



Assessment of Chemical and Bacteriological Parameters of Leek in Sulaymaniyah City, Iraq

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Abstract

In the current study, leek samples were collected seasonally from October 2021 to July 2022 from three sites; site 1 (Tanjaro aera), site 2 (Kanaswra), and site 3 (Aziz Awa) in Sulaymaniyah city, Iraq. Cl^- rate recorded the maximum mean values in leek, which was 0.05 mg/kg. The Na^+ level recorded the highest mean value, at 393.00 mg/kg. The K^+ ratio showed the highest mean value, measuring 3614 mg/kg. SO_4^{2-} concentration had a mean value of 762.33 mg/kg. PO_4^{3-} level recorded the maximum mean value at 16.13. Regarding heavy metals in vegetables, the results indicated no contamination in leek, except for Pb, which had a high level in the vegetables. In terms of bacteriology, leek samples exhibited total aerobic bacteria and total coliform counts above the normal range. Based on the biochemical test findings, *E. coli* and *Salmonella* species were not detected in leek. Different species were isolated from leek, including *Citobacter freundii*, *Citobacter braakii*, *Acinetobacter baumannii* complex, *Enterobacter cloacae* complex.

There is a notable correlation between total aerobic bacteria and lead ($r = 0.60$) in leek. Copper shows a negative correlation with total aerobic bacteria ($r = -0.59$), while iron also exhibits a negative relationship with total aerobic bacteria ($r = -0.60$). Total coliform demonstrates a positive relationship with manganese ($r = 0.64$).

Keywords: Tanjaro River; Leek; Heavy metals; Coliform; Kanaswra

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1. Introduction

Fresh fruits and vegetables are health-promoting components in the human diet. Vegetables are high in vitamins, minerals, dietary fibers, and phytochemicals¹. Leek is the most widely commercially cultivated vegetable in the world, belonging to the *Allium* genus (family Alliaceae). The leaves with a long white blanched stem are eaten cooked or utilized in appetizers². The *Allium* genus is distinguished by the presence of bioactive substances such as flavonoids, polysaccharides, and

glycosylates, as well as various organosulfur compounds³. Leek efficiency has been demonstrated to enhance gastrointestinal health, lower the incidence of heart disease, stroke, and chronic illnesses including diabetes and several types of cancer¹. However, vegetables are a high-risk source for microbial diseases to spread⁴. In recent years, the number of food-borne diseases linked to the consumption of tainted fresh produce has increased. It has been demonstrated that foodborne bacterial pathogens may live on plant surfaces and, under some circumstances, can internalize into plant tissues⁵. The survival and growth of enteric bacteria introduced from manure may be affected by organic compounds released via plant roots or microorganisms within the rhizosphere, and it has been proposed that the persistence of enteric pathogens in the rhizosphere is the result of interactions between pathogens, soil microbes, soil, and plant roots⁶. It was discovered by⁷, *E. coli* O157:H7 remained in soil for up to 76 days after infected irrigation water was applied. Enterohemorrhagic *Escherichia coli* (EHEC) has also been found to live in soil for 154 to 217 days after being exposed to polluted water or compost. *Salmonella enterica* serovar Typhimurium, meanwhile, was demonstrated to persist for up to 231 days in contaminated compost amended soil in which lettuce and parsley were grown; the pathogen was discovered on the surface of lettuce and parsley for up to 63 and 231 days, respectively⁸. Contaminated vegetables also may contain different type of heavy metals, they pose a severe stress factor for plants when present in large concentrations. Their plant toxicity manifests itself in a variety of ways⁹. According to the Environmental Protection Agency (EPA), arsenic, cadmium, lead, and mercury represent the most dangerous metals in the environment¹⁰. The quality of the environment is often assessed according to the source of its chemical, physical, and biological characteristics¹¹.

