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Contamination Alert: Microbial and Heavy Metal Levels in Green Vegetables

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Abstract. *This study investigated microbial and heavy metal contamination in leeks, a staple green vegetable, focusing on seasonal variations and contamination sources. One hundred leek samples were analyzed for Escherichia coli, Klebsiella, and heavy metals such as zinc, copper, and lead. Findings indicated contamination was higher in district areas compared to city centers, with the highest concentrations of zinc reaching 28.41 ppm during the summer. Results highlight the urgent need for improved contamination prevention measures and increased health awareness to ensure vegetable safety.*

Keywords: *vegetable safety, microbial contamination, heavy metals, seasonal variation, public health awareness*

I.INTRODUCTION

Food safety is one of the main factors required for social and economic stability. As a result of changing environmental conditions, the need to produce an appropriate amount of food to feed a growing population represents a serious challenge. Vegetables are the chief dietary feeding which are consumption by people in every place in the world. They are very moist plants and have nutritional benefits which make them essential part of eaten in parts, whole, raw or cooked as a part of our main food .Fresh produce, especially leafy vegetables that are consumed raw, can be considered important vehicles for the transmission of human pathogens that have traditionally been associated with foods of animal origin, which is a serious public health and economic concern [1]. Because fresh produce is generally grown in open fields, it is vulnerable to contamination with foodborne pathogens (such as the use of poor animal manure and contaminated irrigation water) during production [2].

Green leafy vegetables, rich in vitamins and minerals such as calcium and iron, in addition to Bio Logia active compounds, constitute a significant part of a healthy diet. Interestingly, eating the green vegetables could avoid a number of chronic and non-communicable diseases and donate protein, minerals, vitamins, fiber and other nutrients [3]. However, unfortunately, they are contaminated with pathogens, pesticide residues and heavy metals during their production and packaging. Identifying the types of pathogens, heavy metals and pesticide contaminants found in leafy vegetables and their health effects on consumers will go a long way in helping to take appropriate measures through which human health can be protected [4]. Food-borne illnesses are a well-known problem caused by food being contaminated with bacteria, viruses, or parasites. One of the most common forms of food poisoning is diarrhea, which can sometimes lead to death [5]. The most People at risk of food-borne illness are children, the elderly, pregnant women, and people infected with HIV [6]. Green leafy vegetables are easily polluted with pathogenic of several stages during the preparing them for composition. The contamination can began with pre-harvest stage when the plant in the soil facing fertilizers, pesticides, dust, and wild or domestic animals. The second stage of contamination is post-harvest when the farmer dealing with mature plant using harvesting apparatus , vehicle box, and carrying vehicles [7], [8].

Basically, soil and water using in irrigation are source of pathogens contaminating which increasing of the spread of food-borne diseases, mainly on fresh leafy vegetables after they are harvested. This problem can be avoided by using treated water, by avoiding direct contact between the leaves and irrigation water through the use of drip irrigation [9], [10]. The overexploitation of soil resources and the use of chemicals significantly lead to the emergence of a network of unexpected consequences, including agricultural contamination and degradation of agricultural lands [11]. Interestingly, eating the green vegetables could avoid a number of chronic and non-communicable diseases and donate protein, minerals, vitamins, fiber and other nutrients.

Contamination of the leafy vegetable by heavy metals is a serious problem cause it create alternative way of exposed humans to heavy metals. Heavy metals are toxic and have ability to bioaccumulation in the food chain [12]. the most important cause of spreading heavy metals is Rapid urban and industrial developments with increasing of the number of factories which raised ranks of heavy metals in ecosystem. They are toxic at low concentrations and can made long term risk to human health and ecosystem [13].

Heavy metals elements can inter Green vegetables take up elements by absorbing them from different sources such as contaminated soils and using waste water for irrigation. In addition to deposits them from pollutant air [14].The heavy metals can cause many disease in the kidney, liver, neurological disorders, and cancers of many part of body.

