Selenium in cereal plants and cultivation soils by radiochemical neutron activation analysis

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Abstract

Selenium is an essential micronutrient for human health but it is deficient in at least one billion people worldwide. Cereals are by far the most significant agricultural crops, not only on a gross tonnage basis, but also by what they represent in terms of energy supply and dietary intake for human nutrition worldwide. Portugal is no exception to such pattern, the Portuguese situation of selenium dietary intake is difficult to assess due to scarce information and lack of consistent studies on the subject, even if it should not be that different from much of Europe, where falls in Se intake have raised a widespread concern. In these terms, we are investigating cereals. Two species of wheat, bread and durum wheat were sown at the end of November 2009, and then sampled in different growth stages. Rye was collected during harvesting season and cultivation soils were analyzed as well. Selenium results were within the range of 100-225ppb for soils; 3-55ppb for durum wheat; 6-80ppb for bread wheat and 4-30ppb for rye. Accuracy of the RNAA procedure was proved by analysis of reference materials NIST-SRM 1515 and NIST-SRM 8433.

Keywords: biofortification; cereals; rye; selenium; wheat; INAA; RNAA

Introduction

An increase in the bioavailability of essential elements through cereal-crop biofortification may contribute to an upgrade in the health status of the Portuguese population. Selenium (Se) is of utmost importance for a healthy immune system, for its protective (specific) effects against the cardiovascular disease, asthma, male sterility, and, especially, certain forms of cancer. Minimum Se human dietary intakes of 40 µg and 30 µg per day for adult males and females, respectively, are internationally suggested average requirements. Portuguese studies on Se are scarce, yet the available data indicate that current daily intakes fail to meet the above requirements [1-3]. As so, and with financial support by the Portuguese Foundation for the Science and the Technology (research contract PTDC/QUI/65618/2006; FCT, Portugal), an Se supplementation project targeting common cereals is currently under way [4].

Back in the early 1980s, sodium selenite was already being used at moderate dosage in breeding farms for prophylactic and therapeutic purposes [5]. More recently, Finland and the UK have also addressed the issue of Se supplementation to crops [6,7]. In what concerns our program, and prior to any supplementation, the Se levels
in the Portuguese crops and in their cultivation soils should be known in the first place. Still, the first attempts to quantify Se in wheat and rye samples through instrumental neutron activation analysis (INAA) at ITN were unsuccessful, regardless of the analytical procedure, that is, via the long-lived $^{75}$Se or by short-time cyclic INAA using $^{77m}$Se.

The major reason for the former drawbacks was a high content of elements, mainly Al, Hf and Ta, which form interfering radionuclides. A high activity of $^{28}$Al, resulting either from the $^{27}$Al(n,$\gamma$)$^{28}$Al reaction with thermal neutrons or from the $^{31}$P(n,p)$^{28}$Al reaction with fast neutrons, adversely affects the Se detection limit when the short-lived $^{77m}$Se is measured, due to an increased background below the 161.9 keV photopeak of $^{77m}$Se. On the other hand, when long-time INAA is used for low-level determinations via $^{75}$Se, the most intense gamma-lines of this radioisotope – 121.2 keV, 136.0 keV, 264.7 keV, 279.5 keV – are interfered by the gamma-lines of $^{152}$Eu (121.8 keV), $^{181}$Hf (136.2 keV), $^{182}$Ta (264.1 keV) and $^{203}$Hg (279.2 keV), respectively.

In this work, two approaches of NAA were taken: 1) an instrumental procedure (INAA), optimized for maximum $^{75}$Se activation through extended irradiation at the highest neutron fluence rate available at ITN, and minimum $^{181}$Hf interference by prolonging the decay time, even if it was not clear whether the long irradiation could induce Se losses by volatilization; and 2) the radiochemical separation of $^{75}$Se, using a slightly modified RNAA procedure developed earlier [8]. This paper is focused on the latter methodology, reporting the Se status of wheat in various stages of plant growth and grain formation, as well as the Se contents in seeds and soil. Selenium levels in mature (harvested) rye plants will also be reported herein. Furthermore, the accuracy of Se determination by INAA will be assessed by comparing RNAA and INAA results.

