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Carcinogenic Polycyclic Aromatic Hydrocarbons in Vegetables Marketed in Mashhad: Levels, Dietary Intakes, and Health Risk

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ABSTRACT

Vegetables and fruit cultivation is a main food source for human population. However, different types of pollutants contaminate vegetables products like polycyclic aromatic hydrocarbons (PAHs). Human exposure to PAHs via potential food sources is not well investigated. Therefore, this study aimed to determine PAHs concentrations, dietary intakes, and health risks through the consumption of vegetables collected from urban areas in Mashhad, Iran. Chemical analysis was conducted on 75 vegetable samples were collected from markets in Mashhad including three root and leafy vegetables. The human health risk assessment (HHRA) model was used to measure the dietary intake and lifetime health risk of PAHs through the consumption of vegetables. The concentrations of total PAHs were ranged from 0.564± 0.162 to 2.211± 0.834 μg kg $^{-1}$ in all vegetables. The level of health risks of PAHs was below the acceptable risk level (HI < 1) for adults, while the health risk for children was higher than the acceptable risk level in some vegetable samples. Among the carcinogenic PAH congeners, BaA, Chr, and DbA were predominant for adult and children populations. Overall, the total health risk of PAHs for both groups was borderline or higher than the acceptable level of US EPA risk, suggesting the possibility of health risk for the adults and children to the PAHs via vegetable ingestion. Therefore, appropriate control measures and intervention programs need to be used to protect the health of the residents in this study area.

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Introduction

The world's urban areas are developing and becoming wider and denser rapidly because of the rapid development of the human population and migration [1]. The rapid urban expansion and relative anthropogenic activities led to the cultivable agricultural products being contaminated with different types of pollutants [2, 3]. Persistent organic pollutants such as PAHs are considered as the carcinogenic, and mutagenic contaminants [4, 5]. Several laboratory animal researches indicate that certain PAHs affect the immune and hematopoietic systems and have also adverse effect on development of neurologic and reproductive systems. The carcinogenic effects of PAHs are well documented. It was evidenced that PAHs increased incidences of lung, bladder, skin, stomach, and liver cancers in community [6, 7]. These types of pollutants are commonly originated from different anthropogenic and natural activities such as sludge application, irrigation with industrial or domestic effluent, transportation emissions, incomplete combustion of carbon-containing fuels, heavy vehicular traffic, atmospheric deposition, and mining activities [8, 9]. More than 100 compounds of PAHs with aromatic rings were identified and carcinogenic PAHs consist three or more aromatic rings [6, 7]. The United States Environmental Protection Agency (USEPA 2002) and the International Agency for Research on Cancer (IARC 2004) have classified PAHs based on their carcinogenic effects on human health. According to their classification, benzo[a]pyrene (BaP), benzo[a] anthracene (BaA), benzo[k] fluoranthene (BkF), benzo[b] fluoranthene (BbF), chrysene (Chr), indeno [1,2,3-c,d] pyrene, and dibenzo [a,h]anthracene (DbA) are considered as carcinogens group of PAHs[6].

In the metropolitan areas of Mashhad, Iran, wastewater is used for irrigation. Based on previous literature, high concentrations of organic pollutants such as PAH are detected in this wastewater[10, 11]. There is a serious concern regarding the PAHs contamination in the

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agricultural land and their bioaccumulation in foodstuffs because of PAH's adverse effects on human health [12, 13]. Food chain contamination with PAH is the major pathway (70% of the total exposure) to human exposure that can reduce the quality of food products and affect human health [11, 14]. Several factors influence PAHs bioaccumulation in vegetables such as concentrations of PAHs, soil characteristics, and vegetables' physiological condition [11, 14]. It was evidence that vegetables are cultivated in contaminated soils with PAH at enough concentration lead to an adverse effect on human health. Carcinogenic PAHs are transferred from soil particles to plant tissues via different mechanisms including sorption, volatilization, uptake rate through redisposition, and transpiration plant surfaces [11, 15, 16].

