Research Highlights

Selenium species present in edible plants, grown in Se-enriched peat, are studied.

Selcote Ultra® and selenium sodium salts are assayed to increase uptake of Se.

Fortification with Se sodium salts increases Se content in plants.

Plants biotransform inorganic Se mainly to SeMet.

Contents over 10 mg Se kg^{-1} in peat can damage or inhibit plant growth.

Selenium uptake by edible plants from enriched peat

Funes-Collado Virginia*, Morell-Garcia Albert*, Rubio R.*,**, López-Sánchez J. Fermín* , **

* *Departament de Química Analítica. Facultat de Química. Universitat de Barcelona*
C/ Martí i Franquès 1-11, E- 08028, Barcelona (Spain)
** *Water Research Institute. Universitat de Barcelona. Barcelona. Spain*
e-mail address: roser.rubio@.ub.edu

Abstract

As a constituent of selenoproteins, selenium (Se) is considered an essential element for human health. The main way that Se enters the body is via the consumption of vegetables, whose concentration of this element depends on soil Se content. We grew cabbage, lettuce, chard and parsley, in peat enriched in Se by means of the additive Selcote Ultra® and Na_2SeO_3 and Na_2SeO_4. Total Se in plants was determined by acidic digestion and Se speciation by an enzymatic extraction. Both were measured by ICP/MS. The concentration ranges were between 0.1 mg Se kg^{-1} and 30 mg Se kg^{-1} for plants grown in Selcote Ultra® media, and between 0.4 mg Se kg^{-1} and 1606 mg Se kg^{-1} for those grown in peat enriched with Se sodium salts. We found Se (IV), Se (VI) and SeMet in all the extracts. Peat fortified with Selcote Ultra® gave slightly higher Se concentration than natural content values. For plants grown with selenium sodium salts, Se content increases with the Se added and part of the inorganic Se was converted mainly to SeMet. A high Se fortification can damage or inhibit plant growth. Cabbage showed the greatest tolerance to Se.

Keywords: edible plants, enzymatic extraction, LC-ICP/MS, Se-amino acids, Selenate, Selenite.

1. Introduction

Selenium (Se) is an essential element for humans and higher animals, since it is present in several selenoproteins that contribute to preventing oxidative cellular degradation (Zeng and Combs, 2008). This element is incorporated into the primary structure of these proteins as the amino acid selenocysteine (SeCys). In the 1970s, it was discovered that Se was a constituent of the anti-oxidant enzyme glutathione peroxidase (GPX). In addition, it is involved in thyroid hormone homeostasis, immunity, and fertility, among other activities (Reilly 2006). Se generates nutritional and toxicological concerns as the difference between its essentiality and toxicity in dose-response curves is very narrow. The European Directive 2008/100/CE determines the RDA (Recommended Daily Amount) of this element at 55 μg day^{-1}, and the maximum Se consumption without risk to health is 300 μg day^{-1} in adults. According with the literature, the NOAEL (Non Observed Adverse Effects Level) is considered to be about 800 μg Se day^{-1} while the LOAEL (Lowest Observed Adverse Effects Level) is 1500 μg Se day^{-1} and symptoms of toxicity are observed with an intake of 6300 μg Se day^{-1} (Scientific Committee on Food, 2000; Wangher et al., 1996).

Se deficiency in humans causes ailments such as Keshan disease, a heart disorder, and Kaschin-Beck disease, a degenerative disorder that affects bone. However, at elevated doses Se can cause toxic effects (Tan et al., 2002; Hartikanen, 2005; Lenz and Lens, 2009). Furthermore, the essential or toxic effect of this element in humans depends on its chemical form (Reilly, 2006). The major source of Se in most human diets is provided by plants. The availability of Se to the plant is determined by soil properties and conditions. Thus Se can occur as inorganic (selenite and selenate) or organic forms. Selenate, which is more soluble than selenite, can pass directly into plant roots; in contrast the uptake mechanism for selenite is unclear (Sager, 2006; Lin, 2009). Selenate competes with sulphate transport in the root plasma membrane and it is much

more abundant in leaves than selenite (Reilly, 2006). Inorganic Se absorbed by plants is metabolised in a variety of ways to organic Se compounds, the distinct molecular structures of which depend on the plant species (Gammelgaard and Jackson, 2011). Soils differ greatly in Se content, and in some geographical zones low concentrations lead to a decrease in plant Se uptake (Moreno Rodriguez et al., 2005; Hawkesford and Zhao, 2007; Spadoni et al., 2007). In some countries, inorganic Se compounds are commonly used as additives in fertilisers to improve the nutritional quality of local foodstuffs. This practice of Se fertilisation has been applied mainly in Finland and New Zealand (Eurola, 2000). A number of studies have addressed the effects of distinct forms of Se and cultivation conditions on edible plants. These studies mainly used selenite and selenate as sodium salts or barium salts (Iwashita and Nishi, 2004; Rayman et al., 2008; Broadley et al., 2010). Other strategies have been proposed to enhance the uptake of Se by plants, thus the generation of wetting and drying cycles in soil can convert more Se into soluble selenate, which is more amenable for uptake by plants (Shrestha et al., 2006). Several dominant species have been identified in plant foods. The main one is SeMet, and its behaviour has been examined widely due to interest in its biological activity (Reilly, 2006; Mechora et al., 2012; Mazej et al., 2007). Here we addressed Se speciation in vegetables. For this purpose, we grew cabbage *(Brassica oleracea),* lettuce *(Lactuca sativa)*, chard *(Beta vulgaris)* and parsley *(Petroselinum crispum)* in peat subjected to two fortifying treatments, namely the additive Selcote Ultra® (Vereinigte kreidewerke Damman KG) (which has a Se content commonly found for non-contaminated soils) and mixtures of sodium salts of selenite and selenate at Se concentrations widely found in seleniferous areas. Several studies of Se speciation in cabbage and lettuce have been reported (Iwashita and Nishi, 2004; Ahmed, 2010). Our study also includes chard and parsley to widen the information in the literature regarding Se speciation. We analysed Se speciation in distinct parts of plants and along the growing period. Moreover, we

