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An investigation of toxic heavy metals (Pb, Cd, Cu, Cr and Zn) in Garlic *(Allium sativum* L.) and soil samples collected from different locations of Punjab, Pakistan using atomic absorption spectrometry

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Abstract: The present study is based on the determination of heavy metal contents (Pb, Cd, Cu, Cr and Zn) in garlic and soil samples collected from ten different locations of Punjab, Pakistan using flame atomic absorption spectrometer. In garlic samples, Pb, Cd, Zn, Cu and Cr ranged from 4.9 to 94.6 mg/kg, 0.625 to 151.4 mg/kg, 3.7 to 56.4 mg/kg, 2.5 to 50.2mg/kg and 56.4 to 111.6 mg/kg respectively, whereas Pb, Cd, Cr, Zn and Cu in investigated soil samples ranged respectively from 57.4 to 99.6 mg/kg, 25.6 to 132.7 mg/kg, 61.7 to 115.1 mg/kg, 9.2 to 324.7 mg/kg and 15.5 to 34.5 mg/kg. Elevated concentration of Pb and Cd was found in garlic samples from Gujranwala, Cr concentration was found to be higher in samples collected from Raiwind while the other metals such as Cu and Zn were predominant in samples from Kasur. Heavy metal content in soil and garlic samples was within the permissible limits proposed by World Health Organization (WHO) except Cd, Cr and Zn which showed elevated levels in almost all soil and garlic samples. Average concentrations determined in all samples represented that metal content in soil samples was in increasing order as Zn<Cu<Pb<Cd<Cr while in garlic samples, this order was as Cu<Cd<Zn<Pb<Cr. In the present study, it was observed that garlic samples from those areas presented relatively higher levels for investigated metals from where the soil samples also showed comparatively elevated levels of these metals.

Key words: Heavy metal contamination; *Allium sativum*; soil samples; atomic absorption spectroscopy.

Introduction

Research has shown that certain heavy metals such as Fe, Cu, Mn, Co, and Zn are nutritionally essential for a healthy life in very small quantity) but toxic effects of these metals increase in relatively higher concentrations, when they are not metabolized by the body and accumulate in the soft tissues[1]. Heavy metals rank high amongst the chief contaminants of leafy vegetables and medicinal plants[2] as heavy metals are non-biodegradable so they can cause serious health hazards in humans and animals.

The main source of metal accumulation in humans is the plants and vegetables consumed by them, grown in polluted areas. Uptake of trace elements by plants varies and depends largely on several factors, such as soil pH and organic matter content[3]. Metal deposition in environment is either due to the natural processes or as a result of human activities.

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Factors responsible for soil contamination are extensive use of chemical fertilizers and pesticides, mining processes, industrialization, vehicular exhausts and several others. Chronic exposure to Lead results in birth defects, mental retardation and potential toxic effects on fetus. Elevated levels of cadmium and chromium are responsible for lung, renal and cardiovascular diseases. Long term exposure to copper cause kidney damage, brain damage and even death. Zinc is an essential element and an integral component of many coenzymes, essential for DNA and RNA synthesis but toxic in higher concentrations[4].

Herbal products are being extensively used in Asian (especially in Pakistan and India), several African and Western Countries in the complementary and alternative system of medicines[5]. Garlic is an important ingredient of almost every meal and is widely used in Pakistan because of its antioxidant, antimicrobial, and antifungal activity. It is used either as a spice or remedy for various disorders.

Garlic is said to be a wonder spice because of its Phytonutrients, which are effective in the treatment of high blood pressure, coronary heart disease, diabetes type 2, reduces bad cholesterol, and prevents cancer[6]. So the main objective of this study was to determine the levels of selected toxic heavy metals (Pb, Cd, Cu, Cr and Zn) in garlic and soil samples from different regions of Pakistan as soil is one of the major factors responsible for contamination of plants and vegetables, which are extensively consumed in different forms. Furthermore, the monitoring of toxic metals such as Pb, Cd, Cr, Cu and Zn in garlic and the soil of garlic fields collected from ten different cities of Punjab is critical for preventing public health against the hazards of metal toxicity.