Soil is an important part of terrestrial ecosystems. It serves a variety of services, including food production, climate and water management, energy generation, and habitat for many living forms¹². The essential function that soils bacterial communities play in carbon sequestration, soil fertility, and nutrient cycling is underscored by their significance for the ecological functions of soil¹³. Soil has the potential to store contaminants such as microbes, heavy metals (HMs), insecticides, and hydrocarbons and their derivatives¹⁴. Heavy metal pollution of soils is mostly caused by anthropogenic activities such as mining, metallurgical enterprises, and smelting operations can move into agricultural soil¹⁵. Heavy metals (HMs) and other types of soil contaminants, as well as the physicochemical characteristics of the soil, have a significant impact on microbial populations¹⁶. The availability of pollutants from wastewater, may boost crop yield as a result of a reduction in the demand for artificial fertilization¹⁷. Fresh fruits, cereals, and vegetables are grown at up to 2000 locations in the Sulaymaniyah Province's polluted regions; these commodities are sold and consumed daily by more over 500,000 city residents¹⁸. Garbage and dangerous metals accumulate and toxicity to the Tanjaro River, on which the surrounding communities rely for irrigation water and livestock farming. Furthermore, the polluted Tanjaro River flows into Darbandikhan Lake, killing several fish each year due to the continual accumulation of garbage in their natural environment¹⁹.

The aim of this research is to assess the quality of a specific vegetable product, such leeks. The samples were investigated chemically and microbiologically seasonally at fields surrounding the Tanjaro River in Sulaymaniyah city of Iraq.

2. Materials and Methods

2.1 Study Area

Sulaymaniyah city located in northeast Iraq, between latitudes $35^{\circ} 31' 26''$ and $35^{\circ} 35' 37''$ N and longitudes $45^{\circ} 28' 48''$ and $45^{\circ} 22' 10''$ E ²⁰. The city is located in a valley at the peak of Goizha Mountain, which spans around 470 km² and extends southward along the Tanjaro River ²¹. The Tanjaro River originates by the meeting of two significant streams: the Kani-Ban in the Tasluja area and the Qiliasan in northern Sulaymaniyah, Iraq (Figure 1). The river passes southwest of Sulaymaniyah through thickly inhabited areas before draining into Darbandikhan Lake ²².

