abatcha, M. G., Tan, P. L., Chuah, L. O., Rusul, G., Chandraprasad, S. R., & Effarizah, M. E. (2020). Evaluation of 3MTM loop-mediated isothermal amplification-based kit and 3MTM ready-to-use plating system for detection of Listeria in naturally contaminated leafy vegetables, chicken, and their related processing environments. *Food Science and Biotechnology/Food Science and Biotechnology, 29*(8), 1141–1148. https://doi.org/10.1007/s10068-020-00762-2

Babic, I., Watada, A., & Buta, J. (1997). Growth of Listeria monocytogenes Restricted by Native Microorganisms and Other Properties of Fresh-Cut Spinach. *Journal of Food Protection, 60*(8), 912-917. https://doi.org/10.4315/0362-028x-60.8.912

Balarastaghi, S., Rezaee, R., Hayes, A. W., Yarmohammadi, F., & Karimi, G. (2022). Mechanisms of Arsenic Exposure-Induced Hypertension and Atherosclerosis: an Updated Overview. *Biological Trace Element Research, 201*(1), 98-113. https://doi.org/10.1007/s12011-022-03153-2

Bawwab, M., Qutob, A., Khatib, M. A., Malassa, H., Shawahna, A., & Qutob, M. (2022). Evaluation of Heavy Metal Concentrations in Soil and Edible Vegetables Grown in Compost from Unknown Sources in Al-Jiftlik, Palestine. *Journal of Environmental Protection, 13*(01), 112-125. https://doi.org/10.4236/jep.2022.131007

Bingöl, M., Yentür, G., Er, B., & Öktem, A. B. (2010). Determination of some heavy metal levels in soft drinks from Turkey using ICP/OES method. *Czech Journal of Food Sciences, 28*(3), 213-216. https://doi.org/10.17221/158/2008-cjfs

Choi, Y., Jiyeon, J., Junghoon, P., (2016). Kinetic behavior of pathogenic Escherichia coli and Staphylococcus aureus in fresh vegetables during storage at constant and changing temperature. *J. Bioanal. Biostat, 1,* 1-6. https://www.avensonline.org/wp-content/uploads/JBABS-2641-8681-01-0004.pdf

Degaga, B., Sebsibe, I., Belete, T., & Asmamaw, A. (2022). Microbial Quality and Safety of Raw Vegetables of Fiche Town, Oromia, Ethiopia. *Journal of Environmental and Public Health, 2022,* 1-10. https://doi.org/10.1155/2022/2556858

Demi-Rezen, D., & Aksoy, A. (2006). Heavy metal levels in vegetables in turkey are within safe limits for cu, zn, ni and exceeded for cd and Pb. *Journal of Food Quality, 29*(3), 252-265. https://doi.org/10.1111/j.1745-4557.2006.00072.x

Domergue, F. L., & Védy, J. C. (1992). Mobility of Heavy Metals in Soil Profiles. *International Journal of Environmental Analytical Chemistry, 46*(1-3), 13-23. https://doi.org/10.1080/03067319208026993

Fröder, H., Martins, C. G., De Souza, K. L. O., Landgraf, M., Franco, B. D., & Destro, M. T. (2007). Minimally Processed Vegetable Salads: Microbial Quality Evaluation. *Journal of Food Protection, 70*(5), 1277-1280. https://doi.org/10.4315/0362-028x-70.5.1277

Gupta, N., Yadav, K. K., Kumar, V., Prasad, S., Cabral-Pinto, M. M. S., Jeon, B. H., Kumar, S., Abdellattif, M. H., & Alsukaibia, A. K. D. (2022). Investigation of Heavy Metal Accumulation in Vegetables and Health Risk to Humans From Their Consumption. *Frontiers in Environmental Science, 10.* https://doi.org/10.3389/fenvs.2022.791052

GSO 1016, (2015). Microbiological Criteria For Foodstuffs. Gulf Technical Regulation, GCC Standardization Organization, Approved on 05 November 2015 · Edition 2.

