Selenium biofortification of rice and radish: effect of soil texture and efficiency of two extractants

K.F.M. Fernandes, R.S. Berton, A.R. Coscione

Agronomic Institute, Campinas, Brazil

ABSTRACT

The addition of essential elements to human health by mineral fertilization is considered a promising strategy for biofortification. A greenhouse experiment was carried out where amounts equivalent to 0.0; 0.5; 1.0 and 2.0 kg/ha of selenium (Se), as sodium selenite, were added to two soils with contrasting textures to evaluate the increase in Se concentration on the edible parts of rice (grain) and radish (roots) plants. Two extractors (KCl and KH_2PO_4) were also evaluated in their efficiency in predicting available Se to the two species. Total Se concentration in plants increased significantly with the amounts of Se added to both soils showing that selenite can be used for biofortification of these crops. Selenium availability was higher on sandy soil than on sandy clay soil. Se extraction with KCl presented better performance than KH_2PO_4 in predicting Se phytoavailability for rice and radish.

Keywords: selenite; clay content; human health; phytoavailability; micronutrient

Selenium (Se) is an essential element for humans and animals. It is a component of selenoproteins such as glutathione peroxidase which has an antioxidant function, protecting the cellular tissue against oxidation damages from free radicals and reactive oxygen species (Pedrero and Madrid 2009). Also, Se can significantly increase the nutritive value of plant tissues such as in potatoes tubes (Ježek et al. 2011) and in winter wheat grain (Ducsay et al. 2006). Selenium deficiency in human and animals is related to the increase in viral infection rates, thyroid dysfunction, cancer and cardiovascular diseases. Optimal cancer protection appears to require a supra-nutritional Se intake (Lyons et al. 2003).

Selenium concentration on food from vegetal origin consumed in Brazil is considered low, when compared to the international standards. It is believed that the determinant factor for this is the low Se concentration in agricultural soils (Ferreira et al. 2002).

Several factors control Se mobility in soils such as pH, clay content and type, oxi-redox potential and soil organic matter content (Gissel-Nielsen 2002, Cartes et al. 2005, Hlušek et al. 2005). Selenium availability increases with soil pH increase due to the predominance of SeO_4^{2-} instead of SeO_3^{2-} and also due to the increase of negative pH dependent charges. However, Se phytoavailability decreases on soils with high organic matter content (Eich-Greatorex et al. 2007) and when Fe oxi-hydroxi is present in the clay fraction due to the retention of SeO_4^{2-} and SeO_3^{2-} (Rajan and Watkinson 1979).

Brazilian research effort to increase Se concentration in edible portions of crop plants is still scarce. According to FAO, rice remains a major staple food for developing countries, where consumption is estimated at 67.6 kg per person in 2012/2013 (FAO 2013). Belonging to the Brassicaceae family, radish is not cropped in large areas but is important as cash crop. It is consumed in many countries usually as salad and when enriched with selenite may represent a good choice as an organoselenium supplement for the human diet and animal feed (Pedrero et al. 2006). Therefore one may ask what the effect of soil texture on Se biofortification of rice and radish will be when Se is supplied as selenite. Which extractor will be more efficient

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in predicting Se phytoavailability for these crops? How much will be the Se (IV) absorption efficiency for these crops?

This study aimed to increase the Se concentration in the edible portions of radish and rice in two soils with contrasting clay content and to evaluate the efficiency of KCl and KH_2PO_4 extracting solutions for predicting Se availability to plants.

MATERIAL AND METHODS

A greenhouse experiment was carried out from January to April 2010 at Campinas, São Paulo, Brazil (22°53'S; 47°03'W; 674 m a.s.l.).

Soil sampling and characterization. The surface (0–20 cm) samples of two soils with contrasting textures (sand: Hapludalf and sandy clay: Typic Haplustox) used in the greenhouse experiment, were collected at Pindorama and Campinas, SP, Brazil, respectively, and analyzed for soil fertility (Raij et al. 1986), 'free' iron and aluminum oxides (sodium citrate-bicarbonate-dithionite extraction), granulometric and bulk density analysis (Camargo et al. 1986) and total Se analysis (USEPA 3051, 1995). The soil characteristics are shown in Table 1.

