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Trace Metals in Vegetables and Cereals- A Case Study of Indian Market-2016

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Background & Aims of the Study: Vegetables and Cereals are considered vital for properly-balanced diet given that they deliver vitamins, minerals, nutritional fiber, and phytochemicals. This study aimed to assess the concentration of As, Cu, Cd, Pb, Cr and Hg in common vegetables and cereals in urban open markets in Varanasi district, India

Materials & Methods: Total 260 edible portions of vegetable samples of 13 species were collected in March to October, 2016 from predefined market sites. These samples classified into roots, stems, leafy vegetables, fruits, and legumes. These samples (unwashed, acetic acid washed and boiled) were assessed using atomic absorption spectrophotometer. The statistical evaluations were carried out using the IBM SPSS 21.

Results: The results obtained reveal that unwashed vegetables and cereals as compared to washed and boiled samples contain higher trace metal concentration. The order of heavy metal concentration was observed in Cu>Pb>Cd>As in vegetable and cereals samples. Hg and Cr were not detected in any samples. The mean value of Cu, Cd and Pb in unwashed and washed vegetables and cereals were lower than PFA standard except As, whereas in boiled vegetables and cereals are lower than PFA standard but the mean value of Cd and Pb were many folds higher than the EU standard at all the market site samples. Leafy vegetables were found to contain the highest metals values especially Spinacia oleracea followed by roots vegetable like Brassica rapa, at all the studied sites. The market sites MS3 located in the vicinity of industrial zone and in proximity to national highway showed elevated levels of trace metals concentration in the vegetables and cereals as compared to other market sites.

Conclusions: The results showed that, the As, Cu, Cd and Pb concentration were reduced to about 12.5%, 5.87%, 11.36% and 10.42% of the initial concentrations by 2% acetic acid washing and to 25%, 21.87%, 20.45% and 16.67% of the initial concentrations by washing followed by boiling. The boiled vegetables and cereals may reduce the risk of trace metal intake from the vegetables and cereals significantly.

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Background

In 21st Century trace metals contamination in vegetables and cereals is a serious growing concern due to their accumulation, persistence and toxicity in nature (1-3). Vegetables and cereals are rich sources of carbohydrates, proteins, vitamins, minerals as well as trace elements and fibers and also have beneficial antioxidative effects (4-6). Eating of polluted vegetables and cereals may encounter human health. One item that should be considered for quality of food is heavy metal contamination. (7, 8). Sudden industrialization is related to increasing of heavy metals in developing countries such as Egypt (9), Iran (10), China (11) and India (12, 13).

Trace metals may be absorbed into the plants tissues from deposits on the surfaces exposed to the air from polluted environments as well as from contaminated soils (14, 15). A number of studies reveal that trace metals are an important fast growing contaminants in the vegetables and cereals (16-19). Trace metal contamination of vegetables and cereals may be due to contaminated water irrigation, pesticides exposures as well as industries and vehicles emissions during their production, transport and marketing (14, 20-23).

The contamination of vegetables and cereals with trace metals due to soil and atmospheric contamination poses a threat to its quality and safety (3, 24, 25). Dietary intake of trace metals also possesses risk to both animals and human health. Long-term consumption of unhealthy foods, which are polluted with heavy metals, may threat human health and eventually leads to cardiovascular, nervous, kidney and bone diseases.(13, 26, 27).

Presence of some trace metals As, Cd, Cr, Hg and Pb have been shown to have carcinogenic effects (28, 29) and associated with the development of abnormalities in children (30-32). High concentrations of trace metals like Cu, Cd and Pb were related to high prevalence of upper gastrointestinal cancer (33). Long term intake of Cd causes severe diseases such as renal, prostate and ovarian cancers (34, 35), tubular growth, excessive salivation, gastrointestinal irritation, kidney damage, diarrhea and vomiting (36, 37). Lead is sequestered in the bones and teeth, affect nervous bone, liver, weakness in the wrist and figure, Pancreas, and gum and also causes blood diseases (36, 37). Among these heavy metals when Cu exceed its safe value concentration cause hepatic and kidney damage, haemolytic anemia and methanoglobinemia (38).

The absorption of trace metals in vegetables and cereals are mainly influenced by some factors such as climate, atmospheric depositions, the concentrations of heavy metals in soil, the nature of soil on which the vegetables and cereals are grown and the degree of maturity of the plants at the time of harvest (5, 39-41). Air pollution may pose a threat to post-harvest vegetables and cereals during transportation and marketing, causing elevated levels of heavy metals in vegetables and cereals (42).