**Experimental**

Table 1 shows the samples prepared from the wheat and rye cultivation, and the abbreviations used in this work: A-B-C-D-nr means consecutively the cereal type (wheat: W; rye: R), the cultivation site (Elvas: E; Guarda: G; Valpaços: V), the wheat variety (Jordão: J; Marialva: M), the sample type (soil: So; seed: Se; root: Ro; straw: St; spike: Sp), and the sampling growth stage (wheat cultivation period-1, tillering-2, booting-3, grain filling-4, rye harvesting-5). Zero is used whenever some of the attributes does not apply.

**Sampling**

Two species of wheat (designated by W) bread and durum wheat, *Triticum aestivum* L. (Jordão variety, J) and *Triticum durum* L. (Marialva variety, M) were sown at the end of November 2009 (period 1), and then sampled in January of 2010 (period 2), at the end of March / beginning of April 2010 (period 3), and in May 2010 (period 4). Samples of the soil were collected when the species were sown; samples of the seed used to cultivate both varieties of the wheat were taken at the same time. Fig. 1 shows the place where the cultivation took place (Caia-Elvas, Alentejo province; mainland Portugal).

Rye samples were collected in July 2009 (period 5) when the rye was ready to be harvested. Fig. 1 shows the location in mainland Portugal where the samples were collected: Ribeira dos Carinhos, Guarda (Beira Alta province); Santiago-Alvarelhos, Valpaços (Trás-os-Montes province). Cultivation soil was collected at the same time.

**Preparation of samples at ITN**
Topsoil samples (0-E-0-So-1, 0-G-0-So-5, 0-V-0-So-5) were collected at depths down to 15 cm, allowed to dry at room temperature, sieved through a 1-mm mesh screen for removal of coarser materials, and ground to a fine powder using a porcelain mortar and pestle.

In this work, not all samples obtained in the three sampling campaigns were analyzed; a selection was done to show the capabilities of RNAA for Se determination at the ng g⁻¹ level. The wheat plants were divided into various parts: roots (W-E-J-Ro-2, W-E-M-Ro-2), straws (W-E-J-St-2, W-E-J-St-2, W-E-M-St-2, W-E-M-St-3, W-E-M-St-4), and spikes (W-E-J-St-2, W-E-J-St-2, W-E-M-St-2, W-E-M-St-3, W-E-M-St-4). After removing the bulk of soil, roots were washed with a solution of 0.1 M HCl for 15-20 s dried in open air, cut into small pieces, weighed, and frozen. Seeds, straw, spike were cut into small pieces, weighed and then washed for 10-15 s with distilled water, and frozen. Prior to elemental analysis, all samples were freeze-dried in an Edwards Modulyo® freeze-dryer (plate at -40°C; vacuum of 40 kPa). Roots were ground to a fine powder in a Sartorius® Mikro-Dismembrator U ball mill with Teflon™ capsules, at 1500 rpm for about 9 min. Seeds (W-E-J-Se-1, W-E-M-Se-1), straws and spikes were milled using a Waring Blender HGB50E2.

The rye samples were also divided into several parts: roots (R-G-0-Ro-5, R-V-0-Ro-5), straws (R-G-0-St-5, R-V-0-St-5) and seeds (R-G-0-Se-5, R-V-0-Se-5). The same preparation procedure as described above for wheat samples was followed.

Loss of water during freeze-drying of the biological samples was calculated in order to be able to convert dry-weight results to a fresh-weight basis.

