Electronic Supplementary Information

Determination of selenium in food and environmental samples by hydride generation high-resolution continuum source quartz furnace atomic absorption spectrometry

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Summary:

- Interest in Se determination in environmental and food matrices resulting from its dual character with beneficial and toxic effects for humans and plants;
- Operation of the HG-HR-CS-QFAAS instrumentation and measurement of the absorption signal;
- Reagents, CRMs, sample digestion and prereduction of Se(VI) to Se(IV);
- Experimental parameters influencing hydride generation efficiency and Se absorption signal;
- Inter-day reproducibility of the calibration curve and LOD using peak height and peak area measurements;
- Comparative data for Se determination by HG-HR-CS-QFAAS and other spectrometric methods;
- Concentration levels of concomitants in samples that do not cause non-spectral interferences on Se determination by HG-HR-CS-QFAAS;
- Se content in test samples;
- Daily intake of Se in adults and teenagers *via* foods and dietary supplements analyzed in this study.

Se role in humans and plants health and toxicity

Chemistry of Se and its metabolism play a crucial role in humans, animals and plants health.[1-5] Selenium is an interesting microelement in terms of the dual biological influence, since it can be beneficial and toxic relying on its content and the safety range is quite narrow. Selenium acts as a modulator, thus exhibiting antioxidant activity at low concentration and pro-oxidant property at high concentration. An intake below 40 µg/day Se is considered deficient for humans, an entry slightly higher than this threshold is essential for health, while a long-term intake even just over 400 µg/day could result in serious physiological problems. An excessive entry of Se in human body causes serious dermatological problems, such as loss of nails and hair, liver diseases or nervous system disorders. An intake of 1 mg Se/kg body weight or concentrations exceeding 300 µg L^{-1} in blood and 170 µg L^{-1} in urine were associated to death.[1-5] Selenium deficiency has been associated to inflammation and weakness of muscle, abnormal skin coloring, fragile red blood cells, dysfunction of heart muscle, Keshan and Kashin-Beck diseases, type 2 diabetes

mellitus and susceptibility to cancer. Therefore, the deficit of Se is considered to be one of the most important health issues for 0.5–1 billion people.

For humans the beneficial effect of Se is due to its conversion to selenocysteine, an essential constituent of 25 antioxidant enzymes, for example glutathione peroxidase and selenoprotein P, with major role in scavenging and regulation of reactive oxygen species (ROS), or in antioxidant defense system against harmful ROS.[6,7] Co-administration of Se(IV) highlighted a decrease in CH_3Hg^+ (MeHg) bioaccumulation factor in muscular tissue of fish, demonstrating the protective role against CH_3Hg-induced oxidative stress.[8] The beneficial effect of Se on human health turns into toxicity because of the pro-oxidative action on organosulfur compounds, resulting in the increase of ROS and lipid peroxidation, responsible for prostate carcinoma and hepatic cancer.[9,10]

Similar to the dual biological influence on human health, Se in low concentrations is important in plant metabolism through its positive effect on photosynthesis, growth, stress reduction, while in high concentration it becomes toxic.[3-5] Depending on the ability to accumulate Se, plant species were classified in three groups, namely Se hyperaccumulators tolerating >1000 mg kg^{-1}, secondary-accumulators corresponding to 100–1000 mg kg^{-1} Se contents and non-accumulators, which do not reach beyond 100 mg kg^{-1}.[1,3,5] The high tolerance of hyperaccumulators to Se is the result of the high translocation occurring from root to shoot, leafs and seeds, high potential to convert Se inorganic species in selenoproteins and their preferential accumulation compared to sulfur compounds.

