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RISK ASSESSMENT OF HEAVY METAL AND MICROBIAL CONTAMINATION IN COMMERCIALLY AVAILABLE SALAD VEGETABLES OF FAISALABAD, PAKISTAN

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Abstract

Vegetables are vital for an efficient functioning of human's body as they build a major constituent of human diet in each family. Irrigation with polluted waters and poor post harvesting management in olericulture are described as the major sources of heavy metal contamination and bacterial infection in vegetables. Intensive use of pesticides may result in enhancing or reducing the above mentioned hazards. It is very important to regularly assess the market vegetables to determine if they are safe for consumption in raw or cooked form. The major focus of current study was to analyze the quality of four highly consumed salad vegetables carrot (*Daucus carota)* tomato (*Solanum lycopersicum*), green chilli (*Capsicum annuum*) and cucumber (*Cucumis sativus*), for their safe utilization as food. This study was conducted to evaluate the presence of the most reported heavy metals (cadmium, lead and chromium) and microbial contamination like *Escherichia coli* in above mentioned vegetables. Samples were collected from three types of commercial vegetable markets in Faisalabad: local vegetable markets (wholesale market), supermarkets and street venders. It was observed that the range of cadmium (Cd), chromium (Cr) and lead (Pb) in vegetables were 1.23-7.23, 1.11-5.33 and 1.22-7.11 µg g^{-1}, respectively. However, the detected mean values of all heavy metals were less than that of acceptable limits by the World Health Organization (WHO). Furthermore, *E. coli* contamination was observed in all the samples of vegetables from roadside retailers but in only few samples from supermarket, however, all the levels were under permissible limits. So, our results showed that these vegetables were safe for human consumption.

Key words: Heavy metal, *Escherichia coli*, Environment, Health.

Introduction

Fresh green vegetables are among the important constituents of healthy human diet and their regular consumption is recommended for the proper nutrition and health of the communities. They provide the major portion of required quantities of minerals, dietary fibres, vitamins, proteins, calcium, iron etc. of daily intake, especially, in developing countries (Ali *et al.*, 2018; Ruel *et al*., 2014; Yousaf *et al.,* 2019). All these minerals and nutrients are essential for human and their deficiency can have very negative impacts on human health (Keatinge *et al*., 2015). Vegetables, which are considered useful for human body functioning may play a detrimental role if they are contaminated, polluted or infected (Aysha *et al*., 2017; Franz, 2018). So, vegetables can also be a significant factor in the intestinal infections in human beings, especially, when they are used as raw (without cooking) and without disinfecting properly (Slifko *et al*., 2000).

Vegetables can be contaminated by heavy metals and/or microbes during cultivation, transportation, production and marketing (Anwar *et al*., 2016; Franz, 2018; Rehman *et al*., 2017a). Heavy metal contamination of vegetables is among the highly reported ecological problems on global scale (Chabukdhara *et al*., 2016; Chiroma *et al*., 2014). This type of contamination, especially, cannot be ignored in salad vegetables because they are mostly consumed raw and even without washing sometimes. So, accumulation of toxic metals in fresh green salad vegetables may hazardous to the human health. Industrial and municipal wastewater irrigation is frequently practiced in three fourth of the cities in Asia, Africa, and Latin America (Becerra-Castro *et al*., 2015; Gul *et al*., 2016; Shakoor *et al*., 2019). Industrial and domestic wastewaters carry variety of toxic heavy metals in significant amounts, which can create a problem for safe utilization of vegetables (Khan *et al*., 2016; Raja *et al*., 2016; Verma *et al*., 2015). Moreover, pesticide spray can be another source of contamination in vegetables. Chemical hazards in green foods come principally from pesticide deposits and these are becoming significant issues in developing nations (Rehman *et al*., 2017b; Santarelli *et al*., 2018). Some recent studies have shown that Cd causes osteomalacia and kidney cancer (Satarug & Moore, 2004). Excessive intake of Pb is associated with respiratory and dermatogenic problems in humans (Nawaz *et al*., 2016; Saghir *et al.,* 2019). Trivalent chromium plays an essential role for human health, while hexavalent chromium is highly carcinogenic (Costa & Klein, 2006). According to Rehman *et al*., (2017b), cadmium, chromium and lead are mostly present in fruits and vegetables. More than 0.2% for cadmium and 0.6% for lead concentration in food items are considered lethal for human health (Llobet *et al*., 2003).

Poor postharvest management such as poor handling, transportation under hazardous conditions and pesticide spray are often considered as the source of microbial contamination (Amoah *et al*., 2006: Pierangeli *et al.,* 2019). Diverse pesticides are regularly applied to vegetables but they are not a source of microbial contamination. Some previous studies have shown that the pesticides might be available for the growth and survival of microorganisms such as *Escherichia coli, Salmonella* and *Shigella* with a suitable environment (Santarelli *et al*., 2018). Hence, postharvest application of pesticides may be itself the solution to control the microbial contamination but could be a major additional source of microbial contamination.

