Evaluation of various soaking agents as a novel tool for heavy metal residues mitigation from spinach

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Abstract

Heavy metals pollution is a global threat to the environment and ecosystem due to various human and natural activities. Heavy metal intake through vegetables and diet leads to numerous ailments such as nervous disorder, kidney damage tubular growth and bone disease. The present study was conducted to mitigate the residue of heavy metals Hg, Pb, Zn and As in spinach collected from self-grown supervised field by using different washing treatments. Amount of trace metals was measured in fresh and chemically washed spinach samples with the help of Atomic Absorption Spectrophotometer (AAS). Results revealed that highest reduction of mercury, lead, zinc and arsenic residues with 10% citric acid was 23%, 28%, 54% and 22% respectively among the tested solutions. However, tap water treatment also reduced 7%, 7%, 15% and 6, respectively. Among various washing solutions citric acid proved maximum reduction potential followed by Lemon extract, sodium carbonate, reddish extract and hydrogen peroxide respectively. The percent reduction by various solutions ranged from 7 to 23%, 7 to 28%, 15 to 54% and 6 to 22% for elimination of mercury, lead, zinc and arsenic, respectively. More reduction was found in zinc followed lead, mercury and arsenic.

Keywords: heavy metals; spinach; wastewater; acidic solutions; alkaline solutions; biological extract.

Practical Application: Washing solutions citric acid, lemon extract, sodium carbonate, reddish extract and hydrogen peroxide can be used to mitigate heavy metal residues in vegetables.

1 Introduction

Agriculture is the backbone of Pakistan that is blessed with plentiful natural resources such as fertile and productive soil, better water irrigation system and climate change from tropical to temperate. From last few years due to shortage of water availability, the use of sewage water has increased tremendously, especially in semiarid region of Pakistan. Vegetables are the valuable source of different ingredients that play a vital role for the prevention of various ailments, maintaining alkaline reserve of the body, repair the body organ and protect human health (Dalal et al., 2006; Marwa et al., 2009). Among different vegetables, spinach is a short duration crop mostly grow in subtropical region with significant mineral profile, upon consumption it provides macro and micronutrients to the body (Bangash et al., 2011).

Heavy metal contamination mainly occurs due the application of use of metal-based pesticides, waste water irrigation, fertilizers, emission and transportation of industrial waste on crops (Agrawal & Marshall, 2003). Soil contamination due to increasing concentration of heavy metals is becoming a very serious problem to environment (Bhutto et al., 2009). In soil, waste water is single largest contributor of heavy metal contents because these effluents are heavily loaded with metallic compounds and ultimately vegetables consumption pose serious threat to human health (Singh et al., 2004; Mapanda et al., 2005; Sharma et al., 2004).

Traditional washing techniques are used to remove waste, dust, dirt and filth prior to use vegetables and they are considered vital tool for the reduction of heavy metals residues. Strong acid, ozonated water and bleach solution has been found successful for mitigation of heavy metal and other chemical residues. Tap water washing for 2-3 times reduced significant amount of heavy metal such as lead and cadmium from 75-100% and 27-55% for copper and zinc in vegetables (Singh & Kumar, 2006).

Heavy metal residues and other particles deposited on the surface of vegetables removes significantly by simple washing (Sharma et al., 2008). In spinach, heavy metal such as Pb, Cd, Cr and Zn reduced at 21%, 21%, 13% and 26% respectively, by simple washing. In coriander, heavy metal reduction was found 11%, 31%, 11%, 5% and 6% for Cd, Pb, Cr, Ni and Zn and for methi reduction pattern 14%, 13%, 15%, 17% and 13%, respectively (Suruchi & Jilani, 2011).

Increasing concentration of heavy metal residues in soil, water and foodstuff is a distressing phenomenon around the world. The pollution of water and soil directly linked with food

chain contamination through irrigation. European Union and United States has already set up strict limits for various heavy metals in water, but current food legislation does not have legal limits for a number of heavy metals in food and this fact causes considerable health hazards to babies, children, pregnant women and all other consumers. Keeping in view the potential toxic effects of heavy metal residues as a result of vegetable consumption, recent project was designed to elucidate heavy metals residues in spinach to explore the effectiveness of various soaking agents to reduce heavy metal residues.

2 Materials and methods

2.1 Procurement of raw materials

Spinach (*Spinacia oleracea* L.) was grown in the experimental field of University of Agriculture Faisalabad, Pakistan. For irrigation purpose sewage water was applied. One kg sample of spinach was obtained at the point of optimum maturity at triplicate form from different locations of the plot. The samples were instantly shifted to the labortary of National Institute of Food Science and Technology, University of Agriculture Faisalabad, Pakistan for subsequent analysis of samples.

