

Mitigation of Arsenic Concentration in Green Leafy Vegetables viz. Cabbage (*Brassica oleracea* var. *capitata*), Spinach (*Spinacia oleracea*), Cauliflower (*Brassica oleracea* var. *botrytis*) through Different Biochemical Washing Techniques

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Authors' contributions

This work was carried out in collaboration among all authors. Author Sneha designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RKK and JM managed the analyses of the study. Authors MKS and RY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was carried out for "Mitigation of Arsenic concentration in green leafy vegetables viz. Cabbage (*Brassica oleracea* var. *capitata*), Spinach (*Spinacia oleracea*) and Cauliflower (*Brassica oleracea* var. *botrytis*) through different biochemical washing techniques". The agrarian farmers of Nathnagar block in Bhagalpur district of Bihar mostly cultivate vegetables such as cabbage, spinach, cauliflower etc. The farmers irrigate their farm by the polluted water of Champa-nala (main source of irrigation) which contain huge amount of highly health hazardous waste. The farmers are unaware of the harmful effect of the polluted water that they are using for irrigating vegetables, they feel happy to get the water free of cost for irrigating their crops. The

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morphology of crop which is irrigated with polluted water seems likely to the crop irrigated with clean water. At maturity, farmers harvest their crops and sell them in their local market which becomes the constituent of our food chain. The results revealed that in cabbage leaf, the maximum concentration of arsenic observed in unwashed samples (T1) was 427.69 ppb, the distance of 50 m away from the contaminated site. The maximum reduction percentage was 27.30% with a mean value of 13.76% observed after employing the treatments T6 (washed with 8% ginger solution). In spinach the maximum concentration of arsenic observed in unwashed samples (T1) was 351.00 ppb, the distance of 50 m away from the contaminated site. The maximum reduction percentage of 30.20% with a mean value of 16.93% of arsenic was observed after employing the treatments T6 (washed with 8% ginger solution) whereas in cauliflower the maximum concentration of arsenic observed in unwashed samples (T1) was 469.06 ppb, the distance of 50 m away from the contaminated site. The maximum reduction of 29.93% with a mean value of 15.37% was observed after employing the treatment T6 (washed with 8% ginger solution). However, the concentrations of Arsenic was higher in unwashed leaves of the vegetables grown in the industrial areas which indicates that industrial discharge causes heavy contamination of soil and eventually their accumulation in plants.

Keywords: Leafy vegetables; mitigation; heavy metal (As).

1. INTRODUCTION

Globally, the population is rising very rapidly which alters our environment by several anthropogenic activities such as mining, industrialization, agriculture as well as increasing in urbanization level Wang et al. [1]. This leads to the generation of waste in huge amount and disposal of industrial, municipal wastes as well as sewage water properly is a very big problem. The untreated waste water is many times drained to the agricultural lands where it is used for growing crops including vegetables. Vegetables are common diet to be taken, being the sources of essential nutrients, antioxidants, and metabolites which have marked health effects Arai, [2]. The vegetables mainly leafy vegetables accumulates higher amount of metals if grown in heavy metals contaminated soils Al Jassir et al. [3]. The vegetables that are grown in soil polluted with toxic metals accumulate them in higher quantities in its edible and non edible portions that may leads to several clinical problems to both human beings as well as animals after consuming the vegetables Alam et al. [4]; Arora et al. [5]. The farmers of Nath-nagar block irrigate their farm with the polluted water of Champa-nala (main source of irrigation) which consists of huge amount of highly hazardous waste that can be visible to our eyes without aid of any instrument. The farmers are unknown towards the harmful effect of using the contaminated water for irrigating their vegetables, they are happy to get the water free of cost for irrigation. The morphology of crop which is irrigated with polluted water seems likely to the crop irrigated

with clean water. At maturity, farmers harvest their crops and sell them in their local market which becomes the constituent of our food chain. The people buy those contaminated vegetables by rational that it will be extremely nutritious and will be healthy for their diet but the reality is something else. The heavy metals conveyed to the plants and vegetables, which further gets channelized with animals and humans by consuming those vegetables. The built up of heavy metal accumulation is consequence of sewage disposal in irrigated soils and produce obtained from sewage irrigated soils is serious to concern while these vegetables are ingested.

2. MATERIALS AND METHODS

2.1 Collection of Plant Sample

From the representative areas, three samples of each leafy vegetables *i.e.*, cabbage, spinach and cauliflower were collected for Arsenic screening at the stage of its optimum maturity or ready to consume stage. The harvesting of these leafy vegetables was done from four corners and the center of the plot. The vegetables were washed with de-ionised water followed by 1N HCl solution to remove any possible foliar contaminants, such as pesticides, fertilizers etc.