Faisalabad is highly industrialized city of Pakistan and many textile industries alongwith other food industries are located within its residential premises. The hazardous waste water discharged by these industries is openly released in the network of unpaved drains, which are managed by Water and Sanitation authority (WASA). In many urban and peri-urban areas of Faisalabad, waste water is frequently used for irrigational purpose (Mahmood & Maqbool, 2006). Local farmers use these drains to irrigate the agricultural land for the cultivation of vegetables and other crops. A large quantity of these vegetables is sold in supply markets of the cities. Recent studies have shown that the major health problems like hepatitis, cancer, asthma may be due to the consumption of toxic metals through food crops irrigated by wastewater (Muchuweti *et al*., 2006).

Escherichia coli along with some other bacteria have been globally reported in many food items including fresh vegetables and fruits and its presence is attributed either to poor post harvest handling or pesticide spray (Santarelli *et al*., 2018). Although *E. coli* are largely considered as commensal bacteria, which may be present in the intestines of many animals including human being without causing any harm but some strains of *E. coli* are reported to be involved in causing fatal diseases in human (Rabinovitz *et al*., 2012). Only one reported harmful strain of *E. coli* includes *E. coli* 0157: H7 that can be present in the digestive system of healthy human. The symptoms of its infection in humans' digestive tract include vomiting, severe abdominal pain and acute diarrhea (Rabinovitz *et al*., 2012; Belanger *et al*., 2011). So, it is important to analyze periodically the market vegetables to quantify the levels of infection carrying pathogens.

Thereby, this study was conducted to assess the heavy metal concentration (Cd, Pb and Cr) and microbial contamination (*E. coli*) in selected market salad vegetables (carrot (*Daucus carota,)* tomato (*Solanum lycopersicum*), green chilli (*Capsicum annuum*) and cucumber (*Cucumis sativus*) collected from different markets located in district Faisalabad.

Materials and Methods

Study area and sampling technique: The current study was carried out in the urban periphery of Faisalabad city, Pakistan. Vegetable samples were collected from three different types of vegetable markets of city Faisalabad namely local vegetable market (wholesale market), supermarket and street venders. Three locations of each type of market were randomly selected and three samples of each vegetable were collected from each location. The Jhang Bazar, Ghulam Muhammadabad and Noorpur were selected for local vegetable market, the Metero, SB store and Al-Fatah store were selected for supermarket category, and the D-ground, Shahbaz town and Civil town were selected in street venders category. So, total of 27 samples were collected for each vegetable. This study was carried out on four salad vegetables: tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), green chilli (*Capsicum annuum*) and carrot (*Daucus carota).* All the samples were collected while wearing gloves to minimize the contamination and, afterwards, these samples were stored in labelled airtight plastic bags at 4°C. Samples were divided into two portions: one for microbial (*E. coli*) analysis and other for heavy metal detection (Pb, Cd and Cr), then they were transported to their relevant laboratories.

Heavy metal analysis: Heavy metals were analyzed by atomic absorption spectrophotometer (AAS) through wet digestion method. The oven dried samples were ground, sieved at 1mm and about 0.5 g of the sample were digested in 10 ml of strong di-acid solution (70 % high purity HNO_3 and 65 % $HClO_4$, 4:1 v v^{-1}) (Allen *et al*., 1986). Samples were digested at about 80°C till the solution became transparent. The solution was cooled down before filtering through Whatman no.42 filter paper. Then the metal (Pb, Cr and Cd) concentrations in the pre-treated vegetable digested samples were examined using an AAS (Hitachi Polarized Zeeman AAS, Z-8200, Japan) in the Hi-Tech Lab of the University of Agriculture, Faisalabad, Pakistan. During these analyses sterilized glass apparatus and highly purified deionized water were used (Anon., 1990). Commercially available aqueous stock solutions were used to prepare calibrated standards for this analysis.

***E. coli* detection and count:** Collected samples were transported within 3 to 4 hours in the microbiology lab, Department of Microbiology in University of Agriculture, Faisalabad for *E. coli* determination. About 10 to 15 g of each sample was used for the analysis of *E. coli*. Presumptive test was conducted for the detection and determination of the most probable number of coliform bacteria (Thompson *et al*., 1990). Lactose fermentation broth containing an inverted durham tube for gas collection was utilized in this test. Tubes of this lactose medium were inoculated with 10 mL, 1 mL and 0.1 mL aliquots of vegetable crushing sample (Soomro *et al*., 2002). The test scheme of *E. coli* presence detection is given Table 1.

Table 1. Biochemical test scheme for *E. coli*.

