Sains Malaysiana 45(2)(2016): 167–176

Phytoaccumulation of Heavy Metals and Protein Expression by Different Vegetables Collected from Various Parts of Khyber Pukhtunkhawa Province, Pakistan

(Fitoakumulasi Logam Berat dan Ekspresi Protein oleh Sayuran Berbeza Diperoleh daripada Bahagian Berbeza di Wilayah Khyber Pukhtunkhawa, Pakistan)

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ABSTRACT

The present study investigates heavy metal uptake and protein expression by different vegetables collected from various districts of Khyber Pakhtunkhawa province of Pakistan. Statistical analysis of the data showed that maximum concentration of Cd, Cr, Ni, Zn were found in radish and spinach, respectively, collected from Peshawar. Maximum Pb and Mg accumulation were found in cauliflower and pea at Swat followed by coriander at Haripur and minimum Pb uptake was noticed in radish taken from Nowshehra. Highest Cu uptake was detected in spinach at Nowshehra. Data regarding Cd, Cr, Pb, Cu, Ni, Mg and Zn concentration in water samples gathered from different sites of KPK indicated that maximum concentration of Cd was observed in Swat. Maximum Cr and Cu concentration were measured in water samples from Peshawar while maximum concentration of Pb and Ni were detected in water samples from Haripur. In case of soil samples, maximum Cd, Cr, Mg and Zn uptake was observed in soil sample at Nowshehra. Maximum Pb and Ni concentration was found in soil samples collected from Peshawar. Cu concentration was observed to be the highest in soil at Swat. Protein profile of different vegetables i.e. cauliflower, radish, carrot, turnip, pea, spinach, coriander and garlic sampled across five different sites showed that uptake of Cd, Cr, Pb, Cu, Ni, Mg and Zn by these vegetables caused the expression of numerous polypeptides.

Keywords: Heavy metals; protein expression; vegetables; WHO

ABSTRAK

Penyelidikan ini mengkaji penyerapan logam berat dan ekspresi protein oleh sayuran berbeza yang diperoleh dari pelbagai daerah di wilayah Khyber Pakhtunkhawa, Pakistan. Analisis statistik data menunjukkan bahawa kepekatan maksimum Cd, Cr Ni, Zn dilihat pada lobak dan bayam yang masing-masing diperoleh dari Peshawar. Pengumpulan maksimum Pb dan Mg dilihat pada kubis bunga dan kacang pea di Swat diikuti ketumbar di Haripur dan penyerapan minimum Pb dilihat pada lobak yang diambil dari Nowshehra. Penyerapan tertinggi Cu dikesan dalam bayam di Nowshehra. Data kepekatan Cd, Cr, Pb, Cu, Ni, Mg dan Zn dalam sampel air diambil dari tapak berbeza di KPK menunjukkan bahawa kepekatan maksimum Cd diperhatikan di Swat. Kepekatan maksimum Cr dan Cu diukur dalam sampel air dari Peshawar manakala kepekatan maksimum Pb dan Ni telah dikesan dalam sampel air dari Haripur. Dalam kes sampel tanah, penyerapan maksimum Cd, Cr, Mg dan Zn diperhatikan dalam sampel tanah di Nowshehra. Kepekatan maksimum Pb dan Ni diperoleh daripada sampel tanah yang dikumpul dari Peshawar. Kepekatan Cu diperhatikan tertinggi di Swat. Sampel profil protein sayuran berbeza seperti kubis bunga, lobak, lobak merah, turnip, kacang pea, bayam, ketumbar dan bawang putih dari lima tapak berbeza menunjukkan bahawa penyerapan Cd, Cr, Pb, Cu, Ni, Mg dan Zn oleh sayuran ini menyebabkan ekpresi pelbagai polipeptides.

Kata kunci: Ekpresi protein; logam berat; sayuran; WHO

INTRODUCTION

Vegetables are edible plant parts and store reserve food in different parts of plant i.e. roots, stem, leaves and fruits (Sobukola et al. 2007). Vegetables (cauliflower, coriander, carrot, garlic, pea, radish, spinach and turnip) constitute of essential diet components by contributing iron, calcium protein, vitamins and other nutrients. These elements are the building blocks of human body and help in the formation of bones, teeth, hair and nails and protects human body against the attack of various diseases (D'Mello 2003). They also act as buffering agents for acidic substances produced during the digestion process. Metal accumulation in vegetables may pose a direct threat to human health (Turkdogan et al. 2003). Leafy vegetables have a high water content of 70-75% and contain cellulose which forms roughage and improves feaecal movement.