Results and discussion

Soil samples

All of the soil samples, collected from ten different cities of Punjab, Pakistan, were analyzed using Flame atomic absorption spectrometry (FAAS). The results revealed the concentration of Pb were ranged from 57.4 to 99.6 mg/kg, Cd ranged from 25.6 to 132.7 mg/kg and Cr ranged from 61.7 to 115.1 mg/kg in all the soil samples. Concentration of Zn in soil samples ranged from 9.2 to 324.7 mg/kg and that of Cu ranged from 15.5 to 34.5 mg/kg. Concentrations of Pb and Cu were within the permissible limits of WHO (Pb 300 mg/Kg, Cd 3 mg/Kg , Cr 100 mg/Kg, Cu 140 mg/Kg, Zn 300 mg/Kg) whereas concentrations of Cd, Cr and Zn were found higher than WHO permissible limits. The concentration of Cd was found relatively higher in almost all soil samples whereas that of Cr and Zn were slightly higher than WHO permissible limit. The presence of higher levels of Cd, Cr and Zn in soil samples can be attributed to contamination from urban sewage water and industrial effluent since some of the studies in literature [7-9] have already been explained that soil irrigated from urban and industrial effluent streams possesses comparatively higher metal contents as compared to that receiving fresh water.

Out of the ten soil samples, two obtained from Jaranwala and Kamuki showed relatively higher concentration of Pb and Cd. Relative higher concentration of Cu was observed in samples taken from Faisalabad, while high contents of Zn and Cr were observed in the samples received from Jaranwala and Raiwind cities, respectively. Average concentration of Lead in soil samples was 80.4 mg/kg while that of Cd, Cu and Zn were 65.8 mg/kg, 25 mg/kg, and 78 mg/kg respectively. Cr was present in all soil samples with an average of 85 mg/kg.

In all soil samples, Pb concentration showed little variations, (57.4 to 99.6 mg/kg), as all samples represented a comparable distribution except those from Rawalpindi and Gujranwala which have shown relatively lower levels of Pb.

While the Cr and Cd levels were with a wide range of 61.7 to 115.1 mg/kg and 25.6 to 132.7 mg/kg respectively. Cu and Zn showed range from 15.5 to 34.5 mg/kg and 9.2 to 324.7 mg/kg respectively.

Soil samples collected from controlled sites (Lahore and Kasur) showed metal concentrations which were below detection limits when treated according to the same digestion procedure as samples from contaminated sites.

Garlic samples

The entire ten rhizome samples were also analyzed for selected heavy metals using FAAS, gathered from the same fields from where the soil samples were collected. Results indicated that Pb and Cd were present in relatively higher concentration in samples taken from Gujranwala, the concentrations of Cu and Zn were higher in samples from Kasur while Cr concentration was found to be higher in samples from Raiwind as compared to the other samples.

In all the analyzed samples, Pb and Cd were found in appreciable amounts, and the Cd concentration exceeded maximum permissible limit for it in herbal spices. Pb content in all samples ranged between 4.9 to 94.6 mg/kg with high variation of individual results. The levels of Cd, Cu and Zn in all garlic samples varied between 0.625 to 151.4 mg/kg, 2.5 to 50.2 mg/kg and 3.7 to 56.4 mg/kg respectively.

The concentration of Cr was ranged between 56.4 and 111.6 mg/kg. Average concentration of investigated metals i.e., Pb, Cd, Cu, Zn and Cr in rhizome samples was found to be 48 mg/kg, 55 mg/kg, 39 mg/kg, 24 mg/kg and 80 mg/kg respectively. WHO has proposed maximum permissible limits (MPL) for some selected metals, such as for Pb, Cd, Cu and Zn as 10 ppm, 0.3 ppm, 20 ppm and 50 ppm, respectively. For chromium MPL is not specified [10].

Lead

Pb is known to be a non-essential metal as it is toxic even at trace levels. Soil analysis based on AAS has revealed that Pb contributed to about 24% of total metal content among all samples. In all garlic rhizomes its contribution was 19%.

Lead was found to be the second most abundant metal in the soil samples. 40% of garlic and 60% of soil samples presented lead concentration more than the average. The concentrations of Pb obtained in investigated soil and garlic samples have been shown in Figure 1(a).

Earlier reports on heavy metals in herbs and spices reveal that in present study, lead content was more than that found in garlic samples in Egypt[11] with the range 0.06 to 0.23mg/kg and more than that found in Turkey between 0.003 to 0.019 mg/L[12]. It was found to be between 0.14 to 1.03mg/kg in garlic available in Polish market [13].

Studies done on other spices include lead detection in ginger rhizomes and found values were in the range of 0.06 to 0.64mg/L[10], and in branded Pakistani herbal products to 70.1mg/kg [14].