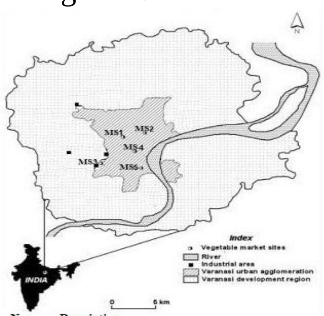
Tracing heavy metals which are exist in vegetables and cereals of markets have been carried out in many developed (43, 44), and developing countries (9, 20, 45), but there are few available data about heavy metals concentrations in the vegetables and cereals from the market sites of India (12, 21, 42, 46).

Aims of the study: The key objective of the present study is to focus on monitoring contamination of trace metals in different vegetables and cereals grown locally in suburban and rural areas and sold in urban open markets and compare the observed concentrations of As, Cu, Cd and Pb in the vegetables with Prevention of Food adulteration (PFA) act (0.2, 30, 1.5 and 2.5 µg/g, respectively; (47) and European Union (EU) (0.1 and 0.3 µg/g, for Cd and Pb respectively) standards of food contamination.

Materials & Methods

Study area and sampling locations

The present study was carried out in March to October, 2016, from Varanasi, India, where the urban latitudes range from 82° 56′ to 83° 03′ E, and the longitudes from 25° 14′ to 25° 23.5′ N. A vigorous survey was conducted to identify locally grown vegetables and cereals and their marketing area in the city. Supply of vegetables and cereals from their production sites, population density, traffic load, residential as well as commercial zone and industrial activities were also considered for selection of sampling sites. Five market sites were identified and selected which provided from specific farmlands around market for collection of samples. The four market sites including Chanduasatti (MS1), Kabirchaura (MS2), Rathyatra (MS4) and Lanka (MS5) are located in a zone dominated by heavy traffic on a narrow road with dense population. The market site Chandpur (MS3) is located in an industrial area having a large number of small-scale industries and heavy traffic on a narrow road. The market sites and their relative positions are shown in Figure 1.



Sites Name	No.	Description
Chanduasatti	MS1	located in a zone dominated by heavy traffic on a narrow road and dense population
Kabirchaura	MS2	located in a zone dominated by heavy traffic on a narrow road and dense population
Chandpur	MS3	Located in an industrial area having a large number of small-scale industries and heavy traffic on a narrow road.
Rathyatra	MS4	located in a zone dominated by heavy traffic on a narrow road and dense population
Lanka	MS5	located in a zone dominated by heavy traffic on a narrow road and dense population

Figure 1) Map of Varanasi showing the relative positions and their description of included market sites.

Collection of samples

Total 260 edible portions of vegetable samples of 13 species were collected in March to October, 2016 from predefined market sites. One Kilogram of vegetables was collected for each sample (48). These samples were classified into roots (Brassica rapa, Daucus carota), stems (Allium cepa, Solanum tuberosum), leafy vegetables (Spinacia oleracea, Brassica oleracea), fruits (Cucumis sativus, Solanum lycopersicum, Lagenaria siceraria), Legumes (Phaseolus vulgaris, Vigna unguiculata) and cereals (Triticum aestivum, Oryza sativa) are shown in table 1. All the samples were stored at 4 °C and immediately carried back to a laboratory for analysis (25).

Preparation and treatment of samples

Composite samples of edible portion of vegetables and cereals collected from the market site were prepared for each species. The 300 grams' composite sample was taken and were separated in three parts, 100g each, that is unwashed, washed (washed with tap water, then soaked in 2% acetic acid solution for half an hour and then washed with distilled water) and boiled (1000C for half an hour). Then these three parts were cut to small pieces using clean knife and kept in air-dried condition for approximately 72 hours. After drying the samples were grinded into a fine powder using a commercial blender. Finally, the samples were taken to the small airtight polythene bags and then kept to the refrigerator for further analysis (49).