The occurrence and accumulation of metals in soil is the result of precise and specific interactions among chemical, biological and environmental parameters (Panuccio et al. 2009). The physical, chemical and biological properties and features of the soil were changed due to soil management and as an outcome, various reactions and responses of biological processes to heavy metal toxicity can be seen. Higher levels and abundance of heavy metals in soil alters the activities of land inhabiting microbes that play a vital role in plant growth (Wani et al. 2007). Heavy metals are also termed as environmental pollutants as they exhibit strong toxic and poisonous effects at higher concentrations (Chehregani et al. 2005). Heavy metal toxicity in plants causes chlorosis, weak plant growth, yield depression along with metabolic disorders, lack of nutrient uptake and reduced or inability of leguminous plants to fix atmospheric nitrogen (Ali et al. 2014; Azmat et al. 2015; Colak et al. 2014; Madiha et al. 2012; Ullah et al. 2011). Heavy metals due to their potential toxicity can cause harmful effects on human and animal health if they enter the food chain. The transmission of heavy metals in food chain through crops grown on heavy metal polluted soil is a potential risk to human health (Fries et al. 2006; Akan et al. 2013; Ata et al. 2013; Basu et al. 2013; Mahmood & Malik 2014). The most important issue related to heavy metals is their transmission and spread via natural ecosystems (Walker et al. 2003). Soil contaminated with heavy metals also adversely affect agricultural yield (Schickler & Caspi 1999).

The presence of elevated levels of heavy metals in the soil was due to different human activities including mining, smelting, application of sewage sludge, addition of manures, pesticides, fertilizers and discharge of wastewaters (Kabata-Pendias & Pendias 2001). In some areas, the anthropogenic activities and advancements in technologies including industrial and agricultural wastes, domestic effluents and natural process of weathering (of rocks) makes water pollution practically difficult and inevitable. High concentrations of heavy metals especially Cd, Pb and Cu were found in waste waters from mining, electroplating and chemical industries and also from chemical laboratories (Sharma et al. 2008). The naturally occurring most toxic heavy metals are Lead (Pb), Mercury (Hg), Arsenic (As), Cadmium (Cd), Selenium(Sn), Chromium (Cr) , Zinc (Zn), Iron (Fe), Silver (Ag) and Copper (Cu). The most toxic and dangerous heavy metals regarding human and other living organisms life are Cd and Pb (Sekara et al. 2005). The present study were carried out to investigate heavy metal content in soil and water collected from the different field grown with vegetable; To determine heavy metal concentration in different vegetables collected from various regions of Khyber Pakhtunkhwa province of Pakistan; and to monitor protein expression of different vegetables collected from various regions of Khyber Pakhtunkhwa by SDS-PAGE.

MATERIALS AND METHODS

The present study was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, Pakistan. The aim of this study was to evaluate the phytoaccumulation potential and to monitor protein expression of eight vegetable samples including cauliflower, coriander, carrot, garlic, peas, radish, spinach and turnip. Plant, soil and water samples were collected from five distant locations of Khyber PakhtunKhwa (KPK) province of Pakistan (Swat, Haripur, Mansehra, Peshawar and Nowshehra).

PROCEDURES FOR HEAVY METAL MEASUREMENT

Vegetable samples were chopped into small pieces, packed in paper bags and dried in oven at 80°C for 48 h. After complete drying, the samples were finely grinded into powdered using an electric grinder. One gram each of the dried samples was digested with 15 mL of concentrated nitric acid (HNO_3) overnight. Digested samples were then heated up to 250°C until white fumes were produced and heating was continued for another 30 min, allowed to cool down to room temperature. 25 mL of distilled water was added to each digested sample. The concentrations of Cd, Cr, Pb, Cu, Ni, Mg and Zn were detected in the samples via Atomic Absorption Spectrophotometer ((Hitachi Z-8100, Japan) at their respective wavelengths.

For soil samples, 1 gram dry soil sample was weighed and digested in 15 mL of concentrated nitric acid overnight followed by acid digestion carried out in a fume hood till the appearance of reddish brown flames. The digested soil samples were allowed to cool down at room temperature and then diluted with 25 mL distilled water and subsequent filtration with filter paper. The concentrations of Cd, Cr, Pb, Cu, Ni, Mg and Zn were detected in the samples via atomic absorption spectrophotometer at their respective wavelengths. For the determination of heavy metals in water sample, 15 mL of water was weighed via graduated cylinder and used directly for the atomic absorption spectrophotometer analysis for the detection of the Cd, Cr, Pb, Cu, Ni, Mg and Zn.

