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Microbiological Quality of Raw Vegetables Grown in Bekaa Valley, Lebanon

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ABSTRACT

The purpose of this study was to assess the microbiological quality of fresh vegetables collected from several regions in Bekaa Valley areas. A total of 63 vegetable samples irrigated from Litani River in Bekaa Valley in Lebanon namely lettuce, parsley and *Malva* samples and other control vegetables samples irrigated from ground water wells were examined to assess microbial contamination level. The microbiological quality of fresh vegetables ranged from 4.3 to 10.4 $\log_{10}$ CFU g^{-1} (aerobic bacteria); 2.0 to 0.71 $\log_{10}$ CFU g^{-1} (coliforns); 1.0 to 8.77 $\log_{10}$ CFU g^{-1} (*E. coli*) and 1.47 to 8.77 $\log_{10}$ CFU g^{-1} (*S. aureus*). Lettuce samples had significantly higher microbial loads including coliforms, *E. coli* and *S. aureus* than parsley samples collected from different locations in Bekaa Valley. Neither *E. coli* nor *S. aureus* had been detected on *Malva* samples. In addition, Barelias had higher microbial loads, coliform, *E. coli* and *S. aureus* than any other location investigated in Bekaa Valley. Moreover, *E. coli* was significantly higher in lettuce samples (42.30%) than in parsley samples (13.8%) and *S. aureus* was significantly more often detected in lettuce samples (51.5%) than in parsley samples (38%). This study demonstrated that lettuce and parsley which are usually consumed raw may contain pathogenic microorganisms and represent a risk for human health.

Key words: *Escherichia coli*, *Staphylococcus aureus*, coliforms, Litani River, Lebanon

INTRODUCTION

Vegetables have been associated with outbreaks of foodborne disease in many countries. Organisms involved include bacteria, viruses and parasites (De Roever, 1999). These outbreaks vary in size from a few persons affected to many thousands. Contamination of vegetables may take place at all stages during pre and post-harvest techniques (De Roever, 1999). Cultivation and operation or preparation the vegetables are responsible for this contamination (Sumner and Peter, 1997). Unsafe water used for rinsing the vegetables and sprinkling to keep them fresh is also a source of contamination (Mensah *et al.*, 2002). Other possible sources of microorganisms include soil, faeces (human and animal origin), water (irrigation, cleaning), ice, animals (including insects and birds), handling of the product, harvesting and processing equipment and transport (Johannessen *et al.*, 2002).

The microorganisms normally present on the surface of raw fruits and vegetables may consist of chance contaminants from the soil or dust. These include bacteria or fungi that have grown and colonized by utilizing nutrients exuded from plant tissues. Among the groups of bacteria commonly found on plant vegetation are those that test positive for coliforms or faecal coliforms, such as *Klebsiella* and *Enterobacter* (Zhao *et al*., 1997). Microorganisms capable of causing human illness and others whose foodborne disease potential is uncertain, such as *Aeromonas hydrophila*, *Citrobacter freundii*, *Enterobacter cloacae* and *Klebsiella* sp. have been isolated in lettuce and salad vegetables (Francis *et al*., 1999).

The main sources of water for irrigation in Lebanon are the Litani-Awali river system and subsurface waters. According to the most recent data, from 1993, 54.3% of all cultivated land was irrigated from surface-water sources compared to 45.7%, from groundwater sources (FAO, 1997).

Plate count of aerobic mesophilic microorganisms found in food is one of the microbiological indicators for food quality (Aycicek *et al*., 2004). These organisms reflect the exposure of the sample to any contamination and in general, the existence of favorable conditions for the multiplication of microorganisms. For various reasons, this parameter is useful to indicate if cleaning, disinfection and temperature control during industrial processing, transportation and storage, have been performed sufficiently (Tortora, 1995). Foodborne bacterial pathogens commonly detected in fresh vegetables were coliform bacteria, *E. coli*, *Staphylococcus aureus* and *Salmonella* sp. (Tambekar and Mundhada, 2006). Coliforms are facultative anaerobic, Gram-negative, non-spore forming rods that ferment lactose with gas formation within 48 h when grown in lactose broth at 35°C. They are commonly-used bacterial indicator of sanitary quality of foods and water and considered as an indicator of microbial pollution and they are common inhabitants of animal and human guts (Tortora, 1995). *Escherichia coli* is the species associated with faecal contamination and is naturally found in the intestines of humans and warm-blooded animals. The presence of these bacteria poses a serious threat to public health with outbreaks arising from food and water that has been contaminated by human or animal feces or sewage. *Staphylococcus aureus* is the third most common cause of confirmed food poisoning in the world (Acco *et al*., 2003) and the illness is due to the ingestion of preformed enterotoxins produced in foods. The present study was undertaken to examine the microbiological quality of fresh vegetables such as lettuce, parsley and *Malva* samples collected from several regions in Bekaa Valley area in Lebanon. Such vegetables are consumed in large quantities as these form main part of national dishes.

