

## Research Article

## Mitigation of heavy metals in different vegetables through biological washing techniques

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### Abstract

*Ample access to nutritious and healthy food is the foremost challenging issue in all over the world. Vegetables constitute integral part of human diet and considered as natural reserves of nutrients. Heavy metals are among the most toxic food pollutants and their intake through diet leads to several disorders. Vegetables get polluted with these toxicants through waste water irrigation, industrial emissions and undue application of metal-based pesticides. This situation prevails more alarming in vegetables grown in peri-urban areas as they have shown high incidence of heavy metals accumulation. Among different heavy metals arsenic, cadmium, chromium and lead are most prevalent metals that can pose threat to humans even at low concentration. In this study effort was made to mitigate these heavy metals (Ar, Cd, Cr and Pb) in cauliflower, spinach, okra and brinjal collected from peri-urban areas through washing with different biological solutions. Heavy metals contents were determined by using Atomic Absorption Spectrophotometry (AAS). A significant reduction in heavy metal contents was observed as a result of washing with different biological solutions. Unwashed vegetable samples were found to be heavily contaminated with heavy metals but washing of vegetables with 8% ginger solution was found to be more effective.*

### 1. Introduction

Food safety has direct impact on healthy life therefore production of safe food is an important aspect of food quality assurance. Now people are demanding fresh, hygienic and quality food. Vegetables are important in human diet because of their beneficial role in growth and development of the body. Vegetables also have some functional constituents such as protein, vitamins and minerals which have health promoting role. The edible portion of vegetable includes stem, leave, fruit, seed and roots that mostly require cooking for maximum digestion. Vegetables also have phytochemicals which act as antioxidant, detoxifying agent, prevent tumor growth and helps to modify metabolic processes (Wargovich, 2000).

Agro-climatic conditions of Pakistan are suitable for the cultivation of variety of vegetables. A range of seasonal vegetables are grown in different parts and seasons in the country (Khalil & Rehman, 2004). In Pakistan only 2 percent of the total cultivated area is under vegetable

cultivation and it contributes about 0.22% in total world vegetable overall export. The per capita daily intake of vegetables is 134g, which is 33-35% below the minimum recommended level of 200g per day (GOP, 2011). Cauliflower, spinach, okra and brinjal are most common vegetables consumed by the people in Pakistan. The vegetables contain high water content, sugars, protein, starch, fat and energy value (Munteanu et al., 2011).

Heavy metals refer to individual metals or metal compounds that can impact human health. Heavy metal contamination of food supply is receiving more and more attentions all over the world. Humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). Dietary sources of heavy metals include contaminated food stuff such as fruits, vegetables and drinking water (Kachenko & Singh, 2006). As vegetables are the source of human consumption so the soil-to-plant transfer quotient is the main source of human exposure (Kalali et al., 2011). Use of waste water for crop cultivation is recognized as important source of heavy

metals in soil (Mapanda et al., 2005). Excessive levels of metals in vegetables are stated due to use of untreated waste water for long time (Sharma, Agarwal, & Marshal, 2008). The existence of metallic compounds in fertilizers imparts an extra source of metal contamination for vegetables. Atmospheric uptake of heavy metals from gas emission has also been recognized as a significant way of heavy metal pollution in vegetable crops (Yusuf, Arwolo, & Bamgbosed, 2003).

Maleki (2008) reported Cu, Cd, Cr and Pb contents at the level of 11.50, 13.60, 0.31 and 7.90ppm respectively in different vegetables grown on sewage water and concluded that these vegetables are unsafe for human consumption. Khan et al. (2010) estimated the levels of different metals in vegetable that had been procured from the peri-urban area of Gilgit. Zinc, lead, copper, cadmium and nickel concentrations were analyzed in different vegetables. The level of cadmium, copper, lead and zinc in these vegetables were found to be in the range of 0.24-2.10, 9-48, 9-44 and 7-35ppm respectively. Because of very high level of these metals in vegetables, these were regarded as unsafe for human consumption.