Acid digestion of vegetable samples for metals determination

Tri-acid mixture (15 ml, 70% high purity HNO_3, 65% $HClO_4$ and 70% H_2SO_4; 5:1:1) was added to the 100 ml beaker separately containing 1.00±0.001 g dry vegetable and cereals sample (4, 5). The mixture was then digested at 80 0C till the transparent solution was achieved (50). After cooling, the digested samples were filtered using Whatman No. 42 filter paper. The resulting filtrate was diluted to 50 ml with double distilled water and was analysed for concentrations of As, Cu, Cd, Pb, Hg and Cr using an atomic absorption spectrophotometer (AAS, Model 2380, Perkin–Elmer, Norwalk, CT, USA). The values of the detection limits for As, Cu, Cd, Pb, Hg and Cr were 0.001, 0.001, 0.005, 0.01, 0.005 and 0.001 µg/ml, respectively.

Experimental procedure for metals determination

The working standard solutions were prepared by diluting a stock solution (1000 ppm, AAS grade standard) with ultra-pure water for heavy metal determination. These solutions were frequently run to construct the calibration curves with the help of AAS. Quality assurance measures included the calculation of method detection limit, inclusion of recovery and analysis of standard reference material. Appropriate drift blank was also taken before the analysis and necessary correlation was made during the calculation of concentration of different elements (49). Hydride Generation technique in Atomic Absorption Spectrometer was used to determine the concentration of As and Hg in the samples.

Statistical analysis

The statistical evaluations (minimum, maximum and mean) were carried out using the computer software, SPSS package (IBM SPSS statistics 21).

Results

Trace metals analysis revealed that the samples contained As, Cu, Cd, and Pb. The descriptive statistics of As, Cu, Cd, and Pb in different vegetables and cereals collected from market of Varanasi district in Uttar Pradesh, India are as given in Tables 2 (unwashed samples), Table 3 (washed samples) and Table 4 (boiled samples). A comparative trace metal (As, Cu, Cd and Pb) concentration in unwashed, washed and boiled samples in the test vegetables and Cereals collected from different market sites of Varanasi are as given in figure 2 to 5. In the present study Hg and Cr were not detected in any of the samples. The concentrations in these samples varied significantly such as As (0.13-0.33, 0.12-0.32 and 0.1-.26 µg/g in unwashed, washed and boiled samples respectively), Cu (1.27-12.32, 1.20-12.2 and 1.12-10.64 µg/g in unwashed, washed and boiled samples respectively), Cd (0.16-1.46, 0.14-1.4 and 0.12-1.28 µg/g in unwashed, washed and boiled samples respectively) and Pb (0.16-1.62, 0.14-1.60 and 0.11-1.53 µg/g in unwashed, washed and boiled samples respectively).

Table 1) List of common vegetables and Cereals included in the present Study

	scientific name	English Name	Common name
Roots			
	Brassica rapa	Turnip	Salgam
	Daucus carota	Carrot	Gajar
Stems			
	Allium cepa	Onion	Pyaaj
	Solanum tuberosum	Potato	Aloo
Leafy			
	Spinacia oleracea	Spinach	Palak
	Brassica oleracea	Cabbage	Patta Gobhi
Fruits			
	Cucumis sativus	Cucumber	Kheera
	Solanum lycopersicum	Tomato	Tamatar
	Lagenaria siceraria	Bottle Gourd	Lauki
Cereals			
	Triticum aestivum	Wheat	Gehu
	Oryza sativa	Rice	Rice
Legume			
	Phaseolus vulgaris	Beans pod	Sem Fali
	Vigna unguiculata	Cowpea	Lobia

Table 2) Trace metals concentrations (µg/g dry weight) of unwashed common vegetables and Cereals in Varanasi district during 2016