1. Aim of Study

Identification of the microbial contamination in green vegetable (Leek) and description the bacteria that infest the plant in addition ton to detect some heavy metal in the soil and Leek .
plant in Thi- Qar city**.**

2. Description Study Area

Thi Qar Governorate is a town in south of Iraq and Nasiriyah is the capital . It is located on the lower Euphrates river , about 360 km south-south east of Baghdad,. The area is located at 31°16′00″ N–30°47′00″ N, 46°00′00″ E–46°28′00″ E the archaeological ruins of Ur Ziggurat (4000 BCE) situated in it . it urban city and It contains a number of agricultural lands.

II.METHOD

1. Soil Samples

Three stations were identified for collecting samples of soil in the agricultural lands of Thi-Qar governorate. The first one was located outside the center of city near Al-Gharf, the second was located near Sharif (not far from the thermal station). The third was located in Sednawia area. 100 samples of vegetables (leeks) were collected using gloves and bags designated for this purpose to prevent contamination. They were transported directly to the laboratory with a container containing ice to maintain normal conditions for the microorganisms in the vegetables. The analysis began immediately after the laboratory arrived. The time between collection and analysis is about 30 minutes, which is a relatively short time so that the samples do not lose a lot of bacteria[15].

Samples were collected from the farm, home gardens, markets, and from vegetable vendors distributed in various places in the center and districts of the city of Nasiriyah during the months from January to May.

2. Preparation of Samples

Each sample was cut into small pieces, then 1 g of it was taken and added to 9 ml of peptone water. They were mixed well. Then, dilutions were prepared in clean, tightly sealed tubes, starting from a 10-1 dilution by adding 1 ml of the suspension that had been prepared previously (from mixing the sample with... Peptone water) to 9 ml of sterile distilled water, and so on, reaching a dilution of 10-6. Then half a ml of each dilution was taken and spread on the surface of the culture media, and then incubated at 37°C for 24 hours [16].

3. Heavy Metals Estimation

a. Collecting t Samples

Plant and leek samples were collected from the three stations with 3 replicators for each station. The samples were kept in paper bags and transferred to the laboratory until complete study requirements.

b. Preparation of Samples for Laboratory Work

Soil samples are sieved with special sieve after drying it in oven at 100°C for two hours. The samples are saved in drying container until the digestion process. For plant samples, they were washed with distilled water and deionized water, the dried at room temperature, the samples were ground and then sieved then kept in the dryer until the heavy metals evaluation .

c. Extraction Heavy Metals

Extraction heavy metals in the soil. The sample (1g)was digested by adding 15 ml of hydrochloric acid HCL with 5 ml of nitric acid 3HNO. Then it sited in a sand bath at a temperature of 80 ° C. For 45-60 minutes, the the cooling and add 5 ml of hydrochloric acid HCL with heating the mixture then cooling it . after that adding 5 ml of hydrochloric acid HCL and 50 ml of distilled water, heat the mixture then filtered into a volumetric bottle, the undissolved precipitate is washed with distilled water and complete to 100 ml and then

sent to analysis by a Flame Atomic Absorption Spectrophotometer to evaluate heavy metals . Extraction heavy metals in the plants, The heavy metals in plant leaves were evaluated according to the method [17].

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III.RESULT AND DISCUSSION

Fresh vegetables are grown and harvested under a wide range of climatic and diverse conditions, and biological, chemical and physical risks may vary greatly from one species to another. The product is exposed to potential microbial contamination at every step including cultivation, harvesting, transportation, packaging, storage, and sale to end consumers.

A. Diagnosis of Isolates

The phenotypic diagnosis was based on the characteristics of the colonies that appeared when grown on MacConkey and Eocene methyl blue media, in terms of the shape of the colony, its color, and whether it was dry or mucous and based on the phenotypic diagnosis of the colonies, *Escherichia coli* appeared in a pink color, fermenting the lactose sugar, dry and small in size on MacConkey medium. On E.M.B, it is green in color with a metallic luster. These results are consistent with [18]. As for the bacteria of the genus *Klebsiella*, their very sticky pink colonies appeared fermenting the sugar lactose on the MacConkey medium, and as for the E.M.B medium, they appeared in a slightly pinkish-orange color. This is consistent with the results of the study [19]. In microbial examination, the isolates were examined in the optical microscope, the results were all Gram-negative bacteria, rod-shaped and short, as shown in Figure (1-1). Therefore, they are identical to what was mentioned in the classification systems that were adopted [20].