Catalase	Coagulate	Indole	Voges proskauer	Methyl red	Citrate	Sugar fermentation	Motility test
Positive	Negative	Positive	Negative	Positive	negative	Positive	Positive

Bacterial count was conducted by using the slides with marked area of 1 cm^2 and using methylene blue as staining agent. An oil immersion lens was used to calculate total coliform counts from randomly selected 15 microscopic fields. Then, microscopic factor (MF) was determined that is characterized as an aggregate number of microscopic fields show in 1 cm^2 prescribed zone of tiny glass slide. With the assistance of stage micrometer, the distance across of one micrometer field at 40 × or 100 × target was measured in microns (µ) = D. One little division of stage micrometer was identical to 10µ in size. Area of one microscopic field was calculated by the following formula:

$a = (D/2)^2\pi$

According to the area of scaled microscope slide (A) = 1cm^2

$A = 1\ cm^2 = 10^8\ \mu$

Hence MF = A/a

Finally, total bacterial count (TBC) or total coliform count may be calculated as:

$$TBC/ml = \frac{\text{Av. Number of bacteria} \times MF \times \text{dilution factor (sample)}}{100}$$

Biochemical tests were used to confirm the *E. coli*. In this Scheme, the eight tests were analyzed: catalase, coagulate, indole, voges Proskauer, methyl red, citrate, sugar fermentation, motility test (Soomro *et al*., 2002).

Statistical analysis

The collected data were statistically analysed by using two-way analysis of variance (ANOVA) in SPSS software version 12 for evaluating the significance difference between heavy metal concentrations in the vegetables collected by different types of vegetable markets of district Faisalabad.

Results

Cadmium (Cd) concentration: The Fig. 1 shows the mean concentrations of cadmium (Cd) in vegetable samples (tomato, cucumber, carrot and chilli) collected from street vender, vegetable market and supermarket. Cd concentration (µg/g) was found relatively higher in cucumber as compared to other vegetables. It was found that Cd concentrations (µg/g) for tomato and cucumber were higher in vegetable market (3.2 and 9.7, respectively). However, higher Cd concentrations for chilli were found in street vender category (7.23 µg/g). Furthermore, no difference was found in Cd concentrations for carrot (1.3 to 1.4 µg/g) when collected from different sources. The minimum values of Cd concentrations for tomato and chilli were noticed in supermarket samples (2.51 and 1.3 µg/g, respectively).

Lead (Pb) concentration: The Fig. 2 shows that the concentration of lead (Pb) in vegetable specimens collected from street vender, vegetable market and supermarket. Pb concentration (µg/g) was higher in the samples of three vegetables (cucumber (4.4), carrot (6.2) and chilli (7.11)), collected from street venders as compared to vegetable market and supermarket. However, for tomato higher Pb concentrations were observed in the samples collected from supermarket (3.3 µg/g). In vegetable market, the concentration of Pb was obtained in tomato (*Solanum lycopersicum)* (1.2 µg/g), in chilli (*Capsicum annuum*) (3.23 µg/g), in carrot (*Daucus carota)* (1.3 µg/g) and in cucumber (*Cucumis sativus*) (2.3 µg/g). The minimum concentration of Pb for three vegetables (tomato, cucumber and chilli) was observed in the samples collected from vegetable market as compared to street venders and supermarket.

Chromium (Cr) concentration: Concentration of Chromium (Cr) in salad vegetable samples collected from street vender, vegetable market and supermarket as shown in Fig. 3. For cucumber (5.5 µg/g) and carrot (3.1 µg/g), Cr concentrations were higher in the samples collected from street vendors as compared to vegetable market (5.1 and 1.1 µg/g, respectively) and supermarket (2.1 and 2.2 µg/g, respectively), respectively. For tomato, higher Pb concentrations were observed in vegetable market (6.1 µg/g); while for chilli higher Pb concentrations were found in supermarket (5.3 µg/g). The minimum Pb concentrations for tomato and cucumber were found in supermarket (1.1 and 2.1 µg/g, respectively), for carrot in vegetable market (1.1 µg/g) and for chilli in street vendor category (1.2 µg/g).

***E. coli* detection and count:** The result of this study showed that *E. coli* was present in salad vegetables in Faisalabad (Table 2). Out of twelve type of samples only three types of samples showed negative *E. coli* test and remaining all samples showed positive result. Among uninfected samples, two samples were from supermarket and only one sample was from vegetable market. All the samples collected from street vendors were found infected.

Table 2. Determination of *E. coli* in vegetables from three sources.

Source	Vegetable	Colony forming units (CFU)/g	E. coli detection/g
Vegetable market	Green chilli	1.32×10^2	Positive
	Carrot	2.3×10^3	Positive
	Cucumber	1.12×10^2	Positive
	Tomato	1.22×10^2	Negative
Supermarket	Green chilli	1.28×10^2	Positive
	Carrot	1.82×10^1	Positive
	Cucumber	1.4×10^1	Negative
	Tomato	1.42×10^1	Negative
Street vender	Green chilli	2.7×10^3	Positive
	Carrot	2.20×10^4	Positive
	Cucumber	1.30×10^2	Positive
	Tomato	1.12×10^2	Positive