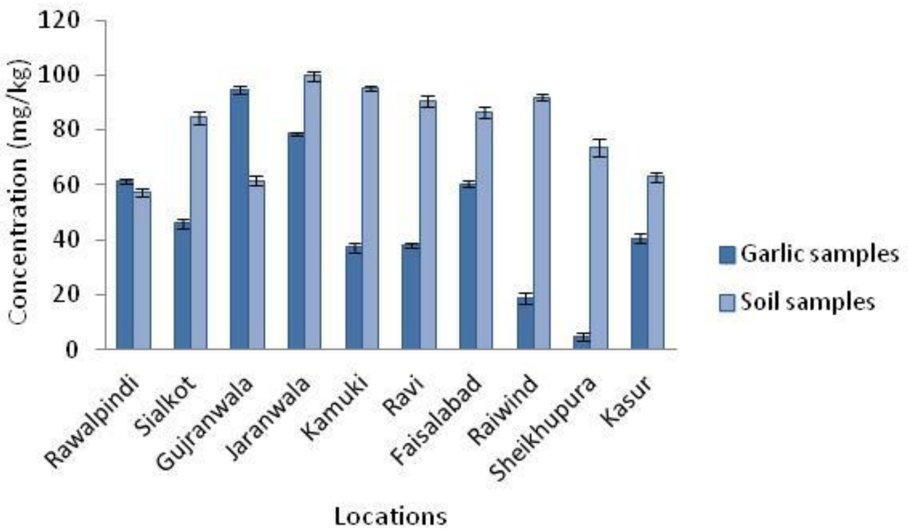

Figure 1(a). Levels of Lead (mg/kg) in garlic and soil samples collected from different locations of Punjab.

Cadmium

It is one of the toxic heavy metal and become hazardous at elevated levels. Cd was detected in the range of 25.6 to 132.7 mg/kg in soil and 0.625 to 151.4 mg/kg in rhizomes. Cd detected level was 22% in garlic and 20% in soil samples among all other metals.

However overall contribution of Cd in both soil and garlic samples was 21% of total metal content. The concentrations of Cd in analyzed soil and garlic samples have shown in Figure 1 (b).

Literature review describes previous studies made on medicinal plants that Cd levels originated in this study were appreciably greater than those found in garlic collected from Polish market [13]. Cd was below detection limit in Turkey [12], and in the range of 0.019 to 0.05mg/kg in Egypt[14].

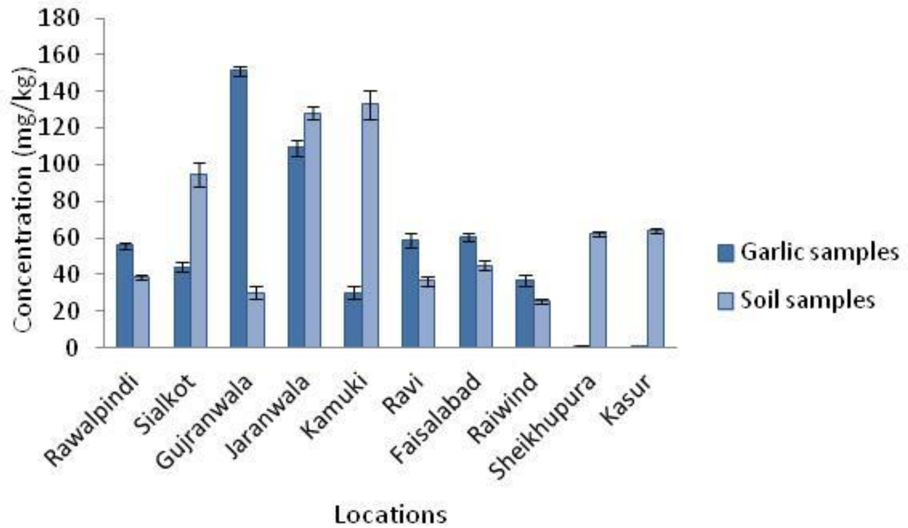

Figure 1(b). Levels of cadmium (mg/kg) in garlic and soil samples collected from different locations of Punjab.

Copper

Cu is one of the essential metals, an important constituent of many enzymes and plays a vital role in oxidative defense system [13]. Cu input resultant of soil analysis was 8% and that of garlic analysis was 16%.

The concentrations of Cu in different investigated soil and garlic samples have been shown in Figure 1(c).

Earlier detection of Cu levels in garlic, were varying between 1 to 2.89mg/kg in Egypt [15], 0.30 to 1.21mg/kg in Turkey [13], 2.23 to 10.76mg.kg in polish market [14]. Pakistani herbal products revealed its level among 1.7 to 26.9mg/kg [16] and in Ginger in the range of 0.002 to 0.03mg/L [15].