Klebsiella

Escherichia coli

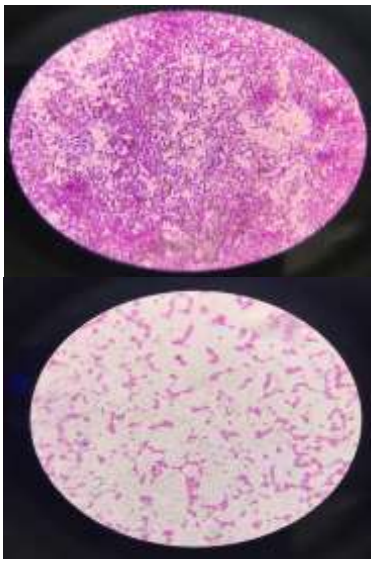

Figure 1. Microscopic Examination by Gram Staining Using an Oil Lens Under 100X Magnification.

A. Diagnosis Using the VITEK 2 Device

The VITEK 2 device is an automated device used to diagnose bacteria, including the Enterobacteriaceae family, as it gives a diagnosis rate of up to 96%. Therefore, it was used in diagnosing the bacterial isolates in this study to ensure their diagnosis.

1. Contamination Rates

Contamination rate based on bacterial species:

a. E.coli

Table 1, It shows that the highest percentage of contamination with *E. coli* bacteria was in the markets in the city center, which amounted to 44.26%, as most of the samples were positive, followed by its percentage in the district farms, which amounted to 11.48%, while the lowest percentage of contamination in restaurants was 3.28% in the city center, and this is consistent with the results of the study, Which may be due to the high rate of contamination in markets compared to farms as a result of the accumulation of pathogens from the time of harvest until they are sold, including the tools used in harvesting and poor storage, in addition to the hands of workers, as well as selling them in open places exposed to insects [21]. In addition, the results of the statistical analysis showed (P>0.05) that there were significant differences in the rates of bacterial contamination with *E. coli* bacteria between the different sample collection areas in the center and districts of the city of Nasiriyah.

The percentage of contamination of *E. coli* bacteria , Table 1.

Table 1. The Percentage of *E. coli* Contamination

Sampling Sources	Geographical location	Total Samples	Positive Samples	Percentage %
Markets	center	36	18	29.51
	Districts	36	27	44.26
Farmer	center	8	3	4.29
	Districts	8	7	11.48
Restaurants	center	6	2	3.28
	Districts	6	4	6.56

X^2 =82.40 , df = 4 , P-value = 0.00

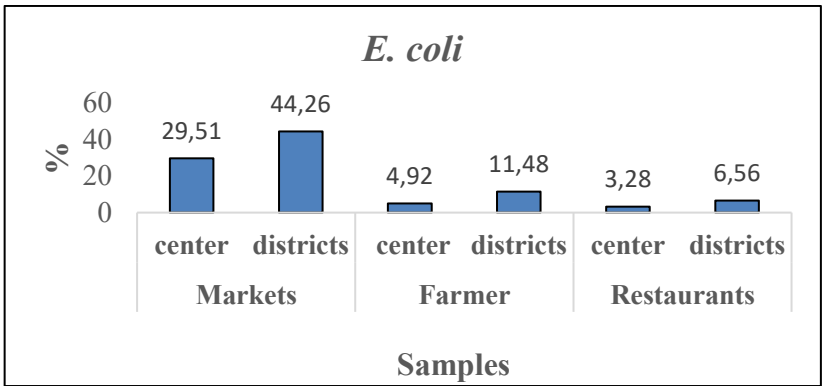

Figure 2. Contamination Rates with *E. coli* Bacteria

b. Klebsiella

Table 2, It shows that the highest percentage of contamination with *Klebsiella* bacteria was in the markets of the districts of the city of Nasiriyah, while the lowest percentage was in the farmers in the city center, 3.13%, followed by that in the restaurants in the city center, then the percentage of contamination in the farmers of the districts of the city of Nasiriyah, which amounted to about 6.25%, and it could be that High levels of contamination in markets are due to several unhealthy practices practiced when vegetables are harvested until they reach the hands of consumers, including transportation, packaging, storage, and sale to final consumers. The results obtained are consistent with the study [22]. The results of the statistical analysis (P>0.05) showed that there were significant differences in the rates of contamination with *Klebsiella* bacteria between the different sample collection areas in the center and districts of the city of Nasiriyah.