RECOVERY STUDIES

Standard addition method was carried out to confirm the validity of our method. Hence, a recovery test was performed using method of standard addition. Standard solutions containing Cd, Cr, Pb, Cu, Ni, Mg and Zn were prepared and spiked with digested samples, after dilution of sample to 50 mL. Ninety six to 98% recoveries was achieved for different heavy metals under study. A blank was run for each digestion procedure to correct the measurements. For sets of every ten samples, a procedure blank and spike sample, involving all reagents, was run to check for interference and cross contamination. The detection limit of AAS was from 0.002 to 0.09 for different heavy metals.

PROTEIN EXTRACTION AND SDS-PAGE

Protein extraction was carried out according to established procedures (Desfrancs et al. 1985). Fresh tissue was harvested, immediately ground with a mortar and pestle in liquid nitrogen and an equal volume of extraction buffer (50 mM Tris–HCl, pH8.5: 4% SDS: 5% mercaptoethanol: 1 mM phenylmethylsulphonyl fluoride: 20 mg mL^{-1}, leupeptin: 4% polyvinylpyrrolidone, average Mr 40 kDa) added to the powder; the mixture was then vortexed for 40 s and centrifuged at 12500 × g for 30 min at 4°C and the supernatant decanted and protein precipitated overnight at −20°C with 10 volumes of ice cold acetone. The precipitate was collected by centrifugation as described above, the pellet was washed in 95% (v/v) ice cold ethanol and then dried down under vacuum. The samples for protein quantification were prepared by mixing 10 µL protein samples with 2 mL CBB solution (CBB powder G250-10%; 95% ethanol; 85% phosphoric acid). The samples were then analyzed for concentration of protein by UV absorption spectrophotometer. Spectrophotometric data was collected for the samples as well as standard protein solution of BSA (Bovine Serum Albumin). Protein samples (50 µg) were then run on 12% polyacrylamide gel containing 4% stacking gel. After electrophoration, protein gels were stained in staining solution (0.25 g CBB powder R250, 125 mL methanol, 25 mL glacial acetic acid and 100 mL dH_2O) for 40 min followed by overnight destaining (30% methanol; 10% acetic acid and 60% dH_2O). Gel documentation was used to observe the banding pattern of proteins on gel through Gel Analyzer 2010 software.

STATISTICAL ANALYSIS

All data are presented as mean values of two replicates. Data were analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez and Gomez (1984). MSTATC computer software was used to carry out statistical analysis (Russel & Eisensmith 1983). The significance of differences among means was compared by using least significant difference (LSD) test (Steel et al. 1997).

RESULTS AND DISCUSSION

Data regarding accumulation of heavy metal Cd by different vegetables collected across various sites of KPK showed that all the investigated heavy metals accumulation was significantly ($p<0.05$) affected by location and vegetables. Maximum concentration of Cd was found in radish followed by turnip at Peshawar whereas minimum concentration of Cd was observed in garlic samples of the same location. Being a root vegetable, accumulation of Cd was found to be highest in radish (Figure 1). Our results showed that in all vegetables and locations, concentration of Cd was well above the permissible limits recommended by WHO/FAO joint. Hamadouche et al. (2012), Bigdeli and Seilsepour (2008) and Lone et al. (2013) reported the hyperaccumulation of Cd by radish. However, the conclusions given by Kumar et al. (2013) were contradictory where Cd accumulation by radish was below the threshold limit. Similarly, Soudek et al. (2009) reported highest Cd uptake by garlic which is not in compliance with our results. The data showed that maximum Cr accumulation was noticed in spinach at Peshawar followed by radish at Swat and spinach sample collected from Mansehra. The hyperaccumulation potential of spinach leaves is due to the fact that it is a leafy vegetable and storage of nutrients along with the heavy metals occurs in leaves (Figure 2). Again, Cr content found in these vegetables and locations was above the allowable limits proposed by WHO/FAO joint. These results agree with Akan et al. (2013), Mahmood and Malik (2014) and Rizwan et al. (2013) who reported hyperaccumulation of Cr by spinach. However, the results reported by Kumar et al. (2013) were contradictory to our findings.