**Analysis of samples at ITN**

All samples were irradiated at the Portuguese Research Reactor (RPI-ITN; Sacavém) for 1 h (soil samples) and 5 h (plant samples), at a thermal-neutron fluence rate of 2.25x10¹² n cm⁻² s⁻¹, together with one disc (thickness: 125 μm; diameter: 5 mm) of an Al-0.1% Au alloy as comparator. Gamma spectra were acquired with a liquid N₂-cooled, high-purity Ge detector (1.85 keV resolution at 1.33 MeV; 30% relative efficiency). Samples were measured 3-4 weeks. The comparator was measured after one week. Elemental concentrations were assessed through k₀-standardized, instrumental neutron activation analysis (k₀-INAA) [9], and calculations were done with the current version of the k₀-IAEA software (version 3.21).

Two samples (soil from the cultivation site Elvas and rye root from the cultivation site Valpaços) and one reference material (GBW07406) were irradiated at the Portuguese Research Reactor (RPI-ITN; Sacavém) for 12 h at a thermal-neutron fluence rate of 5x10¹³ cm⁻² s⁻¹. The mass of each sample was about 200 mg. The samples were counted for 7 h after a decay time of 5 months with a low energy (planar) HPGe detector. Elemental concentrations were assessed using relative standardization using the reference material as the standard.

**Analysis of samples at NPI**

Sample aliquots of about 150 mg were weighed into silica ampoules, which were sealed. The ampoules were cleaned prior to the use by leaching in dilute HF (1:6) for 24 h, leaching in aqua regia for 3 days, and by rinsing with deionized water several times. Reference materials NIST-SRM 1515 ‘Apple Leaves’ and NIST-SRM 8433 ‘Corn Bran’ were prepared in the same way as the samples. A Se standard solution was prepared from 23.15 mg of elemental Se (99.995 %; Fluka™), which was dissolved in approximately 5 mL of dilute HNO₃ (1:1) under reflux and made up to 25 mL with deionized water in a measuring flask. For irradiation, a 100
µL of the prepared solution containing 92.96 µg±0.45 µg of Se was pipetted and weighed into the Si ampoule, which was subsequently sealed.

The samples, reference materials, blank ampoules and standards were irradiated in the LVR-15 reactor for 20 h at a thermal neutron fluence rate of 6x10^{13} cm^{-2} s^{-1}. Thirteen ampoules, each wrapped in a thin Al foil, were accommodated in one Al irradiation container. Each ampoule carried a Fe wire at half height of the sample for monitoring of the axial neutron flux gradient.

The irradiated samples were allowed to cool for 1 month. The ampoules were cleaned on their surface by leaching in aqua regia and washing with distilled water. Then, the ampoules were cooled in liquid nitrogen, wrapped in paper tissue, inserted into a PE bag, and crushed. The samples together with silica splinters were transferred to a Hostaflen flask to which 5-8 mL of conc. HNO₃ and 1 mL of a Se carrier solution (5 mg·mL⁻¹) were added. A pressurized, microwave-assisted digestion system ERTEC® Magnum II (Poland), with pressure and temperature computer control, was employed for sample decomposition.

The silica splinters were removed from the flask and its content was washed out with several mL of conc. HNO₃, and filtered through a glass-fiber filter to separate the possibly present mineral fraction. The filtrate was transferred to a quartz Kjehldahl flask, 3 mL of conc. HClO₄ was added and heated over the flame of gas burner until copious fumes of HClO₄ appeared. Then, 5 mL of dilute HCl (1:1) and 1 mL of saturated solution of MgCl₂ were added. Finally, about 250 mg of ascorbic acid were added to precipitate elemental Se, and the precipitate was left overnight to coagulate properly. The precipitate was filtered off with a Pragopor (Pragochema spol. s r.o.; Czech Republic) nitrocellulose membrane filter (pore size: 1.5 µm; diameter: 35 mm), using a Sartorius® vacuum filtration unit.

After drying, the filter with the precipitate was sealed into a polyethylene bag for counting with a well-type HPGe detector (active volume: 150 cm³; well diameter: 16 mm; well depth: 40 mm; FWHM resolution: 2.02 keV for the 1332.5 keV photons of ^{60}Co), for 2 h. This separated fraction obtained by sample decomposition in conc. HNO₃ is further denoted as “organic Se fraction”.