77 also measured Se in the original seeds. Our results provide further knowledge of the
78 effectiveness of soil Se amendment and of the transformations and further availability of Se
79 species in plants for human consumption.
80
81 **2. Materials and methods**
82
83 2.1 Plant culture
84 A commercial peat provided by Plantaflor Humus Verkaufs-GMbH was used. This peat
85 (composed by perlite and vermiculite) contains more than 90% organic matter (dry matter), 1%
86 N (dry matter), and 60% moisture. It is free from Cl^- and its conductivity is less than 175 μS/cm.
87 Seeds of lettuce, cabbage, chard and parsley were sowing in multi-pots (depth 6cm). The
88 cultivation was carried out in a plant growth chamber (Ibercex, Spain) in a walk-in configuration,
89 for three weeks, under controlled environmental conditions with relative humidity of 70%,
90 temperature 22ºC and 16 h of photoperiod (110 μmol m^{-2} s^{-1} photosynthetically active reaction
91 (PAR)). Next, at a greenhouse (temperature range was 18-30ºC), individual plants were
92 transplanted in individual pots (14 cm upper diameter, 9.5 cm lower diameter, 16 cm in height)
93 of 2 L volume, filled with peat, and the pots were then placed on a tray to collect irrigation water.
94 All vegetables were irrigated on the basis of their water demands.
95
96 2.2 Exposure of plants to selenium
97 Three series of cultivation media were prepared: peat without fortification, as a control; peat
98 fortified with Selcote Ultra® (which contains 10 g Se kg^{-1} with a minimum of 90% as Se (VI) and
99 a maximum of 10% as Se (IV), $BaSeO_4$ (1-5%), Na_2SeO_4 (< 2.5%), Na_2SeO_3 and $BaSeO_3$
100 (~ 0.5%)), and peat fortified with Se sodium salts. Furthermore, the peat was fortified with

101 Selcote Ultra® at two concentration ranges, namely level A: 0.05-0.08 mg Se kg^{-1}, which is the
102 recommended concentration, and level B: 0.21-0.27 mg Se kg^{-1}. In contrast, the peat was
103 fortified with sodium selenite and sodium selenate (1:9) at three concentration ranges, namely
104 level C: 2-8 mg Se kg^{-1}, level D: 9-17 mg Se kg^{-1}; and level E: 83-158 mg Se kg^{-1}. All of these
105 concentrations were prepared in triplicate. Three pots without Se fortification were prepared as
106 controls for each plant species. In order to improve growth, 1 g of an NPK fertiliser (which
107 contains NO_3^-, P_2O_5 and K_2O at the same ratio (15%)) was added to all the growth media 3 times
108 every 2 months. The vegetables were harvested at 4 or 6 months, depending on the growth cycle
109 of each species.
110 After collection, vegetable samples were cleaned, and leaves, stems and roots were separated.
111 This material was then dried at 40ºC. Then the samples were milled with a glass mortar,
112 transferred to a HDPE bottle, and stored at room temperature until analysis (from 2 days to 2
113 weeks).
114
115 2.3 Characterisation of Selcote Ultra® and peat
116 A Phillips PW 2400 X-ray spectrometer with Rh and Au excitation tubes was used to measure
117 the main compounds in the commercial additive Selcote Ultra® and in the peat. After drying at
118 500ºC, samples were diluted (1:20) with lithium tetraborate and melted at 1125ºC in a radio-
119 frequency inductive oven (Panalytical PERLE'X3 Micro-processing System) to obtain pearls
120 with a 30-mm diameter. Major elements were determined by means of a series of international
121 geological reference samples for calibration.
122
123 2.4 Total selenium

Extractable Se in seeds, peat and Selcote Ultra® by aqua regia. A P/Selecta model RAT 4000051 with temperature control was used. The method was applied following ISO 11466 1995 using 1 g of sample. The reagents were HCl 35% and HNO3 69% (Hiperpur Panreac). Once at room temperature, the resulting suspension was passed through an ashless filter (Whatman 40), and the solid residue was washed several times in 0.5 mol L^{-1} HNO_3. The resulting filtrate, together with the washings, were diluted to 50 mL, transferred to a HDPE bottle and stored at 4ºC until analysis.

Acidic microwave digestion of plants. 0.2 g of vegetable samples were weighed in PTFE vessels containing 8 mL of HNO_3 (Hiperpur Panreac) and 2 mL of H_2O_2 (Prolab). The resulting mixture was digested using a microwave (Milestone Ethos Touch Control, 1000 W) by the following program: 10-min ramp from room temperature to 90ºC; 5 min at 90ºC; 10-min ramp from 90ºC to 120ºC; 10-min ramp from 120ºC to 190ºC; and 10 min at 190ºC. After digestion, the samples were filtered (Whatman 40) and diluted to 20 mL with double deionised water, transferred to a HDPE bottle and stored at 4ºC until analysis.

Total Se measurement. Se was measured by a 7500ce series Octopole Reaction System inductively coupled plasma mass spectrometer (ICP/MS) with a concentric micro-flow nebuliser (Agilent Technologies, Waldbronn, Germany). Hydrogen was used as reaction gas to prevent possible interferences, and Rh was used as internal standard. The ion intensity at m/z 78 (^{78}Se) was monitored by time-resolved analysis software.