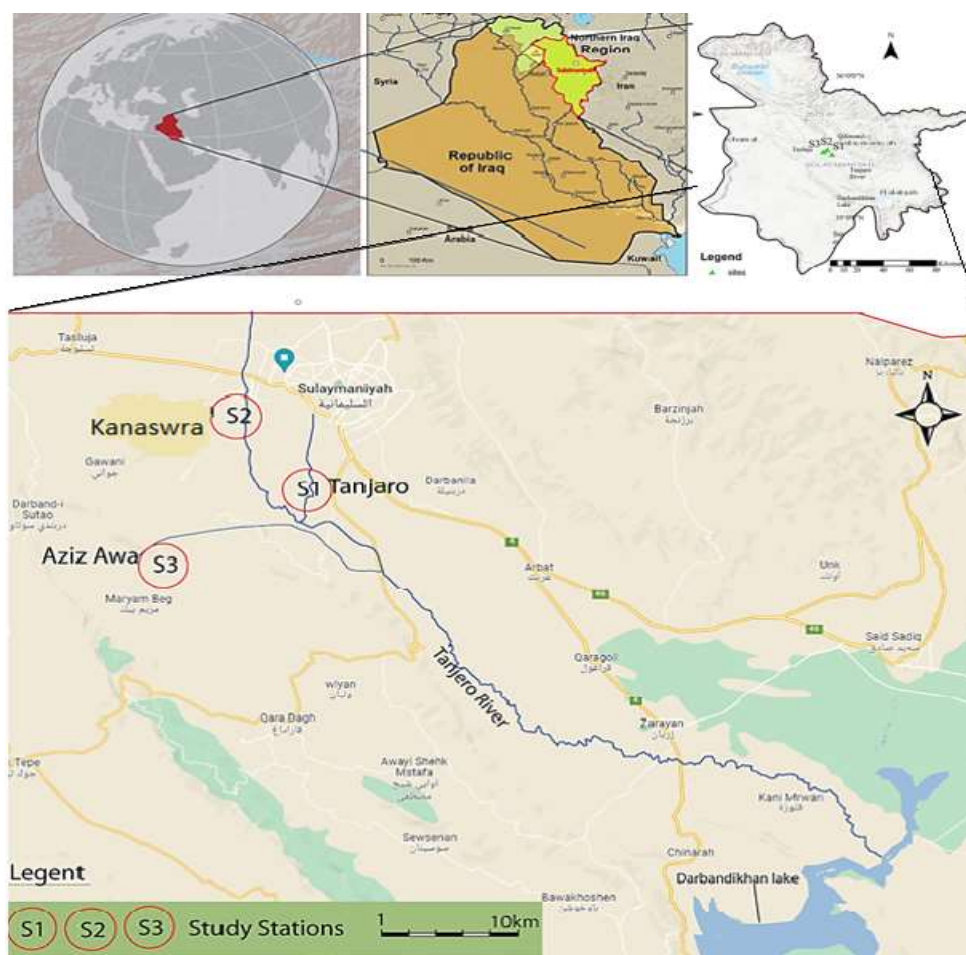


Figure 1. Map of Iraq and location of the leek samples collected in the studied area: (S1) Tanjaro, (S2) Kanaswra, and (S3) Aziz Awa.

The climate of Sulaymaniyah is similar to that of the Middle East. The average summer temperature is 45 °C, while the minimum winter temperature in certain places is 3.5 °C. The average maximum temperature ranges from 7.9 degrees Celsius in January to 38.9 degrees Celsius in August. The average low temperature in January ranges from -0.20 °C to 24.1 °C in August²³. The first location investigated in the present study was along the Tanjaro river at 35°2946 N 45°2455 E. The second Kanaswra site is located at 35°3204.8 N 45°2259 E, while the third Aziz Awa site is located at 35°3110 N 45°2122 E (Figure 2).



Figure 2. Location of leek farms: (A) in Tanjaro, (B) in Kanaswra, (C) in Aziz Awa.

The Tanjaro River flows through several farms and serves as a source of irrigation and cattle water, as well as a washbasin for untreated urban and industrial wastes²⁴. The area surrounding the farms that were chosen for the study is full of factories, power plants, vehicles, and agricultural equipment that emits emissions from burning fuel, as well as used fertilizer, which may fully or partially contribute to raising the percentage of pollutants in the air, soil, or vegetables.

2.2 Sampling

Leek samples were taken seasonally from the three previously mentioned locations from September 2021 to June 2022. Samples were taken in four different seasons: autumn (October and November), winter (December and January), spring (March and April), and summer (May and June). Three times each season, samples were obtained. Triplicate samples were analyzed for chemical properties, heavy metal presence, and content of microbes. 36 samples of leek were collected from

each location. Sterilized polyethylene bags were used for leek collection, ensuring the samples' purity. Following that, all of the samples were sent to the Ecology lab at the University of Sulaimani/ Biology Department for additional examination. To preserve and avoid deterioration, the materials were kept in a refrigerator at 4 °C until they could be evaluated using standardized procedures.

2.3 Samples Preparation and Analysis

To determine the range of contamination levels within each farm, 500 g of leek samples were collected three times during each season. Immediately upon collection, the leek samples were rinsed with distilled water to remove any visible soil particles²⁵. Vegetative parts of leek sample, such as the leaves and stems, were analyzed using the wet digestion technique²⁶.

2.4 Chemical Parameters Analysis of Leek Samples

Leek parameters such as Chloride (Cl^-), potassium (K^+), sodium (Na^+), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}) were measured²⁶. Chloride (Cl^-) concentration was determined by argentometric method²⁷, with some modification. 5 g of the crushed vegetables sample transferred to a 200 ml-volumetric dark flask and diluted to the volume 50 ml with distilled water. 2 ml of K_2CrO_4 indicator were added and then titrated with 2 ml of AgNO_3 . K^+ and Na^+ concentrations were determined using a Flame Photometer (Jenway 500801, UK), at a wavelength of 766.5 nm. The. The Turbidimetric technique was used to determine SO_4^{2-} , which includes the creation of Barium Sulphate turbidity. In order to perform this technique, 100 mL of each sample was put into a 250 mL Erlenmeyer flask. After that, 20 mL of buffer solution was added and well mixed with steady stirring. PO_4^{3-} concentration was determined using the Ascorbic Acid Reduction technique. A 0.45 m filter paper was used to filter the samples. Ultraviolet (UV) Spectrophotometer (Shimadzu 1800, Japan) was utilized to measure the sulphate and phosphate concentration in leek.

2.5 Detection of Heavy metals

The digested leek samples were then determined by the Direct Air-Acetylene Flame Method via Flame Atomic Absorption Spectrophotometer. The device used for this analysis was Buck Scientific's Flame Atomic Absorption Spectrophotometer from the USA, as presented²⁶, were employed in the current research to determine the concentrations of Pb, Zn, and Fe metals.