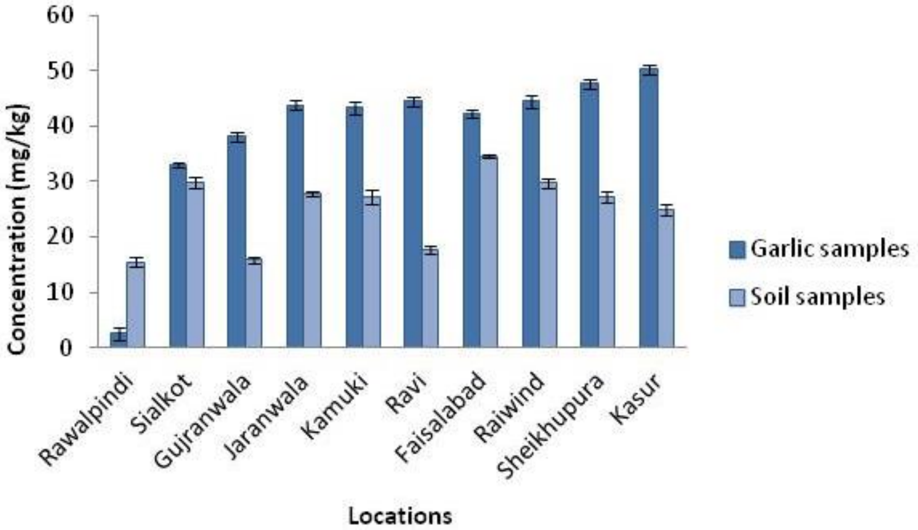

Figure 1(c). Levels of copper (mg/kg) in garlic and soil samples collected from different locations of Punjab.

Chromium

In our present study, Cr content was determined as 33% of all metals in garlic and 25% in soil samples. The concentrations of Cr present in various analyzed soil and garlic samples has been shown in Figure 1 (d).

Previous research on Cr levels in various herbs, spices and herbal products discussed its levels ranged from 55.8 to 131.5mg/kg in herbal products[15] and 14.05 to 18.31 in *Datura stramonium* and *Amaranthus spinosus* [17].

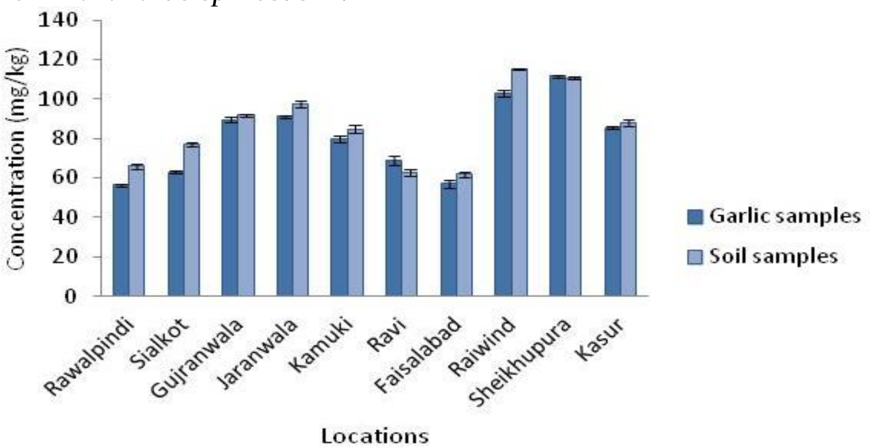

Figure 1(d). Levels of chromium (mg/kg) in garlic and soil samples collected from different locations of Punjab.

Zinc

Zinc acts as a co-factor of approximately 200 enzymes required in metabolism reactions but its higher levels effect the normal copper metabolism [13]. Toxic metal content found to be consisting of 10% of Zn in garlic and 23% in soil samples.

The obtained concentrations of Zn in investigated soil and garlic samples have depicted in Figure 1 (e). According to previous reported data, Zn levels were found to be varied from 12.67 to 18 mg/kg in garlic [16] and 6.43 to 33.44mg/kg in Polish market [13], whereas appreciably higher concentrations of Zn has been detected in herbal products [15].