2.2 Plant Sample Analysis

The plant samples of cabbage, spinach and cauliflower at optimum maturity were collected in paper bags. A particular identification number was given to each sample. The collected plant

samples except control were washed with distilled water and were soaked for 30 minutes in different solutions and after soaking, the samples were wiped and dried in oven at 60°C for 24 hours. After drying, the samples were ground into fine powder using mortar and pestle.

2.3 Tri Acid Digestion of Plant Sample

1 g of each plant sample was kept in a conical flask of 100 mL and 15 mL of tri acid ($\text{HNO}_3:\text{HClO}_4:\text{H}_2\text{SO}_4$ in the ratio 9:3:1) was added to each sample. The conical flask with sample was kept on the hot plate and was heated gently at first. After that it was heated more vigorously till white fumes ceases to come out or a clear colorless solution was obtained. The samples were put out from the hot oven when the volume was reduced to 3-4 mL. The samples were then kept for cooling and after cooling, 20 mL of double distilled water was added. The sample was filtered through Whatman no.1 filter paper and the volume was maintained in 100 mL volumetric flask. The analysis was done by using Agilent GTA 120 Graphite Furnace atomic absorption spectrophotometer.

3. RESULTS

The leaf samples of green leafy vegetables *i.e.* cabbage, spinach and cauliflower were collected from sewage irrigated areas of the three different villages Kalupur, Basantpur and Mathurapur in Nathnagar block at a distance of 50 m, 150 m, and 400 m away from the contaminated site. The six treatments advocate the washing of vegetables for reduction of heavy metal load (As) *viz.*, Unwashed samples (T1), Hot Water Treatment (T2), 5N HCl (T3), 1% Citric acid (T4), 2% Tartaric acid (T5) and 8% Ginger solution

(T6). Results indicated that, there was a significant differences observed in heavy metal load after the implementation of various treatments.

3.1 Mitigation of as (ppb) after Biochemical Washing Technique in Cabbage Grown in Sewage Irrigated Areas

The maximum concentration of arsenic observed in the unwashed samples (T1) was 427.69 ppb at a distance of 50 m away from the contaminated site. The maximum reduction % was 27.30% with a mean value of 13.76% observed after employing the treatments T6 (washed with 8% ginger solution). Whereas, similar results were observed at distance of 150 m and 400 m away from the contaminated site by employing the treatment T6 (washed with 8% ginger solution) which was 19.35 and 25.32% with a mean value of 10.65 and 13.05% respectively.

3.2 Mitigation of as (ppb) after Biochemical Washing Technique in Spinach Grown in Sewage Irrigated Areas

The maximum concentration of arsenic was observed in unwashed samples (T1) *i.e.* 351.00 ppb, the distance of 50 m away from the contaminated site. The maximum reduction percentage *i.e.* 30.20% with a mean value of 16.93% of arsenic was observed after employing treatments T6. Whereas, similar result was observed at distance of 150 m and 400 m away from the contaminated site by employing the treatment T6 which was 25.93% and 25.50% with a mean value of 13.15% and 13.09% respectively.

Table 1. The different treatment details used for reducing the heavy metal (As) load from the test crops Cabbage, Spinach and Cauliflower

Treatment	Treatment details
T1	No washing (Control)
T2	Hot water treatment (HWT)
T3	5 NHCl
T4	1% Citric acid solution (1% C.A)
T5	2% Tartaric acid solution (2% T.A)
T6	8% Ginger solution (8% G.S)
Replication	3
Treatment combination	18
Design	Completely Randomized Design (CRD)

Table 2. Treatment procedure for cabbage grown under sewage irrigated areas

Treatment	Cabbage					
	At 50 m distance from contaminated site		At 150 m distance from contaminated site		At 400 m distance from contaminated site	
	As (ppb)	Percentage (%)Reduction	As (ppb)	Percentage (%)Reduction	As (ppb)	Percentage (%)Reduction
T1-Control	427.69	0.00	285.12	0.00	259.20	0.00
T2-HWT	413.33	3.36	270.00	5.31	248.50	4.13
T3-5N HCl	373.33	12.71	258.88	9.20	223.26	13.87
T4-1% C. A	359.48	15.95	245.65	13.84	217.87	15.95
T5-2% T. A	328.31	23.23	238.87	16.22	209.81	19.06
T6-8% G. S	310.91	27.30	229.94	19.35	193.58	25.32
Mean	368.85	13.76	254.75	10.65	225.37	13.05

Table 3. Treatment procedure for spinach grown under sewage irrigated areas

Treatment	Spinach					
	At 50 m distance from contaminated site		At 150 m distance from contaminated site		At 400 m distance from contaminated site	
	As (ppb)	Percentage (%)Reduction	As (ppb)	Percentage (%)Reduction	As (ppb)	Percentage (%)Reduction
T1-Control	351.00	0.00	233.99	0.00	212.73	0.00
T2-HWT	330.26	5.91	225.17	3.77	204.16	4.03
T3-5N HCl	298.35	15.00	210.89	9.87	190.82	10.30
T4-1% C. A	267.35	23.83	195.23	16.57	182.03	14.43
T5-2% T. A	257.53	26.63	180.68	22.78	161.08	24.28
T6-8% G. S	244.98	30.20	173.32	25.93	158.47	25.50
Mean	291.58	16.93	203.21	13.15	184.88	13.09