2.6 Microbiological Analysis

Total aerobic count (TAC) and total coliform (TC) in leek were measured by made a serial dilution method as specified by²⁸, with some modification. 0.1 mL of appropriate dilutions (10^{-4} - 10^{-8}) have been spread on Plate Count Agar (Liofilchem, Italy) for total bacterial aerobic bacteria and Violet Red Bile Lactose Agar (HiMedia, India) for total coliform. The plates were then left to incubate at 37 °C for 24-48 hours. Colonies were enumerated and represented by colony forming units (CFU/ g). To identify fecal *Escherichia coli* and *Salmonella*, 25 g of leek were placed in 225 mL of pre-enrichment Buffered Peptone Water (Microbiology Granu Cult-Germany). The flasks were then incubated at 37 °C for 18-24 hours. A 0.1 mL of pre-incubated sample has been streaked on HiCrome™ Improved Salmonella Agar (HiMedia, India). The plates were then incubated at 37 °C

for 24 hours. All Gram-negative colonies on HiCrome™ Improved Salmonella Agar plates were detected with a Gram-negative VITEK-2 ID card using a VITEK-2 (BioMerieux, USA) equipment.

2.7 Statistical Analysis

Instrument were calibrated before the analytical analysis began, regarding to the manufacturing company and method blank (MB) was utilized to confirm the validity of the reagents. The data was analyzed using XLSTAT (2019), TWO WAY ANOVA (multiple comparisons Duncan test) analysis of variance, which was adopted to analyze the data to determine significant difference between the parameters over all seasons and locations. The averages were compared at probability level ($p < 0.0001$) and ($= 0.05$).

3. Result and Discussion

3.1 Analysis of Chemical Parameters

As shows in table 1, there were five parameters tested for the leek samples, with concentrations compared with the standard limits. Chloride concentration in Tanjaro found the highest chloride, rate was 0.04 mg/kg during autumn. Kanaswra recorded the highest mean value 0.05 mg/kg over autumn, winter, and summer. Aziz Awa recorded the maximum mean values of chloride ion 0.04 mg/kg during winter. No significant difference was observed between locations and seasons. Chloride concentrations in leek was under the normal range²⁹. The low concentration of the chlorine ion is attributed to the low concentration of chloride in the water which irrigated by it³⁰, as saw in the irrigation water sample which analyzed in the current study (Result not shown). Na^+ registered the maximum mean value in Tanjaro 393.00 mg/kg during autumn and the minimum mean value was 364.67 mg/kg during spring, with a significant difference between seasons at ($p < 0.0001$). Kanaswra recorded the maximum mean values of sodium concentration 195.33 mg/kg during autumn, and minimum mean value was 176.33 mg/kg during winter, respectively. Aziz Awa recorded the maximum mean value 113.00 mg/kg during summer and the minimum mean value 96.33 mg/kg during winter, a significant difference between autumn and spring with other seasons were observed at probability level (< 0.0001). All the mean values of leek were under permissible limits. Sodium level in plant tissue depend on the soil, where the salt content of the plants increased dramatically in response to a decrease in soil humidity³¹.

Tanjaro registered the lowest mean value of potassium concentration in leek was 1970.33 mg/kg in winter, while Kanaswra registered the highest mean value 3614 mg/kg in autumn. There is a significant difference between seasons and locations at probability level (< 0.0001). Mean values recorded in the three sites during seasons were under the threshold range. Irrigation, drainage, and erosion from adjacent regions, sowing, cuttings, transplanting, and residues, organic fertilizer treatments, and commercial fertilizer additions all contribute to K^+ concentration in soil. The whole reasons are made up of the total of all of these inputs. These Sources can be applied directly to the plant or to the soil, as with foliar fertilizers applications³². The maximum average of SO_4^{2-} through summer season in Tanjaro, Kanaswra, and Aziz Awa were 559, 762.33, 734.33 mg/kg, consecutively. A significant difference was noted between seasons and sites ($p < 0.0001$). All the mean values were under the threshold limit. Higher SO_4^{2-} intake may be causes an increase in

sulphate in the roots and leaves. Plants grown with a higher amount of sulphate in their diet tend to store the extra sulphates in their cells as SO_4^{2-} or other organic compounds ³³.