Washing of vegetables prior to consumption not only removes debris and dirt but also expected to decrease heavy metal and pesticide contents. Solutions of chlorine, ozonated water and strong acids have been proven effective in the removal of heavy metals and pesticides residues for vegetable washing (Pugliese et al., 2004). Keeping in view the toxic potential and tenacious nature, there was a dead need to find out the most efficient way to get rid of heavy metals from vegetables. In the present study, firstly samples were analyzed to detect heavy metals concentrations in unwashed vegetables. Secondly biological solutions were used at different concentrations for washing vegetables to reduce their heavy metal load. Results of the present study explore the heavy metal reduction potential of different biological solutions as the most efficient way to mitigate these toxic metals.

## 2. Materials and methods

Two winter (cauliflower, spinach) and two summer (okra and brinjal) vegetables were selected for the study. These vegetables were selected because each vegetable represent different vegetable group such as flower (cauliflower), leafy (spinach), pod (okra) and fruit (brinjal). 3 kg of each vegetable was collected from peri

urban area of Faisalabad. Samples were washed with tap water and biological solutions (radish and ginger) at different concentrations i.e. 4 & 8%.

### 2.1. Vegetable sample procurement

Six samples of each vegetable were obtained at the stage of optimum maturity i.e. stage at which vegetable is ready to consume. The samples were kept in polythene bag and transferred to the laboratory of National Institute of Food Science and Technology, University of Agriculture, Faisalabad.

### 2.2. Sample preparation

Samples were prepared by following sequence of steps such as size reduction, soaking of vegetables for 10 minutes in solutions followed by blending in commercial blender and oven drying at 70-80 °C. Dried vegetables were grinded into fine powder (80 mesh). Powdered samples were subjected to wet digestion by using nitric acid and perchloric acid by following the method as described by Nwajei (2009).

### 2.3. Preparation of standards

The standard solutions (2, 4, 6, 8 and 10ppm) of all the metals (Ar, Cd, Cr and Pb) were prepared from the stock standard solutions containing 1000ppm in distilled water.

### 2.4. Determination of metals

The heavy metals in unwashed, tap water and biologically washed vegetable samples were determined by using Varian GTA 120 AA 240 Graphite Atomic Absorption Spectrophotometer (AOAC, 2006). The data obtained was analyzed and represented using standard statistical procedures i.e. completely randomized design (CRD) as described by Steel, Torrie, & Dicky (1997).

## 3. Results and discussion

In this study, concentrations of arsenic, cadmium, chromium and lead were analyzed in six samples (with three replicates) of each vegetable i.e. one unwashed and five washed. The analysis of variance for different heavy metals (Ar, Cd, Cr, Pb) concentration in cauliflower, spinach, okra and brinjal indicated that our results were highly significant. Difference in the heavy metal reduction potential of different treatments was due to difference in concentration and type of the biological agent used for the vegetable washing.

**Table 1.** Arsenic and cadmium contents in different vegetables (ppm)

Treatment	Arsenic MRLs value= 0.10ppm				Cadmium MRLs value= 0.05ppm			
	Cauliflower	Spinach	Okra	Brinjal	Cauliflower	Spinach	Okra	Brinjal
T <sub>0</sub>	0.130±0.08 <sup>a</sup>	0.122±0.01 <sup>a</sup>	0.113±0.08 <sup>a</sup>	0.105±0.11 <sup>a</sup>	0.054±0.03 <sup>a</sup>	0.061±0.01 <sup>a</sup>	0.057±0.09 <sup>a</sup>	0.059±0.11 <sup>a</sup>
T <sub>1</sub>	0.119±0.09 <sup>b</sup>	0.113±0.21 <sup>b</sup>	0.106±0.01 <sup>b</sup>	0.097±0.23 <sup>b</sup>	0.050±0.04 <sup>b</sup>	0.058±0.09 <sup>b</sup>	0.052±0.05 <sup>b</sup>	0.055±0.33 <sup>b</sup>
T <sub>2</sub>	0.113±0.04 <sup>c</sup>	0.107±0.13 <sup>c</sup>	0.097±0.03 <sup>c</sup>	0.089±0.12 <sup>c</sup>	0.047±0.01 <sup>c</sup>	0.055±0.03 <sup>c</sup>	0.049±0.22 <sup>c</sup>	0.050±0.52 <sup>c</sup>
T <sub>3</sub>	0.106±0.09 <sup>e</sup>	0.097±0.05 <sup>e</sup>	0.087±0.45 <sup>e</sup>	0.077±0.05 <sup>e</sup>	0.044±0.08 <sup>e</sup>	0.050±0.11 <sup>d</sup>	0.044±0.19 <sup>e</sup>	0.045±0.31 <sup>e</sup>
T <sub>4</sub>	0.107±0.13 <sup>d</sup>	0.102±0.23 <sup>d</sup>	0.093±0.03 <sup>d</sup>	0.086±0.11 <sup>d</sup>	0.045±0.06 <sup>d</sup>	0.047±0.07 <sup>e</sup>	0.046±0.03 <sup>d</sup>	0.049±0.11 <sup>d</sup>
T <sub>5</sub>	0.089±0.04 <sup>f</sup>	0.087±0.44 <sup>f</sup>	0.078±0.04 <sup>f</sup>	0.074±0.05 <sup>f</sup>	0.039±0.04 <sup>f</sup>	0.042±0.03 <sup>f</sup>	0.037±0.43 <sup>f</sup>	0.042±0.21 <sup>f</sup>