The glass-fiber filter with the possible present mineral fraction was dissolved in a mixture of 5 mL of conc. HNO₃ and 1-2 mL of conc. HF in the above micro-wave digestion system, and the resulting solution was repeatedly evaporated in a Teflon™ beaker almost to dryness to get rid of HF. Then, the same procedure for separation of Se was employed as described above. The separated fraction was counted with the well-type HPGe detector for 8 h, and is further denoted as “mineral Se fraction”.

The Se separation yield was determined by reactivation of the added carrier by short-time irradiation (30 s) with the aid of a pneumatic facility and counting of the ^{77m}Se radioisotope with a 20.8 % relative efficiency coaxial HPGe detector.

For quantification of Se contents in the samples, the irradiated liquid ^{75}Se standard was washed out from the ampoule into a 5 mL measuring flask, and 25 µL of this solution was deposited onto a filter paper of the same size as the above membrane filters for counting.

**Results and discussion**

*Gamma-ray spectrometry*

Several detector types were tested in preliminary experiments at NPI for counting of separated fractions with ^{75}Se. Fig. 2 shows a comparison of the efficiency of counting of the most intensive gamma-lines with three
coaxial HPGe detectors with relative efficiency of 20.8 %, 52.9 % and 78.0 % (FWHM resolution in the range of 1.75 – 1.85 keV for the 1332.5 keV photons of $^{60}$Co), the above mentioned well-type HPGe detector, and a planar HPGe detector (500 mm$^2$ active area, 15 mm thickness, FWHM resolution 550 eV for the 122.1 keV photons of $^{57}$Co. The highest sensitivity (in counts·s$^{-1}$) of measurement of the same amount of $^{75}$Se on the top of the coaxial and planar HPGe detectors and inside the well HPGe detector was obtained for the sum peak of 400.66 keV. Therefore, measurement of this peak was used for quantification of Se in RNAA at NPI.

In INAA, the selection of the most suitable detector is not so straightforward, because the most intensive gamma-lines of 121.2 keV, 136.0 keV, 264.7 keV, and 279.5 keV are interfered. When a well-type HPGe detector is excluded, the highest sensitivity of $^{75}$Se measurement is achieved using the 136.0 keV gamma-line, which is interfered with a minor 136.17 keV gamma-line (5.8 % intensity) of $^{181}$Hf ($T_{1/2}$=42.39 d). This interference can be alleviated employing a sufficiently prolonged decay time. Therefore, the 136.17 keV gamma-line was selected for quantification of Se in the INAA procedure at ITN and counting with the above described planar HPGe detector was carried out after 5 months of decay. Counting with a planar HPGe reduces significantly background below the 136.17 keV photopeak compared with a coaxial HPGe detector. The result obtained for the soil sample (0-E-0-So-1) was 150±40ppb and for the root sample (R-V-0-Ro-5) 27±18ppb (fresh weight). In this case the error is considerable high so this is not a suitable analytical technique to get selenium contents for these samples. The samples were also analyzed by INAA with 3-4 weeks of decay but values for selenium were not achieved, only the detection limits that were around 2,000ppb for soil, 450ppb for roots and straw, 250 ppb for spikes and 150ppb for seeds.

Radiochemical separation

The yield of chemical separation of Se in the RNAA procedure employed was very high and well reproducible (mean: 95.0 %; standard deviation: 3.8 %; N=52). Nevertheless, in each determination correction for the actual yield was employed to achieve the lowest uncertainty. Concerning radiochemical purity of the separated fractions, unexpectedly, co-precipitation of $^{233}$Pa was found in most of the wheat and rye samples analyzed, which due to an appreciable content of Th in some samples yielded formation of a doublet of the 398.66 keV peak of $^{233}$Pa and the 400.66 keV sum peak of $^{75}$Se. This interference, well resolved by the interactive peak analysis procedure of the Canberra Genie 2000 software, was most noticeable in root samples as depicted in Fig. 3. No occurrence of the 398.66 keV peak was observed in the NIST-SRMs. No detectable amount of Se was found in blank ampoules, which were processed as the samples. The detection limit for Se of 0.3 ng·g$^{-1}$ was achieved with the RNAA procedure employed for counting of the separated fractions for 8 h. Accuracy of the RNAA procedure was proved by analysis of low-level reference materials NIST-SRM 1515 ‘Apple Leaves’ and NIST-SRM 8433 ‘Corn Bran’. Table 2 shows that agreement was found between results of this work and NIST values within uncertainty margins.

