Environmental Informatics Archives, Volume 1 (2003), 606-613
EIA03-063
ISEIS Publication #002
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Uptake of Applied Selenium by Turnip and Radish and Its Influence on their Consumability Potentials

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Abstract. To study the influence of the accumulation of selenium (Se) on the food quality of root vegetables, radish and turnip, pot experiment was performed. The plants were raised from seeds in pots containing garden soil amended with sodium selenite to make the final concentration of Se as 1,5,10.20 and 25 ppm in soil. Plants raised on garden soil alone served as control. In both of the plants Se uptake was more in the roots than the tops when grown on pots having 1-20 ppm Se in soil. At higher concentration of Se there was more accumulation of Se in the tops than the roots. The quantity of Se accumulated in the edible parts of both the plants is definitely on the risk side. The effect of metal uptake on various nutrient components like total protein, carbohydrate, vitamin C and crude fiber content in the edible parts was also studied. The roots showed a dose dependent increase in the carbohydrate content and decrease in crude fiber. There was no much influence on protein and vitamin C content. Selenium is reported to be a week mutagenic agent. The acquired mutagenicity due to accumulation of selenium by the root vegetables was evaluated by performing Ames *Salmonella* assay. The water extracts of roots of turnip and radish showed no mutagenic response in TA 98 and TA 100 strains of *S.typhimurium*`.

Keywords: Ames assay, Anti-mutagenicity, Selenium, Mutagenicity, Food quality, Radish, Turnip

1. Introduction

Food represents an important pathway for the movement of various elements from soil to human beings. Contaminant metal/metalloids can often accumulate in considerable amounts in plant tissues and exceed the level that is toxic to human and animal system.

Many workers had focused their attention on selenium (Se) toxicity via food chain (Grant, 1965; Smith and Watkinson, 1984; Carter *et al.*, 1968). Cattle and buffaloes feeding on plants growing in Se rich soil of India exhibited symptoms of selenosis (Singh and Kumar, 1976; Dhillon and Dhillon, 1991). Selenium has been reported to show mutagenic properties depending upon concentration and its chemical form (Noda *et al.*, 1979, Shamberger 1985). Selenium on the other hand is absolutely necessary for human and animal health (ATSDR, 1996). It has also been reported as anti-mutagenic and anti-carcinogenic (Jacobs *et al.*, 1977; Martin *et al.*, 1981; El-Bayoumy, 1985).

A toxic substance absorbed and stored in a plant or plant part may make that plant or plant part unsafe for consumption. The transference of genotoxicity through this route i.e. by consumption of plants which bioconcentrate genotoxic heavy metals and other mutagens have attracted little attention (Boyd *et al.*, 1982; Sterner *et al.*, 1982; Fiedler *et al.*, 1991). Selenium is readily absorbed by plants. It is not essential element for growth of plants but its level in crops is critical for animal as well as animal health. Though there are some reports of less incidence of cancer in animals and human exposed to selenium rich diet. No attempt has been made to practically examine the mutagenic or anti-mutagenic potentials of crops grown over selenium rich soils. Keeping this lacuna in view the

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present problem was taken up to know selenium uptake capabilities of common root vegetables turnip and radish, and also to evaluate effects of metal uptake on consumability especially genotoxic consequences in the consumers.

2. Material and Methods

To study the effect of selenium uptake on two root vegetables, namely, radish (*Raphanus sativus* cv. Punjab safed) and turnip (*Brassica rapa* cv. L-1), pot experiment was carried out at Botanic Gardens, Punjabi University, Patiala. The seeds of the crops were procured from authorized seed shop of Punjab Agricultural University, Ludhiana, Punjab, India. Fresh earthen pots of one design and uniform size (25 cm diameter) with sealed drainage were selected. 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 garden soil alone served as the control.

2.1. Biochemical estimation

To study the influence of selenium on food quality of both the root vegetables, plants were uprooted at edible stage i.e. 45 days old plants. The fresh roots of both the vegetables were washed thoroughly to remove all traces of soil particles. The fresh plant material was used for estimation of total soluble proteins (Lowry *et al.*, 1951), carbohydrates (Ashwell, 1957), Vitamin C (Chinoy *et al.*, 1976) and crude fiber content (AOAC, 1980).