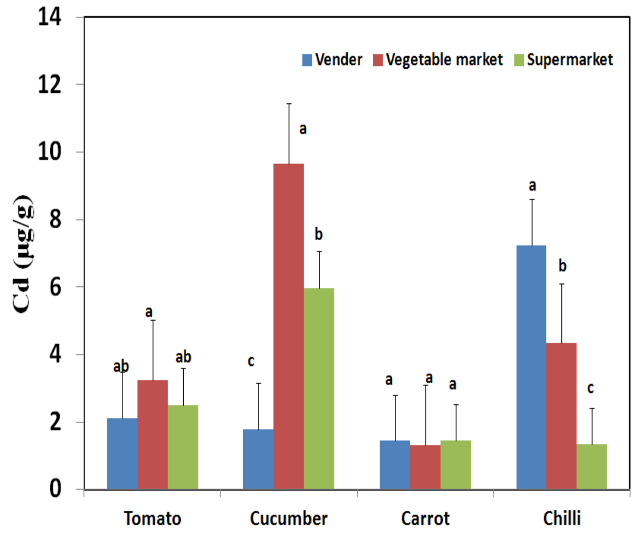

Fig. 1. Cd concentration in vegetables collected from different markets of Faisalabad city.

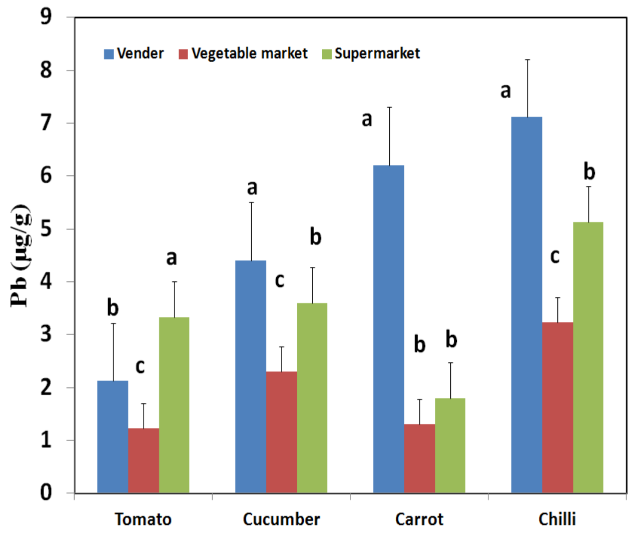

Fig. 2. Pb concentration in vegetables collected from different markets of Faisalabad city.

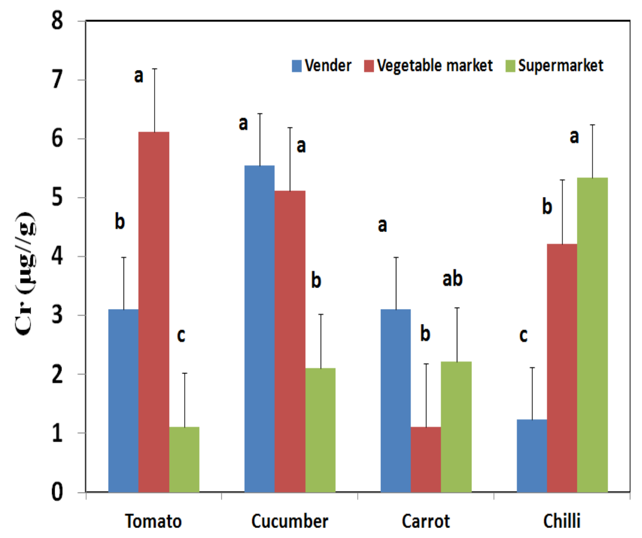

Fig. 3. Cr concentration in vegetables collected from different markets of Faisalabad City.

Discussion

The results of this study indicate that mean concentration of heavy metals (Pb, Cr and Cd) in different salad vegetables (tomato, carrot, cucumber and chilli) collected from three types of vegetable markets are in the decending order of Cd > Pb > Cr. The variations in the heavy metal concentration in different vegetables depend on water, soil composition, metal permissibility, nutrient balance and greatly among plant species (Yadav *et al.*, 2013: Rashid *et al.*, 2019). In this present study, the result showed that the Cd concentration (µg/g) was found relatively higher in cucumber as compared to other vegetables. It was found that Cd concentrations (µg/g) for tomato and cucumber were higher in vegetable market (3.2 and 9.7, respectively). However, higher Cd concentrations for chilli were found in street vender category (7.23 µg/g). These concentrations were found below than acceptable limits of cadmium (20 µg/g) in salad vegetables set by the World Health Organization (WHO). Despite this, several past studies reported the higher level of Cd in various ready to eat vegetable (fruits, leafy, legumes and cereals) samples (Ali & Al-Qahtani, 2012). Air Cd depositions and accumulation of higher concentrations of Cd from contaminated soils in vegetables may cause detrimental effects on human health as reported in previous studies (Santarelli *et al.*, 2018).

In another study, it was found that the levels of cadmium in green vegetables collected from Aseer Region, Saudi Arabia (Oteef *et al.*, 2015) were below than permissible limits and these results were comparable to the results found in current study. However, there are also some other studies, which have reported higher levels of Cd in green vegetables from same country but different locations (Al Jassir *et al.*, 2005). The heavy metal levels found in current study fit excellent with the reports from other areas in the world such as Liberia (Ngumbu *et al.*, 2017), Saudi Arabia (Shabbaj *et al.*, 2017), Sri Lanka (Kananke *et al.*, 2014), Lebanon (Al-Chaarani *et al.*, 2009) and Nigeria (Iwuanyanwu & Chioma, 2017).