MATERIALS AND METHODS

Sample collection: A total of 63 vegetable samples (Table 1) (lettuce, parsley and *Malva parviflora*) were collected from various regions along the Litani River in Bekaa Valley. In addition, eight vegetable samples irrigated from ground water were used as a control sample. All samples were obtained between February 2008 and June 2008 and transported to the laboratory in a cool box. Analysis of these samples was conducted immediately.

Table 1: Vegetable samples collected from different locations and irrigated from different sources

Sample type	No. of samples (n)	Sample locations	Source of irrigation
Lettuce	13	Barelias	Litani River
Lettuce	10	Rawda	Litani River
Parsley	12	Barelias	Litani River
Parsley	13	Jib-Jneine	Litani River
Malva	8	Barelias	Litani River

Microbiological analysis: A 50 g of each vegetable sample were weighed and blended in 100 mL of sterile saline solution for 2 min under sterile conditions. The blender was carefully disinfected to prevent any cross contamination. The homogenates were collected in sterile bottles and stored at -20°C until needed. Aliquots (0.5 mL) of each homogenate were serially diluted in sterile saline solution. Plate count agar (Oxoid CM463) was used for detecting total aerobic bacterial count after incubation at 30°C for 48 h (Bio-Rad, France). Typical colonies on the plates were enumerated and colony counts per 1 g sample were determined. Colony counts were converted into $\log_{10}$ CFU g^{-1}. Total coliforms were determined by using MacConkey agar medium (Oxoid) (Tambekar and Mundhada, 2006). *Escherichia coli* cells were enumerated using trypton bile X-glucouronide (TBX) agar following incubation at 30 and 44°C. Only blue/green colonies were included in the calculations (Tendekayi *et al.*, 2007).

Isolation and enumeration of *S. aureus* was carried out by growing on Baird Parker Agar containing egg yolk emulsion. Inverted Petri dishes were incubated at 35°C and counts were made after 24 h followed by a coagulase test to test for the presence of coagulase-positive staphylococci (Soriano *et al.*, 2000).

Statistical analysis: Statistical analysis, including geometric means, standard deviations, ranges and medians were performed using MINITAB statistical software (Centers for Disease Control and Prevention and World Health Organization). One way ANOVA tests were done using. Tukey comparisons to derive statistical differences ($p<0.05$) of microbial levels between all the studied samples.

Geographical localizations of sampling sites along bekaa valley: The samples collected from Bekaa Valley which were irrigated from Litani River and ground water were localized using a Global Positioning System.

RESULTS

Comparison of microbial quality of lettuce and parsley samples from different locations: The overall microbiological quality of lettuce samples vs. parsley samples was calculated using geometric mean bacterial indicator concentrations of each of vegetable sample. In almost all instances, lettuce samples had higher microbial loads of coliform and aerobic count (Fig. 1)