Results for selenium in plants and soils and observed selenium patterns

Selenium contents in various parts of wheat and rye plants, and in cultivation soils determined by RNAA are given in Table 1. It can be seen from Table 1 that most Se was contained in the organic fractions. The mineral fractions contained non-detectable Se contents or only 1-1.8 % of that in organic fractions of most plant samples. Rather low Se portions (in the range of 0.2 % to 7.2 %) were also found in mineral fractions of soils.
compared with organic fractions. A similarly high Se value determined in the mineral fraction of rye straw from site 3 (27.9 % of that in the organic fraction) was not seen in rye straw from site 1.

The baseline Se concentration of wheat grain used in UK breads has been analyzed in samples from 1982, 1992 and 1998, and a minimal difference in mean concentrations between samples has been found: 0.025, 0.033 and 0.025 mg Se per kg, respectively, with interquartile ranges from 0.015 to 0.019 mg Se per kg [10]. Worldwide, the Se content of wheat grain may vary from 0.001 to 30 mg kg$^{-1}$, even if most values are between 0.02 and 0.60 mg kg$^{-1}$ [11].

Transfer coefficients relative to soil

Transfer coefficients (TC_Soil) from soil to root, straw, spike and seed of wheat and rye are shown in Fig. 4. These coefficients were obtained by assessing the ratios between the selenium concentrations in plants' parts (root, straw, spike or seed, on a fresh weight basis; $[\text{Se}]_{\text{plant}}$) and in soil ($[\text{Se}]_{\text{soil}}$):

$$\text{TC}_\text{Soil} = \frac{[\text{Se}]_{\text{plant}}}{[\text{Se}]_{\text{soil}}} \quad (1)$$

The coefficients increase as the wheat plant goes through the growing stages (tillering < booting < grain filling), and Jordão variety features lower values than Marialva. The TC_Soil values for rye were lower than the corresponding ones for wheat in all plants' parts, with the exception of roots.

Transfer coefficients relative to seed

Transfer coefficients (TC_Seed) from seed to root, straw and spike of wheat are shown in Fig. 4. These coefficients were obtained by assessing the ratios between the selenium concentrations in plants' parts (root, straw or spike, on a fresh weight basis; $[\text{Se}]_{\text{plant}}$) and in the sowing seeds ($[\text{Se}]_{\text{seed}}$):

$$\text{TC}_\text{Seed} = \frac{[\text{Se}]_{\text{plant}}}{[\text{Se}]_{\text{seed}}} \quad (2)$$

The trend of TC_Seed is similar to TC_Soil, that is an increase along the growth stages (tillering < booting < grain filling), with Jordão variety featuring lower values than Marialva.

Conclusions

RNAA is a very sensitive technique that can achieve selenium detection limit as low as 0.3ppb and the yield of chemical separation of Se was very high. INAA technique was not successful to get selenium due to the interference Al, Hf and Ta in the samples. Accuracy of the RNAA procedure was showed by analysis of low-level reference materials NIST-SRM 1515 ‘Apple Leaves’ and NIST-SRM 8433 ‘Corn Bran’.

In this work is shown that selenium transfer coefficient increases as the wheat plant goes through the growing stages, and that transfer coefficients from soils were lower for rye than the corresponding ones for wheat in almost plants' parts.