2.5 Selenium speciation in vegetables

Water extraction of Se from Selcote Ultra®. 1 g of Selcote Ultra® was placed in a 40 mL HDPE tube with 25 mL of double deionised water in an end-over-end system. The mixture was continuously shaken for 3 months. In order to determine Se species over time, aliquots were

148 periodically extracted and analysed. The total volume of the extractant was completed with
149 doubly deionised water throughout the experiment.
150 Enzymatic digestion of plants. 0.3 g of vegetable samples and 30 mg of Protease XIV (Sigma
151 Aldrich) were placed in a 40 mL HDPE tube with 10 mL of a 25 mmol L^{-1} $NH_4H_2PO_4$ solution
152 at pH 7.5. The mixture was shaken for 16 h in a thermo-agitator water bath (Clifton NE5-28D) at
153 37ºC. The resulting solution was firstly centrifuged for 10 min at 3000 rpm, then passed through
154 0.45 μm filters and then through 0.20 μm filters (to prevent chromatographic column damage).
155 Se species were measured immediately after extraction. The extraction was performed by
156 enzymatic digestion using Protease XIV, as recommended in the literature (Kahakachchi et al.,
157 2004).
158 Se species measurement. 1000 mg L^{-1} of Se stock solutions was prepared from selenite 99%
159 Na_2SeO_3 (Aldrich, Milwaukee, WI, USA) and selenate 99% Na_2SeO_4 (Aldrich). 1000 mg l^{-1} of
160 the Se stock solutions was prepared from selenocystine ($SeCys_2$) and selenomethionine (SeMet)
161 with HCl 0.5% and kept at 4ºC. All the standard solutions were prepared daily by dilution.
162 Measurements were carried out by LC-ICP/MS (Quaternary Agilent Technologies 1200 series
163 LC system and 7500ce series Octopole Reaction ICP/MS System). An anion exchange pre-
164 column and column (Hamilton PRP-X100 (Reno, NV, USA)) were used. The mobile phase
165 comprised 40 mmol L^{-1} of $(NH_4)H_2PO_4$ buffer (Merck Suprapur) adjusted at pH 7.0.
166 We considered that most organic Se species are oxidised during the extraction, as reported in
167 some studies (Ayouni et al., 2008). Thus the standard of SeMet was oxidised with hydrogen
168 peroxide (H_2O_2 33%) to identify the chromatographic peak of SeOMet.
169
170 **3. Results**
171

172 Moisture content was determined gravimetrically for all the samples stored (dried at 40ºC). At
173 105ºC, seeds showed 9-19% of moisture content while plants showed 10-23%. All the results are
174 expressed as the range of values and as mg Se kg^{-1} dry mass.
175 Seeds, Selcote Ultra® and peat were analysed for total Se contents. Seeds showed very low
176 values (seeds from cabbage, 212 ± 14 µg Se kg^{-1}; from lettuce, 57 ± 2 µg Se kg^{-1}; from chard, 30
177 ± 7 µg Se kg^{-1}; and from parsley, 82 ± 18 µg Se kg^{-1}). Se content in peat was 251 ± 4 µg Se kg^{-1}
178 of Se. For Selcote Ultra®, total Se was 11 ± 2 g Se kg^{-1} and only Se (VI) was found in the water
179 extracts of this additive.
180 Leaves, stems and roots were analysed in vegetables. Table 1 shows the total Se content and
181 speciation results for the samples. Data are shown for different parts of the plants and according
182 to enriched peat concentration ranges: control samples (not fortified), Selcote Ultra® and Se
183 sodium salts. The results are expressed as a range of the Se content of each medium (control, A,
184 B, C, D and E) in triplicate. In some cases, mainly in cabbage, the wide ranges were attributed to
185 biological variability within the specimens. Figure 1 also presents some examples of
186 chromatograms from vegetables (leaves were selected) grown in control, Selcote Ultra® and
187 soluble salt media.
188
189 **4. Discussion**
190
191 *Preliminary studies*. The present study required previous characterisation of the materials used.
192 We considered it relevant to measure the Se content of the seeds in order to evaluate their
193 contribution to the presence of Se in the plants. Few studies report data of this kind. Furthermore,
194 there are several data on Se-enriched seeds (Ferri et al., 2000, Thavarajah et al., 2008;

195 Lintschinger et al., 2000). The seed Se contents were, in all cases, very low and were considered
196 to reflect natural values.
197 The Se content of peat (251 ± 4 μg Se kg^{-1}) fell within the range of those found in non-
198 contaminated soils, these showing averages between 0.05 mg Se kg^{-1} to 1.27 mg Se kg^{-1},
199 depending on soil composition (Kabatas-Pendias, 2001). We used Selcote Ultra® as an additive
200 to increase the Se content in peat. This practice is commonly used in regions with soil Se
201 deficiency. To determine the extent to which the Se species of Selcote Ultra® could be extracted
202 over time, we performed an extraction with water over 90 days. The maximum percentage (35-
203 40%) of total Se was reached at day 5, and this element was present only as Se (VI). Se (IV) was
204 not detected in the extracts because of the low water solubility of $BaSeO_3$. Thus, when Selcote
205 Ultra® is used in field conditions, it releases Se to the soil solution at a very slow rate and mainly
206 as selenate. The Se content in Selcote Ultra® reported here is consistent with the technical data
207 sheet of this product.
208 Soluble salts were added into the peat to increase the Se concentration to levels similar to those
209 found in seleniferous areas. Reported values for these areas are between 1.3 mg Se kg^{-1} – 138 mg
210 Se kg^{-1} (Kabatas-Pendias, 2001).
211 Total Se and Se species in edible plants growing in the different media (see Table 1) are
212 discussed below.
213 *Plants grown on non-amended peat (controls).* Total Se are natural values from plants grown in
214 non-contaminated soils (Ellis and Salt 2003). Control vegetables showed mainly Se (VI) in all
215 the parts analyzed.
216 *Plants grown in peat fortified with Selcote Ultra®*. In the literature, a range of concentrations of
217 this additive has been studied in pasture, cereals and forage crops (Valle et al., 2002); however,
218 in the present study, this additive was used for the first time to spike peat.