Food is considered the main source of Se for humans (nearly 80%) and intake depends on the individual diet. The demand for Se may be covered by the consumption of protein-rich foods such as game meat or meat from farm-raised animals, fish muscle and food of marine origin (0.1–3 mg kg^{-1}), egg yolk (0.2–0.6 mg kg^{-1}), cereals, for example corn and rice (<0.1–0.8 mg kg^{-1}). Vegetables and fruits provide <0.1 mg kg^{-1}, with some exceptions such as onion (0.15 mg kg^{-1}), melon (1.7 mg kg^{-1}), grapes (up to 4 mg kg^{-1}), mustard beans and herbal tea (0.2 mg kg^{-1}). Milk and dairy products are quite poor sources of Se (<0.03 mg kg^{-1}).[5,11,12] Content of Se in food and its intake in human body are dependent on natural factors such as Se in soil and plant ability to accumulate it. There are significant differences in terms of Se level in soil all over the globe and Se-excessive or Se-deficient areas are encountered.[3] European Food Safety Authority (EFSA)/Scientific Committee of Food (SCF) have set the Adequate Intake (AI)/Tolerable Upper Intake Level (UL) for Se of 70/300 µg/day for adults, 70/250 µg/day for children aged 15 to 17 years and 15/60 µg/day for children aged 1 to 3 years.[13]

In case of Se deficiency the habitual diet needs to be supplemented *via* multivitamin/multimineral tablets or biofortified cereals or vegetables. This could be achieved through the application of foliar feeding with sodium selenate, combined or not with amendment of depleted soil with selenate solution and/or mineral fertilizer containing selenate.[14-17]

Operation of the batch HG-HR-CS-QFAAS equipment

The ContrAA 300 spectrometer used in the experimental study is equipped with a high-intensity xenon short-arc lamp as a continuum radiation source covering the spectral range 189–900 nm, a high-resolution double monochromator (2 pm FWHM) and a charge coupled device (CCD) with 512 pixels, 200 of which were used to record the absorption spectrum in the range ±0.1 nm in the vicinity of Se line at 196.026 nm. A number of 5 pixels in the center of the spectral window were assigned to measurement of absorption signal at the analytical line of Se. The net absorption signal was obtained after background correction using a reference spectrum recorded for 3% (v/v) HCl solution as blank after pre-washing the reaction cell and quartz tube with 6 L h^{-1} Ar for 20 s. The hydride generation system consisted of a PTFE reaction cell, a single-channel variable speed peristaltic pump and a set of three-way valves for controlling the Ar stream used to carry hydrogen selenide to the quartz atomizer. The operation of the HS55 batch hydride generation system involved pipetting aliquot volumes of 5 or 10 mL sample in the reaction cell, air purging from the reaction cell and quartz tube with 6 L h^{-1} Ar for 20 s and pumping of 1.5–5.5 mL of 1.5% (m/v) $NaBH_4$ stabilized in 0.1% (m/v) NaOH as derivatization reagent. The SeH_2 produced was purged from the liquid with 6 L h^{-1} Ar and carried through the Nafion tube to the quartz atomizer pre-heated at 950 ± 10 °C, where Se atoms were generated in Ar-H_2 atmosphere. The net transient signal of Se at 196.026 nm was obtained in the absorption spectrum recorded for 7 s and 20 s in peak height and peak area measurement, respectively. The memory effect was overcome by purging the HG system with Ar for 30 s after analyte measurement. Five successive measurements were performed in each case to evaluate the mean of analytical signal and relative standard deviation. The HG-HR-CS-QFAAS analytical system was operated using ASpects CS 2.2.1, Analytik Jena, software. The working conditions are presented in Table S1.

Reagents, CRMs, sample digestion and prereduction of Se(VI) to Se(IV)

All reagents were analytical or superior grade. Hydrochloric acid 37% (m/m), nitric acid 65% (m/m), H_2O_2 30% (m/m), $NaBH_4$ (<0.0002% Se), NaOH (>98%), Se ICP standard 1000 µg mL^{-1} stabilized in 0.5 mol L^{-1} HNO_3 from Merck (Darmstadt, Germany) were used. Ultra-pure water (18 MΩ cm) obtained from a Milli-Q water purification system Millipore (Bedford, USA) was used for the preparation of samples and all standard solutions. Eight calibration standards (0–10 µg L^{-1} Se) in 3% (v/v) HCl were prepared. A solution of 3% (v/v) HCl was used as blank and diluent. For the optimization of derivatization conditions, the following solutions were prepared: 5 µg L^{-1} Se in 0.5–6% (v/v) HCl, 0.5–3% (m/v) $NaBH_4$ stabilized in 0.1% (m/v) NaOH and 1.5% (m/v) $NaBH_4$ stabilized in 0.02–0.2% (m/v) NaOH.