Table 2. The Percentage of Contamination of *Klebsiella* Bacteria

Sampling Sources	**Geographical Location**	**Total Samples**	**Positive Samples**	**Percentage %**

Markets	**center**	**36**	**24**	**37.50**
	Districts	**36**	**26**	**40.63**
Farmer	**center**	**8**	**2**	**3.13**
	Districts	**8**	**4**	**6.25**
Restaurants	**center**	**6**	**3**	**4.69**
	Districts	**6**	**5**	**7.81**

X^2 = 92.60 , df = 4 , P-value = 0.00

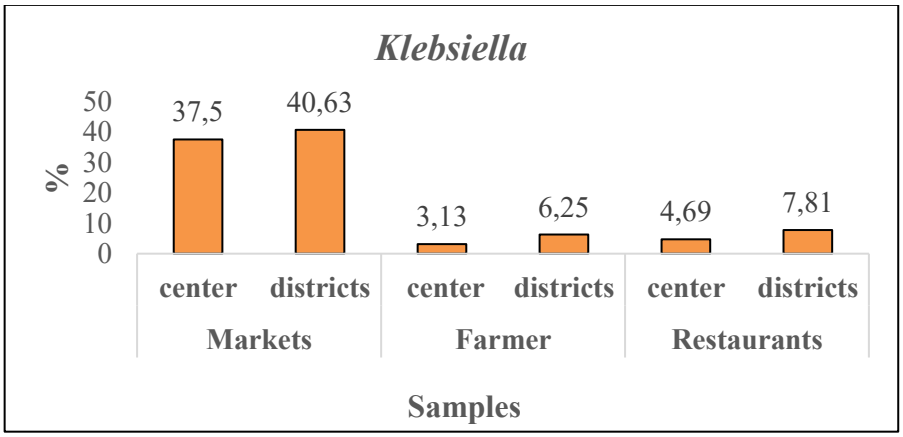

Figure 3. Contamination Rates with *Klebsiella* Bacteria

B. Heavy Metals in Soil

The results of the present study was showed a high concentration of heavy elements in soil and arranged as (94.99, 44.82, 20.68) ug/mg for (Ni, Cu, Pb) in winter season (fig. 4) while in summer was arranged as (101.03.62.10, 21.25) ug/mg respectively (fig. 5). The mean concentration of studied elements was high. This was happened because of the difference in the nature of soil and its content of organic material [23] the mean concentration of heavy metals was high in summer season comparing to winter due to use of fertilizers and pesticides, high traffic density and various industrial activities.

The founding Pb concentration in soil may reflect the ratio of wastes emitted from many industrial plants such as the thermal electric station. Obviously the mean concentrations within allowed concentration (100) µg / gm dry weight.

For cu, the mean concentration was high in summer season due to excessive emissions of gases from cars and small factories spread throughout the city. The results consist with [24]. The value of our results fall within recommended limits of WHO. For Zinc, there was high value in summer and winter season due to use large amount of pesticides and farming waste. The result was agree with [25]. The concentration exceeds the permissible limits. All studied element were high in St. 2 due to its closely distance to the source of pollution (thermal station, traffic emission and small active industrial factory.