In the present study, the concentration of Pb (lead) was found higher in the samples of three vegetables (cucumber (4.4), carrot (6.2) and chilli (7.11), which were collected from street venders as compared to vegetable market and supermarket. However, for tomato higher Pb concentrations were observed in the samples collected from supermarket (3. 3 µg/g). These concentrations are below the acceptable level of lead (30 µg/g) in ready to eat vegetables set by the World Health Organization (WHO). However, several studies reported that the higher level of Pd in eight different vegetable markets from industrial site of Dhaka city of Bangladesh. The level of Pb was 0.06 to 3.5 mg kg^{-1} in vegetables (Islam & Hoque, 2014). The Pb concentrations found in this study observed in salad vegetables are comparable to the values reported in the Aseer Region, Saudi Arabia (Oteef *et al.*, 2015). In this previous survey, lead was detected in the green vegetables in the range of 0.008 to 0.15 and 0.02 to 0.17 mg/kg fresh weight, respectively; these values were below the permissible limits set in the Saudi Arabia and international food standards. It is reported that the higher level of Pb found in some vegetables could be attributed to deposition from vehicle exhaust and industries (Pandey *et al*., 2012). Likewise, Singh *et al*. (2010) has discussed in detail about the possible health hazards because of contamination of Cd, Pb and Ni in privately contaminated vegetables in Varanasi, India. Regular examinations of metals in vegetables and other food things should be performed and appropriate security measures should be taken during the season of vegetable transportation and stockpiling. Moreover, the origin of vegetables should be likewise too observed and appropriate measures should be brought to manage the contamination.

For cucumber (5.5 µg/g) and carrot (3.1 µg/g), Cr concentrations were higher in the samples collected from street vendors as compared to vegetable market (5.1 and 1.1 µg/g, respectively) and supermarket (2.1 and 2.2 µg/g, respectively), respectively. These concentrations are lower than permissible limits of chromium (0.02 mg/g) in fresh green vegetables (salad vegetables) set by the World Health Organization (WHO) and FAO. However, several studies reported that the higher levels of chromium in five different vegetables from two different sites of Guangzhou, South China. The level of chromium was higher in soil when compared with the aerial parts of vegetables and roots which showed that lower amount of heavy metals were bio- aggregated from soil (Li *et al*., 2015; Liu *et al*., 2015). Another study explored contents of toxic heavy metals in eatable portion of vegetables and found that Pb, Ni, Zn, Cu, Cd and Cr contents were greater than limits (Ahsan *et al*., 2009).

Furthermore, most of the vegetables are not properly washed, so, chemicals like pesticides and the microorganisms cannot be removed from the surface of the vegetables. Furthermore, coliform bacteria are the gathering of moderately innocuous microorganisms that live in huge number in the stomach related frameworks and digestion tracts of individuals and cold and warm creatures. Microbial contamination in vegetables depends on many factors that include use of sewage water or waste water for irrigation, post harvest practices and natural condition amid washing/flushing, transportation etc. (Ofor *et al*., 2009). In the current study, *Escherichia coli* were found in all salad vegetables which were collected from street vender. Out of twelve sample types only three types showed negative *E. coli* test and remaining all samples showed positive result. Bacterial counts of all the samples were well below the acceptable limit set to World health organization (WHO) for food standards (10 to 10^2) fresh weight vegetables and as per FAO (<$10g^{-1}$ coliform bacteria (CFU)/g) (Huss *et al*., 2003). The presence of microorganisms in vegetables is a direct reflection of the sanitary quality of the harvesting, cultivation water, storage, processing and transportation of the produce (Ray & Bhunia, 2013). According to Peterside & Waribor (2006), during storage, bacterial load on verdant vegetables increase with time. The presence of *Escherichia coli* was sign of poor hygienic practices by both the agriculturists and venders. The sales conditions make the vegetables vulnerable to contamination particularly as practices in Zaria, where the origin of vegetables and water in the home grassy plot and in the market is hazardous (Abdullahi & Abdulkareem, 2010).

In Faisalabad, the wastewater comes from different sources (hospitals, industries and household) and collected in an urban wastewater treatment plants (UWTPs). Most of the UWTPs present in district Faisalabad were out of order. So, there was no treatment of wastewater. The wastewater came from hospitals may be one of the major birthplace of antibiotic resistant bacteria. When they came in UWTPs, they may spread resistant plasmid to other bacteria by means of conjugation. These microorganisms can reach to the fields and can contaminate the vegetables. The literature demonstrated that both living creatures and/or people could be origin of fecal microorganism's pollution of delivered vegetables. To the safety of purchaser's health, appropriate treatment of waste water grown vegetables is basic to avoid or decrease pathogen contamination and heavy metal contamination at all and each point.