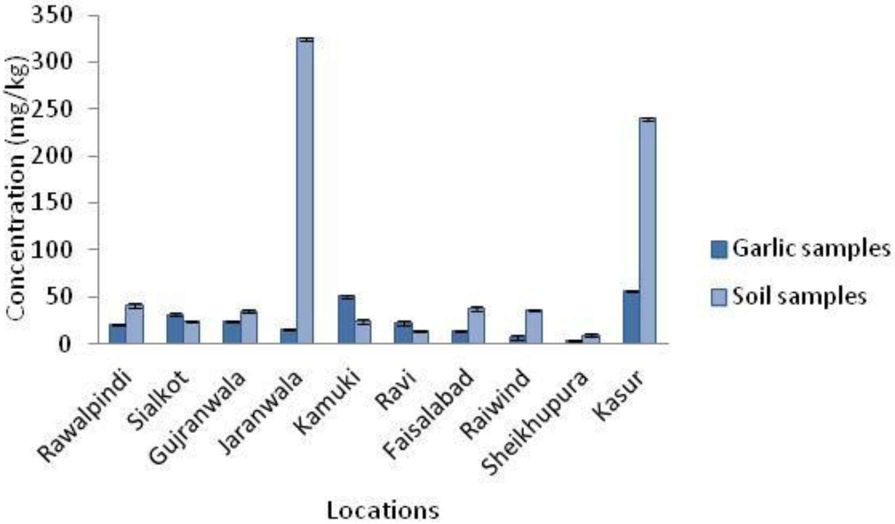

Figure 1(e). Levels of Zinc (mg/kg) in garlic and soil samples collected from different locations of Punjab.

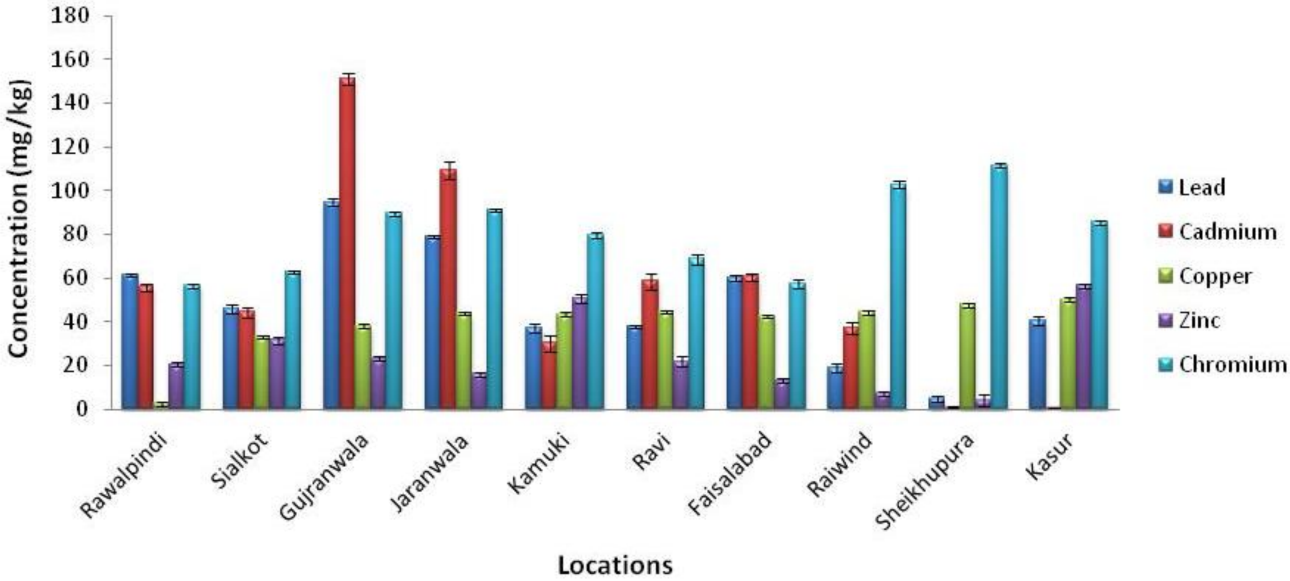

Figure 2(a). Comparison of concentrations of heavy metals in Soil samples collected from different locations of Punjab.

A comparison of concentration of lead, cadmium, copper, chromium and zinc in garlic and soil samples has been shown in Figure 2 (a) and Figure 2 (b). Highest concentration of zinc was found to be present in soil samples procured from Jaranwala and Kasur while highest levels of Cd were found to be present in garlic samples procured from Gujranwala city.