Data presented in Figure 3 showed that maximum Pb accumulation was found in cauliflower sample at Swat followed by coriander at Haripur, whereas minimum Pb uptake was noticed in radish sample taken from Nowshehra. Pb content was found to be highest in the curd part of the cauliflower as it is the main storage site and is an essential part of human diet. Comparison with the safe levels given by WHO/FAO joint, concentration of Pb in all vegetables and locations exceeded the safe limits. The same conclusions related to highest uptake of Pb by spinach and coriander were also given by Basu et

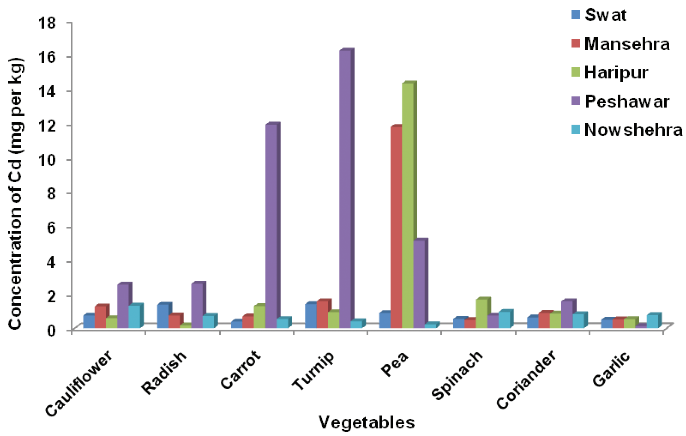

FIGURE 1. Concentration of Cd (mg kg^{-1}) in different vegetables collected from different locations (WHO safe limits for Cd= 0.20 mg kg^{-1})

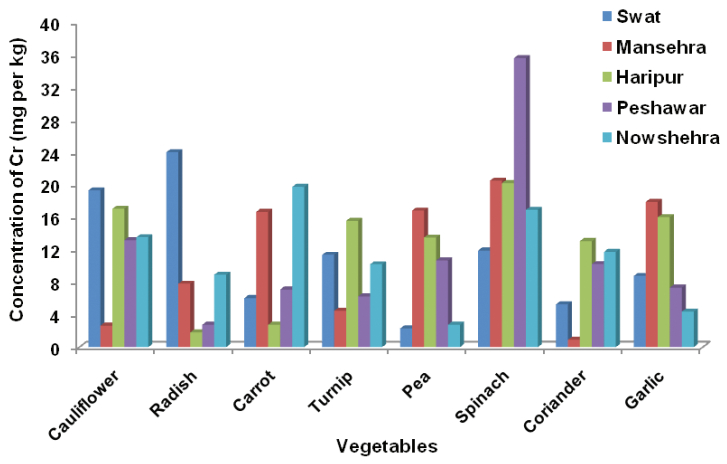

FIGURE 2. Concentration of Cr (mg kg^{-1}) in different vegetables collected from different locations (WHO safe limits for Cr= 2.30 mg kg^{-1})

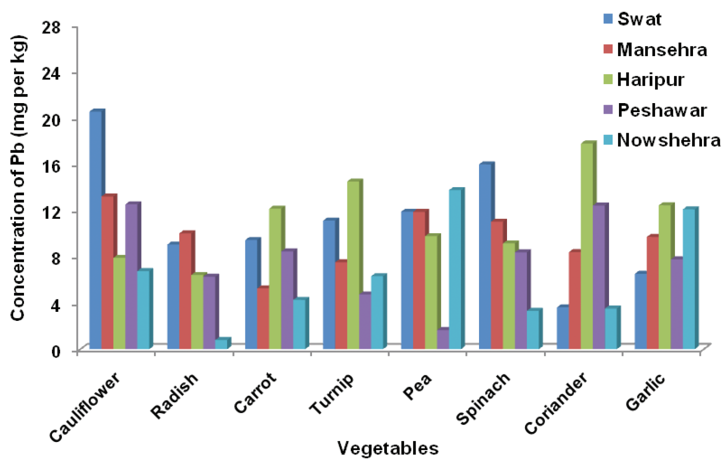

FIGURE 3. Concentration of Pb (mg kg^{-1}) in different vegetables collected from different locations (WHO safe limits for Pb= 0.30 mg kg^{-1})

al. (2013), Khan et al. (2013) Shakya and Khwaounjoo (2013) and Singh et al. (2012). However, Rehman et al. (2013) reported that Pb accumulation was below the threshold value in cauliflower. Data concerning heavy metal accumulation of Cu by different vegetables sampled across different locations of KPK showed that maximum Cu uptake was found in spinach at Nowshehra followed by garlic and spinach samples at Haripur, while minimum value was recorded in coriander and turnip at Peshawar (Figure 4). Hyperaccumulation of Cu in spinach accounts for its ability to store food along with different heavy metals in its leaves and garlic being a bulb also accumulates higher quantities of metals in its cloves. These results indicated that concentration of Cu by all the vegetables and locations is below the threshold value proposed by WHO/FAO joint. Mahmood and Malik (2014) and Akan et al. (2013) reported maximum Cu uptake by spinach that was above the safe limits. Parveen et al. (2013) recorded maximum uptake of Cu by turnip which does not correlate to our results.