2.2. Estimation of selenium accumulation

For the estimation of total selenium accumulation in the roots the samples were digested as suggested by Sokkaro and Ohn (1977). The well washed roots were oven dried at 55 ± 5^0 C for 24 h, ground and kept in air tight containers. The powdered samples were digested in conical flasks covered with water coolers in a mixture of nitric acid and perchloric acid. From these acid digests selenium was determined using Hydride Generation Atom Absorption spectroscopy method given by Clinton (1977).

2.3. Mutagenicity testing

The mutagenic potential of water extract (homogenate) of roots of radish and turnip raised on selenium amended soil were tested using *Salmonella* based Ames assay. Water extract of the roots were made by homogenizing two grams of fresh roots 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. Similarly the extracts were made from radish and turnip roots representing all the treatment sets i.e. control as well as containing selenium amended soils

Two histidine deficient (His^-) tester strains of *Salmonella typhimurium*, TA98 and TA100, used in the present study were obtained from Institute of Microbial Technology, Chandigarh, India. The plate incorporation assay as proposed by Maron and Ames (1983) was used. To check the mutagenic potential of the root extracts of plants raised on selenium contaminated soil, the root extract (0.1 ml) 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. It 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.

Each set of root extract was also further tested for anti-mutagenic potential by performing two types of experiments. For a 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 root extract were added in same order. From the mixture 100 ul of the mixture was spotted on minimal agar plates and number of revertant colonies were counted. For pre-incubation experiment in 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 root 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) was calculated as follows:

Inhibitory activity (%) = (a-b/a-c) x 100

Where a = number of revertants induced by mutagen

b = number of revertants induced by mutagen in presence of root extract

c = number of revertants induced by root extract alone

3. Results

3.1. Accumulation of selenium in plants

To study the effect of selenium accumulation on food quality of radish and turnip, pot experiment was performed. Both vegetables were grown in pots filled with garden soil amended with different concentrations of selenium salt. In radish roots from the control pots no uptake of Se was detected (Table 1). In the roots of plants raised over Se amended soil, a maximum of 40.00 ppm dry wt. of Se uptake was observed in plants from 25 ppm set. The plants up to 20 ppm of Se in the soil showed more accumulation of Se in the roots than the tops but at higher concentration of Se in soil (25 ppm) there was more accumulation in the tops.

In turnip plants also the Se accumulation showed the same trend as observed for radish i.e. the plants obtained from 1-20 ppm of Se in soil showed more uptake of Se in the roots than the shoots (Table 1). Turnip roots harvested from pots with 25 ppm of selenium showed maximum accumulation of selenium.

Table 1 Accumulation of Se in plants of radish and turnip raised in selenium amended soils**.**

Se added to soil	Radish		Turnip	
	Root	Tops	Root	Top
Control	ND	ND	ND	ND
1 ppm	6.80	4.20	6.70	4.00
5 ppm	15.80	11.60	11.80	9.80
10 ppm	28.70	23.50	15.00	15.80
20 ppm	35.00	31.70	40.00	26.60
25 ppm	40.00	43.50	27.80	37.60
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

3.2. Biochemical estimates

Roots of both the plants are the edible parts. Therefore different biochemical parameters have been studied from the roots of various plants raised over Se amended soils.

Total soluble proteins: The roots of radish and turnip have low protein content (Tables 2, and 3). Interestingly, the amount of total soluble proteins is same in both the crops, control as well from Se amended pots.