Table S1. Working conditions for Se determination by HG-HR-CS-QFAAS equipped with a HS55 batch hydride generation system

Parameter	Setting
Analytical wavelength (nm)	196.026
Number of pixels used for absorption measurement at analytical wavelength	5
Transient signal measurement	Peak height or peak area
Time period for recording transient absorption spectrum for peak height/peak area measurement (s)	7/20
Ar flow rate (L h^{-1})	6
Pre-heating temperature of the quartz atomizer (°C)	950 ± 10
Pre-wash time of reaction cell and quartz atomizer with Ar (s)	20
Auto-zero time (s)	20
Volume of $NaBH_4$ solution (mL)/pumping time (s)	1.5–5.5/5–20
Washing time of reaction cell and quartz atomizer (s)	30
Sample volume (mL)	5 and 10
Calibration	External standards
Se concentration in calibration standards (µg L^{-1})	0; 0.5; 1; 2; 4; 6; 8; 10
Number of repeated measurements of standards and samples	5

The accuracy of the method was checked by analyzing the following certified reference materials (CRMs): ERM-BB422 Fish muscle, ERM-CE278K Mussel Tissue, BCR-185R Bovine liver, ERM-BB184 Bovine Muscle, ERM-BB186 Pig Kidney, ERM-CA713 Wastewater, ERM-CA011b Hard Drinking Water (Institute for Reference Materials and Measurements, Geel, Belgium), CRM025050 Metals in soil (Resource Technology Corporation, Laramie, USA), LGC6141 Soil Contaminated with Clinker Ash (Department of Trade and Industry, Teddington Midlesex, UK), Tort-2 Lobster Hepatopancreas Reference Material for Trace Metals (National Research Council Canada, Ottawa, Ontario Canada), CSM-3 Mushroom Powder (*Boletus edulis*) (Institute of Nuclear Chemistry and Technology, Warsaw, Poland), SRM 3280 Multivitamin, SRM 2976 Mussel Tissue (National Institute of Standards and Technology, Gaithersburg, USA), GBW 10018 Chicken (Institute of Geophysical and Geochemistry Exploration, Langfang, China), NMIJ CRM 7202-b River Water (National Metrology Institute of Japan, Tsukuba, Japan).

Sample preparation involved two steps, digestion and prereduction of Se(VI) to Se(IV). Amounts of up to 0.5 g CRM or dried test sample were subjected to microwave assisted digestion in closed PTFE vessels at high pressure. Samples of powdered meat, organs, onion and multivitamin and multielement preparations were digested in a mixture of 9 mL 65% HNO_3 and 3 mL H_2O_2 30%, while soil samples in 10 mL 65% HNO_3. The prereduction of Se(VI) to Se(IV) was carried out in 6 mol L^{-1} HCl by adding 15 mL 37% HCl and 5 mL ultrapure water to soil digest and 3 mL water in the other digests. The microwave heating program for sample mineralization and prereduction with concentrated HCl is presented in Table S2. After cooling, the solution was filtered and diluted to 50 mL in volumetric flask with ultrapure water. Then, 5 or 10 times dilution was performed to yield 3–6% (v/v) HCl in the final solution, optimal for the determination of Se by the batch HG-HR-CS-QFAAS method. Prereduction in non-spiked water samples was conducted under the procedure described previously. Two spiking levels were prepared, 1 and 5 µg L^{-1} Se in 3–6%

(v/v) HCl medium. Non-/ spiked samples were analyzed and recovery of Se was calculated for 95% confidence level.