The data presented in Figure 5 shows that maximum Ni uptake was recorded in spinach collected from Peshawar followed by carrot at Haripur and radish at Mansehra. Higher Ni content was found in the leaves of spinach as the storage solely occurs in leaves, while radish and carrot being root vegetables have accumulated higher levels of Ni. Concentration of Ni was below the allowable limits proposed by WHO/FAO joint. Maximum Ni uptake was reported by Khan et al. (2013), Mahmood and Malik (2014) and Rizwan et al. (2013) by spinach and carrot above the safe levels.

Maximum Mg accumulation was noticed in pea at Swat followed by coriander at Haripur and spinach at Nowshsehra, while minimum Mg uptake was found in coriander at Peshawar followed by garlic at Nowshehra. Pea being a leguminous plant has accumulated higher Mg content while coriander and spinach have accumulated maximum Mg content in their leaves (Figure 6). Similarly, maximum Zn accumulation was detected in spinach sample at Peshawar followed by spinach at Swat while minimum

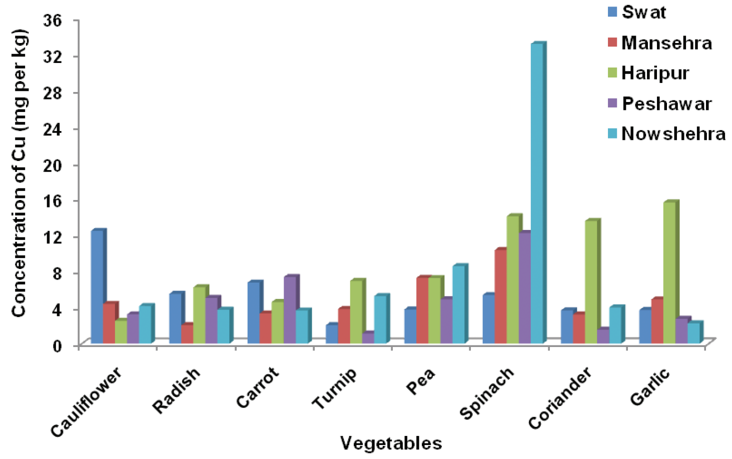

FIGURE 4. Concentration of Cu (mg kg^{-1}) in different vegetables collected from different locations (WHO safe limits for Cu= 40 mg kg^{-1})

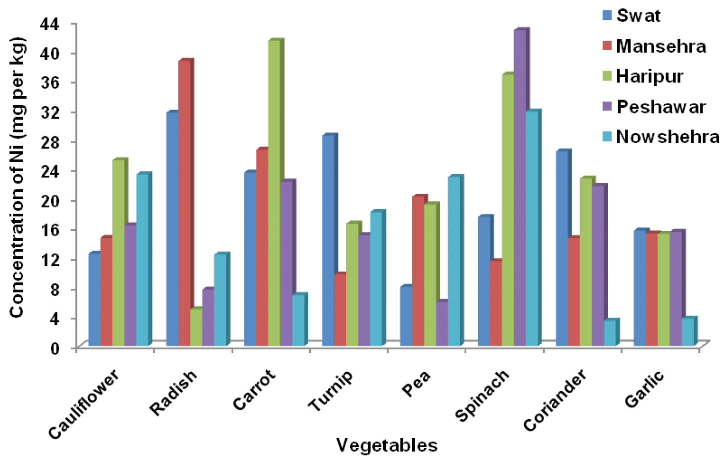

FIGURE 5. Concentration of Ni (mg kg^{-1}) in different vegetables collected from different locations (WHO safe limits for Ni= 67 mg kg^{-1})

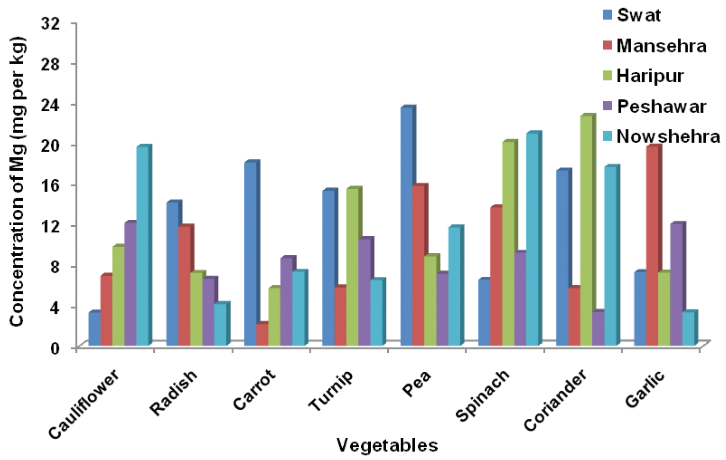

FIGURE 6. Concentration of Mg (mg kg^{-1}) in different vegetables collected from different locations

