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Evaluation of consumability potentials of leafy vegetables harvested from selenium rich soil

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Abstract. To study the effect of selenium uptake on consumability of two leafy vegetables, spinach and mustard, pot experiment was conducted. Earthen pots with sealed holes were filled with garden soil amended with sodium selenite to make the final concentration of Se as 1, 5, 10, 20 and 25 ppm in soil. Seeds of both vegetables were sown to raise the plants. Plants raised on garden soil alone served as the control. Whole experiment was performed in triplicate. It was observed that in both the vegetables Se uptake was more in roots than leaves and the amount of Se accumulated by the plants increased with increase of Se concentration in the soil. The quantity of Se accumulated by leaves of both the vegetables was more than the recommended dose from point of human safety. Leaves of both crops harvested from plants from se rich soils had higher carbohydrate content than the respective controls. The Se uptake adversely affected the dietary parameters like total proteins, vitamin C, crude fibre content, etc. of both the vegetables. Leaf homogenates of both the vegetables were subjected to Ames assay using TA 98 and TA 100 strains of *Salmonella typhimurium* to study the acquired mutagenicity, if any, due to Se in the leaves. No mutagenic response was detected. However, the leaves of mustard plants raised over Se rich soils showed a medium to high anti-mutagenic activity against sodium azide induced mutations.

Key words : Ames assay, Selenium, Mutagenicity, Food quality, Mustard, Spinach

1. Introduction

Selenium is a unique element. It is essential element for animals and human and plays an important role in many enzymatic reactions. Disorders like white muscles disease in cattle, exudative diathesis of poultry, infertility of sheep, etc. are associated with selenium deficiency (Grant,1965). On the other hand animals fed on diet containing more than 5 ppm of selenium (Se) show typical symptoms of Se toxicity disease selenosis or alkali disease (Singh and Kumar, 1976; Dhillon and Dhillon, 1991). Selenium has been reported to show genotoxic properties depending upon concentration and its chemical form (Noda *et al.,* 1979, Shamberger 1985). It has also been reported as anti-mutagenic and anti-carcinogenic (Jacobs *et al.,* 1977; Martin *et al.,* 1981; El-Bayoumy, 1985).

Plants can accumulate good amount of Se in their tissues though it is not required for their metabolism. Food plants are important source of its entry into animals and human beings but its level in crops is critical for animal as well as human health. Since there is no specific information available regarding status of nutritive constituents and genotoxic hazards of the food plants grown over Se rich soil, the present investigation was undertaken. Two common leafy vegetables, mustard and spinach, were studied. Both are the important ingredients of a popular Indian dish (*sarson da saag*) cherished by Punjabi community throughout the world. It is intended to evaluate the impact of Se accumulation on consumability potentials especially metal load and genotoxic consequences.

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2. Material and Methods

Presently, pot experiment was carried out at Botanic Gardens, Punjabi University, Patiala, Punjab India, to study the effect of selenium uptake on two leafy vegetables, namely, mustard (*Brassica campestris* cv RL 1359) and spinach (*Spinacea oleracea* Linn. Cv Poona spinach) The seeds of the crops were procured from authorized seed shop of Punjab Agricultural University, Ludhiana, Punjab, India. For the experiments fresh earthen pots of uniform size (25 cm diameter) with sealed drainage were selected. The soil for the pots was collected from the conservatory area of the Botanic gardens which has no record of irrigation and use of agrochemicals. The soil of the pots was amended with different concentrations of sodium selenite to make the final concentration of 1, 5, 10, 20 and 25 ppm of selenium. Plants raised in pots containing un-amended garden soil served as the control.

2.1. Estimation of selenium accumulation

The plant samples from the control as well as the treated pots were randomly selected and washed first with tap water and then with double distilled water. For the estimation of total selenium accumulation the leaves were digested with a mixture of nitric acid and perchloric acid as suggested by Sokkaro and Ohn (1977). From these acid digests selenium was determined using Hydride Generation Atom Absorption spectroscopy method given by Clinton (1977).