Conclusion

This current study was conducted to determine the presence of three heavy metals (Cd, Pb and Cr) in Faisalabad vegetable markets. The level of all three toxic metals (Cd, Pb and Cr) found in the vegetables from Faisalabad selling points (street venders, vegetable markets and supermarkets) were within the acceptable limit and were not harmful for ultimate human consumption. Similarly, almost in all the samples, the levels of *Escherichia coli* were also well below the maximum level set by WHO/FAO. So, these vegetables were found safe to consume. However, there is urgent need to establish a controlling authority to examine the quality of these vegetables periodically (may be monthly) because irrigation practices and post-harvest handling of vegetables may not be same for whole year and would be dependent on irrigation water availability and climatic conditions.

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References

Abdullahi, I. and S. Abdulkareem. 2010. Bacteriological quality of some ready to eat vegetables as retailed and consumed in Sabon-Gari, Zaria, Nigeria. *Bay. J. Pure Appl. Sci*., 3: 173-175.

Ahsan, M.R., K.M. Islam, I.J. Bulbul, M.A. Musaddik and E. Haque. 2009. Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. *Eur. J. Sci. Res*., 37: 302-310.

Al Jassir, M., A. Shaker and M. Khaliq. 2005. Deposition of heavy metals on green leafy vegerables sold on roadsides of Riyadh City, Saudi Arabia. *Bull. Environ. Contam. Toxicol*., 75: 1020-1027.

Al-Chaarani, N., J.H. El-Nakat, P.J. Obeid and S. Aouad. 2009. Measurement of levels of heavy metal contamination in vegetables grown and sold in selected areas in Lebanon. *Jordan J. Chem*., 4: 303-315.

Ali, M.H. and K.M. Al-Qahtani. 2012. Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. *Egypt. J. Aquat. Res*., 38: 31-37.

Ali, R. and M. Adnan. 2018. Nutritional potential of Pakistan medicinal plants and their contribution to human health in times of climate change and food insecurity. *Pak. J. Bot*., 50: 287-300

Allen, S., H. Grimshaw and A. Rowland, (eds.) 1986. Chemical analysis, pp. 1-285-344. Blackwell Scientific Publication, Oxford, London.

Amoah, P., P. Drechsel, R. Abaidoo and W. Ntow. 2006. Pesticide and pathogen contamination of vegetables in Ghana- urban markets. *Arch. Environ. Contam. Toxicol*., 50: 1-6.

Anonymous. 1990. Official methods of analysis of the Association of Official Analytical Chemists Arlington, VA, USA.

Anwar, S., M.F. Nawaz, S. Gul, M. Rizwan, S. Ali and A. Kareem. 2016. Uptake and distribution of minerals and heavy metals in commonly grown leafy vegetable species irrigated with sewage water. *Environ. Monit. Assess*., 188: 541.

Aysha, M., H. Zakir, R. Haque, Q. Quadir, T.R. Choudhury, S. Quraishi and M. Mollah. 2017. Health risk assessment for population via consumption of vegetables grown in soils artificially contaminated with arsenic. *Arch. Cur. Res. Int*., 10: 1-12.

Becerra-Castro, C., A.R. Lopes, I. Vaz-Moreira, E.F. Silva, C.M. Manaia and O.C. Nunes. 2015. Wastewater reuse in irrigation: A microbiological perspective on implications in soil fertility and human and environmental health. *Environ. Int*., 75: 117-135.

Belanger, L., A. Garenaux, J. Harel, M. Boulianne, E. Nadeau and C.M. Dozois. 2011. Escherichia coli from animal reservoirs as a potential source of human extraintestinal pathogenic E. coli. *FEMS Immunol. Med. Mic*., 62: 1-10.

Chabukdhara, M., A. Munjal, A.K. Nema, S.K. Gupta and R.K. Kaushal. 2016. Heavy metal contamination in vegetables grown around peri-urban and urban-industrial clusters in Ghaziabad, India. *Hum. Ecol. Risk Assess*., 22: 736-752.

Chiroma, T., R. Ebewele and F. Hymore. 2014. Comparative assessment of heavy metal levels in soil, vegetables and urban grey waste water used for irrigation in Yola and Kano. *Int. Ref. J. Eng. Sci*., 3: 01-09.

Costa, M. and C.B. Klein. 2006. Toxicity and carcinogenicity of chromium compounds in humans. *Crit. Rev. Toxicol*., 36: 155-163.

Franz, E. 2018. Quantifying Human Health Risks Associated with Microbiological Contamination of Fresh Vegetables, p. 161-173 Quantitative Methods for Food Safety and Quality in the Vegetable Industry. Springer.

Gul, S., M.F. Nawaz, M. Azeem and M. Sabir. 2016. Interactive effects of salinity and heavy metal stress on eco-physiological responses of two maize (*Zea mays* L.) cultivars. *FUUAST J. Biol*., 6: 81-87.

Huss, H., L. Ababouch and L. Gram. 2003. Pathogenic bacteria. Assessment and Management of Seafood Safety and Quality, *In* R. Food and Agricuture Organization of the United Nations, Italy. FAO Fisheries Technical Paper 444, (ed.).