uptake was noticed in garlic sample from Nowshsehra. Zn accumulation was highest in spinach as storage of food and other nutrients takes place in leaves in green leafy vegetables (Figure 7). Comparison of these values with the safe limits for Zn suggested that uptake of Zn by all vegetables at all locations were below the threshold value. Doherty et al. (2012), Mahmood and Malik (2014), Rapheal and Adebayo (2011), Shakya and Khwaounjoo (2013) and Singh et al. (2012) also reported maximum uptake of Zn by spinach, however, it was below the standard values set by WHO.

Data presented in Table 1 relating to the heavy metal accumulation of Cd, Cr, Pb, Cu, Ni, Mg and Zn by water samples gathered from different sites of KPK showed that maximum concentration of Cd was observed in water sample collected from Swat and minimum in Peshawar. Maximum Cr accumulation was observed in water sample from Peshawar and minimum in Nowshehra. Maximum Pb uptake was noticed in water sample from Haripur whereas minimum in Mansehra water. In case of Cu, maximum accumulation among the means was observed in water sample of Peshawar and minimum in Haripur. Accumulation of Ni was maximum in water sample from Haripur and minimum in Peshawar water samples. Our results showed that concentration of Cd was higher in water samples from Swat, Cr and Cu in water samples collected from Peshawar and Ni in all the locations when compared with the safe limits for these heavy metals determined by WHO/FAO joint. The presence of highest concentration of different heavy metals may have contributed to the maximum accumulation of these metals by the subject vegetables as this water was used for their irrigation. The data also suggested that concentration of Zn was below the allowable limits in all the locations and samples. Similarly, the results were also reported by Perveen et al. (2012) who concluded maximum concentrations of Pb, Ni and Cd in water samples that were well above the safe limits given by WHO. Khan et al. (2013) reported maximum concentration of Cu, Mn, Ni and Cd in water that was above the standard values recommended by WHO while Pb and Zn concentrations were below the safe limits.

Data presenting heavy metal accumulation of Cd, Cr, Pb, Cu, Ni, Mg and Zn by different soil samples collected across different areas of KPK showed that maximum Cd uptake was observed in soil samples collected from Nowshehra while minimum in Swat (Table 2). Similarly, maximum Cr was detected in soil sample from Nowshsehra followed by soil samples from Mansehra whereas minimum concentration was detected in soil at Haripur. Maximum Pb accumulation was found in soil at Peshawar

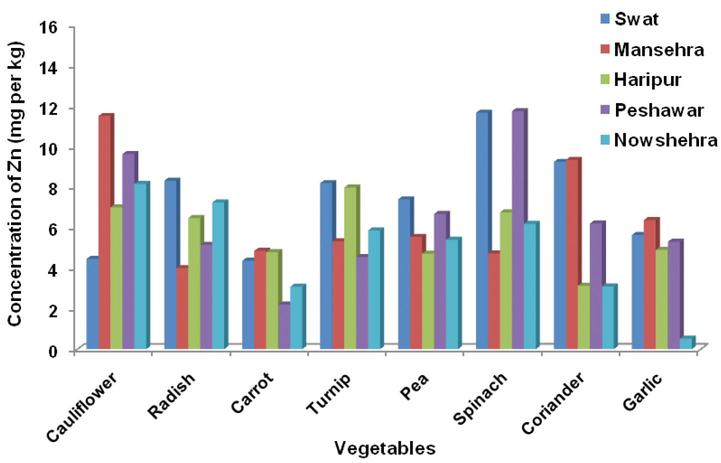

FIGURE 7. Concentration of Zn (mg kg^{-1}) in different vegetables collected from different locations (WHO safe limits for Zn= 60 mg kg^{-1})

TABLE 1. Heavy metals concentration of Cd, Cr, Pb, Cu, Ni, Mg and Zn (mL.L^{-1}) in water samples collected from different locations of KPK Pakistan