2.2. Biochemical estimation

To study the impact of selenium accumulation in the leaves of both the vegetables on their food quality samples were collected at edible stage i.e. 45 days old plants. The fresh leaves of both the vegetables were washed thoroughly to remove all traces of soil particles and were used for estimation of various nutritive constituents like total soluble proteins (Lowry *et al.*, 1951), carbohydrates (Ashwell, 1957), Vitamin C (Chinoy *et al.*, 1976) and crude fiber content (AOAC, 1980).

2.3. Mutagenicity testing

The Ames Salmonella plate incorporation assay (Maron and Ames, 1983) was use to study the mutagenic potential of water extract (homogenate) of leaves of mustard and spinach plants raised on selenium amended soil. Two histidine deficient (His^-) tester strains of *Salmonella typhimurium*, TA98 and TA100, used in the present study were obtained from MTCC, Institute of Microbial Technology, Chandigarh, India.

Water extract of the leaves were made by homogenizing two grams of fresh leaves in 10 ml of distilled water, kept overnight (24 h) and centrifuged at 6000 rpm for 30 min. The supernatant was made 20 ml by addition of more distilled water. The autoclaved supernatant was used as plant extract for genotoxic screening.

To check the mutagenic potential of the leaf extracts of plants raised on selenium contaminated soil, 0.1ml of the leaf extract was added to test tubes (in triplicate) containing 0.1 ml of bacterial culture (TA98 or TA100) and 2 ml of soft agar supplemented with histidine and biotin. Then the mixture was quickly inverted over minimal agar plates which were incubated at $37\ ^0$ C in an inverted position for 48 h and number of His+ revertant colonies was determined. The mean of three plates was taken in each case.

The acquired anti-mutagenic potential by the plants grown over Se rich soil was evaluated by performing two types of experiments. In the first experiment i.e. co-incubation experiment, to a sterile test tube containing 2 ml of soft agar, 0.1 ml of bacterial culture, 0.1 ml of direct acting mutagen (0.1% 4-nitro-o-phenylenediamine or 0.02% sodium azide) and 0.1 ml of the leaf extract were added in same order. From this mixture 100 µl of the mixture was spotted on minimal agar plates and number of revertant colonies were counted. During pre-incubation experiment to a sterile test tube 0.1 ml of test mutagen and 0.1 ml of the root extract were added and mixture was incubated at 37^0 C for 30 min. After incubation 2 ml of molten agar was added to the test tube along with 0.1 ml of bacterial culture and mixture was spotted on minimal agar plates. Similar experiments were performed for all the leaf extracts belonging to different treatment sets. Direct acting mutagens 4-nitro-o-phenylenediamine (NPD) and sodium azide (SA) were used as positive controls for TA98 and TA100 strains, respectively. Anti-mutagenic activity (inhibitory activity of the plant exrtact) was calculated following Saggoo and Kour (2003).

3. Results and Discussion

3.1. Accumulation of Selenium

The leafy vegetables, mustard and spinach, raised over control soil showed no accumulation of Se in their leaves or shoots. In plants collected from Se amended soil there was a clear dose depended increase in Se contents (Table 1). Mustard tops showed 2.0 to 31.6 ppm dry weight of Se accumulation with maximum in the plants of 25 ppm pots. Similar trend was observed in the spinach tops from Se rich soils. Here Se ranged between 5.1 to 32.6 ppm dry on weight bases. However, level of accumulated selenium in this study was much lower than reported earlier. Hurd-Karrer (1935) reported on DW basis 715 ppm Se in leaves and 250 ppm Se in stem when 4 ppm of Se was added to soil. Upon addition of 20 ppm of Se in soil, Hamilton and Beath (1963) also observed much higher accumulation of Se in rape plants (84.6 ppm) than the present findings. Terry *et al.* (1992), Lauchi (1993) and Banuelos and Flohe (2001) reported Se volatilization by plants and crucifers are considered as good volatilizers of Se into atmosphere. Loss of some quantity of selenium due to volatilization might have contributed towards low Se

Table 1. Accumulation of Se in plants of mustard and spinach raised in selenium amended soils**.**

Se added to soil	mustard		spinach	
	Root	Tops	Root	Top
Control	ND	ND	ND	ND
1 ppm	3.30	2.00	5.00	5.10
5 ppm	7.50	11.00	15.60	14.60
10 ppm	20.10	16.90	26.80	27.40
20 ppm	31.00	27.80	35.00	31.30
25 ppm	38.80	31.60	41.90	32.60
r	0.955	0.986	0.903	0.993

ND : Not detected
r : Correlation coefficient between ppm of Se added to soil and Se accumulated in plant
All values significant at 0.01 level

content in presently studied crucifers. Mustard samples from different parts of Punjab showed, however, only 0.11 to 1.40 ppm Se (Dhillon *et al.,* 1977).