Table S2. Microwave heating program for sample digestion and prereduction of Se(VI) to Se(IV)

Parameter	Digestion				
	Step1	Step 2	Step 3	Step 4	Step 5
Temperature (°C)	100	170	200	150	100
Hold time (min)	1	17	25	5	1
Ramp time (min)	2	5	2	1	1
Power (%)[a]	35	80	80	80	30
	Prereduction in 6 mol L^{-1} HCl				
Temperature (°C)	100	150	100		
Hold time (min)	1	15	5		
Ramp time (min)	5	1	1		
Power (%)[a]	35	80	30		

[a]100% power corresponds to 1450 W

All test samples and CRMs were subjected to analysis by HG-HR-CS-QFAAS and ICP-OES for the determination of total Se and metals in the multimineral matrix, respectively.

Experimental parameters influencing hydride generation efficiency and Se absorption response

Influence of HCl concentration

The influence of HCl concentration on hydride generation from Se(IV) was investigated over the range 0.5–6% (v/v) HCl and results are presented in Fig. S1.

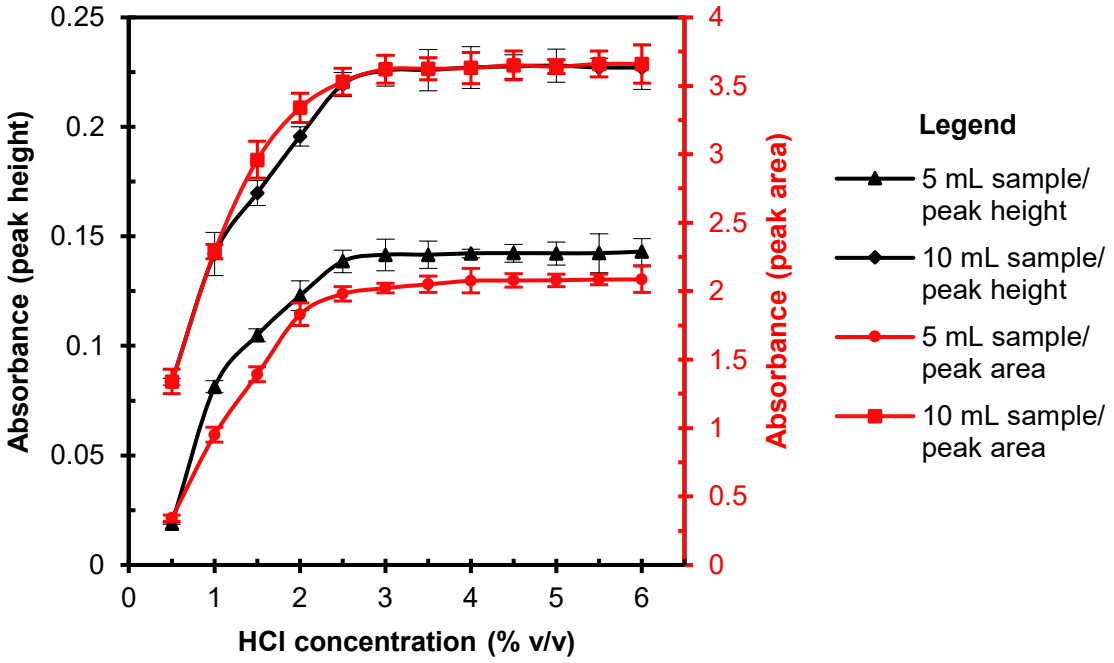

Fig. S1. Effect of HCl concentration on the Se response from aliquot volumes of 5 mL and 10 mL standard containing 5 µg L^{-1} Se(IV) in peak height and peak area measurement of absorption transient signal. Volume of 1.5% $NaBH_4$ solution stabilized in 0.1% NaOH: 4 mL (15 s pumping time). Error bars correspond to RSD for five successive measurements.