Locations/ Heavy metals	Cd	Cr	Pb	Cu	Ni	Mg	Zn
Swat	0.24 A	0.26C	0.35A	0.12AB	1.11A	0.40A	0.01A
Mansehra	0.01 B	0.39	0.14B	0.07AB	0.42B	0.33A	0.01A
Haripur	0.01 B	0.41B	0.90B	0.03B	1.20A	0.39A	0.00A
Peshawar	0.00 B	0.85A	0.33A	0.29A	0.40B	0.36A	0.01A
Nowshehra	0.01 B	0.08D	0.17B	0.07AB	0.63B	0.41A	0.01A
WHO safe limits	0.01	0.10	5.0	0.20	0.20	-	2.0

Means followed by different letter in a column are statistically significant at $p<0.05$

while minimum in soil samples taken from Haripur. Among the Cu means, maximum level was noted in soil samples at Swat whereas minimum in at Haripur. Ni concentration was highest soil samples collected from Peshawar followed by Mansehra and Nowshehra. Highest Mg accumulation was observed in soil samples from Nowshsehra and minimum at Mansehra. Zn maximum levels were maximum in soil samples from Nowshsehra and minimum at Haripur. The highest concentrations of different heavy metals are due to the maximum amount of heavy metals in water which were used for irrigation of these soils. Comparison of the heavy metal content found in these soil samples showed that concentration of Cd was above the permissible limits in all the locations except Swat and Ni uptake exceeded the safe limits only at Peshawar. The accumulation of Cr, Pb, Cu and Zn were below the safe limits given by WHO/FAO. Similar results were also reported by Adah et al. (2013) and Khan et al. (2013) who concluded that concentrations of Zn, Cu, Pb, Ni and Cd in soil were below the permitted values given by WHO. Doherty et al. (2012) reported that concentrations of toxic heavy metals (Zn, Cu, Pb, Cd) were below the safe limits in soil. Our results also agree with Khan et al. (2015).

The most commonly used techniques to analyze trace and heavy metals are inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma atomic emission spectrometry (ICP-AES), atomic absorption spectrometry (AAS) and X-ray fluorescence spectroscopy (XFS). However, ICP-MS, ICP-AES and XFS are usually very costly and their operation is not as simple and convenient as AAS. In the present investigation, a simple, more reliable, sensitive and convenient AAS method was employed for the quantitative estimation of heavy metals in different vegetables.

Protein profile of different vegetables cauliflower, radish, carrot, turnip, pea, spinach, coriander and garlic sampled across five different sites Swat, Haripur, Mansehra, Peshawar and Nowshehra of Khyber Pakhtunkhwa through SDS-PAGE and corresponding analysis of protein bands expressed on Gel Analyzer 2010 is shown in Tables 3-7. Our results showed that uptake of Cd, Cr, Pb, Cu, Ni, Mg and Zn by these vegetables caused the expression of numerous polypeptides. A 200 kDa polypeptide was expressed in radish and carrot at Mansehra and Haripur, in cauliflower, radish, carrot and turnip at Swat, in pea, spinach, coriander at Peshawar and in turnip at Nowshehra. A 130 kDa polypeptide was expressed in spinach at Mansehra and Haripur, in coriander and garlic at Peshawar and in coriander at Nowshehra (Tables 3, 5, 6 & 7). Polypeptide of 124 kDa was expressed in cauliflower, turnip and coriander at Mansehra, in cauliflower at Haripur, in all samples of Peshawar except cauliflower, in carrot, turnip coriander and garlic in Nowshera while there is no expression of 130 kDa polypeptide in Swat vegetables (Tables 3-7). A 108 kDa polypeptide is abundantly expressed in garlic and coriander at Swat while its expression is also observed in carrot, radish and pea in Mansehra, in turnip, pea and garlic at Haripur, in turnip, spinach and garlic at both Peshawar and Nowshsehra (Tables 3, 5, 6 & 7). Abundant expression of a 70 kDa

TABLE 2. Heavy metals concentration of Cd, Cr, Pb, Cu, Ni, Mg and Zn (mg.kg^{-1}) in different soil samples collected from different locations of KPK Pakistan

Locations/ Heavy metals	Cd	Cr	Pb	Cu	Ni	Mg	Zn
Swat	0.78 A	24.96B	19.11C	27.98A	40.88D	3.76BC	3.04C
Mansehra	1.50 A	42.83A	24.02B	14.65B	69.13B	2.90C	7.07B
Haripur	15.96A	8.03 C	5.03E	9.06C	32.39E	4.84AB	0.19E
Peshawar	1.70 A	42.82A	32.48A	24.81A	127.7A	5.70A	1.28D
Nowshehra	15.99A	43.76A	14.39D	17.80B	61.17C	5.15A	12.92A
WHO safe limits	3.0	150	300	140	75	-	300