In both the presently studied plants the roots showed more Se content than in the tops and are positively and significantly correlated with Se in soil (Fig. 1). Earlier, in *Brassica* raised over nutrient solution with 0-9 mg/l of Na_2SeO_4 , Kospell and Randle (1999) recorded the Se accumulation in order of leaf>root>stem.

3.2. Effect of Se on Nutritive Constituents

Plants can take up heavy metals besides other elements from the soil. This can disturb the plant metabolism effecting quantity and quality of various storage products and metabolites. This aspect of selenium toxicity was studied by evaluating the some of the nutrient components like total protein, carbohydrate, vitamin C and crude fibres, and acquired toxicity and mutagenicity in edible parts of plants.

***Total soluble proteins* :** From mature leaves total soluble protein were estimated using Lowry's (1951) method. Mustard and spinach leaves from the control plants showed the presence of 9.52 and 9.04 mg/g FW of total soluble protein, respectively (Table 2,3). Spinach grown on soil amended with Se exhibited decrease in the total soluble

proteins from 6.28 to 3.33 mg/g FW in a dose dependent manner. Similar trend was seen in case of mustard plants. The plants from 25 ppm of Se in soil showed minimum amount of soluble proteins. Similar trend of dose dependent decrease in protein content has been reported in wheat (Singh and Singh, 1978) and celery (Lee *et al*. 1999). Presence of Se in soil showed no effect on amount of protein in turnip and radish roots (Saggoo and Kour, 2003). Singh and Singh (1978) correlated reduced protein content with non-availability of phosphorous (P) while Peterson and Butler (1962) attributed lower protein content in presence of Se to the fact that Se decreased N content and also checks the incorporation of amino acids into proteins. In the leaves of mustard and spinach a significant and negative correlation was observed between Se in soil and protein contents and Se content in the edible part and the total soluble proteins.

***Carbohydrate content* :** The amendment of soil by Se was found to increase the carbohydrate content in the leaves of both the plants. Maximum carbohydrate content was observed in spinach as well as mustard leaves from pots with 10 ppm of Se (7.50mg/g and 13.80mg/g FW, respectively). The carbohydrate content decreased with the further increase in concentration of Se in soil. The effect of selenium on carbohydrate content has not been a favourable topic with earlier workers. Selenium was reported to enhanced the sugars in leaves and stem of lettuce cultivars (Shang *et al.,* 1998).Correlation analysis between carbohydrate content and ppm of Se added to soil and accumulated in plants depict a negative correlation.