Gezahegn, W. W. (2017). Study of Heavy Metals Accumulation in Leafy Vegetables of Ethiopia. *IOSR Journal of Environmental Science, Toxicology and Food Technology, 11*(05), 57-68. https://doi.

org/10.9790/2402-1105015768

Hardaway, C. J., Sneddon, J., Sneddon, E. J., Kiran, B., Lambert, B. J., McCray, T. C., Bowser, D. Q., & Douvris, C. (2016). Study of selected metal concentrations in sediments by inductively coupled plasma-optical emission spectrometry from a metropolitan and more pristine bayou in Southwest Louisiana, United States. *Microchemical Journal, 127,* 213-219. https://doi.org/10.1016/j.microc.2016.02.016

Hashemi, S. M. B., Abhari, K., & Khaneghah, A. M. (2019). The combined effects of ultrasound and lactic acid in inactivating microorganisms on fresh radish (Raphanus raphanistrum subsp. sativus): Microbiological and quality changes. *Food Science & Nutrition, 8*(1), 162-169. https://doi.org/10.1002/fsn3.1287

Hanif, R., Zafar, I., Mudassar, I., Shaheena, H., & Masooma, R. (2006). Use of vegetables as nutritional food: role in human health. *Journal of Agricultural and Biological Science, 1*(1), 18-22. https://www.arpnjournals.com/jabs/research_papers/jabs_0706_03.pdf

Ismail, A., & Sook, F. C. (2003). Determination of vitamin C, β-carotene and riboflavin contents in five green vegetables organically and conventionally grown. *Malaysian journal of nutrition, 9*(1), 31-39. https://pubmed.ncbi.nlm.nih.gov/22692530/

Jean-Lou Dorne, George EN Kass, Luisa R Bordajandi, Billy Amzal, Ulla Bertelsen, Anna F Castoldi, Claudia Heppner, Mari Eskola, Stefan Fabiansson, Pietro Ferrari, Elena Scaravelli, Eugenia Dogliotti, Peter Fuerst, Alan R Boobis, Philippe Verger (2010). Human risk assessment of heavy metals: principles and applications. *Metal Ions in Life Sciences, 8,* 27-60. https://pubmed.ncbi.nlm.nih.gov/21473375/

Khalil, R. K. (2016). Effect of abusive storage temperatures on growth and survival of Escherichia coli O157:H7 on leafy salad vegetables in Egypt. *Lebensmittel-Wissenschaft + Technologie/Food Science & Technology, 65,* 954-962. https://doi.org/10.1016/j.lwt.2015.09.034

Kothe, C. I., Pessoa, J. P., Malheiros, P. S., & Tondo, E. C. (2019). Assessing the growth of Staphylococcus aureus and Escherichia coli on fruits and vegetables. *Journal of Infection in Developing Countries, 13*(06), 480-486. https://doi.org/10.3855/jidc.10573

Kananke, T., Wansapala, J., & Gunaratne, A. (2014). Heavy Metal Contamination in Green Leafy Vegetables Collected from Selected Market Sites of Piliyandala Area, Colombo District, Sri Lanka. *American Journal of Food Science and Technology, 2*(5), 139-144. https://doi.org/10.12691/ajfst-2-5-1

Li, W., Pires, S. M., Liu, Z., Ma, X., Liang, J., Jiang, Y., Chen, J., Liang, J., Wang, S., Wang, L., Wang, Y., Meng, C., Huo, X., Lan, Z., Lai, S., Liu, C., Han, H., Liu, J., Fu, P., & Guo, Y. (2020). Surveillance of foodborne disease outbreaks in China, 2003-2017. *Food Control, 118,* 107359. https://doi.org/10.1016/j.foodcont.2020.107359

Lieu, P. T., Heiskala, M., Peterson, P. A., & Yang, Y. (2001). The roles of iron in health and disease. *Molecular Aspects of Medicine, 22*(1-2), 1-87. https://doi.org/10.1016/s0098-2997(00)00006-6

Liu, W. H., Zhao, J. Z., Ouyang, Z. Y., Söderlund, L., & Liu, G. H. (2005). Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China. *Environment International, 31*(6), 805-812. https://doi.org/10.1016/j.envint.2005.05.042

Mensah, E., Kyei-Baffour, N., Ofori, E., & Obeng, G. (2009). Influence of Human Activities and Land Use on Heavy Metal Concentrations in Irrigated Vegetables in Ghana and Their Health Implications. In *Springer eBooks* (pp. 9–14). https://doi.org/10.1007/978-1-4020-9139-1_2

McBride, M. (2003). Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks? *Advances in Environmental Research, 8*(1), 5–19. https://doi.org/10.1016/s1093-0191(02)00141-7

Ma, L., Yang, Z., Kong, Q., & Wang, L. (2017). Extraction and determination of arsenic species in leafy vegetables: Method development and application. *Food Chemistry, 217,* 524-530. https://doi.org/10.1016/j.foodchem.2016.09.015