Islam, M.S. and M. Hoque. 2014. Concentrations of heavy metals in vegetables around the industrial area of Dhaka city, Bangladesh and health risk assessment. *Int. Food Res. J*., 21: 2121-2126.

Iwuanyanwu, P.K. and N. Chioma. 2017. Evaluation of Heavy Metals Content and Human Health Risk Assessment via Consumption of Vegetables from Selected Markets in Bayelsa State, Nigeria. *Biochem. Anal. Biochem*., 6: 2161-1009.1000332.

Kananke, T., J. Wansapala and A. Gunaratne. 2014. Heavy metal contamination in green leafy vegetables collected from selected market sites of Piliyandala area, Colombo District, Sri Lanka. *Am. J. Food Sci. Technol*., 2: 139-144.

Keatinge, J., J. Wang, F. Dinssa, A. Ebert, J.D.A. Hughes, T. Stoilova, N. Nenguwo, N. Dhillon, W. Easdown and R. Mavlyanova. 2015. Indigenous vegetables worldwide: their importance and future development. *Acta Hortic*., 1102: 1-20.

Khan, Z.I., K. Ahmad, M. Ashraf, S. Yasmeen, A. Ashfaq and M. Sher. 2016. Metal accumulation in a potential winter vegetable mustard (*Brassica campestris* L.) irrigated with different types of waters in Punjab, Pakistan. *Pak. J. Bot*., 48: 535-541.

Li, N., Y. Kang, W. Pan, L. Zeng, Q. Zhang and J. Luo. 2015. Concentration and transportation of heavy metals in vegetables and risk assessment of human exposure to bioaccessible heavy metals in soil near a waste-incinerator site, South China. *Sci. Total Environ*., 521: 144-151.

Liu, J., Z. Zhuo, S. Sun, X. Ning, S. Zhao, W. Xie, Y. Wang, L. Zheng, R. Huang and B. Li. 2015. Concentrations of heavy metals in six municipal sludges from Guangzhou and their potential ecological risk assessment for agricultural land use. *Pol. J. Environ. Stud*., 24: 165-174.

Llobet, J., G. Falco, C. Casas, A. Teixido and J. Domingo. 2003. Concentrations of arsenic, cadmium, mercury, and lead in common foods and estimated daily intake by children, adolescents, adults, and seniors of Catalonia, Spain. *J. Agric. Food Chem*., 51: 838-842.

Mahmood, S. and A. Maqbool. 2006. Impacts of wastewater irrigation on water quality and on the health of local community in Faisalabad, Pakistan. *Pak. J. Water Resour*., 10: 19-22.

Muchuweti, M., J. Birkett, E. Chinyanga, R. Zvauya, M.D. Scrimshaw and J. Lester. 2006. Heavy metal content of vegetables irrigated with mixtures of wastewater and sewage sludge in Zimbabwe: implications for human health. *Agr. Ecosys. Environ*., 112: 41-48.

Nawaz, M.F., S. Gul, M.A. Tanvir, J. Akhtar, S. Chaudary and I. Ahmad. 2016. Influence of NaCl-salinity on Pb-uptake behavior and growth of River Red gum tree (*Eucalyptus camaldulensis* Dehnh.). *Turk. J. Agr. For*., 40: 425-432.

Ngumbu, R., F. Sumo, J. Kiazolu and P. Humphrey. 2017. Assessment of lead and cadmium residues in assorted vegetables collected from markets in Monrovia, Liberia. *Int. J. Sci. Res. Sci. Technol*., 3: 54-58.

Ofor, M., V. Okorie, I. Ibeawuchi, G. Ihejirika, O. Obilo and S. Dialoke. 2009. Microbial contaminants in fresh tomato wash water and food safety considerations in South-Eastern Nigeria. *Life Sci. J*., 1: 80-82.

Oteef, M.D., K.F. Fawy, H.S. Abd-Rabboh and A.M. Idris. 2015. Levels of zinc, copper, cadmium, and lead in fruits and vegetables grown and consumed in Aseer Region, Saudi Arabia. *Environ. Monit. Assess.*, 187: 676.

Pandey, R., K. Shubhashish and J. Pandey. 2012. Dietary intake of pollutant aerosols via vegetables influenced by atmospheric deposition and wastewater irrigation. *Ecotoxicol. Environ. Safety*, 76: 200-208.

Peterside, F.N. and O. Waribor. 2006. Bacteria associated with spoilage of fluted pumpkins leaves and their effect on the chlorophyll content. *Nigerian J. Microbiol*., 20: 751-756.

Pierangeli, G., R. Windell, A. Joseth and F. Jude. 2019. Microbiological assessment of fresh, minimally processed vegetables from open air markets and supermarkets in Luzon, Philippines, for food safety. *Environ Dev Sustain,* 21(1): 51-60.