Table 4. Treatment procedure for cauliflower grown under sewage irrigated areas

Treatment	Cauliflower					
	At 50 m distance from contaminated site		At 150 m distance from contaminated site		At 400 m distance from contaminated site	
	As (ppb)	Percentage (%)Reduction	As (ppb)	Percentage (%)Reduction	As (ppb)	Percentage (%)Reduction
T1-Control	469.06	0.00	312.70	0.00	284.27	0.00
T2-HWT	436.06	7.03	298.71	4.48	263.28	7.39
T3-5N HCl	411.79	12.21	284.53	9.01	249.57	12.21
T4-1% C. A	375.04	20.04	260.02	16.85	229.29	19.34
T5-2% T. A	361.19	23.00	250.79	19.80	221.90	21.94
T6-8% G. S	328.66	29.93	229.10	26.73	205.18	27.82
Mean	396.97	15.37	272.65	12.81	242.25	14.78

3.3 Mitigation of as (ppb) after Biochemical Washing Technique in Cauliflower in Sewage Irrigated Areas

The maximum concentration of arsenic was observed in unwashed samples (T1) i.e. 469.06 ppb, the distance of 50 m away from the contaminated site. The maximum reduction was

29.93% with a mean value of 15.37% of arsenic was observed after employing treatments T6. Whereas, similar results was observed at distance of 150 m and 400 m away from the contaminated site by employing the treatment T6 which was 26.73% and 27.82% with a mean value of 12.81% and 14.78% respectively.

4. DISCUSSION

The best approach to reducing the load of arsenic within safe limit was found to be in T₆ (washing with 8% ginger solution) by washing the leaf samples of test crop and reduction % of heavy metals were found to be highest than any other treatments. Elevated levels of heavy metal in soils may lead to their uptake by plants, which depends not only on heavy metal content in soils but is also determined by soil pH, metal content in soil, organic matter and clay contents and fertilizers. These parameters cannot change the total amount of heavy metals but can significantly affect the bio available part. The highest proportion of As reduction was 27.84% in spinach. The accumulation pattern of As in cauliflower, cabbage and spinach (leafy vegetables) was high and it might be due to the large surface area of their leaves, their high transpiration and faster growth rate, which enhances the metal translocation in leafy vegetables sensitizes them to be recipient of dust and rain water splashes Luo et al. [6]. The significant reduction % of As was highest for the plant sample washed with 8% ginger solution was chiefly because of presence of gingerol in ginger solution which exhibit metal chelating properties sattar et al. [7]. Masuda et al. [8] analyzed an antioxidant activity of gingerol-related compounds isolated from the dichloromethane extract of the ginger rhizomes. Gingerols, shogaols, gingerdiols, gingerdiones, and dehydrogingerdiones (with an alkyl group bearing 10-, 12-, or 14-carbon chain length) showed antioxidant activity(Masuda, [8]. Their results suggested that the substituents on the alkyl chain might contribute to both radical scavenging effect and inhibitory effect of autoxidation of oils. Stoilova et al. [9] evaluated the antioxidant effect of ginger and its CO₂ extract. So due to the presence of these superior possessions in ginger emphasis that with the increment in the concentration of ginger solution, there was increment in the percent reduction of heavy metal in various leafy vegetables. In perpetuation of leafy vegetables, several mechanisms have been used to reduce the As load by establish the scavenging species pledge peroxidation, chelating metal ions able to impotent to generate reactive species or decompose peroxides, flouting the auto-oxidative chain reaction and reducing localized O₂ concentrations. Asimi et al. [10] reported that the reduction of heavy metals may be due to several mechanisms like interaction of scavenging species that initiate peroxidation, chelating metal

ions so that they are unable to generate reactive species or decompose peroxides, reducing localized O₂ concentrations and breaking the auto-oxidative chain reaction. Their result suggested that the substituent present on the alkyl chain might contribute to both radical scavenging effect and inhibitory effect of autoxidation of oils.

5. CONCLUSION

Reduction of Arsenic concentration from the leaves of cabbage, spinach and cauliflower was done by using different washing techniques and it was observed that maximum percentage reduction of As in leafy vegetables was obtained in treatment T₆ (washing the leaves with 8% ginger solution). So due to the presence of some special properties in ginger we can conclude that with the increment in the concentration of ginger solution there was increment in the percent reduction of arsenic in different leafy vegetables. Hence washing the vegetable leaves with 8% ginger solution was observed better in the reduction of arsenic concentration among all other treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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