2.2 Application of washing solutions to treated spinach samples

The collected spinach samples were reduced to appropriate size by using knife to facilitate the analysis. The study was designed with different washing treatments by using tap water and following concentrations of chemical, alkaline and biological solutions along with one unwashed sample shown in Table 1.

After solution preparation spinach samples were soaked in solution for ten minutes at 30 °C for washing purpose. The washed spinach samples were blended by using commercial blender to consistent paste of pulp and juice. Spinach samples in three replicates of each washing technique were kept in hot air oven at 70-80 °C till complete dryness. After drying, vegetable samples were grounded in to fine powder (80 mesh) using commercial blender and preserved in polythene bag for further analysis.

2.3 Sample digestion

The grounded spinach sample (0.5-1.0) was placed in to Pyrex beaker, added 10 mL of concentrated HNO_3 to it and kept for night without heating. Then it was heated on hot plate near to dryness, cooled and add 5 mL of $HCLO_4$ and again heated till digestion was completed, then digested sample was filtered in to clean volumetric flask and diluted to 50 mL with deionized water (Nwajei, 2009).

2.4 Preparation of standards

The standard solutions (5, 10, 15 and 20 ppm) of all the metals (Hg, Pb Zn and As) were prepared from the stock solutions of 1000 ppm in distilled water.

2.5 Quantification of heavy metals through atomic absorption spectrophotometer

The heavy metals Hg, Pb, Zn and As in unwashed, tape water, chemically washed, dried and digested spinach samples were determined by using Varian GTA 120 AA 240 Graphite Atomic Absorption Spectrophotometer (Horwitz, 2006). The data obtained was analyzed and represented using standard statistical procedures i.e. completely randomized design (CRD) as described by (Steel et al., 1997).

3 Results

The concentrations of mercury, lead, zinc and arsenic were analyzed in different samples of spinach collected from supervised field at the stage of optimum maturity. The results regarding these heavy metals concentration in spinach showed significant variation and linear reduction of heavy metals as presented in Table 2, 3, 4 and 5, respectively. The concentrations of heavy metals in unwashed spinach samples were higher than samples subjected to different washing treatments. The result

Table 1. Different washing treatments.

Reagents	Concentrations	Treatments
Unwashed	-	T_1
Tap water	-	T_2
Washing Treatments		
Reddish	5%	T_3
	10%	T_4
Sodium Carbonate	5%	T_5
	10%	T_6
Hydrogen Peroxide	5%	T_7
	10%	T_8
Lemon	5%	T_9
	10%	T_{10}
Citric Acid	5%	T_{11}
	10%	T_{12}

Table 2. Mean ± S.D value and percent reduction of mercury in spinach.

	Spinach	
Treatments	Mean ± S.D mg/kg	Percent Reduction (%)
T_1 Unwashed	0.0481 ± 0.00081[a]	
T_2 Tap water washed	0.0447 ± 0.0023[b]	7
T_3 Reddish extract 5%	0.0436 ± 0.0016[bc]	9
T_4 Reddish extract 10%	0.0408 ± 0.0007[de]	15
T_5 Sodium Carbonate 5%	0.0429 ± 0.0013[bcd]	11
T_6 Sodium Carbonate 10%	0.0399 ± 0.0008[ef]	17
T_7 Hydrogen Peroxide 5%	0.0446 ± 0.0013[b]	7
T_8 Hydrogen Peroxide 10%	0.0422 ± 0.0005[cd]	12
T_9 Lemon extract 5%	0.0429 ± 0.0017[bcd]	11
T_{10} Lemon extract 10%	0.0374 ± 0.0019[f]	22
T_{11} Citric Acid 5%	0.0410 ± 0.0016[de]	15
T_{12} Citric Acid 10%	0.0370 ± 0.0017[f]	23

The letters indicate level of significance between different washing techniques, if same letter then there is no variation.

Table 3. Mean ± S.D value and percent reduction of lead in spinach.

Spinach		
Treatments	Mean ± S.D mg/kg	Percent Reduction (%)
T_1 Unwashed	0.9838 ± 0.0055^a	
T_2 Tap water washed	0.9127 ± 0.0017^b	7
T_3 Reddish extract 5%	0.8638 ± 0.0039^d	12
T_4 Reddish extract 10%	0.8034 ± 0.0029^i	18
T_5 Sodium Carbonate 5%	0.8541 ± 0.0043^e	13
T_6 Sodium Carbonate 10%	0.8235 ± 0.0032^g	16
T_7 Hydrogen Peroxide 5%	0.9035 ± 0.0037^c	8
T_8 Hydrogen Peroxide 10%	0.8548 ± 0.0012^e	13
T_9 Lemon extract 5%	0.8340 ± 0.0038^f	15
T_{10} Lemon extract 10%	0.7267 ± 0.0029^j	26
T_{11} Citric Acid 5%	0.8121 ± 0.0105^h	17
T_{12} Citric Acid 10%	0.7067 ± 0.0032^k	28

The letters indicate level of significance between different washing techniques, if same letter then there is no variation.