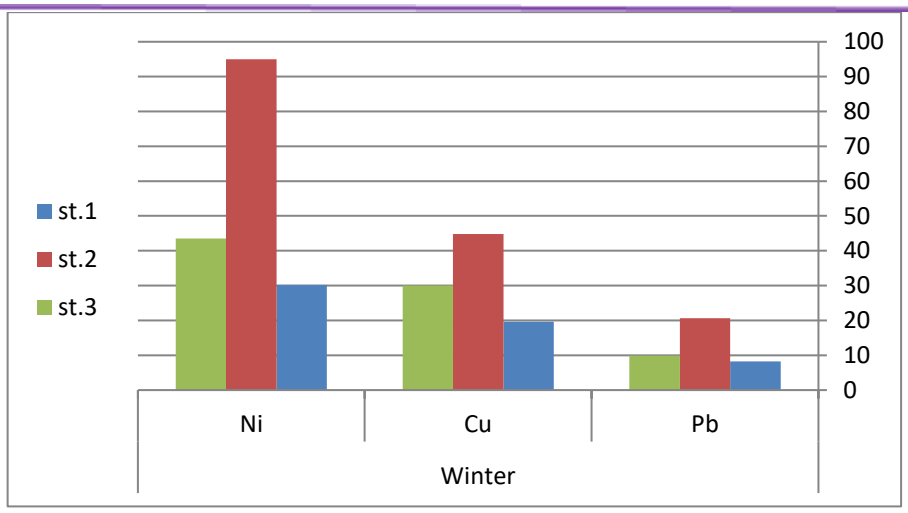

Figure 4. Mean Concentrations of Ni, Cu, Pb level µg / gm during Winter Season in Soil.

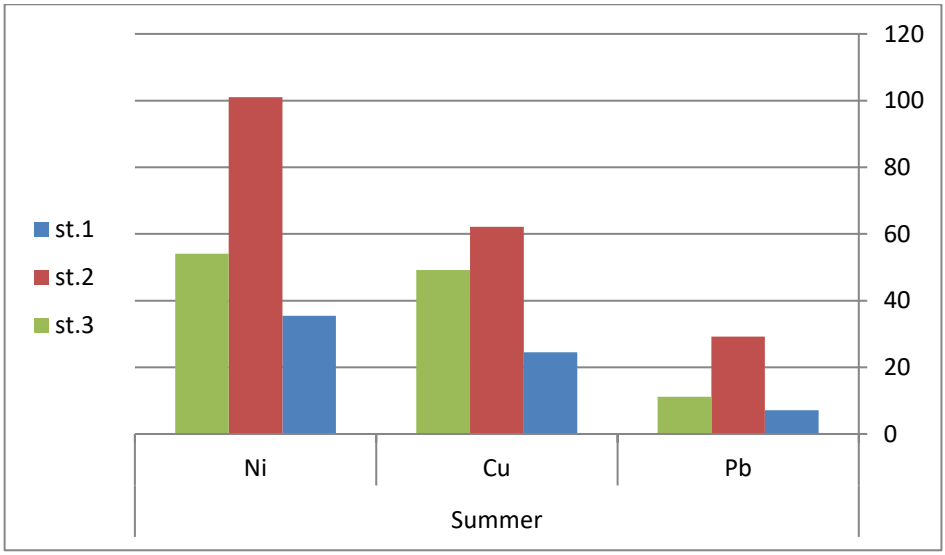

Figure 5. Mean Concentrations of Ni, Cu, Pb level µg / gm during Summer Season in Soil.

The results were indicate that heavy metal have a high mean concentration in leek vegetable and the arrange as market > restaurant > farmer. the highest concentration of the element during summer season. (fig 6,7). The highest concentration of the heavy element was (28.41, 22.08, 6.73) µg/gm dry weight in market for Ni, Cu, Pb. while the lowest concentration of the element (18.05, 15.42, 4.25) µg/gm dry weight in farm during Winter. For Pb, the mean concentration was high in both season and higher than the allowable level offered by WHO of (5) µg/gm dry weight.

The heavy metal value was high in leek vegetable cause the contamination of heavy metal in the soil and the value was raised by the increasing of traffic movement and vehicular in addition to the dust from re-suspended road. This result agree with [26], [27] stated that leek has higher capability to accumulation of Pb more than radish For Cu and Ni element, there were increasing in mean concentration in leek vegetable from deposit of element on serval part of plant [28]. The concentrations do not exceed the allowable level. Mean concentration in summer was high more than winter since there was many factors such as temperature and wind [29].