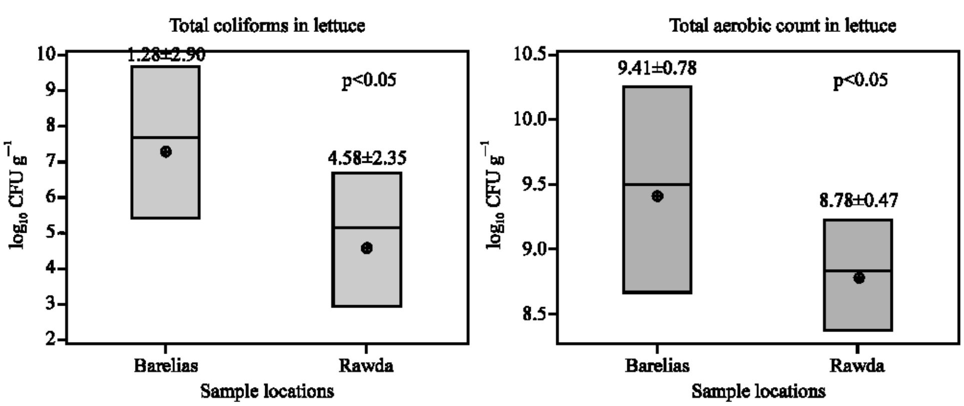

Fig. 1: The geometric mean ($\log_{10}$ CFU g^{-1}) of total aerobic count, total coliforms isolated from lettuce samples that were collected from different regions in Bekaa valley (Barelias and Rawda (El Establ)). The number above the box plot indicates the geometric mean

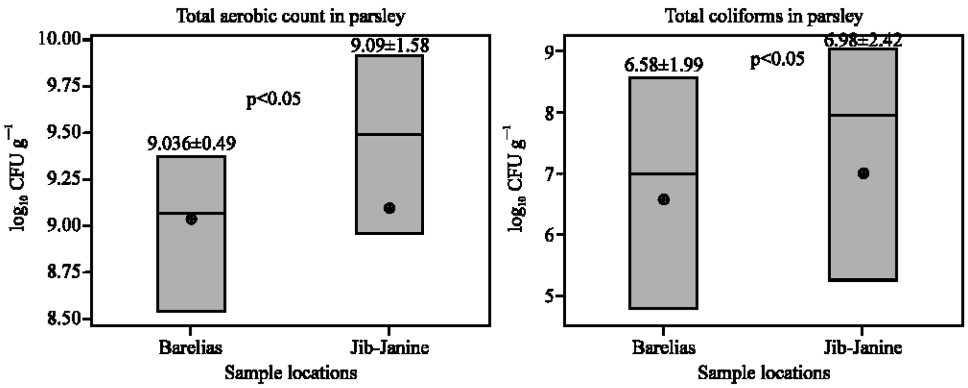

Fig. 2: The geometric mean ($\log_{10}$ CFU g^{-1}) of total aerobic count, total coliforms, viable count of *E. coli* and viable count of *S. aureus* isolated from parsley samples that were collected from different regions in Bekaa valley (Barelias and Jib-Janine). The number above the box plot indicates the geometric mean

compared to those obtained from parsley samples (Fig. 2). The data was compared statistically involving the total aerobic count, total coliforms between different locations. The geometric means of aerobic count on lettuce samples collected from Barelias (n = 13) and Rawda (El Establ) (n = 10) were 9.41 and 8.78 $\log_{10}$ CFU g^{-1}, respectively (Fig. 1). This difference in the level of aerobic microorganisms among different locations (Barelias and Rawda) on lettuce samples were statistically significant ($p<0.002$), But the aerobic levels on parsley samples collected from different locations had a different results from those on lettuce sample where the geometric means of aerobic microorganisms in Barelias (n = 12) and Jib-Janine (n = 13) were 9.03 and 9.09 $\log_{10}$ CFU g^{-1}, respectively and there was a significant statistical difference between different locations (Barelias and Jib-Janine) (Fig. 2). A similar comparison was made on the coliform levels between lettuce and parsley samples in all sampling locations. In this case, the geometric mean values of lettuce samples in Barelias and Rawda (El Establ) were 7.28 and 4.58 $\log_{10}$ CFU g^{-1}, respectively which were statistically significant between the two areas ($p<0.02$). The coliform level on parsley samples did not differ significantly compared to lettuce samples in all sampling locations that had mean values of 6.58 and 6.98 $\log_{10}$ CFU g^{-1} in Barelias and Jib-Janine, respectively (Fig. 2).