Sample	As			Cu			Cd			Pb		
	Mean	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**
Brassica rapa	0.28	0.22	0.32	4.05	3.22	4.62	0.71	0.62	0.83	0.49	0.41	0.66
Daucus carota	0.26	0.18	0.33	3.10	2.4	3.8	0.37	0.27	0.52	0.39	0.19	0.62
Allium cepa	0.25	0.21	0.31	1.53	1.4	1.8	0.50	0.24	0.72	0.23	0.18	0.27
Solanum tuberosum	0.21	0.17	0.26	1.41	1.27	1.53	0.31	0.17	0.44	0.43	0.35	0.51
Spinacia oleracea	0.26	0.19	0.31	9.00	7.42	12.32	1.01	0.68	1.46	1.16	0.86	1.62
Brassica oleracea	0.24	0.18	0.27	4.44	3.52	6.52	0.28	0.23	0.42	0.72	0.68	0.76
Cucumis sativus	0.24	0.19	0.27	3.60	2.98	4.72	0.27	0.19	0.36	0.34	0.24	0.42
Solanum lycopersicum	0.27	0.16	0.33	3.76	2.38	5.96	0.37	0.25	0.46	0.59	0.46	0.72
Lagenaria siceraria	0.22	0.18	0.26	4.59	3.92	5.32	0.46	0.39	0.58	0.39	0.24	0.76
Triticum aestivum	0.25	0.21	0.29	2.62	2.18	3.34	0.39	0.16	0.64	0.38	0.28	0.49
Oryza sativa	0.25	0.19	0.32	2.87	2.38	3.52	0.26	0.18	0.33	0.41	0.28	0.61
Phaseolus vulgaris	0.24	0.13	0.29	3.97	3.34	4.47	0.40	0.32	0.48	0.27	0.16	0.42
Vigna unguiculata	0.20	0.15	0.23	3.85	2.76	5.32	0.37	0.26	0.48	0.42	0.29	0.53

*Hg and Cr were not detected in any samples and market sites included in present study

Table 3) Trace metals concentrations (µg/g dry weight) of washed common vegetables and Cereals in Varanasi district during 2016

Sample	As			Cu			Cd			Pb		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Brassica rapa	0.23	0.16	0.28	3.25	2.46	3.8	0.65	0.58	0.71	0.44	0.36	0.61
Daucus carota	0.22	0.16	0.28	2.5	1.8	3.2	0.30	0.21	0.42	0.34	0.14	0.58
Allium cepa	0.21	0.16	0.26	1.33	1.2	1.6	0.44	0.23	0.62	0.19	0.14	0.24
Solanum tuberosum	0.19	0.16	0.23	1.34	1.2	1.46	0.26	0.14	0.4	0.37	0.32	0.46
Spinacia oleracea	0.23	0.16	0.28	8.74	7.3	12.2	0.94	0.6	1.4	1.08	0.8	1.6
Brassica oleracea	0.21	0.16	0.24	4.32	3.4	6.4	0.25	0.2	0.36	0.68	0.64	0.72
Cucumis sativus	0.2	0.16	0.23	3.46	2.83	4.6	0.23	0.16	0.32	0.29	0.21	0.36
Solanum lycopersicum	0.24	0.14	0.32	3.66	2.3	5.8	0.33	0.22	0.43	0.54	0.43	0.68
Lagenaria siceraria	0.2	0.16	0.23	4.44	3.82	5.2	0.42	0.36	0.52	0.36	0.2	0.73
Triticum aestivum	0.2	0.16	0.23	2.53	2.1	3.2	0.35	0.14	0.6	0.34	0.26	0.46
Oryza sativa	0.21	0.16	0.28	2.78	2.3	3.4	0.21	0.14	0.28	0.34	0.2	0.52
Phaseolus vulgaris	0.21	0.12	0.26	3.84	3.26	4.36	0.33	0.26	0.4	0.23	0.14	0.36
Vigna unguiculata	0.18	0.12	0.22	3.75	2.68	5.2	0.32	0.22	0.43	0.36	0.22	0.46

*Hg and Cr were not detected in any samples and market sites included in present study

Table 4) Trace metals concentrations (µg/g dry weight) of boiled common vegetables and Cereals in Varanasi district during 2016

Sample	As			Cu			Cd			Pb		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Brassica rapa	0.18	0.12	0.23	2.91	2.23	3.3	0.59	0.52	0.66	0.39	0.28	0.56
Daucus carota	0.19	0.13	0.24	2.22	1.72	2.82	0.26	0.18	0.39	0.30	0.12	0.52
Allium cepa	0.17	0.13	0.21	1.24	1.12	1.48	0.4	0.18	0.58	0.29	0.11	0.8
Solanum tuberosum	0.16	0.12	0.19	1.27	1.16	1.38	0.22	0.12	0.36	0.33	0.28	0.38
Spinacia oleracea	0.2	0.14	0.24	6.91	5.2	10.64	0.87	0.56	1.28	1.03	0.74	1.53
Brassica oleracea	0.18	0.14	0.21	3.32	2.68	4.81	0.22	0.17	0.3	0.62	0.56	0.68
Cucumis sativus	0.16	0.14	0.18	2.89	2.12	3.8	0.20	0.14	0.28	0.25	0.18	0.32
Solanum lycopersicum	0.20	0.12	0.26	2.96	1.88	4.56	0.3	0.18	0.38	0.49	0.38	0.62
Lagenaria siceraria	0.18	0.14	0.21	3.58	2.72	4.12	0.38	0.32	0.48	0.33	0.18	0.68
Triticum aestivum	0.18	0.14	0.21	2.10	1.64	2.64	0.30	0.12	0.52	0.30	0.22	0.38
Oryza sativa	0.19	0.14	0.24	2.29	1.84	2.76	0.19	0.12	0.25	0.30	0.18	0.46
Phaseolus vulgaris	0.18	0.1	0.22	3.18	2.76	3.64	0.28	0.22	0.33	0.20	0.12	0.32
Vigna unguiculata	0.15	0.1	0.18	3.16	2.26	4.32	0.28	0.18	0.36	0.32	0.21	0.38