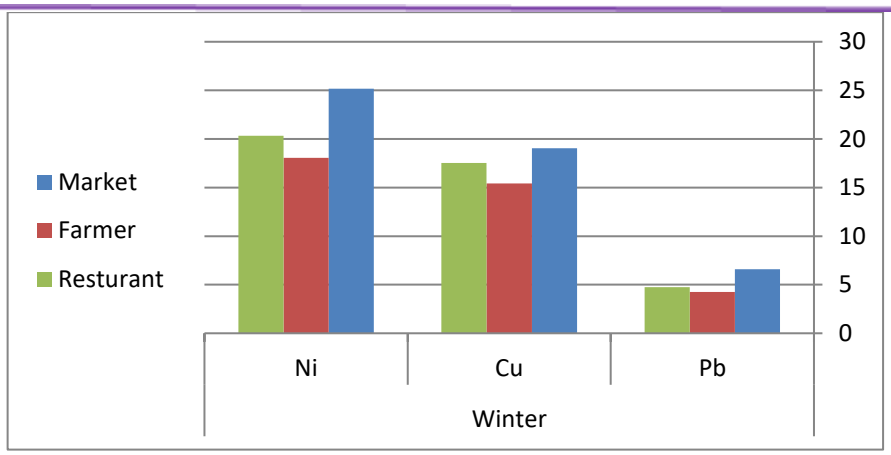

Figure 6. Mean Concentrations of Ni, Cu, Pb level µg / gm in Leek Vegetables in Winter Season.

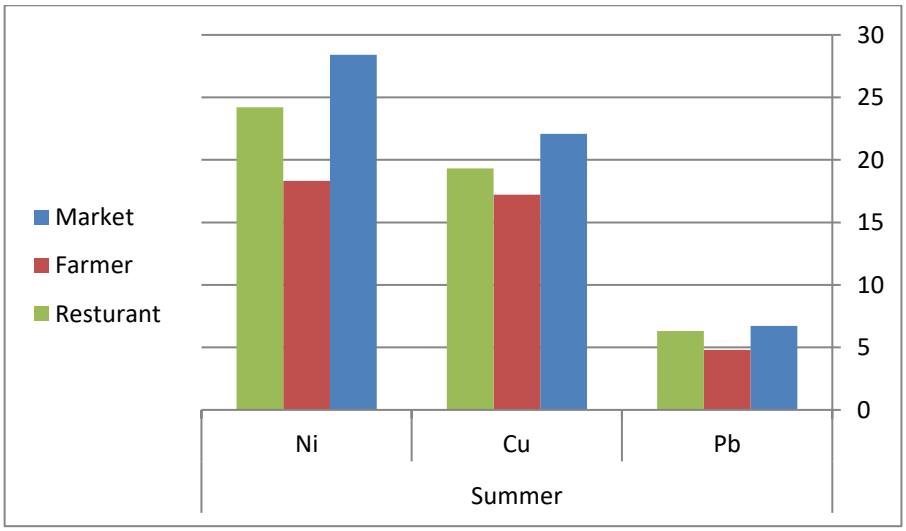

Figure 7. Mean Concentrations of Ni, Cu, Pb level µg / gm in Leek Vegetables during Summer Season.

IV. CONCLUSION

In conclusion, the study identified several causes of contamination of fresh vegetables, represented by sources of contamination before and after harvest. Also, the lack of health guardians among farmers, sellers and consumers can lead to an increase in the spread of food-borne diseases as a result of food contamination. Despite the high microbial counts obtained for some of the samples in this study, it is important to note that these samples did not show any obvious signs of spoilage. Therefore, the external appearance may not be a good criterion for judging whether it is free of microbes.

Recommendations

In light of the results of this study, , the estimating of heavy element and microbial should done to reduce the health risks of consuming fresh vegetables. Recommendations specifically aimed at reducing food contamination with pathogens include Prevent the worker from handling ready-to-eat foods with their hands without wearing gloves;, farmers should choose the location of the agricultural lands in the safest distance far away from factories and road. The need to washing and sterilizing tools and surfaces in contact with food. Separating the tools used in preparing raw animal products from those used for fresh vegetables and other foods.

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