Comparison of the microbiological quality of vegetables irrigated by litani river water with those irrigated by ground water: The geometric mean bacterial indicator concentration was calculated for each kind of vegetable sample that was irrigated from either the Litani River or ground water. The mean the total aerobic count and total coliforms on lettuce and parsley samples was calculated for each kind of samples irrigated from the two different sources. The level of aerobic bacteria on lettuce irrigated by Litani River showed statistically a higher bacterial load than samples irrigated using ground water ($p<0.05$) with a mean value 9.418 $\log_{10}$ CFU g^{-1} compared to those irrigated by ground water (4.292 $\log_{10}$ CFU g^{-1}) (Fig. 3). A similar comparison was made for parsley samples irrigated from different sources which showed a significant level ($p<0.05$) of contamination with a mean value 9.03 and 4.99 $\log_{10}$ CFU g^{-1} for parsley samples irrigated from Litani River and underground water, respectively (Fig. 4). The level of coliforms on lettuce samples showed a statistically significant ($p<0.05$) difference between lettuce samples irrigated by either Litani River or ground water with a mean value of 7.28 and 1.42, respectively (Fig. 3). Also the

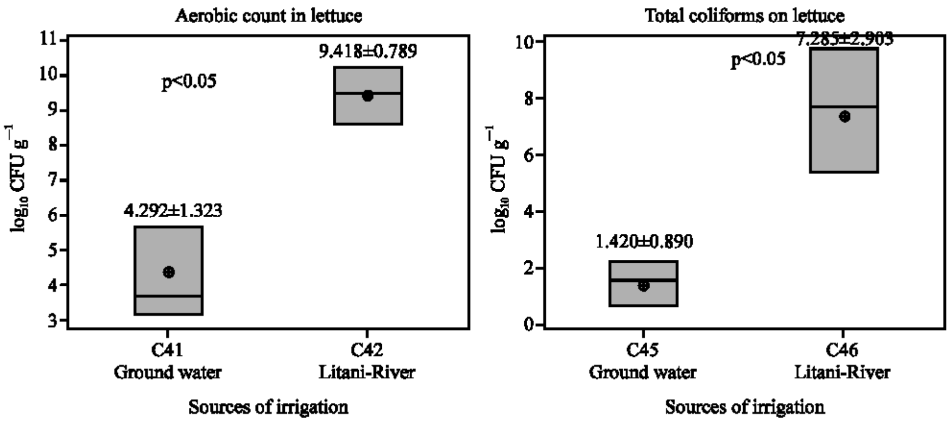

Fig. 3: The geometric mean values ($\log_{10}$ CFU g^{-1}) of total aerobic count and total coliforms isolated from lettuce samples that were irrigated from different sources (Litani-River and Ground water). The number above the box plot indicates the geometric mean. All microbial loads of lettuce samples were statistically significant ($p<0.05$)

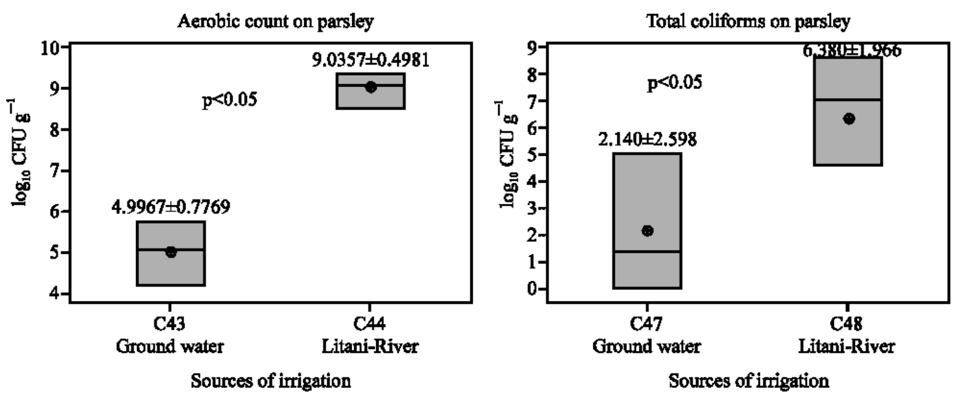

Fig. 4: The geometric mean values ($\log_{10}$ CFU g^{-1}) of total aerobic count and total coliform isolated from parsley samples that were irrigated from different sources (Litani River and Ground water). The number above the box plot indicates the geometric mean

mean values of coliforms on parsley samples irrigated using Litani River (6.380 $\log_{10}$ CFU g^{-1}) appeared to be statistically significant ($p<0.01$) compared with those irrigated by ground water (2.140 $\log_{10}$ CFU g^{-1}) (Fig. 4).