*Hg and Cr were not detected in any samples and market sites included in present study

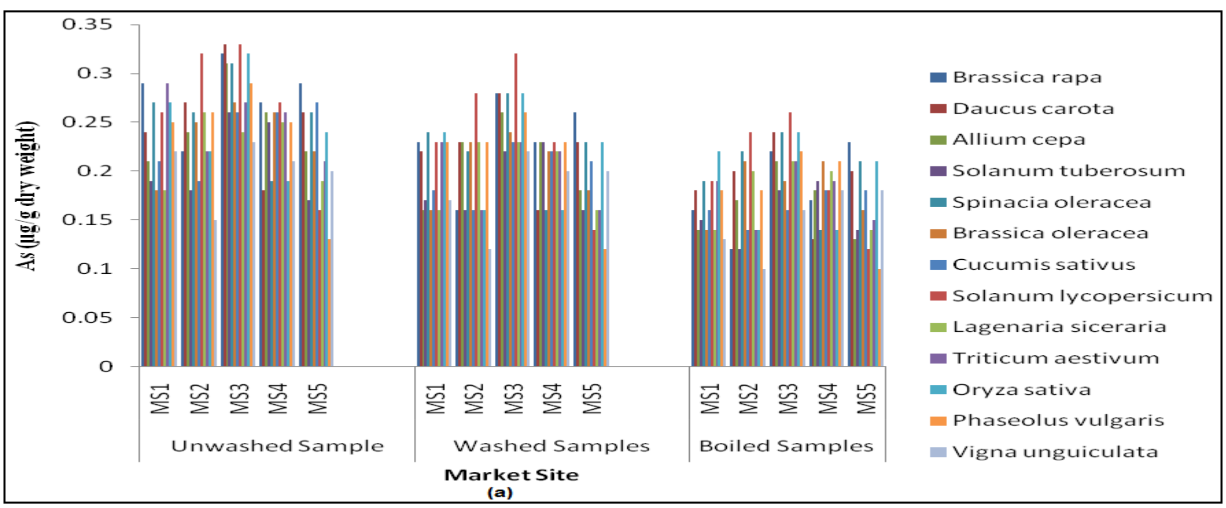


Figure 2) Concentration of As in unwashed, washed and boiled vegetables and Cereals collected from market sites in Varanasi