Rabinovitz, B.C., E. Gerhardt, C.T. Farinati, A. Abdala, R. Galarza, D. Vilte, C. Ibarra, A. Cataldi and E.C. Mercado. 2012. Vaccination of pregnant cows with EspA, EspB, Î³-intimin, and Shiga toxin 2 proteins from Escherichia coli O157: H7 induces high levels of specific colostral antibodies that are transferred to newborn calves. *J. Dairy Sci*., 95: 3318-3326.

Raja, S., H.M.N. Cheema, S. Babar, A.A. Khan, G. Murtaza and U. Aslam. 2016. Socio-economic background of wastewater irrigation and bioaccumulation of heavy metals in crops and vegetables. *Agric. Water Manag*., 158: 26-34.

Rashid, M.H.U, M. Asif, T.H. Farooq, N.P. Gautam, M.F. Nawaz, I. Ahmad, M.M. Gilani and P. Wu. 2019. Cuttings growth response of Dalbergia sissoo (Shisham) to soil compaction stress. *Appl. Ecol. Environ. Res.*, 17: 1049-1059.

Ray, B. and A. Bhunia. 2013. Fundamental food microbiology. 5th Edition CRC press, USA. pp 663.

Rehman, M.Z.U., M. Rizwan, S. Ali, Y.S. Ok, W. Ishaque, M. Nawaz, F. Akmal and M. Waqar. 2017a. Remediation of heavy metal contaminated soils by using *Solanum nigrum*: A review. *Ecotoxicol. Environ. Safety*, 143: 236-248.

Rehman, Z.U., S. Khan, M.L. Brusseau and M.T. Shah. 2017b. Lead and cadmium contamination and exposure risk assessment via consumption of vegetables grown in agricultural soils of five-selected regions of Pakistan. *Chemosphere*, 168: 1589-1596.

Ruel, G., Z. Shi, S. Zhen, H. Zuo, E. Kroger, C. Sirois, J.F. Levesque and A.W. Taylor. 2014. Association between nutrition and the evolution of multimorbidity: the importance of fruits and vegetables and whole grain products. *Clin. Nutr*., 33: 513-520.

Saghir, A.S., B.N. Mirani, S.M. Nizamani, A.A. Panhwari and M. Memon. 2019. Nutitional composition of wild-non traditional vegetables of sindh, under different postharvest processing methods. *Pak. J. Bot*., 51: 45-47.

Santarelli, G.A., G. Migliorati, F. Pomilio, C. Marfoglia, P. Centorame, A. D'Agostino, R. D'Aurelio, R. Scarpone, N. Battistelli and F. Di Simone. 2018. Assessment of pesticide residues and microbial contamination in raw leafy green vegetables marketed in Italy. *Food Control*, 85: 350-358.

Satarug, S. and M.R. Moore. 2004. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. *Environ. Health Perspect.*, 112: 1099-1103.

Shabbaj, I.I., M.A. Alghamdi, M. Shamy, S.K. Hassan, M.M. Alsharif and M.I. Khoder. 2017. Risk assessment and implication of human exposure to road dust heavy metals in Jeddah, Saudi Arabia. *Int. J. Environ. Res.Pub. Health*, 15:36.

Shakoor, M.B., N.K. Niazi, I. Bibi, M. Shahid, Z.A Saqib, M.F Nawaz, S.M. Shaheen, H. Wang, D.C.W Tsang, J. Bundschuh, Y.S. Ok and J. Rinklebe. 2019. Exploring the arsenic removal potential of various biosorbents from water. *Environ. Int.*, 123: 567-579.

Singh, A., R.K. Sharma, M. Agrawal and F.M. Marshall. 2010. Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi, India. *Trop. Ecol*., 51: 375-387.

Slifko, T.R., H.V. Smith and J.B. Rose. 2000. Emerging parasite zoonoses associated with water and food. *Int. J. Parasitol*., 30:1379-1393.

Soomro, A., M. Arain, M. Khaskheli and B. Bhutto. 2002. Isolation of Escherichia coli from raw milk and milk products in relation to public health sold under market conditions at Tandojam. *Pak. J. Nutr*., 1: 151-152.

Thompson, J.S., D.S. Hodge and A.A. Borczyk. 1990. Rapid biochemical test to identify verocytotoxin-positive strains of Escherichia coli serotype O157. *J. Clin. Microbiol*., 28: 2165-2168.

Verma, P., M. Agrawal and R. Sagar. 2015. Assessment of potential health risks due to heavy metals through vegetable consumption in a tropical area irrigated by treated wastewater. *Environ. Sys. Decis.,* 35: 375-388.

Yadav, A., P.K. Yadav and D. Shukla. 2013. Investigation of heavy metal status in soil and vegetables grown in urban area of Allahabad, Uttar Pradesh, India. *Int. J. Sci. Res. Pub*., 3: 1-7.

Yousaf, M.T.B., M.F. Nawaz, H.F. Khawaja, S. Gul, S. Ali, I. Ahmad, F. Rasul and M. Rizwan. 2019. Ecophysiological response of early stage *Albizia lebbeck* to cadmium toxicity and biochar addition. *Arab. J. Geosci.*, 12(4): 134.

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