Soil pH of sandy clay soil was adjusted to the same soil pH as that of the sandy soil (pH 5.1) by adding 2 g/pot of dolomitic limestone (Ca = 22.1%, Mg = 10.8%).

Experimental design. The treatments were arranged in a completely randomized factorial, with four replicates. Air-dried soil samples were sieved trough a 4-mm-mesh screen and portions of 3 dm^3 were transferred to plastic pots. Selenium was applied as sodium selenite (Na_2SeO_3 – Sigma-Aldrich, Dorset, UK) at rates (mg/kg): 0.0; 0.19; 0.38; 0.76 for sandy soil and 0.0; 0.24; 0.48; 0.96 for sandy clay soil, which are equivalent to 0.0; 0.5; 1.0 and 2.0 kg/ha. The crops selected were rice (*Oryza sativa* L. cv. IAC 202) and radish (*Raphanus sativus* L. cv. Saxa), totalizing 64 experimental units. After Se addition, the soils were fertilized (mg/pot) with: P: 600 (simple superphosphate); K: 600 and S: 245 (K_2SO_4); N: 40, Ca: 28.6 and Mg: 18.9 as $Ca(NO_3)_2$ and $Mg(NO_3)_2$; B: 3 (H_3BO_3), Cu: 6 ($CuSO_4{\cdot}5\,H_2O$), Zn: 12 ($ZnSO_4$), Fe: 18 (Fe-EDTA). Soil water was monitored daily by weighing the pots and adding deionized water to the soil surface up to 70% of the maximum soil water holding capacity.

For the rice trial each pot received 10 seeds and only four seedlings were left twenty days after emergence. During plant growth, each pot received 300 mg of S as $(NH_4)_2\,SO_4$ and a total of 500 mg of N, using a solution containing $(NH_4)_2\,SO_4$ (260 mg), $Ca(NO_3)_2{\cdot}4\,H_2O$ (120 mg), and $Mg(NO_3)_2{\cdot}6\,H_2O$ (120 mg). At 114 days after emergence, roots and shoots were harvested and seeds were separated from stem.

For the radish trial, each pot received 10 seeds and only three seedlings were left eight days after emergence. During plant growth each pot received a total of 500 mg N as NH_4NO_3. Shoots and roots were harvested at 30 days after emergence.

Table 1. Basic physico-chemical soil characteristics and total selenium content in the experimental soils

	Soil	
	sand	sand clay
Particle size distribution (%)		
Clay	4.4	35.9
Silt	5.7	4.5
Sand	89.9	59.6
Bulk density (g/dm^3)	1.32	1.04
CEC ($mmol_+/dm^3$)	38.5	57
pH ($CaCl_2$ 0.01 mol/L)	5.1	4.4
C_{org} (%)	1.16	1.57
Base saturation (%)	63	33
Available nutrients – Resin extraction (mg/kg)		
P	26.5	5.8
K	80.3	146
Ca	228	192
Mg	55	59
Micronutrients – DTPA extraction (mg/kg)		
B	0.13	0.18
Cu	0.3	1.1
Fe	10.6	52.9
Mn	13.5	1.44
Zn	1.21	0.4
Fe and Al oxides – CDB extraction (g/kg)		
Fe_2O_3	12.6	25.2
Al_2O_3	2.2	10.0
Se_{total} – USEPA 3051 (mg/kg)	0.24	0.32

CEC – cation exchange capacity

Analytical determinations. After harvesting, rice and radish parts were washed with tap water and oven-dried at 60°C to constant weight. In sequence, the shoot, roots and seeds dry matter weights were recorded, ground and saved for Se analysis.

The extraction procedure to predict phytoavailable selenium was performed by incubating both soils with the same amounts of Se used in the greenhouse experiment for 60 days in plastic containers of 200 mL. Soil water holding capacity was maintained at 70% as described earlier. The extraction methodology used well-mixed subsamples of 1 g air dried soil and 50 mL extracting solution (KH_2PO_4 0.1 mol/L or KCl 0.25 mol/L) as described by Huang and Fujii (1996). The Se concentration was determined by ICP-OES with hydride generation (HGAAS) as described by Welsch et al. (1990).