Phosphate mean values in Tanjaro was recorded in order of 6.9, 6.33, 5.7, 4.7 mg/kg over summer, spring, autumn, winter, respectively. Kanaswra, it had mean values ranged 13.43, 12.47, 12.33, 10.77 mg/kg during summer, spring, autumn, and winter, consecutively. Aziz Awa was recorded 16.13, 15.23, 13.8, then 13.03 mg/kg over summer, spring, winter, autumn, respectively (Table 1). There is a significant difference between sites and seasons ($p < 0.0001$). The mean values of phosphate among all sites were under the acceptable limit ²⁹. The phosphate concentration can be affected by agriculture condition, where these agricultural aspects are affected by a variety of factors, including temperature, precipitation, and the amount of sunlight. The effects of climate change on these components are a major concern ³⁴. The quality of the soil would be adversely impacted by an increase in temperature and an increase in precipitation ³⁵. The amount of nutrients in the soil can vary depending on the area of the soil, as well as the chemistry of the soil, which can change depending on the temperature and humidity of the air and soil ³⁶.

Table 1. Leek parameters in three different sites, data represented seasonally as means $\pm$ S.D.

Parameter (mg/kg)	Season	Location			Standard limit ²⁹
		Tanjaro	Kanaswra	Aziz Awa	
Cl^-	Autumn	0.04 $\pm$ 0.04 ^{ab}	0.05 $\pm$ 0.01 ^{ab}	0.01 $\pm$ 0.00 ^b	100
	Winter	0.01 $\pm$ 0.00 ^b	0.05 $\pm$ 0.00 ^a	0.04 $\pm$ 0.06 ^{ab}	
	Spring	0.02 $\pm$ 0.00 ^{ab}	0.04 $\pm$ 0.01 ^{ab}	0.02 $\pm$ 0.00 ^{ab}	
	Summer	0.03 $\pm$ 0.00 ^{ab}	0.05 $\pm$ 0.00 ^a	0.03 $\pm$ 0.00 ^{ab}	
Na^+	Autumn	3614 $\pm$ 3.61 ^a	195.33 $\pm$ 3.51 ^e	102.67 $\pm$ 2.52 ⁱ	500
	Winter	371.3 $\pm$ 1.53 ^c	176.333 $\pm$ 4.51 ^g	96.33 $\pm$ 2.08 ^j	
	Spring	364.67 $\pm$ 3.51 ^d	183.33 $\pm$ 3.06 ^f	96.33 $\pm$ 3.51 ^j	
	Summer	377.67 $\pm$ 3.51 ^b	191.67 $\pm$ 4.51 ^e	113 $\pm$ 4.58 ^h	
K^+	Autumn	1980 $\pm$ 3.00 ^g	3601.33 $\pm$ 1.53 ^b	3480 $\pm$ 2.00 ^d	10,000
	Winter	1970.33 $\pm$ 1.53 ^h	3594.33 $\pm$ 2.52 ^c	3472.67 $\pm$ 4.51 ^e	
	Spring	1981 $\pm$ 4.58 ^g	3602 $\pm$ 2.65 ^b	3473 $\pm$ 4.36 ^e	
	Summer	1992.33 $\pm$ 3.21 ^f	3614 $\pm$ 2.58 ^a	3470 $\pm$ 4.58 ^e	
SO_4^{2-}	Autumn	533 $\pm$ 2.65 ^h	749.33 $\pm$ 3.06 ^b	715.33 $\pm$ 4.73 ^e	1000
	Winter	524 $\pm$ 3.00 ⁱ	736.67 $\pm$ 4.51 ^c	722.67 $\pm$ 2.52 ^d	
	Spring	552.67 $\pm$ 3.79 ^g	755.33 $\pm$ 2.52 ^b	725 $\pm$ 3.61 ^d	
	Summer	559 $\pm$ 4.58 ^f	762.33 $\pm$ 3.06 ^a	734.33 $\pm$ 4.73 ^c	
PO_4^{3-}	Autumn	5.7 $\pm$ 0.60 ^g	12.33 $\pm$ 0.32 ^d	13.03 $\pm$ 0.15 ^{cd}	2000
	Winter	4.7 $\pm$ 0.56 ^h	10.77 $\pm$ 0.40 ^e	13.8 $\pm$ 0.56 ^c	
	Spring	6.33 $\pm$ 0.35 ^{fg}	12.47 $\pm$ 0.51 ^d	15.23 $\pm$ 0.32 ^b	
	Summer	6.9 $\pm$ 0.56 ^f	13.43 $\pm$ 0.25 ^c	16.13 $\pm$ 0.31 ^a	

*Value in each row with same letters are not significantly difference at ($p < 0.0001$), value with different letters is significantly different.