219 The total Se contents of the vegetables were slightly higher than those of controls, except for
220 cabbage which did not show any difference. Slight differences were observed for the total Se
221 content in plants grown at the two levels of fortification (A and B). These results indicate that
222 Selcote Ultra® releases Se very slowly as a result of poorly soluble components. We did not
223 measure Se species in most of the enzymatic extracts. Se (VI) was quantified in all vegetables
224 except cabbage. Chard and parsley were the plants that contained Se (IV), Se (VI) and SeMet,
225 although the values were close to the limit of detection.
226 *Plants grown in peat fortified with soluble Se salts*. All plants grew in peat media fortified at
227 levels C and D. However some toxic effects, resulting in withering and rotting on leaves and a
228 decrease of biomass with respect to control plants, were observed in plants subjected to level D.
229 Moreover, plants presented growth inhibition at the highest Se fortification level (E). Lettuce and
230 parsley were the vegetables most affected and SeMet was present in the enzymatic extracts from
231 these plants, as shown by the chromatograms in Figure 1. As a detoxification mechanism, these
232 kinds of plants usually convert inorganic Se to SeMet by volatilization to form dimethyl selenide
233 (Tapiero et al., 2003; Dumont 2006). Our results support the findings of those studies. Se toxicity
234 in non-accumulator plants in the present study (lettuce, chard and parsley) was also due to the
235 incorporation of SeCys and SeMet into proteins in place of Cystine (Cys) and Methionine (Met),
236 respectively (Terry et al. 2000). However, although cabbage was the plant with the higher Se
237 content, no toxic symptoms were observed. The tolerance of accumulator plants to inorganic Se
238 is attributed to its conversion to non-protein seleno amino acids (Terry et al., 2000).
239 The total Se content in plants increased with the concentration of Na_2SeO_4 and Na_2SeO_3 in the
240 peat. Among all plants grown with soluble Se salts, cabbage was the plant with the highest Se
241 content. These results are consistent with Se accumulation reported in *Brassica* species which
242 generally accumulate several hundred milligrams of Se per kilogram of dry weight (Lin, 2009;

243 Ximènez-Embn et al., 2004; Seo et al., 2008). For cabbage, Se concentration in leaves was
244 higher than in roots. This is in agreed with other studies about Se accumulator plants grown in Se
245 enriched soil (Dumont et al., 2006). Regarding speciation, the concentration of Se species rose
246 with increased Se fortification of the growth media. For all the vegetables, Se (VI) was one of
247 the main inorganic species present. This finding is attributed to the high fortification in peat. A
248 high percentage of inorganic species were converted to SeMet, which was the major organic
249 species in the enzymatic plant extracts as in other studies (Polatajco et al., 2006; Mazej et al.,
250 2007; Mechora et al., 2012). The occurrence of $SeCys_2$ in enzymatic extracts has been also
251 studied and the results showed that the concentration of this species was lower than its detection
252 limit (0.03 mg kg^{-1}).
253 Considering all supplemented media, for a global discussion on the results Figure 2 and 3 are
254 showed. Results from those plants which presented growth inhibition or concentrations lower
255 than limit of detection have been not considered in these Figures. Presented data correspond to
256 the higher Se content of the obtained ranged of values (Table 1). Figure 2 shows how increased
257 the Se content in leaves from different vegetables grown respect the Se added in peat. In general,
258 Se was more available to plants and was absorbed faster than Selcote Ultra® by roots. Cabbage
259 (*Brassica oleracea*) had the highest Se concentration and in case of parsley (*Petroselinum*
260 *crispum*), the Se uptake by plant was lower than for others. Figure 3 indicates the percentage of
261 (a) SeMet and (b) Se (VI) in the enzymatic extracts from leaves of cabbage and parsley, which
262 obtained the highest and lowest Se content according to Se added in peat, respectively. This
263 Figure shows that extracts from parsley contained high SeMet concentration and a low Se (VI)
264 content and for cabbage the species distribution was reversed.
265
266 **5. Conclusions**

Here we report on the total Se and its species content for cabbage, lettuce, chard and parsley grown in peat fortified with Selcote Ultra® and sodium selenite and selenate mixtures. In all cases, leaves and roots (also stems for lettuce) were considered.

Selcote Ultra® is commonly used in fertilisers for fodder crops or for forage for animal diets. Here we used this additive for the first time to increase Se in plants for human consumption. Peat fortified with Selcote Ultra® gave slightly higher total Se and Se species than natural content values, even when twice the recommended amount of Selcote Ultra® was added.

For plants grown in peat fortified with selenium sodium salts at concentrations similar to those found in seleniferous soils, the content of Se increases with the supplementation. During plant growth, part of the inorganic Se was converted mainly to SeMet.

Soluble salts are the fastest strategy to enrich peat with Se. However, Se concentrations of approximately 10 mg Se kg^{-1} or higher in fortified peat can damage or inhibit plant growth. Cabbage, which was the vegetable with the highest Se content, showed the greatest tolerance to Se, among the plants studied.

Acknowledgements The authors thank Dr. Toni Padró from the *Serveis Científico-Tècnics* of the University of Barcelona (UB) for his valuable help with the ICP/MS measurements and the Experimental Fields Service of the UB for providing the greenhouse. The authors also thank the German Company Vereinigte Kreidewerke Dammann KG for providing the commercial additive Selcote Ultra®. This study was funded by the DGICyT (Project No. CTQ2010-15377/BQU), and by the *Generalitat de Catalunya* (project SGR2009-1188). Virginia Funes received a predoctoral grant from the UB.

References

Ahmed H K 2010 Differences between some plants in selenium accumulation from supplementation soils with selenium. Agric. Biol. J. N. Am 1(5), 1050-1056.

Ayouni L, Barbier F, Imbert J L, Gauvrit J Y, Lantéri P, Grenier-Loustalot M F 2006 New separation method for organic and inorganic selenium compounds based on anion exchange chromatography followed by inductively coupled plasma mass spectrometry. Anal Bioanal Chem. 385, 1504-1512.

Broadley M R, Alcock J, Alford J, Cartwright P, Ian Foot I, Fairweather-Tait S J, Hart D J, Hurst R, Knott P, McGrath SP, Meacham M C, Norman K, Mowat H, Scott P, Stroud J L, Tovey M, Tucker, M, White P J, Young S D, Zhao F J 2010 Selenium biofortification of high-yielding winter wheat (Triticum aestivum L.) by liquid or granular Se fertilization. Plant and Soil. 332, 5–18.

Dumont E, Vanhaecke F, Cornelis R 2006 Selenium speciation from food source to metabolites: a critical review. Anal Bioanal Chem. 385, 1304-1323.

Ellis R, Salt D 2003 Plants selenium and human health. Current opinion in plant biology 6:273-279.

Eurola M, Alfthan G, Aro A, Ekholm P, Hietaniemi V, Rainio H, Rankanen R, Venäläinen E-R. Results of the Finnish selenium monitoring program, 200-2001 Agrifood Research Finland. *Agrifood Research Reports*, 36.