The Se content in plant material was digested according to the method EPA3051a (USEPA 1995). Then, the extracts were submitted to volume reduction on a heating plate at 150–200°C to a volume of 5 mL. After cooling, each sample received 5 mL of concentrated HCl, heating following by filtering with Whatman Nº 42 filter paper and the volume was made up to 50 mL. The samples still underwent a Se pre-reduction treatment by staying for 1 h in hot bath at 70°C and received 0.5 mL of 1.1 mol/L o-phenanthroline ($C_{12}H_8N_2 \cdot H_2O$) to each 25 mL of extract. Then, aliquots of the solution were taken for Se determination by ICP-OES with HGAAS as described previously. The Se reference standard, spinash leaves SRM 1570a (Se content 0.117 ± 0.009 mg/kg) was obtained from the NIST-National Institute of Standards and Technology and included in each analytical run as a quality assurance of the results.

Table 2. Total dry matter (g/pot) produced by rice plants (roots, shoots and grain) and radish (roots and shoots) grown in soils with contrasting clay content as a function of selenium (Se) rates applied

Se rate (kg/ha)	Rice		Radish	
	S	SC	S	SC
0	42.14^A	30.94^A	2.83^A	3.61^A
0.5	40.77^A	27.96^A	2.81^A	3.54^A
1	38.29^A	28.28^A	2.96^A	3.48^A
2	44.33^A	28.93^A	3.13^A	3.40^A

Means followed by the same letter in the same column do not differ by the Tukey's test (P < 0.05). S – sandy soil; SC – sandy clay soil

Selenium absorption efficiency (%) was calculated as 100[(mg Se absorbed on Se treatments – mg Se absorbed on control treatments)/mg of applied Se].

All data was subjected to ANOVA at 95% confidence, using SISVAR 5.0 software (Build 67, Lavras, Brazil). Linear and polynomial regression analyses were carried out for Se rates applied (as independent variable) and the Tukey's test ($P \leq 0.05$) was performed to compare means.

RESULTS AND DISCUSSION

Selenium addition to both soils did not affect dry matter yield of rice and radish (Table 2). Also, toxicity symptoms were not observed at any Se

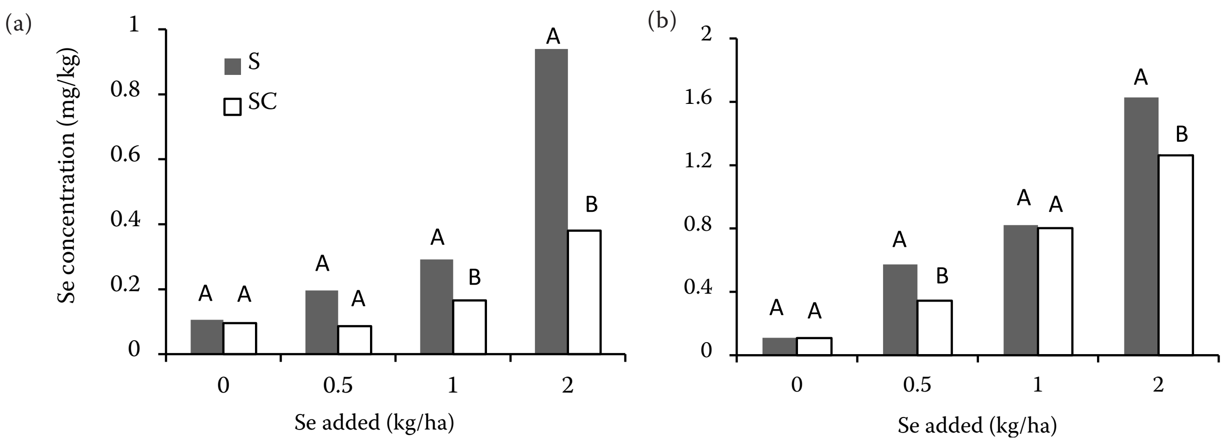

Figure 1. Selenium (Se) concentration in rice (a) and radish (b) shoots grown in two soils with contrasting clay content and treated with increasing concentrations of Se. Within each rate, values followed by the same letter do not differ by the Tukey's test (P < 0.05). S – sandy soil; SC – sandy clay soil