3.2 Heavy Metals Analysis

Tanjaro recorded a high level of Pb concentration 0.92 mg/kg in summer (Table 2). Kanaswra recorded the mean value 0.02 mg/kg during summer, spring, and autumn, while in winter the mean

value was 0.01 mg/kg. Aziz Awa 0.02 mg/kg during autumn and summer, the lowest mean value in spring and winter was 0.01 mg/kg. A significant difference between locations and seasons ($p < 0.0001$). All the mean values above the threshold limits in Tanjaro as reported by WHO/FAO, while the other mean values are under the normal range. The presence of lead in vegetables may be due to air pollution. Industrialization has had a major impact on the distribution of industrial pollutants containing heavy metals. These pollutants increase the number of heavy metals in soil and in the environment, thus transferring them to plants through the absorption of these metals from contaminated soil³⁷. The variability of soil characteristics, including pH, clay and organic matter levels, as well as crop management practices, such as irrigation water and fertilizers, and crop reactions, all contribute to the concentration of potentially hazardous trace elements in vegetables³⁸. High levels of heavy metals can be caused by air deposition of contaminated dust on leaves. Vegetables and fruits may contain heavy metals due to the presence of cement dust from brick-making. The dust from the cement industry can be blown by the wind, and land on the soil and on crops. Vehicles can also be a source of heavy metal pollution³⁹.

Zinc was recorded in Tanjaro a highest mean during summer 4.30 mg/kg, the value in winter season significantly different with the rest seasons at probability level (< 0.0001). Kanaswra the highest mean was during summer 3.09 mg/kg. Aziz Awa registered 4.05 through summer season. With a significant difference were statically investigated between seasons and locations at probability level ($p < 0.0001$). The mean values of leek were under the acceptable level, as presented the WHO/FAO standard limit. Leek was found that Tanjaro, Kanaswra, and Aziz Awa had the highest values of iron concentration during summer were 27.16, 26.27, and 19.23 mg/kg, 19.20 mg/kg, respectively as represented in (Table 2). There is a significant difference have been showed between locations and seasons ($p < 0.0001$). All the mean values of leek were recorded under the acceptable limit according to WHO/FAO. The low presence of other hazardous metals in leeks may be attributed to the fact that the farms are situated away from the sources of the irrigation water rivers. Additionally, this could be due to the permanent elimination of heavy metals by the crops grown in these regions, as well as the presence of heavy metals in the deep layers of soil⁴⁰.

Table 2. Heavy metals concentration in leek from different three sites. The data represented seasonally as means $\pm$ S.D.

Metal (mg/kg)	Season	Location			Standard limit
		Tanjaro	Kanaswra	Aziz Awa	
pb	Autumn	0.9 $\pm$ 0.02 ^a	0.02 $\pm$ 0.00 ^a	0.02 $\pm$ 0.00 ^a	0.3
	Winter	0.88 $\pm$ 0.01 ^a	0.01 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a	
	Spring	0.89 $\pm$ 0.02 ^a	0.02 $\pm$ 0.00 ^a	0.01 $\pm$ 0.00 ^a	
	Summer	0.92 $\pm$ 0.01 ^a	0.02 $\pm$ 0.00 ^a	0.02 $\pm$ 0.00 ^a	
Zn	Autumn	4.2 $\pm$ 0.09 ^{ab}	3.00 $\pm$ 0.12 ^e	4.01 $\pm$ 0.00 ^{cd}	99.4
	Winter	3.9 $\pm$ 0.20 ^d	2.94 $\pm$ 0.03 ^e	3.97 $\pm$ 0.00 ^{cd}	
	Spring	4.1 $\pm$ 0.12 ^{bc}	3.04 $\pm$ 0.05 ^e	4 $\pm$ 0.00 ^{cd}	
	Summer	4.3 $\pm$ 0.06 ^a	3.09 $\pm$ 0.01 ^e	4.05 $\pm$ 0.02 ^{bcd}	
	Autumn	27.15 $\pm$ 0.01 ^a	26.2 $\pm$ 0.06 ^c	19.2 $\pm$ 0.05 ^{de}	
	Winter	27.12 $\pm$ 0.00 ^a	26.18 $\pm$ 0.00 ^c	19.17 $\pm$ 0.00 ^e	

Fe	Spring	27.14± 0.01 ^a	26.2±0.04 ^c	19.19±0.01 ^{de}	425.5
	Summer	27.16± 0.00 ^a	26.27±0.00 ^b	19.23±0.00 ^d	

*Value in each row with same letters are not significantly difference at ($p < 0.0001$), value with different letters is significantly different.