Acknowledgements

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References

Table 1. List of samples analyzed and results for Se determined by RNAA (for “Code” see text)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Code</th>
<th>Origin</th>
<th>Variety</th>
<th>Fraction</th>
<th>Sampling date</th>
<th>“Organic fraction”(^a), ng g(^{-1})</th>
<th>“Mineral fraction”(^a), ng g(^{-1})</th>
<th>Total Se(^a), ng g(^{-1}), dry mass</th>
<th>Total Se(^a), ng g(^{-1}), fresh mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>0-E-0-So-1</td>
<td>Elvas</td>
<td>-</td>
<td>&lt; 1 mm</td>
<td>End of Nov. 2009</td>
<td>115 ± 6</td>
<td>3.1 ± 0.3</td>
<td>118 ± 6</td>
<td>118 ± 6</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-J-Se-1</td>
<td>Elvas</td>
<td>Jordão</td>
<td>Seed</td>
<td>End of Nov. 2009</td>
<td>57 ± 3</td>
<td>&lt; 0.3</td>
<td>57 ± 3</td>
<td>54 ± 3</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-J-Ro-2</td>
<td>Elvas</td>
<td>Jordão</td>
<td>Root</td>
<td>19.01.2010</td>
<td>39 ± 2</td>
<td>&lt; 0.3</td>
<td>39 ± 2</td>
<td>6.1 ± 0.3</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-J-St-2</td>
<td>Elvas</td>
<td>Jordão</td>
<td>Straw</td>
<td>19.01.2010</td>
<td>22 ± 1</td>
<td>&lt; 0.5</td>
<td>22 ± 1</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-J-St-3</td>
<td>Elvas</td>
<td>Jordão</td>
<td>Straw</td>
<td>13.04.2010</td>
<td>26 ± 1</td>
<td>0.46 ± 0.05</td>
<td>26 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-M-Se-1</td>
<td>Elvas</td>
<td>Marialva</td>
<td>Seed</td>
<td>End of Nov. 2009</td>
<td>41 ± 2</td>
<td>&lt; 0.3</td>
<td>41 ± 2</td>
<td>38 ± 2</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-M-Ro-2</td>
<td>Elvas</td>
<td>Marialva</td>
<td>Root</td>
<td>19.01.2010</td>
<td>35 ± 2</td>
<td>0.39 ± 0.05</td>
<td>35 ± 2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-M-St-2</td>
<td>Elvas</td>
<td>Marialva</td>
<td>Straw</td>
<td>19.01.2010</td>
<td>64 ± 3</td>
<td>0.62 ± 0.04</td>
<td>65 ± 3</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-M-St-3</td>
<td>Elvas</td>
<td>Marialva</td>
<td>Straw</td>
<td>30.03.2010</td>
<td>83 ± 4</td>
<td>&lt; 0.3</td>
<td>83 ± 4</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-M-St-4</td>
<td>Elvas</td>
<td>Marialva</td>
<td>Straw</td>
<td>05.05.2010</td>
<td>53 ± 3</td>
<td>&lt; 0.3</td>
<td>53 ± 3</td>
<td>9.4 ± 0.5</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-M-Sp-3</td>
<td>Elvas</td>
<td>Marialva</td>
<td>Spike</td>
<td>30.03.2010</td>
<td>32 ± 2</td>
<td>&lt; 0.3</td>
<td>32 ± 2</td>
<td>5.6 ± 0.4</td>
</tr>
<tr>
<td>Wheat</td>
<td>W-E-M-Sp-4</td>
<td>Elvas</td>
<td>Marialva</td>
<td>Spike</td>
<td>05.05.2010</td>
<td>25 ± 1</td>
<td>&lt; 0.3</td>
<td>25 ± 1</td>
<td>22 ± 1</td>
</tr>
<tr>
<td>Rye</td>
<td>0-G-0-So-5</td>
<td>Guarda</td>
<td>-</td>
<td>Soil</td>
<td>14.07.2009</td>
<td>97 ± 5</td>
<td>0.20 ± 0.03</td>
<td>97 ± 5</td>
<td>97 ± 5</td>
</tr>
<tr>
<td>Rye</td>
<td>R-G-0-Se-5</td>
<td>Guarda</td>
<td>-</td>
<td>Seed</td>
<td>14.07.2009</td>
<td>29 ± 2</td>
<td>0.51 ± 0.06</td>
<td>30 ± 2</td>
<td>23 ± 2</td>
</tr>
<tr>
<td>Rye</td>
<td>R-G-0-Ro-5</td>
<td>Guarda</td>
<td>-</td>
<td>Root</td>
<td>14.07.2009</td>
<td>3.3 ± 0.2</td>
<td>0.92 ± 0.06</td>
<td>4.3 ± 0.3</td>
<td>4.2 ± 0.3</td>
</tr>
<tr>
<td>Rye</td>
<td>R-G-0-St-5</td>
<td>Guarda</td>
<td>-</td>
<td>Straw</td>
<td>14.07.2009</td>
<td>7.0 ± 0.4</td>
<td>&lt; 0.3</td>
<td>7.0 ± 0.4</td>
<td>6.7 ± 0.4</td>
</tr>
<tr>
<td>Rye</td>
<td>0-V-0-So-5</td>
<td>Valpaços</td>
<td>-</td>
<td>Soil</td>
<td>14.07.2009</td>
<td>210 ± 10</td>
<td>15.1 ± 0.8</td>
<td>225 ± 11</td>
<td>225 ± 11</td>
</tr>
<tr>
<td>Rye</td>
<td>R-V-0-Se-5</td>
<td>Valpaços</td>
<td>-</td>
<td>Seed</td>
<td>14.07.2009</td>
<td>39 ± 2</td>
<td>&lt; 0.3</td>
<td>39 ± 2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Rye</td>
<td>R-V-0-Ro-5</td>
<td>Valpaços</td>
<td>-</td>
<td>Root</td>
<td>14.07.2009</td>
<td>5.0 ± 0.3</td>
<td>&lt; 0.3</td>
<td>5.0 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Rye</td>
<td>R-V-0-St-5</td>
<td>Valpaços</td>
<td>-</td>
<td>Straw</td>
<td>14.07.2009</td>
<td>7.2 ± 0.5</td>
<td>&lt; 0.3</td>
<td>7.2 ± 0.5</td>
<td>6.8 ± 0.5</td>
</tr>
</tbody>
</table>