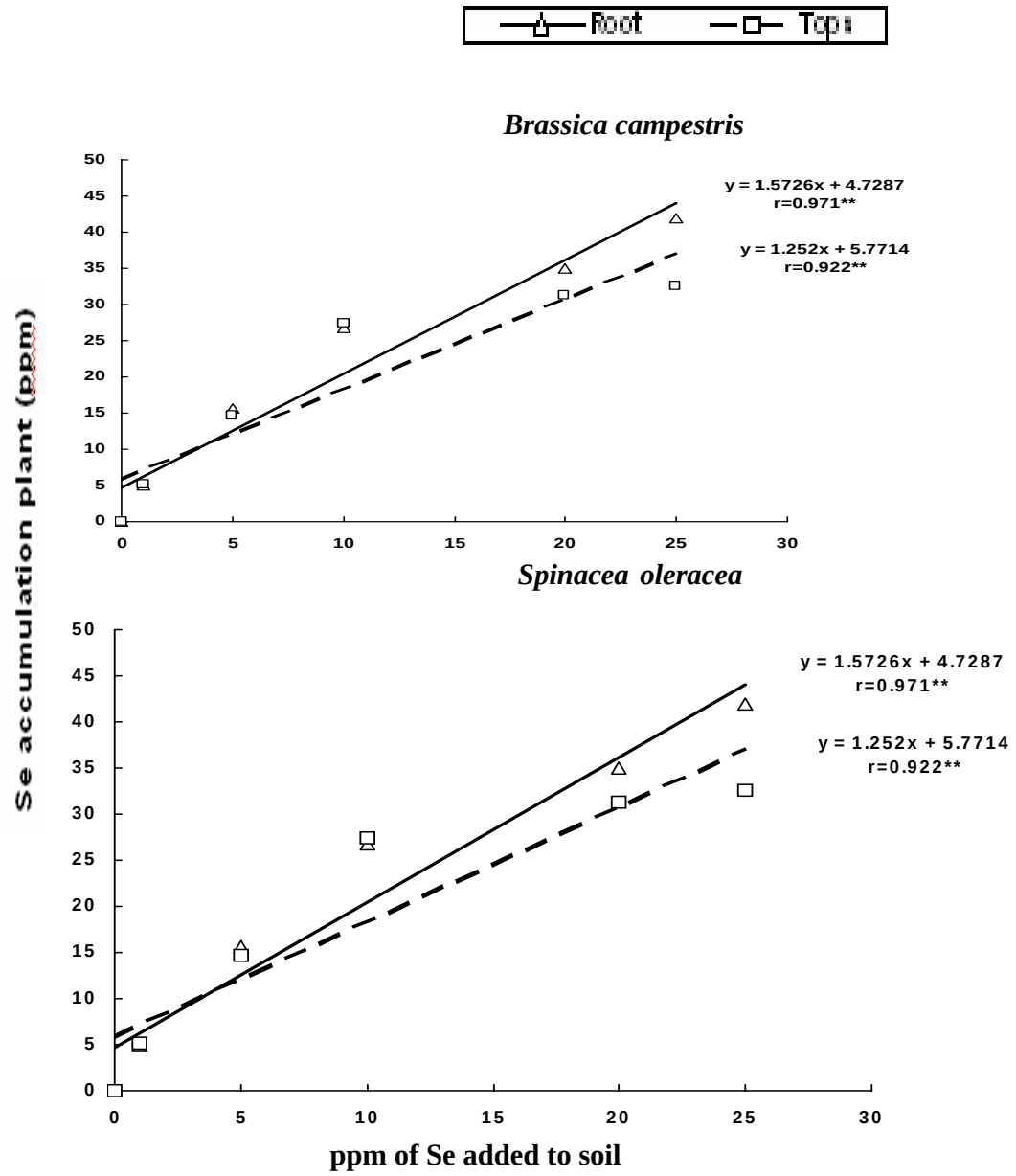

Figure 1 Metal accumulation in leaf crops and correlation coefficient between ppm of selenium added to soil and selenium accumulated in leaves raised on selenium amended soils.

Table 2 Data on nutritive constituents of leaves of mustard plants raised on soil amended with different concentrations of selenium.

Treatment	Total soluble proteins (mg/g FW)	Carbohydrate (mg/g FW)	Vitamin C (mg/g FW)	Crude fiber content (%)
Control	9.52	10.50	0.28	27.39
1 ppm	9.35	10.80	0.36	22.60
5 ppm	9.04	12.00	0.39	19.73
10 ppm	8.66	13.80	0.44	17.85
20 ppm	7.14	10.95	0.50	10.14
25 ppm	5.71	09.00	0.53	08.60
r1	-0.978**	-0.348	0.951**	-0.974**
r2	-0.940**	-0.212	0.942**	-0.983**

r1: Correlation coefficient between ppm of Se added to soil and quantity of nutritive constituent
r2: Correlation coefficient between ppm of Se accumulated and quantity of nutritive constituent
** values significant at 0.01 level

***Vitamin C content* :** The mature leaves of the control mustard plants showed 0.28 mg/g FW of vitamin C (Table 2). Increase in the Se in soil resulted in an increase in amount of vitamin C content in the leaves. A maximum of 0.53 mg/g FW vitamin C was estimated in the leaves of 25 ppm plants. In the spinach leaves it first increased with increase in concentration of Se in soil from 0.19 mg/g FW in control to 0.39 mg/g FW in pots with 20 ppm of Se and then decreased in 25 ppm (Table 3). Present findings are in line with earlier studies. Lee *et al.* (1999) and Shang *et al.* (1998) noticed increase in total vitamin C with increase in concentration of selenium in celery and lettuce cultivars. Statistical analysis exhibit a positive correlation between vitamin C and ppm of Se added to soil as well as Se accumulated in the edible portion of the plant.