3.3 Bacteriological Analysis

The highest average of total aerobic bacteria in leek was $43.33 \times 10^5 \pm 2.08$ CFU/g recorded in Tanjaro during summer, the lowest average was $30.33 \times 10^4 \pm 0.58$ CFU/g in Kanaswra during winter, and in Aziz Awa during summer. There is a significant difference ($p < 0.0001$) between seasons and locations. According to ⁴¹, standard limit of total aerobic bacteria in vegetables (1.0×10^2 CFU/ml). The mean values of total aerobic bacteria were above the standard limits. The presence of aerobic bacteria in vegetables may be the result of direct contamination from vegetable farms, as well as other sources such as mice, flies and domestic food animals ⁴².

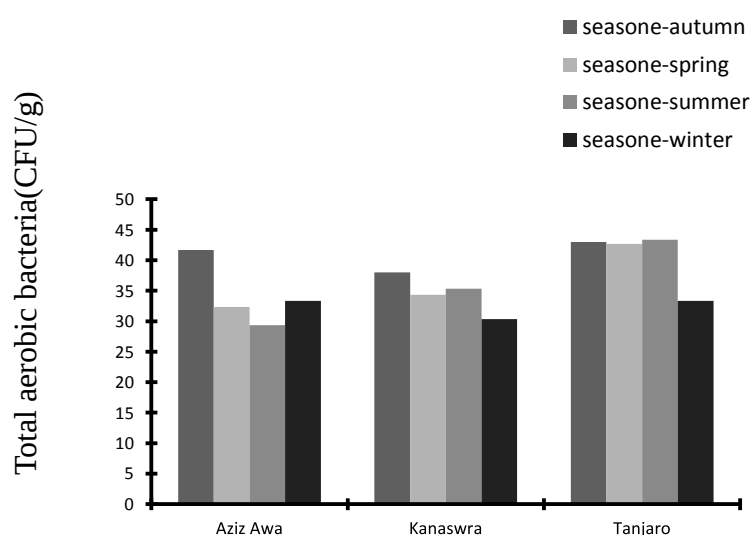


Figure 3. Seasonal variation of total aerobic bacteria in leek.

Leek recorded the highest average of total coliform $35.33 \times 10^4 \pm 2.89$ CFU/g in Kanaswra during summer. Kanaswra registered the lowest mean value in leek $29.33 \times 10^3 \pm 2.52$ CFU/g through winter. The values significantly difference between seasons and locations at ($p < 0.0001$). Total coliform leek was above the normal limits (1.0×10^1 CFU/g) ⁴¹. Factors such as animal excrement, dirty storage areas, insufficient markets, animal transport, and irrigation with wastewater should never be disregarded when considering coliform pollution ⁴³. In addition, During the preharvest period, untreated manure (of either cattle or sheep origin) was routinely applied to the fields may be led to load the coliform in the leek ⁴⁴.

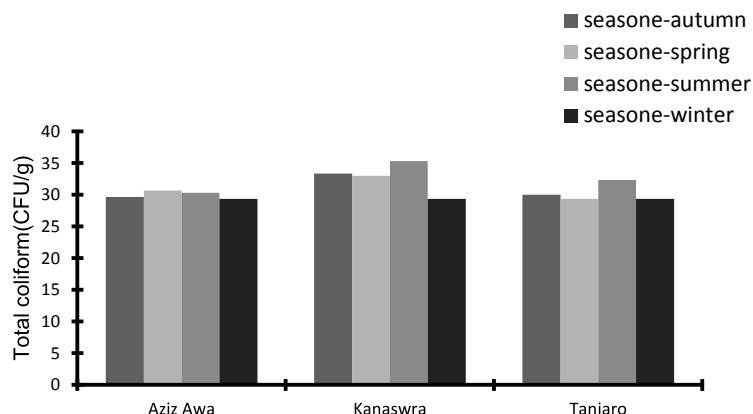


Figure 4. Sessional variation of total coliform in leek.