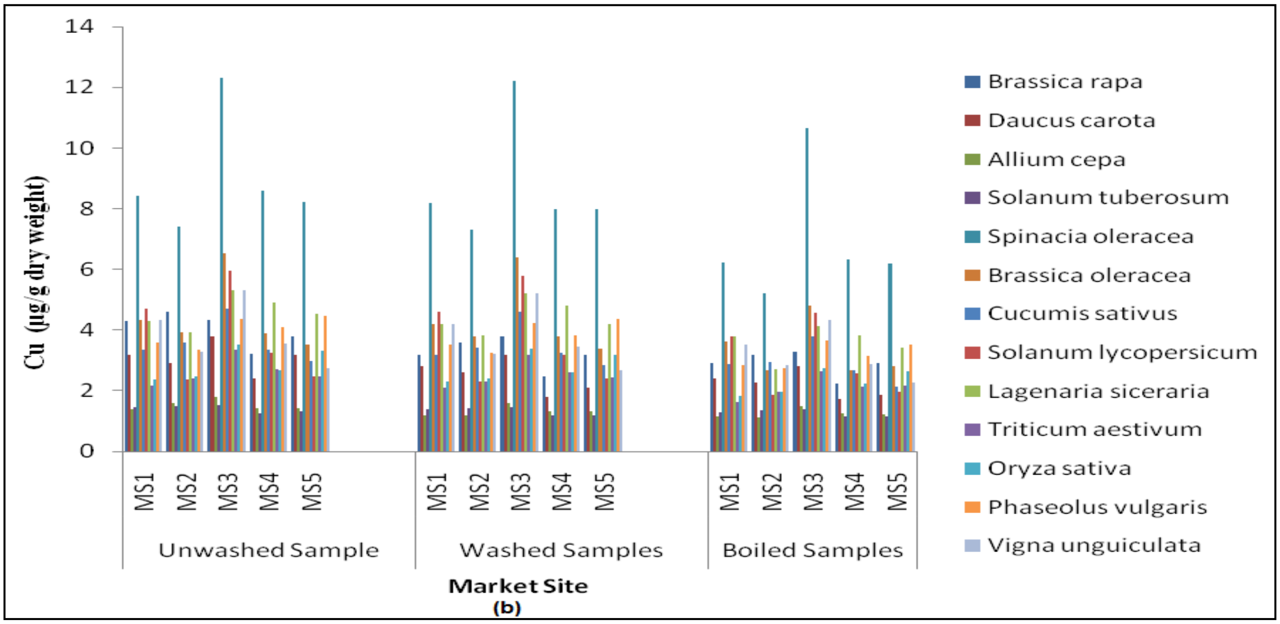


Figure 3) Concentration of Cu in unwashed, washed and boiled vegetables and Cereals collected from market sites in Varanasi (a) As (b) (c) Cd (d) Pb

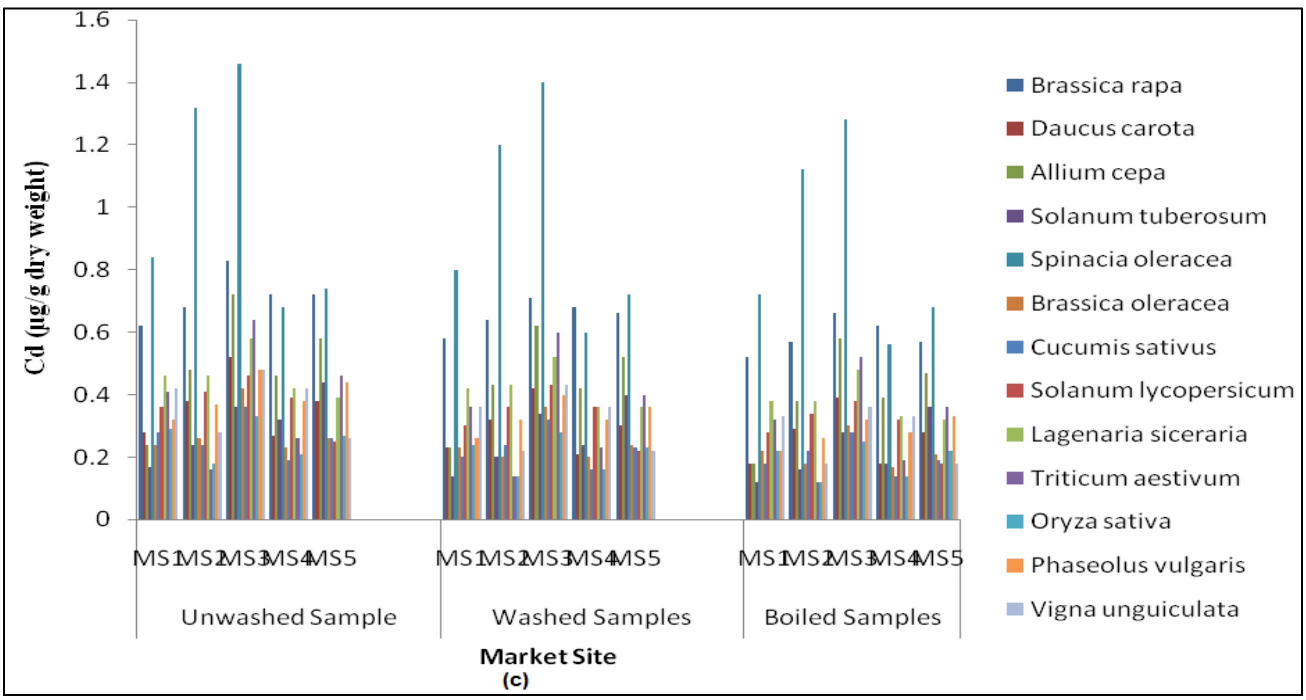


Figure 4) Concentration of Cd in unwashed, washed and boiled vegetables and Cereals collected from market sites in Varanasi