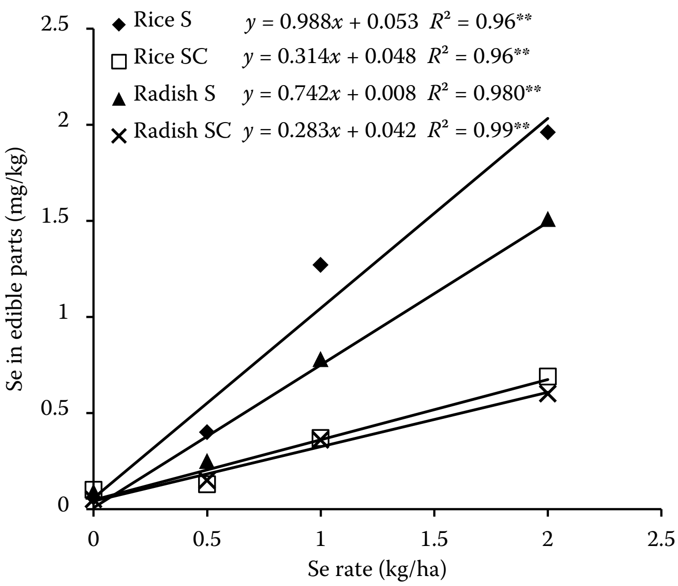

Figure 2. Selenium (Se) concentration in edible parts of rice and radish grown in two soils with contrasting clay content as affected by Se rates applied. S – sandy soil; SC – sandy clay soil; $^*P < 0.05$; $^{**}P < 0.01$

rate applied for both crops during their vegetative growth in the greenhouse. Boldrin et al. (2012) reported an increase in root dry matter of rice plants when Se(IV) was added to a medium textured soil (23% clay) up to 1.5 mg/kg and a decrease in shoot dry matter in all rates applied.

Concentrations of Se on rice and radish shoots varied from 0.10 to 0.94 mg/kg and from 0.11 to 1.63 mg/kg, respectively (Figure 1). The addition of increasing Se doses as sodium selenite significantly increased Se concentrations in both crops under investigation. Selenium concentrations were also significantly higher in sandy soil than in sandy clay soil with increases of 264% and 24% for rice and radish crops, respectively. The decrease in Se availability in the sandy clay soil is associated to its higher adsorption (Mouta et al. 2008), organic matter content, presence of competing ions and microbiological activity which are characteristic of this soil type (Hopper and Parker 1999, Wang and Gao 2001).

The regression analysis for Se concentrations in the edible parts of rice and radish (grain and roots, respectively) showed a significant linear increase for the Se rates applied (Figure 2) and also for the type of soil. Higher Se concentrations in the edible parts of both crops were observed in the sandy soil than in the sandy clay soil. These results support Se addition to soil as a strategy of staple food and vegetable biofortification, and hence, to supplement this element on human diet. Moreover, they also indicate that Se addition to soil for food biofortification will be specific for each type of soil and cultivated crop in order to avoid Se phytotoxicity. This is why some authors recently evaluated selenium levels in grain of different genotypes, species and varieties of wheat (spring, emmer and einkorn) with the aim to find wheat accessions with better selenium accumulation (Lachman et al. 2011).

The Se absorption efficiency showed, as expected, greater percentages of Se absorbed from the sandy soil and also for the rice crop, probably due to the cropping period which was 84 days longer than radish and, hence, gave more time for rice roots to absorb Se (Table 3). Selenium uptake was less than 2% which suggests that the remaining Se in the soil could be used in some extent by the next crop, since this element accumulates in the plowed layer and it is not easily leached (Watkinson 1983).