Based on the results of the identification gram negative bacteria by VITEK -2 shows in Table 3. *E. coli* and *Salmonella* were not detected in leek. The study identified several bacterial species present in the leek sample using HiChrome™ Improved Salmonella Agar, which is developed to detect *Salmonella* species. However, other gram-negative species appeared on this medium such as, *Citrobacter freundii*, *Acinetobacter baumannii* complex, *Enterobacter cloacae* complex, and *Citrobacter braakii*, were recovered from leek. The most prominent indicator of fecal contamination is *Escherichia coli*, as it is present in both warm-blooded animals and humans and is most closely associated with potential contamination from enteric pathogens ⁴⁵.

Table 3. Gram negative bacteria cultivated on chromogenic agar.

Locations	Samples	Species	Percentage
Tanjaro	leek	<i>Citrobacter freundii</i>	98%
Kanaswra	leek	<i>Acinetobacter baumannii</i> complex	99%
Aziz Awa	leek	<i>Enterobacter cloacae</i> complex	97%
Kanaswra	leek	<i>Citrobacter braakii</i>	95%

3.5 Correlation Test

The statistical correlation matrix of leek shows in Table 4, there is a relation between total aerobic bacteria and lead ($r = 0.60$). The author has previously commented on the fact that the majority of heavy metal concentrations are highly interrelated. Although the highest concentrations of cadmium, pb, and zinc were observed in the transects analyzed, it is expected that microorganisms will be able to adapt to each of these metal levels ⁴⁶. Negative correlation between copper and total aerobic bacteria ($r = -0.59$). This may be attributed to the potential for copper to form thiol groups that may bind to the cell surface and impede the activity of respiratory enzymes ⁴⁷.

Negative relation between Iron and total aerobic bacteria ($r = -0.60$). Due to the ability of iron ions to inhibits bacterial development at high concentrations, and because Fe^{3+} is also a dangerous

chemical, Fe ion exhibited a negative relationship with total aerobic bacteria. High iron levels restrict bacterial development and had an inhibitory effect on *E. coli* ⁴⁸. Total coliform had a positive correlation with Manganese ($r = 0.64$). This might be due to the Manganese element, which is essential for living species to reproduce and live. It serves as a cofactor for numerous enzymes and is required for various metabolic activities, including those involving sugar, proteins, and lipids. Pathogenesis, signal transduction, severe reaction, catabolism. Manganese Acidobacteria sporulation homeostasis ROS detoxification is a well-known and investigated Mn activity in which it acts as a redox-active cofactor in free radical detoxifying enzymes such as Mn-superoxide dismutase (MnSod) and mangani-catalase ⁴⁹.

Table 4. Correlation matrix between heavy metals and bacteria in leek samples.

Variables	Total aerobic bacteria	Total coliform	Pb	Zn	Fe
Total aerobic bacteria	1				
Total coliform	0.06	1			
Pb	0.60	-0.35	1		
Ni	-0.51	0.42	-0.72		
Cu	-0.59	0.18	-0.92		
Cr	-0.28	-0.41	-0.21		
Zn	-0.45	-0.21	-0.54	1	
Fe	-0.60	0.17	-0.91	0.83	1
Mn	-0.38	0.64	-0.81	-0.04	0.53
Co	0.28	-0.02	0.14	0.54	0.19

Values in bold are different from 0 with a significance level $\alpha=0.05$.

4. Conclusion

In the current investigation leek analysis uncontaminated with some parameters such as: Cl^- , K^+ , SO_4^{2-} , and PO_4^{3-} they did not exceed the permissible limit. leek vegetables highly contaminated with Pb metals in Tanjaro and Aziz Awa during different seasons, while other heavy metals within the safe limits. Leek were found seasonally highly loaded with total count bacteria and coliform in the three sites. Different species were isolated from leek sample. Proper treatment is required to decrease pollutant accumulation in the Tanjaro River and to limit pollution concerns. Avoid utilizing Tanjaro River effluent water for irrigation, especially for raw crops. Waste from factories is processed and treated before being disposed of in the Tanjaro River.

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