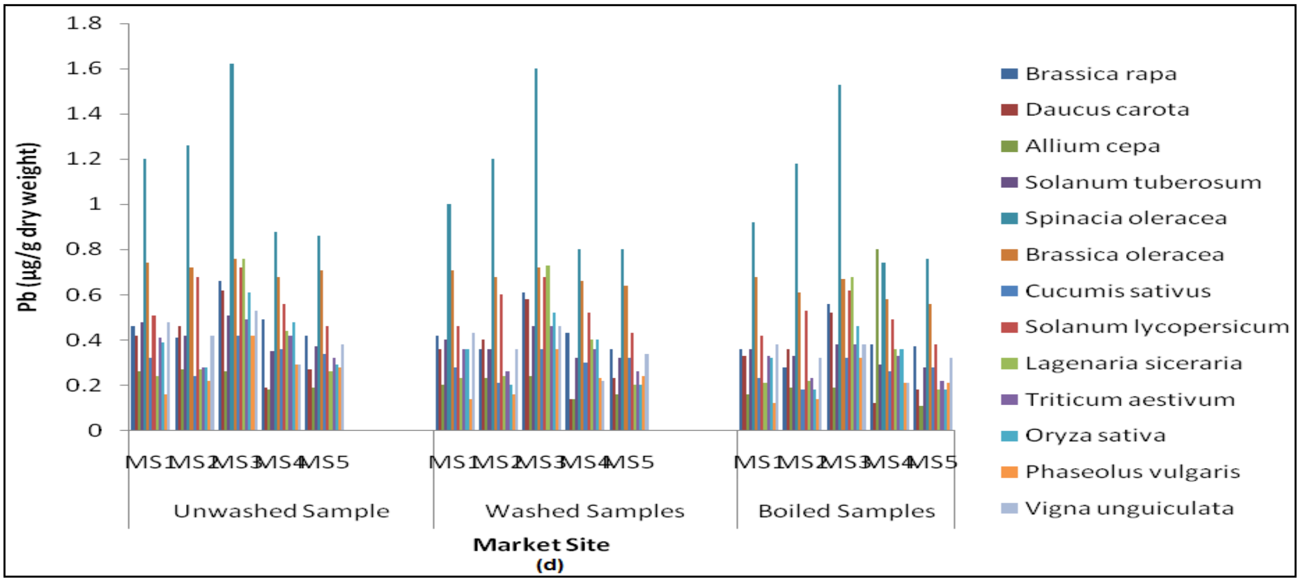


Figure 5) Concentration of Pb in unwashed, washed and boiled vegetables and Cereals collected from market sites in Varanasi

Discussion

High trace metals concentration was observed in unwashed vegetables and cereals as compared to washed and boiled samples (fig. 2). The mean value of Cu, Cd and Pb in unwashed and washed vegetables and cereals are lower than PFA standard except As where as in boiled vegetables and cereals are lower than PFA standard but the mean value of Cd and Pb was many folds higher than the EU standard at all the market sites. The sampling sites MS3 showing elevated levels of trace metals in the vegetables and cereals as compared with other sampling sites may be ascribed to their location in the vicinity of industrial zone and proximity to national highway. The vegetables and cereals growing in vicinity of industrial zone or proximity to national highway are vulnerable to atmospheric pollution, in the form of metal containing aerosols. These aerosols can penetrate the soil and be absorbed by vegetables, or alternatively be deposited on leaves and adsorbed (49). Air pollution may pose a threat to post-harvest vegetables during transportation and marketing, causing elevated levels of heavy metals in vegetables (5). Higher trace metals concentration especially in leafy vegetables observed during the present study. A number of studies suggest that leafy vegetables absorb these metals through their leaves (20, 51).