\(^a\) - combined uncertainties (coverage factor k=1) are given
Table 2. Results for Se in NIST-SRM 1515 ‘Apple Leaves’ and NIST-SRM 8433 ‘Corn Bran’, in ng·g⁻¹, (dry weight).

<table>
<thead>
<tr>
<th>Material</th>
<th>This work ⁴</th>
<th>NIST value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIST-SRM 1515</td>
<td>43 ± 4 (6)</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>NIST-SRM 8433</td>
<td>44 ± 3 (4)</td>
<td>45 ± 8</td>
</tr>
</tbody>
</table>

⁴ mean ± S.D. (number of replicates)

Figure 1. Outline of mainland Portugal, showing the approximate location of the rye and wheat fields for soil and plant sampling – Site G: Ribeira dos Carinhos, Guarda (Beira Alta); site V: Santiago-Alvarelhos, Valpaços (Trás-os-Montes); site E: Caia, Elvas (Alentejo).

Figure 2. Comparison of detection efficiency for counting of the same amounts of ⁷⁵Se with various detectors.
Figure 3. Sections of gamma-ray spectra of RNAA “organic fractions”: (a) NIST-SRM 8433, 400.66 keV gamma-line of $^{75}$Se only; (b) wheat straw, 398.66 keV + 400.66 keV gamma-lines of $^{233}$Pa + $^{75}$Se, respectively; (3) wheat root, 398.66 keV + 400.66 keV gamma-lines of $^{233}$Pa + $^{75}$Se, respectively.

Figure 4. Transfer coefficients from soil and from seed to plant parts (fresh weight).