T<sub>0</sub>: Unwashed; T<sub>1</sub>: Tap water washed; T<sub>2</sub>: Radish 4% ; T<sub>3</sub>: Radish 8% ; T<sub>4</sub>: Ginger 4% ; T<sub>5</sub>: Ginger 8%  
MRLs= maximum residual limits as established by FAO

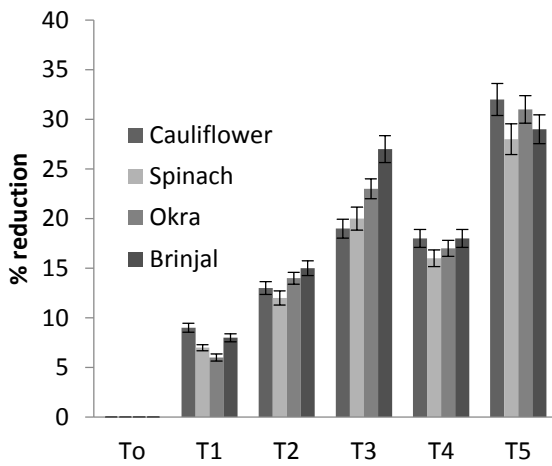
**Table 2.** Chromium and lead contents in different vegetables (ppm)

Treatment	Chromium MRLs value= 1.30ppm				Lead MRLs value= 0.10ppm			
	Cauliflower	Spinach	Okra	Brinjal	Cauliflower	Spinach	Okra	Brinjal
T <sub>0</sub>	0.994±0.03 <sup>a</sup>	0.907±0.19 <sup>a</sup>	1.011±0.10 <sup>a</sup>	0.901±0.20 <sup>a</sup>	0.491±0.45 <sup>a</sup>	0.770±0.02 <sup>a</sup>	0.392±0.16 <sup>a</sup>	0.475±0.21 <sup>a</sup>
T <sub>1</sub>	0.904±0.09 <sup>b</sup>	0.843±0.09 <sup>b</sup>	0.950±0.04 <sup>b</sup>	0.837±0.11 <sup>b</sup>	0.461±0.03 <sup>b</sup>	0.732±0.12 <sup>b</sup>	0.364±0.09 <sup>b</sup>	0.446±0.11 <sup>b</sup>
T <sub>2</sub>	0.864±0.04 <sup>c</sup>	0.798±0.04 <sup>c</sup>	0.859±0.05 <sup>c</sup>	0.783±0.45 <sup>c</sup>	0.427±0.02 <sup>c</sup>	0.701±0.09 <sup>c</sup>	0.348±0.05 <sup>c</sup>	0.408±0.09 <sup>c</sup>
T <sub>3</sub>	0.785±0.04 <sup>e</sup>	0.725±0.10 <sup>e</sup>	0.768±0.19 <sup>e</sup>	0.702±0.29 <sup>e</sup>	0.397±0.16 <sup>e</sup>	0.670±0.05 <sup>d</sup>	0.321±0.11 <sup>e</sup>	0.370±0.22 <sup>e</sup>
T <sub>4</sub>	0.834±0.05 <sup>d</sup>	0.752±0.06 <sup>d</sup>	0.818±0.04 <sup>d</sup>	0.774±0.07 <sup>d</sup>	0.407±0.08 <sup>d</sup>	0.647±0.16 <sup>e</sup>	0.333±0.13 <sup>d</sup>	0.389±0.13 <sup>d</sup>
T <sub>5</sub>	0.725±0.08 <sup>f</sup>	0.671±0.07 <sup>f</sup>	0.717±0.11 <sup>f</sup>	0.675±0.01 <sup>f</sup>	0.378±0.09 <sup>f</sup>	0.608±0.09 <sup>f</sup>	0.294±0.07 <sup>f</sup>	0.351±0.05 <sup>f</sup>