Table 4. Mean ± S.D value and percent reduction of zinc in spinach.

Spinach		
Treatments	Mean ± S.D mg/kg	Percent Reduction (%)
T_1 Unwashed	7.4747 ± 0.0227^a	
T_2 Tap water washed	6.3577 ± 0.0220^b	15
T_3 Reddish extract 5%	5.8498 ± 0.0164^d	22
T_4 Reddish extract 10%	5.0962 ± 0.0992^{ef}	32
T_5 Sodium Carbonate 5%	5.9077 ± 0.0099^{cd}	21
T_6 Sodium Carbonate 10%	4.4114 ± 0.3785^h	41
T_7 Hydrogen Peroxide 5%	6.0669 ± 0.0296^c	19
T_8 Hydrogen Peroxide 10%	5.0091 ± 0.0003^{fg}	33
T_9 Lemon extract 5%	5.2344 ± 0.0181^e	30
T_{10} Lemon extract 10%	3.5816 ± 0.0362^i	52
T_{11} Citric Acid 5%	4.8476 ± 0.0811^g	35
T_{12} Citric Acid 10%	3.4316 ± 0.0274^i	54

The letters indicate level of significance between different washing techniques, if same letter then there is no variation.

Table 5. Mean ± S.D value and percent reduction of arsenic in spinach.

Spinach		
Treatments	Mean ± S.D mg/kg	Percent Reduction (%)
T_1 Unwashed	0.1048 ± 0.0105^a	
T_2 Tap water washed	0.0987 ± 0.0013^b	6
T_3 Reddish extract 5%	0.0965 ± 0.0006^{bcd}	8
T_4 Reddish extract 10%	0.0913 ± 0.0007^{def}	13
T_5 Sodium Carbonate 5%	0.0976 ± 0.0005^{bc}	7
T_6 Sodium Carbonate 10%	0.0928 ± 0.0005^{cdef}	11
T_7 Hydrogen Peroxide 5%	0.0976 ± 0.0014^{bc}	7
T_8 Hydrogen Peroxide10%	0.0945 ± 0.0015^{bcde}	10
T_9 Lemon extract 5%	0.0943 ± 0.0006^{bcde}	10
T_{10} Lemon extract 10%	0.0889 ± 0.0008^f	15
T_{11} Citric Acid 5%	0.0903 ± 0.0009^{ef}	14
T_{12} Citric Acid 10%	0.0818 ± 0.0011^g	22

The letters indicate level of significance between different washing techniques, if same letter then there is no variation.

regarding mercury showed progressive increase in reduction of residues of mercury with the increase of concentration of treatments (Table 2). Out of all the washing treatments, it was observed that the minimum reduction of mercury residues 7% was recorded when T_1 tap was applied to spinach. The spinach treated with T_{12} 10% citric acid yielded the maximum reduction of mercury which was 23% followed by lemon extract 22%, sodium carbonate 17%, reddish extract 15% and hydrogen peroxide 12%. Furthermore, the maximum reduction of lead residues 28% was observed when T_{12} 10% citric acid was applied to spinach followed by lemon extract 26%, reddish extract 18, sodium carbonate 16% and hydrogen peroxide 13% depicted in Table 3. Similarly, the maximum reduction of zinc residues 54% was recorded when T_{12} 10% citric acid was applied to spinach followed by lemon extract 52%, sodium carbonate 41%, hydrogen peroxide 33% and reddish extract 32% depicted in Table 4. Moreover, spinach treated with T_{12} 10% citric acid yielded the maximum reduction of arsenic which was 22% followed by lemon extract 15%, reddish extract 13%, sodium carbonate 11%, and hydrogen peroxide 10% shown in Table 5. The tap water reduces the mercury, lead, zinc and arsenic contents by 7, 7, 15 and 6% in spinach and T_{12} 10% citric acid was found to be more effective in all the spinach samples to reduce mercury, lead, zinc and arsenic contents.

4 Discussion

The results pertaining from the current study reveals that the heavy metal residues declined extraordinary when spinach samples were given washing treatment irrespective to the type of washing, different treatments declined the mercury residues ranges from 7 to 23%, lead 7 to 28%, zinc 15 to 54% and arsenic 6 to 22% in spinach. More reduction was found in zinc followed by lead, mercury and arsenic, respectively.

Among all heavy metals zinc exhibited higher mitigation owing to their more dissolving power and physiochemical properties as compared to other heavy metals. During washing operation, variation in reduction of heavy metal residues may be due to their change in the behavior against washing treatments. The treatment of spinach with tap water removes maximum of the heavy metal residues attached to the outer surface of the spinach as well as in the skin of the vegetable due to its dissolving power because that is considered a universal solvent.