***Crude fibre content* :** Mustard leaves showed maximum crude fibres in the control leaves (27.39%) while all the treatments showed decrease in percentage of crude fibre content in a dose dependent manner (Table 2). In spinach the per cent of crude fibre content in the control as well as plants grown over 1 to 5 ppm of Se in soil was almost same. The plants from higher concentration of Se in soil possessed reduction in fibre content (Table 3).

Limited efforts have been made by earlier workers to study the effect of Se on fibre content. In lettuce cultivars, crude fibre content decreased in the presence of selenium (Shang *et al.*, 1998).

3.3. Acquired Genotoxic Potentials due to Se uptake

Studies conducted by earlier workers like Nakamuro *et al.* (1976), Noda *et al.* (1979), Shamberger (1985), etc. revealed that Se is a week mutagen. At the same time it has been also established that selenium salts possess anti-mutagenic potentials against certain established mutagens (Jacobs *et al.* 1977, Martin *et al.* 1981, El-Bayoumy 1985). In order to check whether the leaves of mustard and spinach have acquired any mutagenic potential due to accumulation of mutagenic selenium, Ames Salmonella assay was conducted. The aqueous leaf extracts of mustard leaves of the control plants (PE_0) and the plants from Se rich soil (PE_1-PE_{25}) were not mutagenic to both the histidine deficient strains of *Salmonella typhimurium* (TA98, TA100). The increase in the number of his^+ revertants than the number of spontaneous revertants was not significant statistically (Table 4). The leaf extract of spinach plants collected from the control as well as selenium amended soils also exhibited no mutagenic potentials in Ames

assay (Table 5). This is in contrary to observation by Babish *et al.* (1979), Boyd *et al.* (1982) and Hopke *et al.* (1984) that mutagens are translocated by plants from soil.

Table 3 Data on nutritive constituents of leaves of spinach plants raised on soil amended with different concentrations of selenium.

Treatment	Total soluble proteins (mg/g FW)	Carbohydrate content (mg/g FW)	Vitamin C (mg/g FW)	Crude fiber content (%)
Control	9.04	4.50	0.19	36.82
1 ppm	6.28	4.65	0.23	37.98
5 ppm	6.16	6.75	0.23	37.02
10 ppm	5.80	7.50	0.26	36.26
20 ppm	5.23	5.25	0.39	35.54
25 ppm	3.33	4.65	0.20	33.89
r1	-0.855*	-0.059	0.395	0.927**
r2	-0.850*	0.311	0.511	-0.799

r1: Correlation coefficient between ppm of Se added to soil and quantity of nutritive constituent
r2: Correlation coefficient between ppm of Se accumulated and quantity of nutritive constituent
* values significant at 0.05 level
** values significant at 0.01 level

Table 4 Genotoxic response of leaf extracts of mustard plants raised over soil amended with selenium in Ames assay.

Dose Treatment	His$^+$ Revertants			
	TA 98	Per cent Inhibition	TA 100	Per cent Inhibition
Spontaneous (0.1ml)	51.00		132.66	
0.02% NaN_3 (0.1ml)	—		354.15	
0.1% NPD (0.1ml)	159.00		—	
Plant Extract Direct				
PE_0	58.04		150.34	
PE_1	60.85		156.82	
PE_5	61.07		161.01	
PE_{10}	63.23		167.97	
PE_{20}	69.37		169.36	
PE_{25}	72.11		173.72	
Extract Co-incubated with mutagen*				
PE_0	110.29	48.24	320.10	16.75
PE_1	111.45	48.44	317.47	18.58
PE_5	110.18	49.85	321.03	17.14
PE_{10}	121.10	39.57	323.65	16.36
PE_{20}	126.89	35.82	326.54	14.94
PE_{25}	133.00	29.92	331.51	12.54
Extract Pre-incubated with mutagen*				
PE_0	109.33	49.19	298.41	27.34
PE_1	98.53	61.60	214.42	70.81
PE_5	101.96	58.24	211.95	73.62
PE_{10}	108.68	52.54	206.84	79.03
PE_{20}	114.09	50.10	202.77	81.92
PE_{25}	115.22	50.38	195.29	88.04

* NaN_3 in TA 100 and NPD in TA 98

PE_0 : Mustard extracts of control plants.