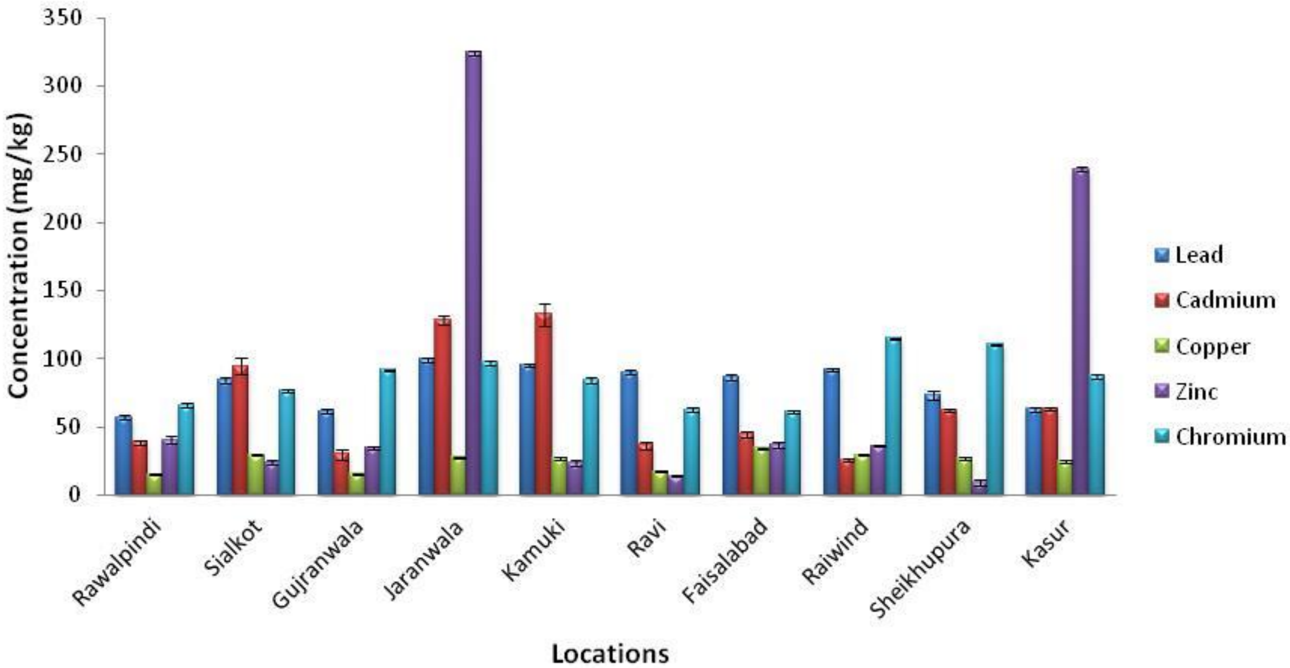

Figure 2(b). Comparison of concentration of heavy metals in garlic samples collected from different locations of Punjab.

Furthermore, the % contribution of selected metals in soil and garlic samples has been shown in Figure 3 (a and b). It can be seen in Figure 3 (a) that Cr contributes highest (33%) to the garlic samples whereas Zn contributes lowest (10%). On the other hand, the Cr, Pb and Zn contributes comparably i.e., 25%, 24% and 23 % respectively whereas Cu contributes only 8% to the soil samples (Fig 3 b).

Figures 3 (a & b) % Concentration of total heavy metal load in Garlic and soil samples collected from Punjab.

Statistical Analysis

Analysis of heavy metal content variability in garlic and soil samples, collected from ten different cities of Punjab, was done by applying one-way ANOVA. Extent of contamination by heavy metals in samples was determined using descriptive statistics.

All the analysis was performed on SPSS 18.0 for Windows and statistical significance level was $P<0.05$ for the analysis. Results of the descriptive analysis for garlic and soil samples are represented in Table 1 and 2.

Analysis of garlic and soil samples showed that garlic samples contained relatively higher levels of these toxic heavy metals as compared to soil samples which may be due to the reason that in addition to soil, some other factors like irrigation of land with industrial and sewage effluents, impact of automobile and industrial emissions, fertilizers etc. which cause the metals to be deposited in the soil selected for cultivation [7-9].

The poisoning associated with the presence of toxic metals in medicinal plants has also been reported earlier in Asia, Europe and the United States.

All of the results obtained in this work were found to be prominently high for lead and cadmium than those previously observed in ginger [15]. Gupta's research work [15] was limited to the only ginger samples, collected from different locations but in the present study, a great focus have also been made on analysis of soil samples for toxic metals, collected from

Table 1. Statistics of heavy metal content in Garlic samples collected from different locations of Punjab and evaluation of contamination extent (n=10).

Metals	Lead	Cadmium	Copper	Zinc	Chromium
Minimum	0.04	0.01	0.02	0.03	0.45
Maximum	0.76	1.21	0.40	0.45	0.89
Mean	0.38	0.40	0.31	0.19	0.64
Range	0.72	1.21	0.38	0.42	0.44
Std.Deviation	0.21	0.27	0.11	0.14	0.15
Variance	0.046	0.072	0.012	0.019	0.023
Skewness	0.191	0.837	-2.508	0.934	0.187
Kurtosis	-0.121	0.947	6.891	0.045	-1.082

Table 2. Statistics of heavy metal content in Soil samples collected from different locations of Punjab and evaluation of Contamination extent (n=10).