Vegetable samples irrigated by litani river and collected from different locations: A total of 33 vegetable samples collected from Barelias and irrigated from Litani River. About 36.36% of the samples were positive for *E. coli* and 51.5% for *S. aureus* compared to 20 and 30% for the Rawda (El Establ) data, respectively. For Jib-Janine samples, 6.66% were positive for *E. coli* and 26.66% for *S. aureus*. Neither *S. aureus* nor *E. coli* were present on vegetable samples collected from Kaaroun (Table 2). From all locations, *E. coli* and *S. aureus* were the predominant species on lettuce (92.30%) followed by parsley samples (51.72%) then *Malva* samples (0%). Using Chi-square analysis, all samples collected from Barelias had significantly higher level of *E. coli* ($p<0.003$) and *S. aureus* ($p<0.001$). 42.3 and 50% of lettuce samples contained *E. coli* and *S. aureus* compared to

Table 2: Percentage of *E. coli* and *S. aureus* isolated from different vegetable samples collected from different locations

	Samples contaminated	
Sample location	*E. coli* (%)	*S. aureus* (%)
Barelias	36.36	51.50
Rawda	20.00	30.00
Jib-Janine	6.66	26.66
Kaaroun	0.00	0.00

Table 3: Incidence of *E. coli* and *S. aureus* in different types of vegetable samples irrigated from Litani River contaminated by untreated waste-water

		Samples contaminated	
Sample type	Locations	*E. coli* (%)	*S. aureus* (%)
Lettuce	Barelias, Jib-Janine, Rawda, Kaaroun	42.30	50.00
Parsley	Barelias, Jib-Janine, Kaaroun	13.80	37.93
Malva	Barelias	0.00	0.00

13.8 and 38% for parsley respectively. However, neither *E. coli* nor *S. aureus* were recovered from Malva samples (Table 3). *Staphylococcus aureus* was the predominant species isolated from all vegetable samples (n = 63) in different locations (38.09%) compared to *E. coli* in all vegetable samples (23.8%).

DISCUSSION

Vegetables may be contaminated with pathogenic microorganisms during growing in the field or during harvesting, post harvesting, handling, processing and distribution. Therefore, vegetables may act as a reservoir for many microorganisms from which they will be colonized inside these vegetables and infect susceptible host. Almost any ready to eat fruits or vegetables that have been contaminated with pathogens either from the environment or from human or animal faeces or through storage, processing and handling could potentially cause disease (Beuchat, 2002). The large number of Aerobic Plate Count (APC), indicator organisms (coliforms and *E. coli*) and pathogens (*S. aureus*) detected in the food samples surveyed in this investigation revealed that the contamination of these foods by pathogenic microorganism may present a potential health hazard to consumers in Lebanon especially in Bekaa area.

In many countries, wastewater used for irrigation of vegetables is based on the value of its content and constituents which are used as fertilizers. In addition, wastewater also contains salts, toxic metallic compounds and pathogenic organisms which may be harmful to the soil, crops, grazing animals and human health (Rosas and Coutino, 1984). The study reported here revealed that the major cause for contamination of vegetables in Bekaa is the irrigation with wastewater where the transported pathogens from wastewater may survive in soil and crops which will in turn be transported to consumers may potentially cause numerous diseases.

Aerobic organisms reflect the exposure of the sample to any contamination and in general, the existence of favorable conditions for multiplication of microorganisms (Tortora, 1995). For export purposes, it is important that fresh vegetables should not have a total aerobic count exceeding 6.69 $\log_{10}$ CFU g^{-1} which is the acceptable limit by some countries (Nguyen-the and Carlin, 1994). Therefore, reducing the total count on the Lebanese products is a priority to ease the economic impact of such contamination. However, other studies showed low levels of contamination in Egypt, Turkey and Taiwan food Products reported by Saddik *et al.* (1985) and Lund (1993) where the upper limit of aerobic count of microorganisms on vegetable samples was 6.69 $\log_{10}$ CFU g^{-1} in addition to Albrecht *et al.* (1995) and Fang *et al.* (2003) who reported an aerobic plate count on vegetable samples in Taiwan ranging from 3.30 to 8.64 $\log_{10}$ CFU g^{-1}. Furthermore, Vural and Erkan (2008) in Turkey had a range of aerobic count of microorganisms from 6.43 to 7.63 $\log_{10}$ CFU g^{-1}. Similarly for salad vegetables collected from Johannesburg in South Africa, an average aerobic plate count of 7 $\log_{10}$ CFU g^{-1} (Christison *et al.*, 2008) was higher than counts for raw salads obtained in other studies which reported a range from 2 to 6 $\log_{10}$ CFU g^{-1} (Angelidis *et al.*, 2006; Ayçiçek *et al.*, 2004; Kubheka *et al.*, 2001; Mosupye and Von-Holy, 2000). The difference in the APC values between these reported results and previous studies may probably be due to the different cultivation areas of the vegetables and different sources of irrigation.