Table 2 Data on biochemical estimates of roots of radish 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	0.47	3.30	0.09	25.53
1 ppm	0.47	4.50	0.09	25.03

5 ppm	0.47	5.25	0.09	25.16
10 ppm	0.47	6.00	0.09	18.61
20 ppm	0.47	5.10	0.14	14.30
25 ppm	0.43	4.50	0.09	14.27

Table 3 Data on biochemical estimates of roots of turnip 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	0.47	1.05	0.03	32.42
1 ppm	0.47	1.35	0.03	05.36
5 ppm	0.47	1.80	0.03	24.27
10 ppm	0.47	2.25	0.03	22.47
20 ppm	0.47	2.70	0.04	16.86
25 ppm	0.45	1.50	0.04	11.28

Carbohydrate content: The carbohydrate content in the turnip roots (mg/g FW) are presented in Table 3. Maximum amount of carbohydrate was estimated in the roots of turnip plants obtained from 20 ppm of Se in the soil. There was dose dependant increase in the carbohydrate content with increase in the selenium content of the soil except in case of 25 ppm of Se in soil. A similar tend in carbohydrate content in radish roots has been observed. It increased from 3.30 mg/g FW in the control to 6.0 mg/g FW in roots obtained from plants grown over soil with 10 ppm of Se. Further increase in the soil selenium decreased the carbohydrate content in the roots.

Vitamin C: Presence or absence of selenium in soil had no significant effect on the vitamin C content in turnip as well radish roots (Tables 2, 3).

Crude fiber content: The data regarding the crude fiber content of both the control and treated roots are presented in Tables 2 and 3. The roots from the control plants of radish and turnip contained maximum amount of fiber i.e. 25.53% and 32.42%, respectively. When the selenium content was increased in the soil a decrease in the fibred content was observed in both the crops.

3.3. Mutagenicity of the root extracts

The extracts of the radish roots of the control (PE_0) and of plants from Se amended soils (PE_1 – PE_{25}) do not show mutagenicity when tested with TA98 and TA100 histidine deficient strains of *Salmonella* (Table 4). From data given in the table 4 it is evident that root extracts has no significant modulatory effect on NPD or SA induced His^+ revertants in co-incubation experiment. On the other hand PE_{20} and PE_{25} extracts inhibited the NPD induced revertant colonies in pre-incubation by 53.38 % and 58.6 %, respectively.

The root extracts of turnip also exhibited similar tend as shown by radish (Table 5). However, the root extracts, PE_5 to PE_{25}, inhibited the SA induced revertant colonies by 55.09 % to 65.38 % in pre-incubation experiment. The PE_{25} alone was able to induce more than 50 % inhibition in NPD induced revertants.

4. Discussion

Selenium uptake and storage in crop of plants is a potential health hazard because consumption of these plants can exert toxic effects on the consumers. In the presently investigated root crops the accumulation of Se ranged between 6.70 ppm to 40.0 ppm in their roots. A positive and significant correlation exists between Se added to the soil and

taken up by radish and turnip plants. These findings are in line with observations of earlier workers (Tripathi and Misra, 1974). In both the vegetables the Se content was more in the roots than the shoots. However, Fleming (1962) and Dhillon and Dhillon (1991) reported less Se in the roots than leaves of these crops.

Heavy metals are known to disturb the physiological processes of plants affecting quantity and quality of various metabolites. In the presently investigated root vegetables there is practically no effect of Se uptake on protein content and vitamin C content. Selenium has been reported to cause toxic effect on protein contents in other plants (Singh and Singh, 1978; Lee *et al.,* 1999). In cultivars of celery and lettuce Shang *et al.* (1998) and Lee *et al.* (1999) noticed increase in total vitamin C with increase in Se uptake. The present observation about increase in the

Table 4 Genotoxic response of root extracts of radish 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)		56.44	144.08	
0.02% NaN_3 (0.1ml)		—	356.82	
0.1% NPD (0.1ml)		183.25	—	
Plant Extract Direct				
PE_0		54.81	146.78	
PE_1		50.64	160.35	
PE_5		67.24	168.62	
PE_{10}		69.02	179.93	
PE_{20}		65.53	172.84	
PE_{25}		61.45	164.12	
Extract Co-incubated with mutagen*				
PE_0	172.41	8.43	344.56	5.83
PE_1	169.59	10.30	327.18	15.08
PE_5	158.33	21.48	320.37	19.36
PE_{10}	152.00	27.35	311.48	25.63
PE_{20}	146.45	31.26	301.62	30.00
PE_{25}	140.23	35.32	290.23	34.50
Extract Pre-incubated with mutagen*				
PE_0	167.97	11.89	338.32	8.80
PE_1	142.81	30.49	300.41	28.71
PE_5	135.13	41.47	293.53	33.62
PE_{10}	128.03	48.34	281.38	42.64
PE_{20}	120.40	53.38	277.76	42.97
PE_{25}	111.86	58.60	262.92	48.72

* NaN_3 in TA100 and NPD in TA 98

PE_0 : Radish extracts of control plants.