The present study results also indicated that citric acid showed maximum reduction of heavy metal residues due its working ability as a chelating agent and resulting made the heavy metal residues unavailable, salt solution sodium carbonate also reduced significant amount of heavy metal residues and biological extract lemon exposed high decrease of heavy metal residues as compared to reddish extract. Acidic washing solutions were more effective in the removal of the zinc, lead, mercury and arsenic under examination than biological extracts and salt solutions.

Vegetables are the valuable source of different ingredients that play a vital role for maintaining the nutritional status of humans and prevention of various ailments, maintaining alkaline reserve of the body, repair the body organ and assure healthy human life. Heavy metals accumulation in the vegetables take place usually

from soil, water and atmosphere. Vegetables intake affected with heavy metals leads to several disorders including nervous disorder, kidney damage, bone diseases and several tubular growths. Uptake of these metals in vegetables from polluted soil by absorbing them as well as from deposits on the exposed vegetable region through air in the prevailing polluted environments.

The results of the recent research are in conformity with previous results of Krol et al. (2000) who also observed residues of cadmium and lead were reduced during washing treatment in vegetables.

The findings of the present study are in line of Parveen et al. (2003) who determined the concentration of cadmium in unwashed vegetables. Reduction pattern of cadmium in this study was similar to the investigation that was carried out by Singh & Kumar (2006) to estimate the heavy metal load of unwashed and washed vegetables in peri-urban, Delhi. In addition, Suruchi & Jilani (2011) also reported the same reduction pattern of cadmium in spinach, okra and brinjal, open to different levels of pollution in the city of India i.e Agra, by washing treatment given with tape water. The arsenic is a toxic element and the humans may get exposure to arsenic through drinking water obtained by wells bored into arsenic contaminated areas or through contaminated water by agro-chemical waste or industrial effluents. The results of the present study regarding arsenic in spinach are similar with the findings of Al-Chaarani et al. (2009). Higher level of lead contents than the recommended (0.2 μgg^{-1}) in humans, causes teeth and blood diseases, weak bones, kidney damage, defects in reproductive system, increased pancreas weight, affects liver, cardiovascular and peripheral nervous system. The results of the present study regarding the lead concentration in normal treatment of vegetables have shown the same results as mentioned by Naser et al. (2009) to estimate concentration of different heavy metals in vegetables. Lead reduction by tap water washing follows the same pattern as reported by the Singh & Kumar (2006) to estimate the different heavy metal contents from unwashed and washed vegetables collected from peri urban region of Delhi. In past, maximum level (0.02 μgg^{-1}) of mercury was observed in fenugreek/methi i.e. leafy vegetables while minimum level (0.001 μgg^{-1}) were reported in root/tuberous vegetables whereas high contents of arsenic (0.083 μgg^{-1}) was observed in fruity vegetables (okra), on the other hand minimum level (0.014 μgg^{-1}) was reported in cauliflower (Nergus et al., 2005).

Traditional washing techniques are used to remove waste, dust and dirt before using vegetables and they are expressed as vital tool for reducing heavy metals residues. Washing tap water for 2-3 times reduced significant amount of heavy metal such as lead and cadmium from 75-100% and 27-55% for copper and zinc in vegetables (Singh & Kumar, 2006).

Finding of the recent study are parallel to the investigation of Sharma et al. (2008) who reported that significant removal of excess heavy metal contents and other particles deposited on the surface of vegetables by simple washing. In spinach, heavy metal such as Pb, Cd, Cr and Zn reduced at 21%, 21%, 13% and 26% respectively, by simple washing. In coriander, heavy metal reduction was found 11%, 31%, 11%, 5% and 6% for Cd, Pb, Cr, Ni and Zn and regarding leafy vegetable i.e methi reduction figures were 14%, 13%, 15%, 17% and 13%, respectively (Suruchi & Jilani, 2011).

5 Conclusions

Spinach samples were contaminated with different heavy metal residues and wastewater irrigation is the sole and important source of heavy metals contamination in soil as well as in vegetables. Due to large surface area, spinach shows higher deposition of different heavy metals and tap water and household chemicals solutions washing significantly decrease dust, dirt, debris and heavy metal residues. These different washing techniques mechanically clear the heavy metals adhered on the surface of the spinach which mostly accumulate through aerial deposition. As an outcome of this study, it is highly recommended that vegetables must not be grown with sewerage water and at kitchen gardening level the vegetables must be treated with tap water moreover washing treatments including citric acid, hydrogen peroxide, sodium carbonate and biological extracts of lemon and radish are useful in the reduction of heavy metal residues. These methods are very simple because they can easily perform at local conditions and beneficial due their working ability to mitigate the metal residues.

Acknowledgements

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