The Se extracted by KCl and KH_2PO_4 was linearly correlated with the Se rates applied to both soils (Figure 3). The results showed that KH_2PO_4 removed about 110% more Se than KCl indicating the ability of KH_2PO_4 in extracting exchangeable

Table 3. Selenium (Se) absorption efficiency (%) by rice and radish grown in soils with contrasting clay content as a function of Se rates applied

Se rates (kg/ha)	Rice		Radish	
	S	SC	S	SC
0.5	0.95	0.00	0.15	0.08
1	1.58	0.24	0.14	0.12
2	1.94	0.36	0.16	0.12

S – sandy soil; SC – sandy clay soil

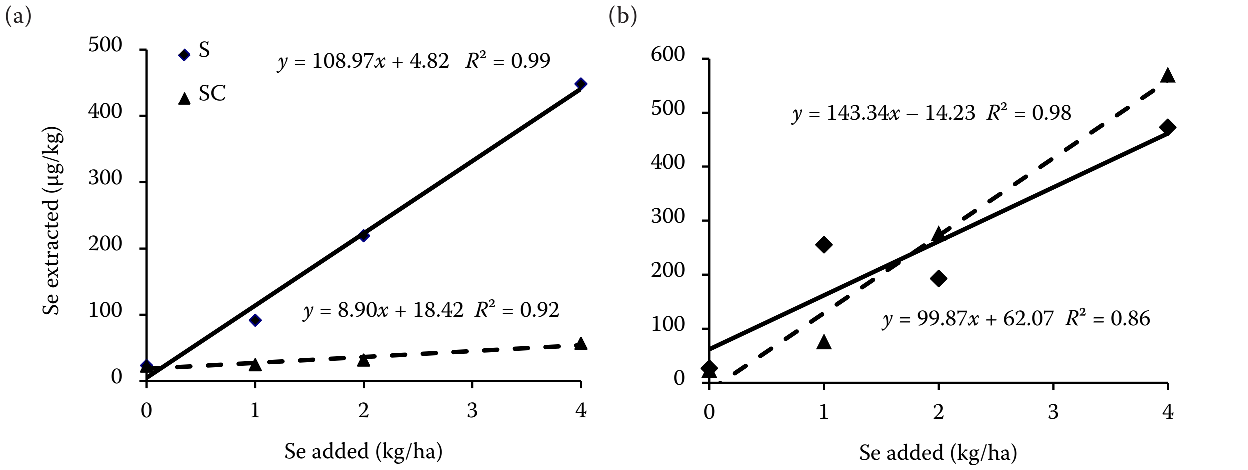

Figure 3. Selenium (Se) extracted by KCl (a) and KH_2PO_4 (b) in soils with contrasting clay content and treated with increasing concentrations of Se as sodium selenite. S – sandy soil; SC – sandy clay soil

and specifically adsorbed Se, as remarked by Huang and Fujii (1996). With respect to soil clay content, a seven-fold decrease was observed in the amounts of Se extracted by KCl on sandy clay compared to the sandy soil. However, KH_2PO_4 extracted similar amounts of Se in both soils suggesting that this solution was efficient in withdraw of Se adsorbed by Fe and Al oxides present in sandy clay soil.

The correlation coefficients obtained for the comparisons between the amounts of Se extracted by KCl and KH_2PO_4 with those absorbed by plants showed a higher agreement for rice than for radish, probably due to the greater amounts of Se removed by the rice crop (Table 4). The correlation coefficients observed for KCl extractant were ≥ 0.94 for all treatments denoting that Se availability is better correlated with its concentration in soil solution, which is considered as readily available to plants.

In conclusion, selenium addition to soil, as sodium selenite, increased the amounts of this element in edible parts of rice and radish indicating its use as a strategy of staple food and vegetable biofortification. The increase of Se concentration in edible parts of rice and radish crops was inversely proportional to soil clay content. Selenium extracted by KCl presented a better potential than KH_2PO_4 for predicting the amounts of phytoavailable Se for both crops under investigation.

Table 4. Correlation coefficients for selenium accumulated in rice and radish plants and extracted by KCl and KH_2PO_4 in two soils with contrasting clay content

Extractant	Rice		Radish	
	S	SC	S	SC
KCl	0.99*	0.99*	0.99*	0.94
KH_2PO_4	0.62	0.99*	0.65	0.94

S – sandy soil; SC – sandy clay soil; *$P < 0.05$

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Corresponding author:

Prof. Ronaldo Severiano Berton, Ph.D., Agronomic Institute, Av. Barão de Itapura 1481, 13020-902, Campinas, Brazil
e-mail: berton@iac.sp.gov.br