Various research examined the level of chemical contamination in different environmental matrices in Mashhad urban area[10, 14]; however, no new databases are available on the status of carcinogenic PAHs through vegetable consumption in this region. Available monitoring data on organic pollutants from the urban areas of Mashhad show symptoms of contamination in agricultural soils and surface water[10, 11]. With regard to the importance of this large city (as a main industrial and tourism location) in Iran, it is necessary to investigate the vegetable uptake of PAHs and the adverse effects of PAH-contaminated vegetables on public health. Therefore, this study aimed to determine PAHs concentrations, dietary intakes, and health risks through the consumption of vegetables collected from urban areas in Mashhad, Iran.

Materials and Methods

Sample collection and preparation

This study was conducted after the approval and permission of Mashhad University of Medical Sciences Research Committee (IR.MUMS.REC.1397.079) and it was conducted with consideration of Helsinki Declaration in all phases of the study. Confidential data treatment was guaranteed. Data from this study will not be openly available until planned publication outputs have been completed.

In this study, 78 samples were collected from local markets from March to April 2017 in Mashhad, Iran. Three varieties of leafy vegetables included 39 samples of spinach, lettuce, and cabbage. Three varieties of root vegetables included 39 samples of potato, carrot, and radish. We collected each variety vegetable at 13 sampling points (supermarket) and three samples of variety vegetable were collected at each sampling point. We collected each variety of vegetable from 13 sampling points (supermarket) and for each variety three samples were collected at every sampling point. Then, a solvent-cleaned food processor was used to ground and homogenize all samples, and finally, all samples were packed in polyethylene covers, and stored at – 20 ◦C until analysis.

Sample extraction and analysis

We used the Soxhlet method to extract PAHs from vegetables [19]. Ten germs of each Freeze-dried sample were mixed with 2 g anhydrous sodium sulfate and extracted with 200 mL dichloromethane (DCM) for 24 hours. We used the rotary evaporator and gentle flow of nitrogen to remove the DCM and decreased the volume to 1 mL, respectively. Then, silica gel and alumina were used for adsorption chromatography. The extracted sample was eluted with a mixture of DCM (7:3, v/v) and hexane (60 ml). This fraction was again decreased the volume to 1 mL under a gentle flow of nitrogen to measure PAH concentration. Gas chromatography–spectrometry was used to measure PAHs concentration from final concentrated extracts. The SIM model was used based on using the molecular ions selective to identify each PAHs.

Quality control

For precision and accuracy of the extraction and analysis procedure, recovery methods were conducted by using PAH standards with a known concentration (2 µg −1) in uncontaminated plant materials [3]. A satisfactory recovery range (92.2±3.2% to 112.9±2.3%) for plant materials was observed in this study.

Health risk assessment:

The daily intake of PAH through consumption of PAH-contaminated vegetable was determined by the following equation 1.

$$ADD_{PAH} = \frac{C_{PAH} * IR_{intake} * C_{factor}}{B_{weight}}$$

[1]

Where ADD_{PAH}, B_{weight}, C_{PAH}, C_{factor}, and IR_{intake}, represent the daily intake of PAH (mg/kg/day), average body weight, concentrations in vegetables (µg/kg), conversion factor, average daily vegetable intake, and the daily intake of the vegetables (kg/day), respectively[17].

Conversion factor was considered of 0.085 for vegetable. This factor was used to convert fresh weight of vegetable into dry weight[3]. Based on National Institute of Nutritional Research and Food Industry report, average child and adult body weights were 32.7 and 72 kg, respectively, and average daily intake of vegetable for children and adults were considered of 0.042 and 0.076 kg/day, respectively [15]. For selected PAH in this study, we calculated the total toxic BaP equivalent (TEQ) using the following equation 2:

$$HQ_{PAHs} = \frac{ADD}{RfD}$$

[2]

where HQ, ADD, and RfD represent the target hazard quotient, the daily intake of PAH (mg/kg/day), and the (mg/kg/day). Oral reference dose was estimated for BkF and Bap (3×10^{-4}) that adopted from USEPA integrated risk information system (IRIS)[17, 18]. The total hazard quotient (HI) estimated by Equation 3:

$$THQ = HI = \sum HQ1 \ldots . HQn$$

[3]

We analyzed all the data for conducting descriptive and bivariate statistical analyses using Microsoft excel (Microsoft 2007) and SPSS software.