Ferri T, Coccioli F, De Luca C, Callegari C V, Morabito R 2004 Distribution and speciation of selenium in Lecythis ollaria plant. Microchemical Journal. 78, 195-203.

Gammelgaard B, Jackson M I 2011 Review. Surveying selenium speciation from soil to cell-forms and transformations. Anal.Bioanal.Chem. 399, 1743-1763.

Hartikainen H 2005 Review. Biogeochemistry of selenium and its impact on food chain quality and human health. J.Trace Elements Med. Biol. 18, 309-31.

Hawkesford M J, Zhao F J 2007 Strategies for increasing the selenium content of wheat. J.Cereal Science. 46, 282-292.

Iwashita Y, Nishi K 2004 Cultivation of selenium-enriches vegetables in large scale. Biomed.Res. Trace Elements.15, 72-75.

Kabatas-Pendias 2001 Trace Elements in soils and plants. 3ª Ed. CRC PRESS. 241-252.

Kahakachchi C, Totoe H T, Uden P C, Tyson J F 2004 Chromatographic speciation of anionic and neutral selenium compounds in Se-accumulating Brassica juncea (Indian mustard) and selenized yeast. J. Chromatogr. A. 1054, 303-312.

339 Lenz M, Lens P N L 2009 The essential toxin: The changing perception of selenium in
340 environmental sciences. Sci.Total Env. 407, 3620-3633.
341
342 Lin Z-Q 2009 Uptake and accumulation of selenium in plants in relation to chemical speciation
343 and biotransformation. In Development and Uses of Biofortified Agricultural Products Eds:
344 Banuelos, Gary Stephan; Lin, Zhi-Qing. CRC Press, Boca Raton. 45-56
345
346 Lintschinger, J, Fuchs, N, Moser, J, Kuehnelt, D, Goessler, W 2000 Selenium-enriched Sprouts.
347 A raw Material for Fortified Cereal-Based Diets. *Journal of Agricultural and Food Chemistry*,
348 48, 5362-5368.
349
350 Mazej D, Osvald J, Stibilj V 2007 Seelenium species in leaves of chicory, dandelion, lamb's
351 lettuce and parsley. Food Chemistry. 107, 75-83.
352
353 Mechora S, Germ M, Stibilj V 2012 Selenium compounds in selenium-enriched cabbage. Pure
354 and Applied Chemistry. 84, 2, 259-268.
355
356 Moreno Rodriguez M J, Cala Rivero V, Jiménez Ballesta R 2005 Selenium distribution in
357 topsoils and plants of a semi-arid Mediterranean environment. Env.Geochem.and Health. 27,
358 513-519.
359
360 Polatajko A, Jakubowski N, Spuznar J 2006 State of the art report of selenium speciation in
361 biological samples. J. Anal. At. Spectrom. 21, 639-654.
362
363 Rayman M P, Goenaga Infante H, Sargent M 2008 Food-chain selenium and
364 humanhealth:spotlight on speciation. Review. British Journal of Nutrition. 100, 238-253.
365
366 Reilly C 2006 Selenium in Food and Health. 2nd ed. Springer, USA.
367
368 Sager M 2002 Selenium in agriculture, food, and nutrition 2006 Pure Appl.Chem. 78, 111-133.
369
370 Tan J, Zhu W, Wang W, Li R, Hou S, Wang D, Yang L 2002 Selenium in soil and endemic
371 diseases in China. Sci.Total Env. 284, 227-235.
372
373 Scientific Committee on Food 2000 Opinion of the Scientific Committee on Food on the
374 Tolerable Upper Intake Level of Selenium (http://ec.europa.eu/food/fs/sc/scf/out80g_en.pdf).
375
376 Seo T C, Spallholz J E, Yun H K, Kim S W 2008 Selenium-Enriched Garlic and Cabbage as a
377 Dietary Selenium Source for Broilers J Med Food. 687-692.
378
379 Shrestha B, Lipe, S, Johnson K A, Zhang T Q, Retzlaff, W, Lin Z Q 2006 Soil hydraulic
380 manipulation and organic amendment for the enhancement of selenium volatilization in a soil–
381 pickleweed system Plant and Soil. 288, 189–196.
382
383 Spadoni M, Voltaggio M, Carcea M, Coni E, Raggi A, Cubadda F 2007 Bioaccessible selenium
384 in Italian agricultural soils: Comparison of the biogeochemical approach with a regression model
385 based on geochemical and pedoclimatic variables Sci.Total Env. 375, 160-177.
386

Thavarajah, D., Ruszkowski, J., Vandenberg, A 2008 High Potencial for Selenium Biofortification of Lentils (Lens culinaris L.). *Journal of Agricultural and Food Chemistry*, 56, 10747-10753.

Tapeiro H, Townsend D M, Tew K D 2003 The antioxidant role of selenium and seleno-compounds. Biomedicine & pharmacotherapy. 57:134-144.

Terry N, Zayed A M, de Souza M P, Tarun A S 2000 Selenium in Higher Plants. Annu. Rev. Plant. Physiol. Plant Mol. Biol. 51:401-32.

Valle G, McDowell L R, Prichard D L, Chenoweth P J, Wright D L, Martin F G, Kunkle W E,, Wilkinson N S 2002 Selenium concentration of fescue and bahiagrasses after applying a selenium fertilizer Commun. Soil Sci. Plant Anal. 1461-1472.

Whanger P, Vendeland S, Park YC, Xia Y 1996 Metabolism of subtoxic levels of selenium in animals and humans. Ann. Clin. Lab. Sci. 26 (2), 99-113.

Ximènez-Embn P, Alonso I Madrid-Albarrn Y, Cámara C 2004 Establishment of Selenium Uptake and Species Distribution in Lupine, Indian Mustard, and Sunflower Plants. J. Agric. Food Chem. 52 (4), 832-838.

Zeng H, Combs, Jr G F 2008 Review: Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion J.Nutritional Biochemistry. 19, 1-7.