PE_1- PE_{25} Mustard extracts of plants from 1ppm–25ppm of Se in soil

Table 5 Genotoxic response of leaf extracts of spinach plants raised over soil amended with selenium in Ames assay.

Dose Treatment	His$^+$ Revertants			
	TA 98	Per cent Inhibition	TA 100	Per cent Inhibition
Spontaneous (0.1ml)	57.00		137.81	
0.02% NaN_3 (0.1ml)	—		350.83	
0.1% NPD (0.1ml)	170.24		—	
Plant Extract Direct				
PE_0	60.86		150.34	
PE_1	66.75		157.82	
PE_5	71.32		166.29	
PE_{10}	76.85		171.76	
PE_{20}	72.15		168.15	
PE_{25}	69.63		172.48	
Extract Co-incubated with mutagen*				
PE_0	165.54	4.29	332.83	8.97
PE_1	156.76	13.02	321.67	15.10
PE_5	148.56	21.91	310.89	21.64
PE_{10}	143.56	28.56	299.52	28.65
PE_{20}	139.67	31.16	287.56	34.63
PE_{25}	130.92	39.08	281.27	39.00
Extract Pre-incubated with mutagen*				
PE_0	160.00	9.36	323.44	13.63
PE_1	152.89	16.76	294.81	29.02
PE_5	146.25	24.25	286.24	35.00
PE_{10}	136.82	35.78	273.61	43.12
PE_{20}	124.12	47.01	260.33	49.54
PE_{25}	118.63	51.29	251.76	55.54

* NaN_3 in TA100 and NPD in TA 98

PE_0 : Spinach extracts of control plants.

PE_1- PE_{25} : Spinach extracts of plants from 1ppm–25ppm of Se in soil.

When the leaf extracts of mustard or spinach were co-incubated with mutagens (4-nitro-*o*-phenylene diamine and sodium azide) none of the extracts from Se rich plants exhibited antimutagenic activity. Selenium salts have been reported to be anti-mutagenic (Jacobs *et al*., 1977).

3.4. Accumulation vs Metal Load

Selenium is an essential trace element for animals and human (Bennett, 1982; Robinson, 1982; WHO, 1984). The most persuasive evidence for essentiality in humans comes from the association of Keshan disease (a juvenile cardiomyopathy) with selenium deficiency in diet (Chen, 1980; Xu *et al.*, 1985). On the other hand high level of Se

in diet is toxic and cause selenosis. The concentration range from trace element requirement to lethality is quite marrow (Wilber, 1983). The minimum nutritional level of Se for animal is 0.05 to 1.0 mg Se/ kg dry forage feed and exposure to levels of 2-5 mg Se/ kg dry forage will lead to toxic problems in animals (Wu *et al.*, 1996). On the basis of various health considerable the U.S. National Academy of Sciences has recommended a safe and adequate range of 0.05 to 0.2 mg per person per day for adults.

4. Conclusion

The present investigations revealed that the leaves of mustard and spinach from Se amended soil had accumulated between 2.00 to 32.60 ppm on dry wt bases. This amount of Se accumulation is definitely on the risk side. The lack of direct mutagenicity in the leaf extracts of the plants from Se rich soil is a good signal for the consumers but the non-mutagenic results may have been obtained due several factors. Selenium metallo-protein or organo-metallic metabolites present in plants, if insoluble in water, cannot give positive mutagenic response. The food quality also deteriorates in plants from Se contaminated soil as is evident from present study. It may be concluded that the consumption of leafy vegetables grown over selenium rich soil is not safe. But careful use of such plants for Se supplementation of fodder in Se deficient areas could not be ruled out.

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