Results

PAH concentrations in vegetables

Relevant data on the concentration of total and individual carcinogenic PAHs in the edible parts of the vegetables were summarized in Table 1. Average total PAHs concentrations in the edible part of root vegetables were ranged from 0.564± 0.162 to 0.69± 0.092 μg kg −1, while in the edible part of leafy vegetables were from 1.143± 0.260 to 2.211± 0.834 μg kg −1. According to the statistical test, significant differences ($p < 0.05$) were observed between the concentration of total PAHs in leafy vegetables and root vegetables. The concentration of total PAHs in the leafy vegetables was significantly higher than the root vegetables and the concentration of carcinogenic PAHs in vegetables was as follows: lettuce > spinach > cabbage > radish>potato> carrot. High concentrations of carcinogenic PAHs were detected in lettuce and spinach.

Table 1. Average concentration concentrations (μg kg $^{-1}$) of PAHs in vegetable samples (n = 78).

	Leafy vegetables (n= 39)			**Root vegetables (n=39)**		
	Spinach	**Lettuce**	**Cabbage**	**Potato**	**Carrot**	**Radish**
BaA	0.445 ± 0.123	0.474± 0.203	0.160± 0.067	0.208 ± 0.022	0.177± 0.061	0.245± 0.104
Chr	0.429 ± 0.083	0.701± 0.350	0.290± 0.081	0.148 ± 0.093	0.18± 0.103	0.173± 0.063
BbF	0.0945± 0.023	0.134± 0.053	0.137± 0.047	0.023± 0.0021	0.027± 0.0103	0.052± 0.032
BkF	0.450 ± 0.349	0.234± 0.062	0.209± 0. 085	0.024 ± 0.0015	0.027± 0.0092	0.045± 0.028
BaP	0.154 ± 0.081	0.180± 0.013	0.187± 0.056	0.053± 0.0015	0.0456± 0.0024	0.0877± 0.00361
DbA	0.474 ± 0.139	0.488± 0.108	0.160± 0.091	0.123± 0.051	0.098± 0.042	0.088± 0.0312
∑6 PAHs	2.042±0.719	2.211± 0.834	1.143± 0.260	0.589± 0.061	0.564± 0.162	0.69± 0.092

Data are calculated as average ± SD of three replicates

Table 2. Daily intake levels of PAHs (ng g $^{-1}$ body weight day^{-1}) via vegetable ingestion for child and adult groups.

DI_{PAHs}	**Leafy vegetables (n= 39)**			**Root vegetables (n=39)**		
	Spinach	**Lettuce**	**Cabbage**	**Potato**	**Carrot**	**Radish**
Child						
BaA	5.32E-05	5.67E-05	1.91E-05	2.49E-05	2.12E-05	2.93E-05
Chr	5.13E-05	8.38E-05	3.47E-05	1.77E-05	2.15E-05	2.07E-05
BbF	1.13E-05	1.60E-05	1.64E-05	2.75E-06	3.23E-06	6.22E-06
BkF	5.38E-05	2.80E-05	2.50E-05	2.87E-06	3.23E-06	5.38E-06
BaP	1.84E-05	2.15E-05	2.24E-05	6.42E-07	5.45E-07	1.05E-06
DbA	5.67E-05	5.84E-05	1.91E-05	1.47E-05	1.17E-05	1.05E-05
∑ADD 6 $_{PAHs}$	2.45E-04	2.64E-04	1.37E-04	6.93E-05	6.63E-05	8.26E-05
Adults						
BaA	3.94E-05	4.19E-05	1.42E-05	1.84E-05	1.57E-05	2.17E-05
Chr	3.80E-05	6.20E-05	2.57E-05	1.31E-05	1.59E-05	1.53E-05
BbF	8.36E-06	1.19E-05	1.21E-05	2.04E-06	2.39E-06	4.60E-06
BkF	3.98E-05	2.07E-05	1.85E-05	2.12E-06	2.39E-06	3.98E-06
BaP	1.36E-05	1.59E-05	1.65E-05	4.75E-06	4.04E-06	7.76E-06
DbA	4.19E-05	4.32E-05	1.42E-05	1.09E-05	8.67E-06	7.79E-06
∑ADD 6 $_{PAHs}$	1.81E-04	1.96E-04	1.01E-04	5.13E-05	4.91E-05	6.11E-05