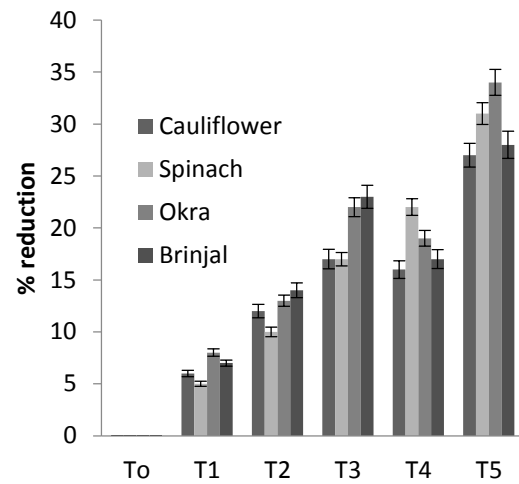
T<sub>0</sub>: Unwashed; T<sub>1</sub>: Tap water washed; T<sub>2</sub>: Radish 4%; T<sub>3</sub>: Radish 8%; T<sub>4</sub>: Ginger 4%; T<sub>5</sub>: Ginger 8%  
MRLs= maximum residual limits as established by FAO

In different vegetables concentrations of arsenic and cadmium are shown in the Table 1 while chromium and lead in Table 2. Unwashed samples of all the vegetables were found to be heavily contaminated with arsenic, cadmium and lead as their contents were more than their established MRLs values by FAO. Whereas washing of vegetables with different biological solutions resulted in significant reductions of heavy metals. Arsenic contents were in the range of 0.089-0.130ppm (cauliflower), 0.087-0.122ppm (spinach), 0.078-0.113ppm (okra) and 0.074-0.105ppm (brinjal) as shown in Table 1. Out of all the washing treatments, maximum arsenic reduction was observed in T<sub>5</sub> i.e. 8% ginger solution, which reduced the arsenic contents to 32% (cauliflower), 28% (spinach), 31% (okra) and 29% (brinjal) as compared to unwashed samples (Fig. 1). Contrarily minimum reduction was observed in T<sub>2</sub> i.e. tap water which resulted in only 6-9% reduction in arsenic concentration in different vegetables. Similarly unwashed samples of vegetables were found to be heavily contaminated with cadmium (Table 1), chromium (Table 2) and lead (Table 2). Concentration of Cd, Cr and Pb were in the range of 0.039-0.054, 0.725-

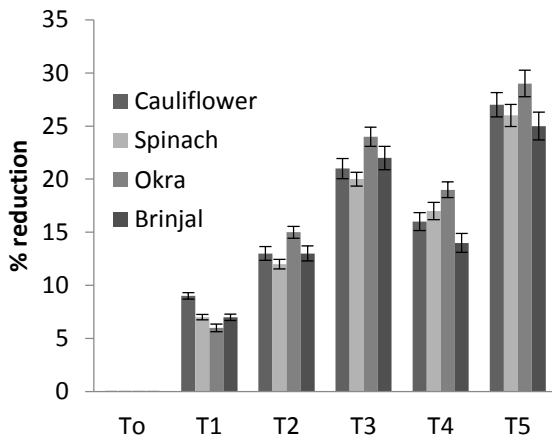
0.994, 0.378-0.491ppm in cauliflower, 0.042-0.061, 0.671-0.907, 0.608-0.770ppm in spinach, 0.037-0.057, 0.717-1.011, 0.294-0.392ppm in okra and 0.042-0.059, 0.675-0.901, 0.351-0.475ppm in brinjal. In all treatments, T<sub>5</sub> i.e. Ginger 8% showed maximum reduction in heavy metal contents as washing with T<sub>5</sub> resulted in 27-34% reduction in Cd (Fig 2), 25-29% in Cr (Fig 3), and 21-26% in Pb (Fig 4) when compared with unwashed vegetable samples. Heavy metal reduction with ginger is mainly due to presence of gingerol in ginger solution which exhibit metal chelating properties. Therefore increase in the concentration of ginger solution increased the heavy metal reduction in different vegetables. However reduction with 8% radish solution i.e. T<sub>3</sub> was also significant as it reduce Ar, Cd, Cr and Pb contents up to 27, 23, 24 and 22% respectively in different vegetable samples. Radish in solution form provides adsorption sites for heavy metals and these sites bind these metals when these were removed by tonic effect (solvent properties) of water. Radish and ginger (4% each) solutions also reduce the metal contamination load but reduction was not significant. However vegetables