Hazard Analysis and Critical Control Points- Total Quality Management (HACCP-TQM) Technical Guide-lines lay down the microbial quality for raw foods (Ayçiçek *et al.*, 2006), where the food containing less than 4, 4-6.69, 6.69-7.69 and greater than 7.69 $\log_{10}$ CFU g^{-1} (aerobic plate count) are rated as good, average, poor and spoiled food, respectively (Ayçiçek *et al.*, 2006). Based on these criteria, the data of the present study showed that 84.12% of our samples could then be regarded as spoiled vegetable food. The consumption of lettuce as a salad vegetable has increased in recent years, partly due to its nutritional value and because of the assumed beneficial health effects (Ortega *et al.*, 1997). However, it is known to serve as a vehicle of foodborne pathogens and toxins, of which the principal sources of contamination are the cultivation, stages of processing and operations for preparation. In the present work, all lettuce samples collected from different locations in Bekaa Valley had higher incidence of aerobic organisms than any other vegetable samples collected from the same locations ($p<0.05$). In Barelias, for instance, the aerobic bacterial count on lettuce ranged from 8.0 to 10.4 $\log_{10}$ CFU g^{-1} compared to Rawda (El Establ), Jib-Janine and Kaaroun where the aerobic count were 8.0 to 9.27, 7.17 and 6.75 $\log_{10}$ CFU g^{-1}, respectively. However, the total aerobic plate count of microorganisms on parsley samples collected from different locations in Bekaa Valley ranged from 4.3 to 10.36 $\log_{10}$ CFU g^{-1}, where in Barelias, the APC on parsley samples ranged from 8.3 to 9.85 $\log_{10}$ CFU g^{-1}. However, samples collected from Jib-Janine had a range from 4.3 to 10.36 $\log_{10}$ CFU g^{-1} compared to Kaaroun samples which ranged from 7.6 to 9.34 $\log_{10}$ CFU g^{-1}. In addition, the total aerobic count of *Malva* samples collected from Barelias ranged from 9.66 to 10.56 $\log_{10}$ CFU g^{-1}. This is the first study to evaluate the microbiological quality of *Malva* species in Lebanon.

Total coliform counts can be considered as a hygiene indicator, especially for faecal contamination. Their presence indicates that pathogens might be present due to fecal contamination by human, animal or irrigation water (Vishwanathan and Kaur, 2001). In this investigation, the level of coliforms in all vegetable samples ranged from 2.0 to 10.71 $\log_{10}$ CFU g^{-1}. Similar findings had been reported by Viswanathan and Kaur (2001) where the coliform counts of salad vegetables ranged from 6.0-9.0 $\log_{10}$ CFU g^{-1}. Moreover, Patterson and Woodburn (1980) reported coliform counts of 6.0 $\log_{10}$ CFU g^{-1} and Albrecht *et al.* (1995) found a range from 4.81 to 6.30 $\log_{10}$ CFU g^{-1}. While Brocklehurst *et al.* (1987), Ercolani (1976) and Garcia-Villanova *et al.* (1987) found values higher than 3.0 $\log_{10}$ CFU g^{-1}. Furthermore, Fang *et al.* (2003) obtained a range from 2.3 to 7.55 $\log_{10}$ CFU g^{-1} of coliforms in all vegetable samples in Taiwan, Nguz *et al.* (2005) in Zambia found a range of coliform counts on vegetable products between 2.2 and 5.9 $\log_{10}$ CFU g^{-1} and Ayçiçek *et al.* (2006) obtained a range of total count of coliforms on vegetable samples from 3.0 to 6.9 $\log_{10}$ CFU g^{-1}. However, the total coliform reported by Soriano *et al.* (2000) ranged from 0.47 to 3.38. Furthermore, Johnston *et al.* (2005) reported a coliform level for all green leafy vegetable and herbs of less than 1 to 4.3 $\log_{10}$ CFU g^{-1} which is in agreement with Johnston *et al.* (2006) which reported a coliform count on vegetable produce collected from United states were less than 1.0 to 4.5 $\log_{10}$ CFU g^{-1}.