Risk assessment of PAH

In this study, the health risks of the carcinogenic PAHs were examined based on daily intake of carcinogenic PAHs through ingestion of leafy vegetables and root vegetables for adult and child populations that were resident in Mashhad. In the adult population, daily intake of PAH through vegetable consumption ranged from 4.91E-05 to 1.96E-04 µg kg $^{-1}$ body weight day $^{-1}$, and for children ranged from 6.63E-05 to 2.64E-04 µg kg $^{-1}$ body weight day $^{-1}$ (Table 2).

Based on the mean value of total ADD (equation 1), children were ~ 1.4 times more exposed to PAHs of vegetables than adults (Table 2). The hazard index (HI) from the ingestion of leafy vegetables and root vegetables for adult and child consumers was shown in Table 3. As data show, the average value of HQ (equation 2) and HI (equation 3) through ingestion of leafy vegetables and root vegetables are lower than the level of concern (HQ < 1) for all of the vegetables. However, our finding showed that the HI level in some samples of leafy vegetable variety such as spinach and lettuce was higher than the level of concern (HQ > 1) in the children population (Figure 1). Total HI in the leafy vegetables and root vegetables were dominated by BAa, Chr, and DbA, which showed average values of 28.3%, 30%, and 19.5% in the sediments, respectively (Figure 2). The three abovementioned PAHs constitute a significant portion of the total PAHs concentrations in most of the vegetables.