TABLES

Table 1. Total Se and Se species in parts of vegetables grown in peat fortified with Selcote Ultra® and sodium salts, at a range of concentrations, as described in the experimental design: **A**, **B**, **C**, **D** and **E** expressed as mg Se kg^{-1}. All measurements were made in duplicate. na: not analyzed.
Total Se:
LOD (mg kg^{-1}): 0.03; LOQ (mg kg^{1}): 0.1
Se speciation:
LOD (mg kg^{-1}) Se (IV): 0.01; SeMet: 0.1; Se (VI): 0.03
LOQ (mg kg^{-1}) Se (IV): 0.04; SeMet: 0.5; Se (VI): 0.09

Plant species	Concentration ranges of fortifier mg Se kg^{-1} (n=3)		Vegetable part	Total Se	Speciation		
					Se (IV)	SeMet	Se (VI)
Cabbage	Control		Leave	0.8 – 1.3	< 0.01	< 0.1	< 0.09
			Root	1.0 – 1.4	< 0.01	< 0.1	< 0.09
	Selcote Ultra®	A	Leave	0.8 – 1.6	< 0.01	< 0.1	< 0.03
			Root	1.3 – 2.4	< 0.01	< 0.1	< 0.03
		B	Leave	0.9 – 1.4	< 0.01- < 0.04	< 0.1	< 0.09
			Root	1.4 -1.7	< 0.01	< 0.1	< 0.09
	Na_2SeO_3 + Na_2SeO_4	C	Leave	11 – 76	0.21 – 1.5	2.4 – 8.11	4.7 – 47.2
			Root	12 - 40	0.03 – 0.22	3.4 – 10.6	0.9 – 2.1
		D	Leave	64 – 98	0.63 – 1.1	2.4 – 10.8	< 0.09 – 43.1
			Root	52 – 72	0.24 – 0.8	10.6 – 15.8	2.1 – 8.2
		E	Leave	952 – 1606	22.7 – 32.8	102 – 168	653 – 1188
			Root	414 - 793	12.9 – 20.8	153 - 194	29.9 - 141
Lettuce	Control		Leave	< 0.1 – 0.1	< 0.01	< 0.1	< 0.09
			Stem	< 0.1 – 4.1	< 0.01	< 0.1	< 0.09
			Root	< 0.1 – 1.1	< 0.01	< 0.1	< 0.09
	Selcote Ultra®	A	Leave	< 0.1 – 2.8	< 0.01 - < 0.04	< 0.1 - < 0.5	< 0.09 – 0.24
			Stem	0.8 – 1.7	< 0.01	< 0.1 - < 0.5	< 0.09 – 0.28
			Root	0.5 – 2.3	na	na	na
		B	Leave	0.8 – 1.0	< 0.01	< 0.1	< 0.09 – 0.1
			Stem	0.6 – 1.0	< 0.01	< 0.1	< 0.09 – 0.1
			Root	1.7 – 2.1	na	na	na
	Na_2SeO_3 + Na_2SeO_4	C	Leave	34 – 113	0.06 – 0.18	4.5 – 12.4	6.9 – 19.9
			Stem	29 – 66	0.15 – 0.24	6.1 – 11.5	3.4 – 9.3
			Root	82 – 197	na	na	na
		D	Leave	108 – 171	0.09 – 0.2	15.8 – 19.7	22.7 – 40.8
			Stem	103 – 117	0.3 – 0.7	13.9 – 19.4	14.3 – 16.4
			Root	175 – 244	na	na	na
		E	Leave	na	na	na	na
			Stem	na	na	na	na
			Root	na	na	na	na
Chard	Control		Leave	< 0.1	< 0.01	< 0.1 - < 0.5	< 0.03 - < 0.09
			Root	< 0.1 - 0.4	< 0.01	< 0.1	< 0.03
	Selcote Ultra®	A	Leave	0.1 - 0.7	< 0.01	< 0.1 – 5.1	< 0.09 – 0.3
			Root	0.3 - 0.5	< 0.01	< 0.1	< 0.09
		B	Leave	0.5 - 0.7	< 0.01 – 0.06	< 0.1 – 12.2	0.36 – 0.37
			Root	0.2 - 0.3	< 0.01	< 0.1 – 0.8	0.1 – 0.8
	Na_2SeO_3 + Na_2SeO_4	C	Leave	26 – 31	0.09 – 0.9	4.8 – 9.9	3.4 – 18.6
			Root	11.2 - 11.8	0.1 – 0.4	3.0 – 5.1	1.2 – 4.4
		D	Leave	0.4 – 59	< 0.01 – 2.0	< 0.1 – 12.3	0.2 – 33
			Root	19 - 26	< 0.01 – 0.2	4.4 – 4.7	5.7 – 14.6
		E	Leave	753 - 817	1.9 – 2.0	187 – 254	621 – 686
			Root	na	na	na	na
Parsley	Control		Leave	< 0.1	< 0.01	< 0.1 – 0.7	< 0.03 – 0.2
			Root	< 0.1	< 0.01	< 0.1	< 0.09
	Selcote Ultra®	A	Leave	< 0.1 - 0.8	0.05 - 0.07	0.4 - 0.9	< 0.09 – 0.1
			Root	< 0.1 - 0.4	< 0.04	< 0.5	< 0.09
		B	Leave	0.3 - 0.6	0.04 - 0.07	0.7 - 1.2	< 0.09 – 0.2
			Root	0.4 - 0.5	< 0.04 - 0.07	< 0.5 - 0.8	< 0.09 – 0.2
	Na_2SeO_3 + Na_2SeO_4	C	Leave	16 -26	< 0.04	13.1 - 21.9	3.5 – 5.1
			Root	12 - 23	< 0.04	6.4 – 16.1	12.8 – 26.7
		D	Leave	21 -74	0.4 - 2.1	25.1	9.7 – 16.7
			Root	20 - 71	< 0.04	9.7	14.6
		E	Leave	na	na	na	na
			Root	na	na	na	na

FIGURE CAPTION

Figure 1. Example of chromatograms corresponding to Se speciation in leaf extracts of vegetables grown in control peat (without Se), in peat with Selcote Ultra®, and in peat with soluble salts (at level C: 10 mg Se kg^{-1}; level D: 100 mg Se kg^{-1}).