The present work showed a statistically significant higher incidence of coliform in all lettuce samples compared to parsley samples ($p<0.05$). Coliform count on lettuce ranged from 2.3 to 10.31 $\log_{10}$ CFU g^{-1}, where in Barelias, the total coliform counts ranged from 5.0 to 10.31 $\log_{10}$ CFU g^{-1}, but in Rawda (El Establ) and Jib-Janine the count was 2.3 to 6.95, 7.71 to 7.74 $\log_{10}$ CFU g^{-1}, respectively. However, Total coliform counts of parsley samples collected from Barelias ranged from 3.6 to 8.77 $\log_{10}$ CFU g^{-1}. On the other hand, in Jib-Janine and Kaaroun the total coliform organisms ranged from 3.3 to 9.39 and 0 to 3.0 $\log_{10}$ CFU g^{-1}, respectively ($p>0.05$). The coliform levels obtained in this study for Malva samples collected from Barelias in Bekaa had a value of 9.91 $\log_{10}$ CFU g^{-1}, the high coliform counts in these samples and other vegetable samples was due to poor hygiene and to pollution by humans, animals or irrigation water. In Barelias, untreated sewage is released in to local rivers including Litani River. This is likely to be the source of contamination.

Staphylococcus aureus is a dangerous pathogen and one of the most common causative agents of hospital infections (nosocomial infections) in human beings. Surface of vegetables may be contaminated by this organism through human handling and other environmental factors and can be able to survive for several weeks (Erkan *et al.*, 2008). Human skin and nasal cavity is the main reservoir of staphylococci (Jablonski and Bohach, 1997). Contamination of food stuffs during distribution and handling may allow bacterial growth and subsequently production of toxins which may represent a potential risk to humans (Erkan *et al.*, 2008). The result of this study showed that lettuce carried higher incidence of *E. coli* and *S. aureus* organisms (42.30 and 50%) than parsley samples (13.80 and 37.93%) ($p<0.05$), the higher microbial loads on lettuce samples than parsley counterpart may be due to the large surface area of the former leaves. Having foliar surfaces with many folds and fissures this provide good shelter for microorganisms and the fragility of leaves allow the penetration and reproduction of bacteria in their inner tissues. Therefore, parsley samples exhibited low microbial loads possibly due to its small surface area.

The microbiological quality of *Malva* species samples showed the absence of *E. coli* and *S. aureus*. No other studies had been done before on *Malva* plants which are considered to be important food used in Lebanon due to its nutritional and medicinal benefits. In the present work, it had been observed that vegetables collected from Barelias and irrigated from Litani River were highly contaminated by *E. coli* (36.36%) and *S. aureus* (51.5%). However, the incidence of *E. coli* in Rawda (El Establ) was about 20% and *S. aureus* about 30%. For Jib-Janine samples, 6.66% were positive for *E. coli* and 26.66% were positive for *S. aureus*. Neither *S. aureus* nor *E. coli* were present on vegetable samples collected from Kaaroun. No previous studies had been done on the microbiological quality of vegetable samples collected from different locations and irrigated from different sources. Overall, going down stream, samples collected from Barelias, contains larger areas of agricultural lands in Bekaa, which carried the highest microbial load. This is likely to be due to the discharge of untreated sewage into Litani River from many villages and towns. Moreover, the Litani River and Kaaroun Lake are seriously threatened by industrial run off from centers of agro processing the sugar beet factory, tanneries and glue factories.

CONCLUSION

In conclusion, the present study revealed the potential hazard of raw vegetables collected from Bekaa Valley area which were irrigated from Litani River water. In Lebanon, this study was the first to evaluate the microbiological quality of vegetables grown in Bekaa Valley, where these vegetables harboured high microbial loads including aerobic bacteria, coliforms, *E. coli* and *S. aureus* due to irrigation by untreated wastewater in that area. The data obtained provide a first-hand indication of which microorganisms might be present in fresh vegetables and due to the potential microbiological risks of these products, it should be treated directly with certain disinfectant before consumption and to develop highly effective treatments for removing pathogens from a wide range of raw produce. As a consequence to our study, the government should impose strict measures to control or at least minimize the risk of microbial contamination by implementing the Hazard Analysis and Critical Control Point (HACCP).

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