The toxicity of Arsenic is related to its chemical form, so inorganic forms is more toxic than the organic form. The mean concentration of As (µg/g) in unwashed samples was recorded minimum in Vigna unguiculata (0.20) and maximum in Brassica rapa (0.28). The lowest value of As (µg/g) in unwashed samples was recorded in Phaseolus vulgaris (0.13) at MS5 whereas the highest value was recorded in Daucus carota and Solanum lycopersicum (0.33) at MS3. Significant presence of arsenic in vegetables and cereals reveals that excess use of chemical pesticides and the crop production sites which were in the vicinity near the national highway and having long-term uses of wastewater for irrigation (47, 52).

Cu act as micronutrient for the growth of animals and human beings when present in trace quantities but significantly trends in increasing level of Cu in vegetables and cereals in the present study was observed especially in leafy vegetable such as Spinacia oleracea and in fruits like Solanum lycopersicum was may be attributed to excessive use of chemical pesticides, chemical fertilizers, and irrigation with mixtures of wastewater and sewage as well as having greater capacity to adsorb trace metals from atmospheric deposition on surface. The mean concentration of Cu (µg/g) in unwashed samples was recorded minimum in Solanum tuberosum (1.41) and maximum in Spinacia oleracea (9.0). Sharma et al. (2009) who also reported the three folds' higher mean concentration of Cu (27.59 µg/g dry weight) in Spinacia olerace grown in industrial areas in Varanasi district (4). The lowest value of Cu (µg/g) in unwashed samples was recorded in Allium cepa (1.4) at MS1 whereas the highest value was recorded in Spinacia oleracea (12.32) at MS3 followed by Brassica oleracea (6.52) at same market site.

Cd is a highly toxic carcinogenic trace metals and long term intake in trace quantity present in vegetables and cereals causes severe diseases such as renal, prostate and ovarian cancers. The present trend of increasing concentration of Cd in vegetables and cereals was observed especially in leafy vegetable such as Spinacia oleracea and in roots such as Brassica rapa. This increase may be due to anthropogenic and natural activity. The role of atmospheric deposition on surface can also be significant. The mean concentration of Cd (µg/g) in unwashed samples was recorded minimum in Oryza sativa (0.26) and maximum in Spinacia oleracea (1.01). Sharma et al. (2009) who also reported the two folds' higher mean concentration of Cd (1.96 µg/g dry weight) in Spinacia olerace grown in industrial areas in Varanasi district. The lowest value of Cd (µg/g) in unwashed samples was recorded in Triticum aestivum (0.16) at MS2 whereas the highest value was recorded in Spinacia oleracea (1.46) at MS3 followed by Brassica rapa (0.83) at same market site.

Increase of Pb levels in vegetables and cereals especially leafy vegetable like Spinacia oleracea and in fruits like Lagenaria siceraria was attributed to heavily traffic in this area (4) and irrigation with mixtures of wastewater and sewage (16,53) which leads to the accumulation of Pb emitted from vehicle exhaustions. The mean concentration of Pb (µg/g) in unwashed samples was recorded minimum in Allium cepa (0.23) and maximum in Spinacia oleracea (1.16). Sharma et al. (2009) who also reported some higher mean concentration of Pb (1.44 µg/g dry weight) in Spinacia olerace grown in industrial areas in Varanasi district. The lowest value of Pb (µg/g) in unwashed samples was recorded in Phaseolus vulgaris (0.16) at MS1 whereas the highest value was recorded in Spinacia oleracea (1.62) at MS3 followed by Brassica oleracea and Lagenaria siceraria (0.76).

Conclusion

The present comparative study has generated data on trace metal pollution in and around an Indian city for consumer's exposure to the trace metals. This study focused on the trace metal monitoring and the effects of washing and boiling on its residues in selected vegetable and cereals collected from open urban market. The most noticeable evil associated with urbanization and industrialization in a haphazard and unplanned manner has resulted in the release of trace metals in the local environment. Elevated level of trace metal contents was observed in vegetables and cereals especially in leafy and root vegetables that sold in the open market that located nearby industrial area or national highway, which could be potential health concern to the local resident. The magnitude of trace metals detected in different kinds of unwashed, washed and boiled vegetables and cereals was arranged Cu>Pb>Cd>As. It is proposed that tracing of heavy metals in vegetables and other foods should be considered in human food chain. Appropriate precautions should also be taken at the time of transportation and marketing of vegetables and cereals as well as during food processing in kitchen.

Footnotes

Acknowledgments:

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Conflict of Interest:

The authors declared no conflict of interest.

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