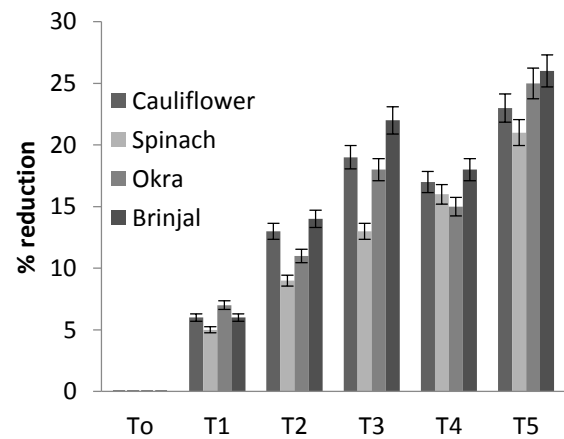
**Figure 1.** Comparison of arsenic reduction (%) by different washing treatments with respect to unwashed vegetables



**Figure 2.** Comparison of cadmium reduction (%) by different washing treatments with respect to unwashed vegetables



**Figure 3.** Comparison of chromium reduction (%) by different washing treatments with respect to unwashed vegetables



**Figure 4.** Comparison of lead reduction (%) by different washing treatments with respect to unwashed vegetables

washing with tap water (T<sub>2</sub>) reduce the Ar, Cd, Cr and Pb contents up to 15, 14, 15 and 14% respectively. During preliminary trials (data not shown) when biological solutions were used in concentration < 4%, they were found to be ineffective in removing these metals from vegetables whereas increase in the concentration of biological solution i.e. >8% with the intentions to get more reduction in heavy metals were found to be adversely affect the sensory properties of these vegetables as it impart taste and flavor.

Nadine et al. (2009) reported the arsenic reduction by washing of vegetable with tap water but that was slightly high than in the present study. The cadmium reduction pattern in this study was parallel to the findings of Singh & Kumar (2006) who estimated the heavy metal load in

exposed to different degrees of pollution in Agra, by washing with tap water. For chromium concentration, results of the present study are well supported by Abbas et al. (2010) who estimated the concentration of chromium in vegetables and found that chromium reduction trend by washing with tap water is the same as described by the Nadine et al. (2009) during their work on vegetables grown and sold in selected areas in Lebanon. However lead concentrations in unwashed vegetables were in the same range as described by Naser et al. (2009). Lead reduction by tap water washing differ from the findings of the Suruchi & Jilani (2011) who assessed the heavy metals concentration in washed and unwashed vegetables exposed to different degrees of pollution in Agra, India.

#### 4. Conclusions

The heavy metal residues were present at varying concentrations in cauliflower, spinach, okra and brinjal. In all vegetables arsenic, lead, cadmium and chromium residue exceeded the prescribed maximum residual limits (MRLs) in vegetables as established by FAO/WHO. Spinach showed the highest accumulation of different metals because of its large surface area as compared to other vegetables. The washing of vegetables with tap water and biological solutions not only removed the dirt and dust particles but also reduced the heavy metals significantly. Difference between washed and unwashed vegetables with regard to heavy metal concentrations suggests that heavy metals reaches on the vegetables by aerial deposition and adhere to them. Washing treatments mechanically remove the heavy metals deposited on the surface of the vegetables. On the basis of present study, it is strongly recommended that vegetables should be washed with house hold biological solutions before cooking to decrease the intake of these toxic metals.

#### Declaration of interest

There is no conflict of interest among authors regarding the submission of this research work

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