Figure 2. Total selenium content in leaves of vegetables, according to selenium fortification in peat.

Figure 3. Percentage of the main species in enzymatic extracts from leaves of cabbage and parsley, respect to the selenium fortification in peat. **(a)**Percentage of SeMet; **(b)**Percentage of Se (VI).

Figure 1

Click here to download high resolution image

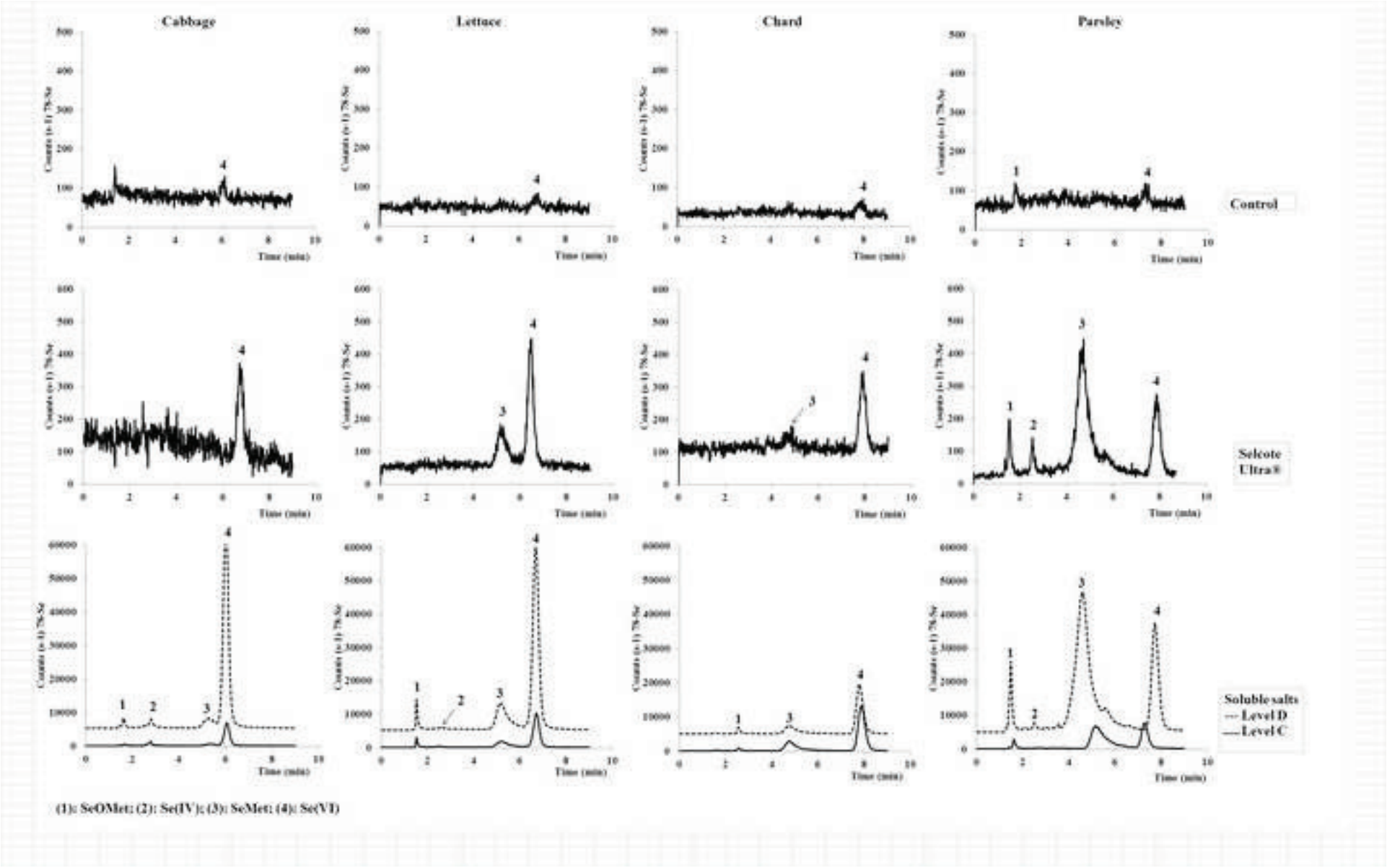

Figure 2

Click here to download high resolution image

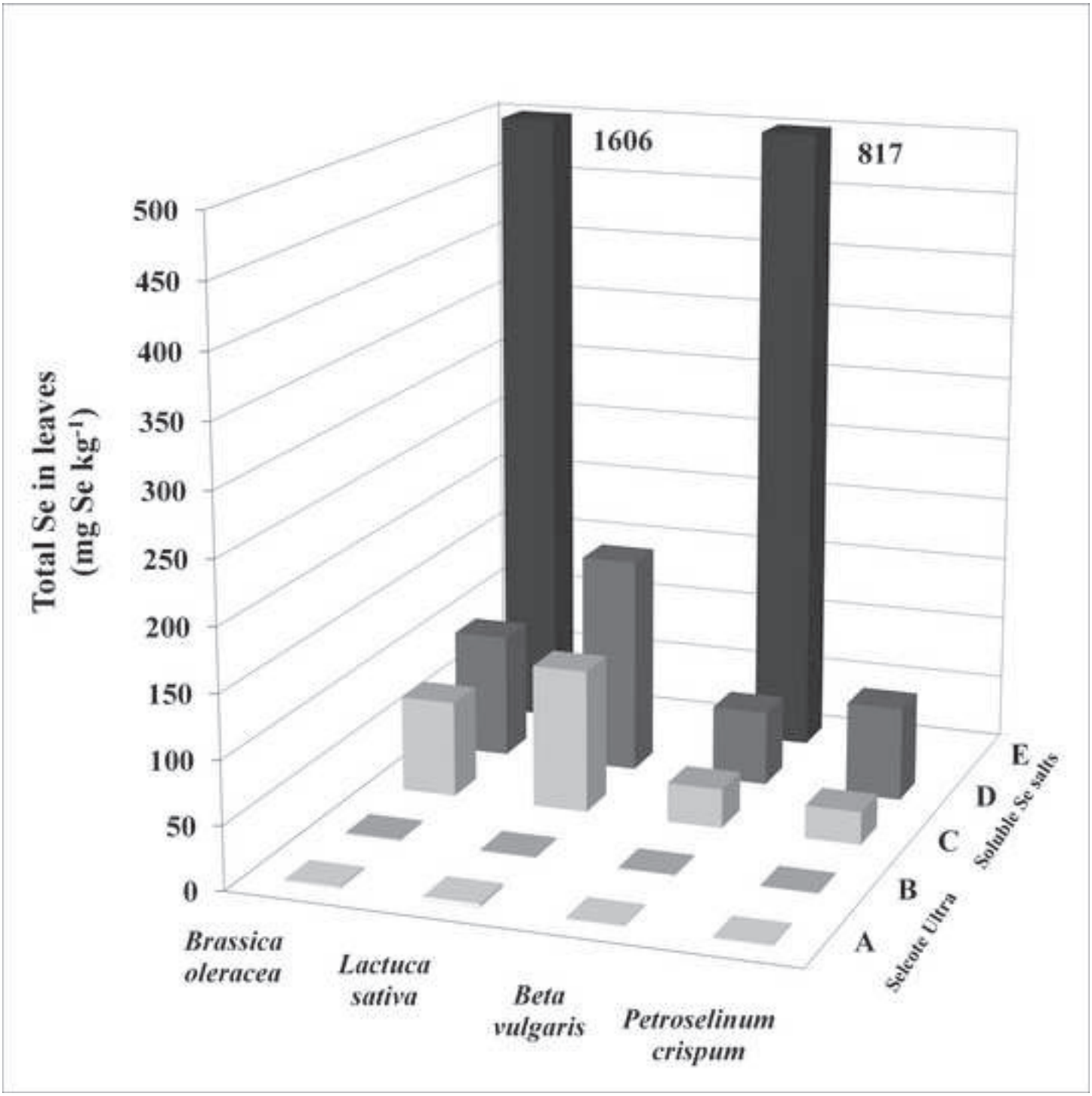

Figure 3a
Click here to download high resolution image

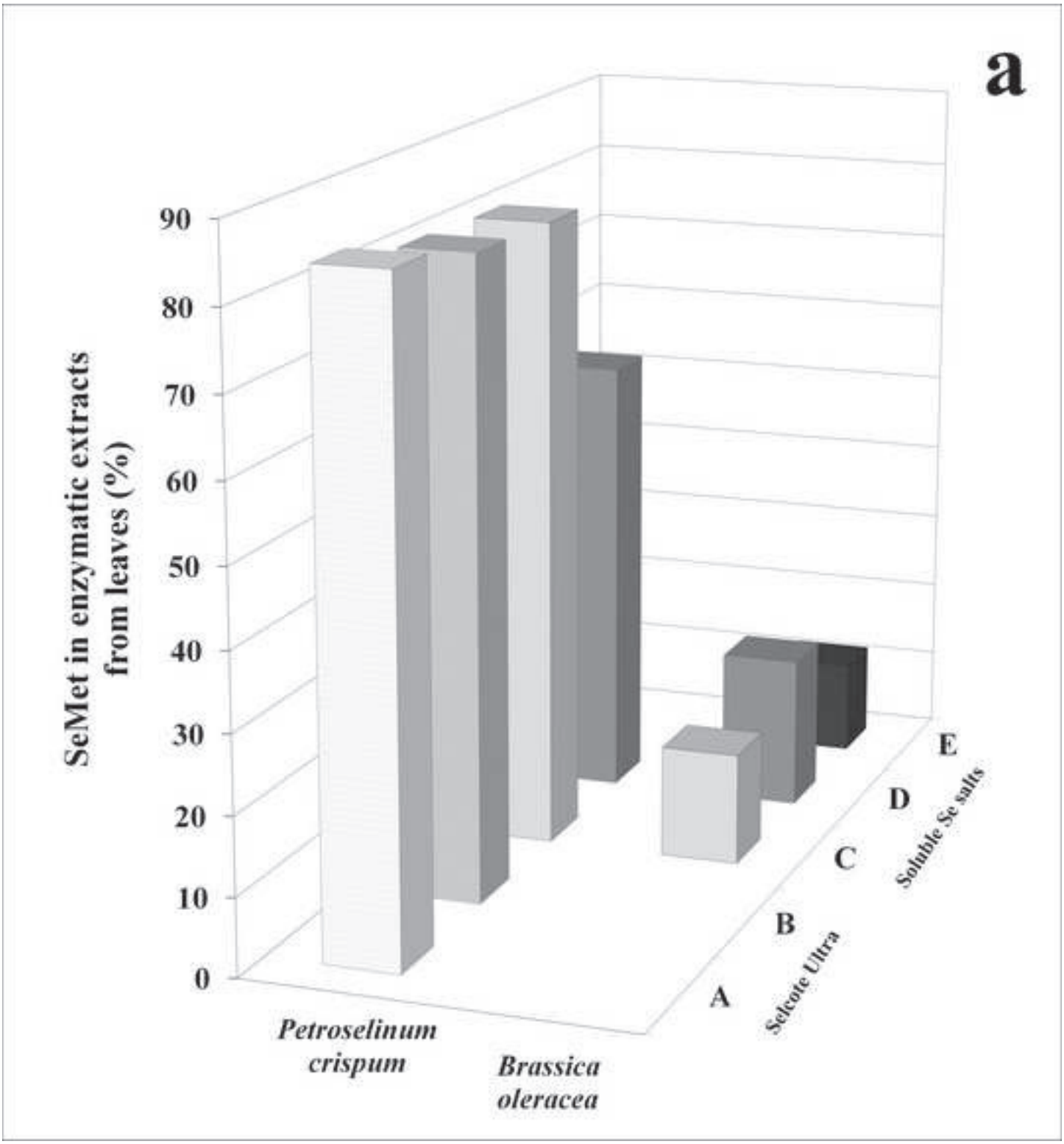

Figure 3b
Click here to download high resolution image