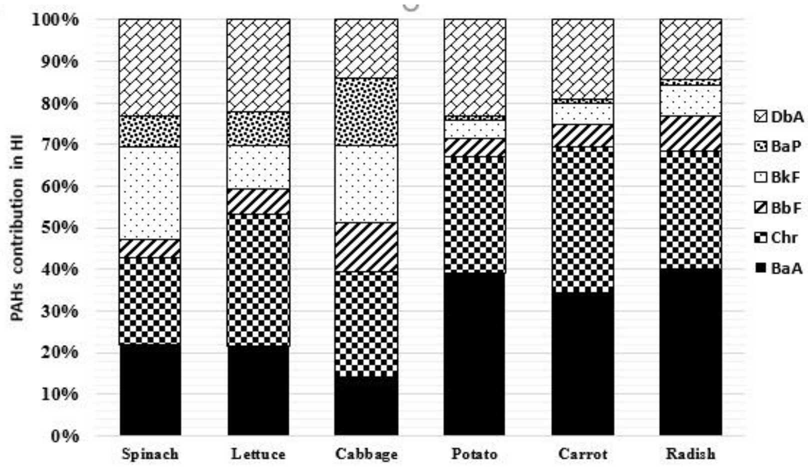

Figure 1. Contribution of individual PAHs on vegetable HI for two target groups

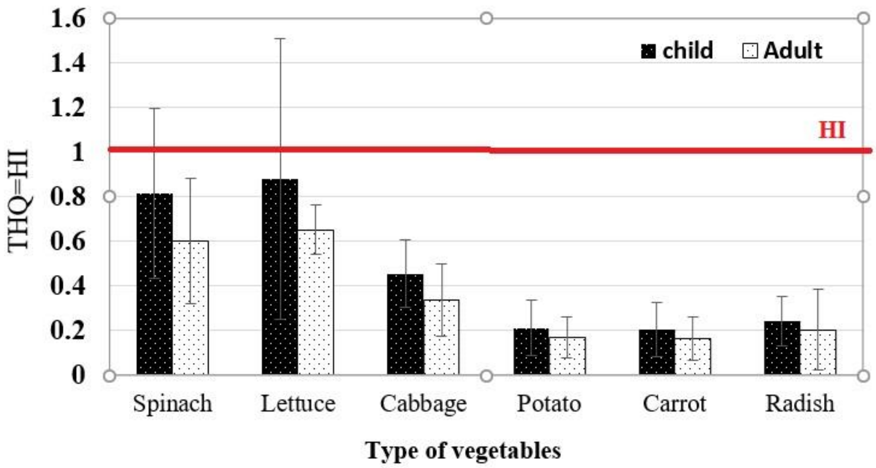

Figure 2. Estimated total health risk for vegetables through ingestion rout by adult and children

Table 3. target hazard quotient level of PAH via vegetable ingestion

THQ_{PAHs}	Leafy vegetables (n= 39)			Root vegetables (n=39)		
	Spinach	Lettuce	Cabbage	Potato	Carrot	Radish
Child						
BaA	0.177± 0.082	0.189± 0.098	0.064± 0.042	0.083± 0.059	0.071± 0.038	0.098± 0.042
Chr	0.171± 0.132	0.279± 0.162	0.116± 0.69	0.059± 0.031	0.072± 0.052	0.069± 0.031
BbF	0.038± 0.021	0.053± 0.058	0.055± 0.022	0.009± 0.0082	0.011± 0.006	0.021± 0.012
BkF	0.179± 0.069	0.093± 0.022	0.083± 0.061	0.010± 0.007	0.011± 0.008	0.018± 0.009
BaP	0.061± 0.042	0.072± 0.049	0.075± 0.049	0.002± 0.001	0.002± 0.001	0.003± 0.002
DbA	0.189± 0.132	0.195± 0.092	0.064± 0.051	0.049± 0.039	0.039± 0.022	0.035± 0.022
$\sum_{HQ=HI}$	0.816± 0.379	0.881± 0.629	0.456± 0.152	0.212± 0.125	0.205± 0. 12	0.244± 0.112
Adult						
BaA	0.131± 0.071	0.140± 0.034	0.047± 0.023	0.061± 0.031	0.052± 0.031	0.072± 0.031
Chr	0.127± 0.062	0.207± 0.1 62	0.086± 0.068	0.044± 0.036	0.053± 0.021	0.051± 0.031
BbF	0.028± 0.011	0.040± 0.024	0.040± 0.026	0.007± 0.002	0.008± 0.002	0.015± 0.002
BkF	0.133± 0.041	0.069± 0.037	0.062± 0.075	0.007± 0.003	0.008± 0.006	0.013± 0.002
BaP	0.045± 0.012	0.053± 0.018	0.055± 0.042	0.016± 0.006	0.013± 0.012	0.026± 0.013
DbA	0.140± 0.073	0.144± 0.093	0.047± 0.032	0.036± 0.012	0.029± 0.011	0.026± 0.016
$\sum_{HQ}{}^{=HI}$	0.604± 0.282	0.652± 0.11	0.337± 0.162	0.171± 0.093	0.164± 0.096	0.204± 0.182

Discussion

In this research we examine concentration of six PAHs including BaA, Chr, BbF, BkF, BaP, and DBA in edible parts of vegetables. These PAHs were considered as possible carcinogenic by USEPA[3, 9]. We hypnotized that bioaccumulation of carcinogenic PAHs in vegetables can be responsible for variety adverse effect on health of consumers. The tolerable limits for PAH are different from country to country, but several countries such as Iran have not defined these benchmark levels for PAHs in vegetables and other food products[10]. Our finding showed that selected carcinogenic PAHs concentrations in vegetables are generally lower than those previously studies in others cities in Iran[19-21], and concentrations of all individuals PAHs in this study were lower than maximum permissible limits set for food product in the Netherlands and world health organization[3]. Likewise, the concentration of total PAHs were higher than Chinese cities[3], and Spain[22], but lower than Pakistan[23]. According to results of this study, concentrations of total PAHs and individuals PAHs in leafy vegetables were significantly higher than root vegetables, similar to the studies that were conducted in China [3] and India [1], which could be caused by atmospheric deposition and dust-fall [2, 22]. Several studies indicated that dust-fall were contaminated with high molecular weight of PAH (BaA, BaP, BkF, Chr, BbF, and DBA)[3, 10], therefore; dust-fall might be contributing to increase PAH concentration in vegetables.

