RISK ASSESSMENT OF HEAVY METAL AND MICROBIAL CONTAMINATION IN COMMERCIALY 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 horticulture 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 vendors. 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 (Sliško 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; Pierangelii 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 (Muchuwi 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 vendors. Three locations of each type of market were randomly selected and three samples of each vegetable were collected from each location. The Jhang Bazar, Gulam 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 vendors 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, C d 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 % HNO3 and 65 % HClO4, 4:1 v: v) (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). Commericially available aqeous 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>
<thead>
<tr>
<th>Catalase</th>
<th>Coagulate</th>
<th>Indole</th>
<th>Voges proskauer</th>
<th>Methyl red</th>
<th>Citrate</th>
<th>Sugar fermentation</th>
<th>Motility test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
<td>negative</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

**Table 1. Biochemical test scheme for E. coli.**
Bacterial count was conducted by using the slides with marked area of 1 cm² 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 cm² 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:

\[ a = \left(\frac{D}{2}\right)^2 \pi \]

According to the area of scaled microscope slide (A) = 1cm²

\[ A = 1 \text{ cm}^2 = 10^4 \mu \]

Hence MF = A/a

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

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

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 vendor, 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 vendor 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 vendor, 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 vendors 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 vendors and supermarket.

**Chromium (Cr) concentration:** Concentration of Chromium (Cr) in salad vegetable samples collected from street vendor, 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, three samples were from supermarket and only one sample was from vegetable market. All the samples collected from street vendors were found infected.

<table>
<thead>
<tr>
<th>Source</th>
<th>Vegetable</th>
<th>Colony forming units (CFU)/g</th>
<th>E. coli detection/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vegetable market</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green chilli</td>
<td>1.32×10⁴</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>2.3×10³</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Cucumber</td>
<td>1.12×10³</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>1.22×10²</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Supermarket</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green chilli</td>
<td>1.28×10²</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>1.82×10¹</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Cucumber</td>
<td>1.4×10¹</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>1.42×10¹</td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Street vendor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Green chilli</td>
<td>2.7×10³</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Carrot</td>
<td>2.20×10⁴</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Cucumber</td>
<td>1.30×10²</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Tomato</td>
<td>1.12×10²</td>
<td>Positive</td>
</tr>
</tbody>
</table>
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 descending 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 (7.1), 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 Pb 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). 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 edible 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 vendor. 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 10^6) fresh weight vegetables and as per FAO (<10^3 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 & Bhumia, 2013). According to Petersides & 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 vendors. 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**


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