TABLE 3. Protein profile of different vegetables collected from Mansehra KPK Pakistan

Location/ Vegetables	Bands (kDa) Newly expressed	Greater abundance	Lesser abundance	Absent	Present but undetected due to noise
Mansehra					
Cauliflower	--	--	--	200	--
Carrot	68	55	200	--	--
Radish	--	--	--	200	--
Turnip	43	25	--	200	--
Pea	--	--	--	200	--
Spinach	68	--	--	--	53
Coriander	68	--	130	200	--
Garlic	--		--	130, 124,108	66, 45, 25

TABLE 4. Protein profile of different vegetables collected from Swat KPK Pakistan

Location/ Vegetables	Bands (kDa)				Present but undetected due to noise
	Newly expressed	Greater abundance	Lesser abundance	Absent	
Swat					
Cauliflower	--	70	--	130	--
Carrot	43	70	--	130	--
Radish	--	70	--	130	
Turnip	43	--	--	200,130	--
Pea	--	--	--	200,130	--
Spinach					
Coriander	--	108	--	200,130	--
Garlic	--	108	124		--

TABLE 5. Protein profile of different vegetables collected from Haripur KPK Pakistan

Location/ Vegetables	Bands (kDa)				Present but undetected due to noise on gel
	Newly expressed	Greater abundance	Lesser abundance	Absent	
Haripur					
Cauliflower	--	--	124,66	200	--
Carrot	--	--	70,50	--	--
Radish	--	--	--	--	
Turnip	--	--	--	200	--
Pea	--	--	--	--	35
Spinach	--	--	--	--	--
Coriander	--	--	--	200	--
Garlic	--	--	--	--	35

TABLE 6. Protein profile of different vegetables collected from Peshawar KPK Pakistan

Location/ Vegetables	Bands (kDa)				Present but undetected due to noise on gel
	Newly expressed	Greater abundance	Lesser abundance	Absent	
Peshawar					
Cauliflower	--	--	--	--	--
Carrot	205	--	--	--	--
Radish	--	--	--	--	
Turnip	--	66,55,45,40,25	200	--	--
Pea	--	25	200	--	--
Spinach	--	--	--	--	--
Coriander	--	--	130	--	--
Garlic	--	--	--	--	55

TABLE 7. Protein profile of different vegetables collected from Nowshehra KPK Pakistan

Location/ Vegetables	Bands (kDa)				Present but undetected due to noise on gel
	Newly expressed	Greater abundance	Lesser abundance	Absent	
Nowshehra					
Cauliflower	--	--	130,108,55,50	200	--
Carrot	--	70	--	200	--
Radish	53	--	--	200	
Turnip	53	108,66,55,50,45, 35,25	--	--	--
Pea	53	--	--	200	--
Spinach	--	--	--	--	--
Coriander	--	108,66	--	200	--
Garlic	--	108,55,50	--	200	--

polypeptide is seen in cauliflower, radish and carrot at Swat location. A 68 kDa polypeptide was newly synthesized in carrot, spinach and coriander samples from Mansehra. A 205 kDa polypeptide is newly expressed in carrot sample at Peshawar. A 66 kDa polypeptide was expressed in all the samples of Swat but was expressed only in pea at Mansehra, in cauliflower, radish, spinach, coriander and garlic in Haripur and Peshawar and in radish, turnip and spinach at Nowshehra. Similarity, in the banding pattern of polypeptides was observed in turnip sample of Peshawar and Nowshehra, where 124, 108, 70, 66, 55, 45, 40, 45, 35 and 25 kDa polypeptides were densely expressed. A 53 kDa polypeptide was newly expressed in radish, turnip and pea sample at Nowshehra. The expression pattern of 45 and 35 kDa is observed in almost all the samples at all locations.

Conclusion

From these results it can be concluded that different vegetables analyzed contained toxic heavy metal content above the permissible limits of WHO which may cause deleterious effects to the human health. High levels of toxic heavy in different vegetables were the result of large quantities of these metals in water and soil samples collected from different locations where these vegetables were grown and irrigated. The presence of elevated levels of heavy metals in the soil and water was due to different human activities in these locations including application of sewage sludge, addition of manures, pesticides, fertilizers and discharge of wastewaters without proper treatment. Therefore, attention should be paid to proper treatment of these sources of heavy metals before they were released to the environment to avoid the bioaccumulation of toxic heavy metals by different crop spices including vegetables.

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Received: 10 October 2014
Accepted: 6 August 2015