Exposure to PAHs through vegetable consumption is a main public health concern. Since no data is available related to the carcinogenic effects of PAHs vegetable consumption among people who lived in Mashhad, ADD and THQ were used to quantify the level of health risk of the selected PAH associated with vegetable consumption. In this study, the daily intakes of the BAa, Chr, and DbA through vegetable ingestion have the main contribution to increase health risk in child and adults groups. This result is consistent with recent researches that highlighted the contribution of BAa, Chr, and DbA as most important congeners of PAHs, which associated with health risk [3, 9, 23]. Likewise, the average of ADD showed that children were ~ 1.4 times more exposed to vegetables than adults. This finding is a consistent result of some studies indicating that the total doses of PAHs intake in children were mainly higher than in adults[2, 5, 23]. For instance, in China and USA, the average total ADD values via the vegetable ingestion in children groups were ~ 1.9 [3] and 2.1[9] times higher than adults, respectively.

As results show, the average values of HQ and HI did not exceed the standard threshold (HQ < 1) for both target groups; therefore, there may not be a health risk to both target groups from PAHs compounds in these vegetables. This result is in agreement with other studies in China and Pakistan [3, 14]. However, our finding was not consistent with several studies in the world. Nie et al. (2014) estimated the concentration of PAHs in 24 food products (including fruit, wheat flour, and vegetables, etc.), and indicated that vegetables contribute around one-third of PAHs in dietary food and the level of health risk from PAHs for individuals who lived in Taiyuan were higher than populations in this study[24]. Ding et al. (2013) also estimated the health risks to PAHs compound via ingestion pathway (including rice, meat, and vegetable) for individuals who lived in Shenzhen, China, and the level of health risk was 2.5 times higher than our finding[25]. Yoon et al. (2007) examined the health risk of PAHs via ingestion of 27 different food commodities for the Korean population, and indicated the level of risk were ranged from 0.7 to 2.3 and vegetables were the main contributor [26]. Furthermore, BAa, Chr, and DbA showed the highest contribution of HI for both target groups. It seems these compounds could be the most carcinogenic PAHs in the case of health risk.

This result highlighted that children are highly exposed groups to the carcinogenic PAHs via ingestion routes, suggesting that children could be more susceptible to health risks from the carcinogenic PAHs compared to adults[3, 22]. World Health Organization (WHO) considered children as a vulnerable group to health risks as they breathe more air and consume more food and water in proportion to their weight, resulting in higher health risks[20]. Since, Child's digestive, immune, nervous, and reproductive systems are still in the early part of development, exposure to carcinogenic contaminants such as PAHs causes irreversible damage.

Likewise, the health risk for children was higher than the acceptable risk level in some vegetable samples, suggesting the possibility of health risk for the children to the PAHs via vegetable ingestion. Therefore, appropriate control measures and intervention programs need to be used to protect the health of the residents in this study area.

Conclusion

In this work, we investigate the health risk of six carcinogenic PAHs for adults and children (as a sensitive population) according to daily intake of vegetables. Total concentration of PAHs in leafy vegetables was significantly higher than those in root vegetables, probably due to the atmospheric deposition. Residents in Mashhad were more exposed to BAa, Chr, and DbA congeners compounds among the other carcinogenic PAH compounds. For both target population, the average level of PAHs risk never exceeded the safety level of risk that US EPA set for management criterion, suggesting there may be no health risk to both target groups from PAHs compounds. Risk evaluation indicated that the health risk from vegetable consumption for child groups was borderline or higher the acceptable level of US EPA risk in some vegetable samples. Therefore, children may experience more health risk via ingestion of vegetables in this study area and more attention should be paid to this population.

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Conflict of Interest

There is no conflicts of interest in this work.

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