Metals	Lead	Cadmium	Copper	Zinc	Chromium
Minimum	0.46	0.02	0.12	0.07	0.49
Maximum	0.80	1.06	0.28	2.60	0.92
Mean	0.64	0.42	0.20	0.63	0.68
Range	0.34	1.04	0.15	2.52	0.43
Std. Deviation	0.12	0.26	0.05	0.88	0.15
Variance	0.015	0.067	0.003	0.769	0.023
Skewness	-0.427	0.750	-0.481	1.886	0.214
Kurtosis	-1.505	0.160	-1.021	2.333	-1.072

Different cities of Punjab, Pakistan which were chosen for garlic collection. So continuous monitoring is required to ensure the lower levels of toxic metals in medicinal plants and vegetables.

Levels of these metals exceed in plants when they are grown in contaminated soil. Contamination results from the use of sewage water in agriculture, smelting and mining processes and false agricultural practices, use of chemical based fertilizers and rapid industrialization etc. [14,16].

Very low concentrations of heavy metals are essential for healthy life. Exceeding concentrations are dangerous because their elevated levels interfere with the proper functioning of the body. So, monitoring of heavy metals in herbal plants and medicines is required to avoid their toxic effects.

Conclusion

Most of the world population utilizes medicinal plants in more than one way but quality monitoring of plants is still in the prelude stage. The study has illustrated that some soil and garlic samples contained high levels of heavy metals although they were within the safe limits except that of Cd which exceeded safe levels in all samples.

This is the first report to access the toxic heavy metals in the garlic, collected from different locations of Punjab, Pakistan. Furthermore, the current study not only compels regulatory and monitoring authorities to think about safety assessment of the finished and raw herbal products and take positive steps in this regard, but also guides farmers in sensible selection of more suitable plant type and site of farming.

Experimental Section

Study area

Punjab is an agriculturally important Province of Pakistan due to its fertile and rich soil. Ten cities of Punjab, Pakistan were selected for the collection of soil and garlic rhizome samples. Selected cities are supposed to be relatively more contaminated by a number of pollutants as a consequence of increasing number of industrial setups, emissions from vehicles and modern agricultural technology, irrigation water and many more other reasons.

These cities are Rawalpindi, Sialkot, Gujranwala, Jaranwala, Kamuki, and areas near the river Ravi, Faisalabad, Raiwind, Sheikhupura and Kasur.

Rawalpindi is a part of Northern Punjab and is located 33°-34° North and 73°-73.7° East. This region is an arid area and no proper irrigation system is yet established which may contribute to the contamination of the environment with the dangerous pollutants like heavy metals.

Sialkot (32°-33°N and 74°-75°E) is known for a large setup of industries which produce sports and surgery articles, Gujranwala (31.8°-32.6°N and 73.7°-74.5°E) is also an industrial city which is famous for manufacturing plastic, metal and house hold things, Kamuki (32°N and 74.2°E) is a town in Gujranwala and is famous for rice production and processing industries, Sheikhupura (31.4°-32°N and 73.6°-74.7°E) is an agricultural area, produces a large number of crops especially cereal and wheat etc., Raiwind and Kasur (30.6°-31.4°N and 73.3°-74.6°E) are located nearby Lahore and they bear fertile and rich soil land in Punjab.

These cities are famous for maize and cereal cultivation etc. and different industries are also working in these areas. All the above-mentioned cities lie in the North, Eastern Punjab.

Faisalabad (30.5°-31.8°N and 72.7°-73.7°E) is known as Manchester of Pakistan because of a large network of textile industries. It is also famous for cotton and sugarcane industry.

Jaranwala (31.3°N and 73.5°E) has the same land and industrial value as Faisalabad. Both these cities are located in Central Punjab.

Therefore, these cities were selected for the collection of garlic and soil samples because of their agricultural importance and at the same time their industrial importance, to evaluate the risk to the people of these areas by the consumption of agricultural products cultivated near the industrial areas.

Controlled samples

Two garlic rhizomes and two soil samples were also collected from the controlled sites of Lahore and Kasur which are opposite to the contaminated or polluted sites in respect to their metal content. Garlic bulbs were simply taken by hand and kept stored in polythene bags until analyzed for the presence of metals.

Collection of soil and garlic Samples

Random soil and garlic samples were taken from the agricultural fields at ten different locations of province Punjab. Each soil sample was collected from five different regions of a selected field consisting of upper layer (05-10cm) and lower layer (15-20cm). All five soil samples were mixed together to constitute a representative sample of a single site. Similarly, five garlic specimens were collected from different parts of a selected field and these five garlic samples were combined together in order to constitute a representative sample of selected site.