Marshall, K. E., Nguyen, T. A., Ablan, M., Nichols, M. C., Robyn, M. P., Sundararaman, P., Whitlock, L., Wise, M. E., & Jhung, M. A. (2020). Investigations of Possible Multistate Outbreaks of Salmonella, Shiga Toxin–Producing Escherichia coli, and Listeria monocytogenes Infections - United States, 2016. *Morbidity and Mortality Weekly Report. Surveillance Summaries, 69*(6), 1-14. https://doi.org/10.15585/mmwr.ss6906a1

Murphy, C. J., & Oudit, G. Y. (2010). Iron-Overload Cardiomyopathy: Pathophysiology, Diagnosis, and Treatment. *Journal of Cardiac Failure, 16*(11), 888-900. https://doi.org/10.1016/j.cardfail.2010.05.009

Nava-Ruíz, C., & Méndez-Armenta, M. (2013). Cadmium, Lead, Thallium: Occurrence, Neurotoxicity and Histopathological Changes of the Nervous System. In *Environmental chemistry for a sustainable world* (pp. 321-349). https://doi.org/10.1007/978-3-319-02387-8_6

Nousiainen, L. L., Joutsen, S., Lunden, J., Hänninen, M. L., & Fredriksson-Ahomaa, M. (2016). Bacterial quality and safety of packaged fresh leafy vegetables at the retail level in Finland. *International Journal of Food Microbiology, 232,* 73-79. https://doi.org/10.1016/j.ijfoodmicro.2016.05.020

Saiwal, N., Dahiya, M., & Dureja, H. (2019). Nutraceutical Insight into Vegetables and their Potential for Nutrition Mediated Healthcare. *Current Nutrition & Food Science, 15*(5), 441-453. https://doi.org/10.2174/1573401314666180115151107

Settaluri, V. S., Al-Mamari, K. M. K., Al-Balushi, S. I. M., Al-Risi, M. K. Z., & Ali, M. B. (2015). Review of Biochemical and Nutritional Constituents in Different Green Leafy Vegetables in Oman. *Food and Nutrition*

Sciences, 06(09), 765-769. https://doi.org/10.4236/fns.2015.69079

Souri, M. K., Alipanahi, N. A., & Tohidloo, G. (2016). Heavy metal content of some leafy vegetable crops grown with wastewater in southern suburb of Tehran-Iran. *Vegetable Science, 43*(2), 156-162. https://doi.org/10.61180/

Sampaio, A. F. S., Silva, M., Dornas, W. C., Costa, D. C., Silva, M. E., Santos, R. C. D., De Lima, W. G., & Pedrosa, M. L. (2014). Iron toxicity mediated by oxidative stress enhances tissue damage in an animal model of diabetes. *BioMetals, 27*(2), 349-361. https://doi.org/10.1007/s10534-014-9717-8

Sant'Ana, A. S., Landgraf, M., Destro, M. T., & Franco, B. D. (2011). Prevalence and counts of Salmonella spp. in minimally processed vegetables in São Paulo, Brazil. *Food Microbiology, 28*(6), 1235-1237. https://doi.org/10.1016/j.fm.2011.04.002

Uriu-Adams, J. Y., & Keen, C. L. (2005). Copper, oxidative stress, and human health. *Molecular Aspects of Medicine, 26*(4-5), 268-298. https://doi.org/10.1016/j.mam.2005.07.015

World Health Organisation (WHO), (1989). Report of 33rd meeting, Joint FAO/WHO Joint Expert Committee on Food Additives, Toxicological evaluation of certain food additives and contaminants No. 24, International Programme on Chemical Safety, WHO, Geneva. https://www.who.int/publications/i/item/9241207760

Xu, W., Chen, H., Huang, Y., & Wu, C. (2013). Decontamination of Escherichia coli O157:H7 on green onions using pulsed light (PL) and PL–surfactant–sanitizer combinations. *International Journal of Food Microbiology, 166*(1), 102-108. https://doi.org/10.1016/j.ijfoodmicro.2013.06.027

Yafetto, L., Ekloh, E., Sarsah, B., Amenumey, E. K., & Adator, E. H. (2019). Microbiological Contamination of some Fresh Leafy Vegetables Sold in Cape Coast, Ghana. *Deleted Journal, 60*(2), 11-23. https://doi.org/10.4314/gjs.v60i2.2

Zwolak, A., Sarzyńska, M., Szpyrka, E., & Stawarczyk, K. (2019). Sources of Soil Pollution by Heavy Metals and Their Accumulation in Vegetables: a Review. *Water, Air and Soil Pollution/Water, Air & Soil Pollution, 230*(7). https://doi.org/10.1007/s11270-019-4221-y