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

Table 5 Genotoxic response of root extracts of turnip 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)	33.67		144.08	
0.02% NaN_3 (0.1ml)	—		356.82	
0.1% NPD (0.1ml)	147.86		—	
Plant Extract Direct				
PE_0	36.16		142.54	
PE_1	39.34		156.13	
PE_5	43.41		169.27	
PE_{10}	47.77		172.81	
PE_{20}	50.95		168.46	
PE_{25}	48.23		160.89	
Extract Co-incubated with mutagen*				
PE_0	133.85	12.54	329.66	12.67
PE_1	128.87	17.49	305.11	25.76
PE_5	123.38	23.43	294.53	33.21
PE_{10}	118.10	29.73	282.26	40.51
PE_{20}	111.00	38.03	271.84	45.11
PE_{25}	107.48	40.52	259.73	49.55
Extract Pre-incubated with mutagen*				
PE_0	127.52	18.20	291.00	18.44
PE_1	120.85	24.88	267.50	44.50
PE_5	116.38	30.13	253.49	55.09
PE_{10}	109.96	37.86	244.38	61.09
PE_{20}	103.28	46.00	237.75	63.21
PE_{25}	96.59	51.46	228.71	65.38

* NaN_3 in TA 100 and NPD in TA 98

PE_0 : Turnip extracts of control plants.

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

carbohydrate content and decrease in the crude fiber content in the roots of radish and turnip with increase in Se uptake confirms the earlier study by Shang *et al.* (1998).

Selenium has been tested for mutagenicity using different test systems. Studies conducted by Nakamuro *et al.* (1976), Noda *et al.* (1979), Shamberger (1985), etc. revealed that Se is a week mutagen. Studies 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 radish and turnip roots have acquired any mutagenic potential due to accumulation of mutagenic selenium, Ames Salmonella assay was conducted. The root extracts of both the vegetables showed no mutagenicity in TA98 and TA100 strains of the bacterium. Earlier, Fiedler *et al.* (1991) failed to observe passage of mutagens in alfa-alfa and bermuda grass grown on mutagen containing sewage sludge amended soil. However, Babish *et al.* (1979), Boyd *et al.* (1982) and Hopke *et al.* (1984) reported translocation of mutagens from soil by plants.

The root extracts were also tested for their potentials to cause prevention of mutagenicity of sodium azide and 4-nitro-o-phenylenediamine in TA100 and TA98 strains. A reduction in mutagenic response of both the mutagens has been observed during co-incubation and pre-incubation mixtures of mutagens and root extract. In both the root vegetables anti-mutagenic activity ranged between insignificant (<30%) to moderate (50-70%) activity as per criteria given by DeFlora and Ramel (1988).

Selenium has been proved to be an essential micronutrient (NAS, 1976; WHO, 1984). On the other hand high level of Se in diet is toxic. The concentration range from trace element requirement to toxicity is quite narrow (Wilber, 1983). On the basis of various health considerations 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 (USEPA, 1984). The minimum amount of Se in drinking water is 0.01 mg/L (WHO, 1984). The amount of Se accumulated in the roots of radish and turnip harvested presently from selenium rich soil is definitely on the risk side.

5. Conclusion

The lack of direct mutagenicity in the extracts of the roots is a good signal for the consumers but it may have resulted from several contributing factors. 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. From above it may be concluded that consumption of root vegetables from selenium rich soil is not safe.

Acknowledgement. The authors wish to thank Head, Department of Botany, Punjabi University, Patiala and University Grants Commission, New Delhi (DRS Project) for providing necessary facilities.

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