Preparation of soil and garlic samples

Collected soil samples were dried, ground to get rid of large soil particles and sieved through 0.3mm diameter sieve to get fine powder by sorting out any kind of large and hard materials such as stones which are not easy to digest. All the collected garlic samples were first washed with tap water and then with the double distilled water in order to remove superficial impurities such as soil salts. Rhizomes of all samples were dried in oven at 70°C for 48 hours. Dried samples were then ground into fine powder, passed through a sieve of 0.3mm diameter and stored in air tight jars until used for analysis.

Analytical strategy for soil and garlic samples

Prior to analysis all the glassware was rinsed first with 10% HNO_3 solution and then washed with double distilled water for quality control purpose. Acid digestion of samples was carried out. The digestion procedure selected for sample decomposition was employed using two different digestion mixtures, one consisting of H_2SO_4/H_2O_2 and the other HNO_3/H_2O_2. About 0.2 g of each sample was weighed in 25 ml conical flask. Samples were digested overnight by making use of 5ml concentrated H_2SO_4 or HNO_3. Solution was then heated at 128°C until the evolution of brown fumes ceased. 2 ml of H_2O_2 were added in the solution and heated at 125°C for one hour with the evolution of intense white fumes. Again 2 ml H_2O_2 was added and solution was then diluted with 0.2 N HNO_3 solution.

AAS analysis was carried out on a Perkin Elmer (Analyst 800) equipped with hollow cathode lamp for investigated elements.

Blank digestions were also carried out in the same way as original samples and all samples were analyzed in triplicates. Instrumental parameters employed in present study are given in Table 3.

Table 3. Instrumental parameters for Atomic Absorption Spectrometer for the analysis of heavy metals in garlic and soil samples.

Metals	Flame gas mix.	Flame type	Wavelength (nm)	Slit width (nm)	Cathode lamp Current (mA)	Acetylene Flow (L/min)	Air Oxide flow(L/min)
Pb	Air-Acetylene	Oxidizing	283.3	0.7	15	2.0	17
Cd	Air-Acetylene	Oxidizing	324.8	0.7	15	2.0	17
Cu	Air-Acetylene	Oxidizing	324.5	0.7	15	2.0	17
Cr	Air-Acetylene	Reducing	357.9	0.7	15	2.5	17
Zn	Air-Acetylene	Oxidizing	213.9	0.7	15	2.0	17

Preparation of working standards

All used reagents were of analytical grade. All working standard solutions of selected metals were prepared by dilution of multielement standard solution (1000 ppm) procured from Merck using 0.2N HNO_3.

Data Validation

Prior to the determination of trace metals in real samples, results of both digestion mixtures were compared with each other. The accuracy of each mixture was evaluated by the percentage recovery of the standard heavy metals added to the soil and garlic samples before carrying out the digestion.

The recoveries in garlic obtained by employing H_2SO_4/H_2O_2 (Mixture A) for digestion were 94% for Pb, 94% for Cd, 96% for Cu, 95% for Cr and 91% for Zn while those observed in soil samples were 91% for Pb, 94% for Cd, 93% for Cu, and 91% for Cr and Zn.

Recoveries observed with HNO_3/H_2O_2 (Mixture B) were 84% Pb, 82% Cd, 81% Cu, 85% Cr and 82% Zn in rhizomes while those obtained for soil samples were 87% for both Pb and Cu, 85% for Cd and Zn and 88% for Cr.

Recoveries obtained from first mixture were appreciably greater than those from second one so the mixture A was applied to all real soil and garlic samples (Table 4).

Table 4. Recovery and % RSD for determination of heavy metals in garlic and soil samples using digestion mixture A and B.

	Mixture A (H_2SO_4/H_2O_2)				Mixture B (HNO_3/H_2O_2)			
Metal	**Garlic**		**Soil**		**Garlic**		**Soil**	
	%Recovery	%RSD	%Recovery	%RSD	% Recovery	%RSD	% Recovery	%RSD
Pb	94	3.383	91	1.787	84	1.598	87	0.894
Cd	94	0.828	94	0.676	82	3.967	85	1.33
Cu	96	1.768	93	0.075	81	2.356	87	0.569
Cr	95	0.669	91	0.077	85	0.415	88	0.079
Zn	91	0.785	91	0.466	82	1.724	85	0.998

Repeatability of the analysis was expressed in terms of SD and % RSD. Limit of detection (LOD) of instrument for Pb, Cd, Cu, Cr and Zn was, 0.01, 0.001, 0.002, 0.003 and 0.002mg/L respectively.

Detection limits for the investigated metals analyzed by AAS were found to be as 0.003mg/L (0.375mg/kg) (Pb), 0.001mg/L (0.125 mg/kg) for (Cd), 0.019mg/L (2.375 mg/kg) (Cu), 0.019mg/L (2.375 mg/kg) for (Zn) and 1.21mg/L (15.12 mg/kg) (Cr).

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