The graph shows the significant influence of the HCl concentration resulting in a considerable increase of the analytical response up to a content of 2% (v/v) HCl in the sample. For 3% (v/v) HCl or more, a stabilization of the absorption signal occurred regardless of the measurement mode. Thus, it was concluded that the sample should contain at least 3% (v/v) HCl and the range 3–6% (v/v) HCl was considered suitable for Se determination. Therefore, the original sample digests were subjected to a minimum 1:5 dilution. For dilutions greater than 1:10 the diluent was 3% (v/v) HCl solution.

Influence of $NaBH_4$ and NaOH concentrations

The influence of $NaBH_4$ and NaOH concentrations on hydride generation from 5 mL and 10 mL standard solution containing 5 µg L^{-1} Se(IV) in 3% (v/v) HCl is presented in Figs. S2 and S3.

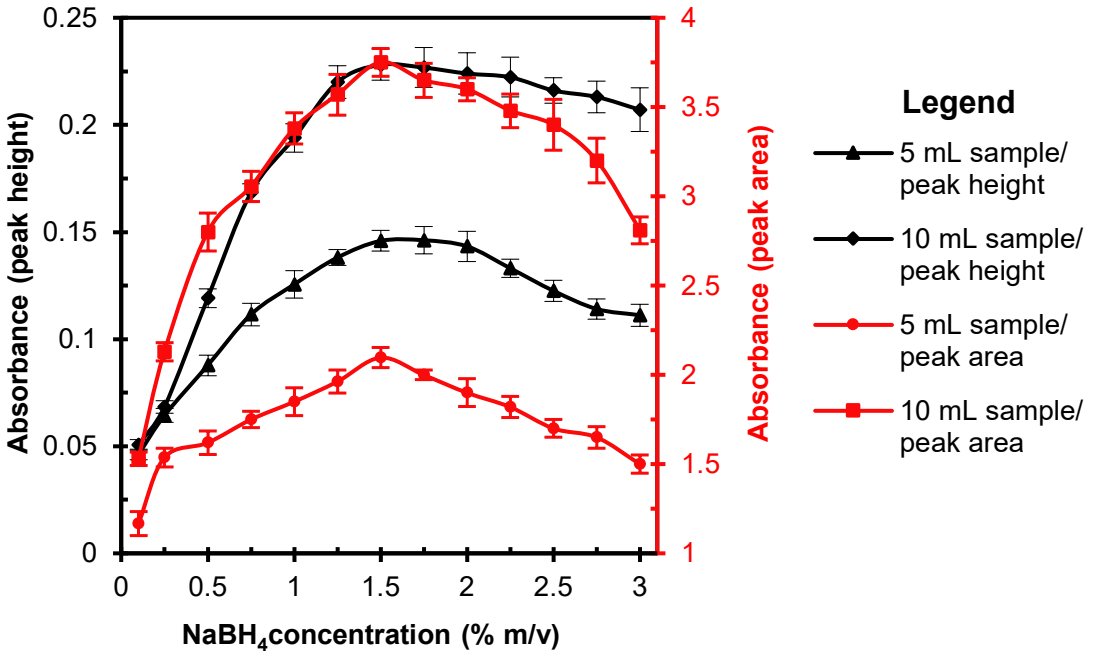

Fig. S2. Effect of $NaBH_4$ concentration stabilized in 0.1% (m/v) NaOH on the Se response from aliquot volumes of 5 mL and 10 mL standard containing 5 µg L^{-1} Se(IV) in 3% (v/v) HCl in peak height and peak area measurement of transient absorption signal. Volume of 1.5% $NaBH_4$ solution stabilized in 0.1% NaOH: 4 mL (15 s pumping time). Error bars correspond to RSD for five successive measurements.

The use of 1.5% (m/v) $NaBH_4$ in 0.1% (m/v) NaOH resulted in the highest response for Se in both measurement modes of absorption. For NaOH concentrations greater than 0.1% a decrease of response was observed as a result of lower efficiency of hydride generation at higher pH values. At the same time, the absorption peak became higher and narrower as the borohydride concentration increased due to the higher reaction rate of hydride generation. Therefore, the maximum signal in the peak height measurement was reached only 7 s after mixing sample and reagent compared to 20 s in the case of peak area measurement.

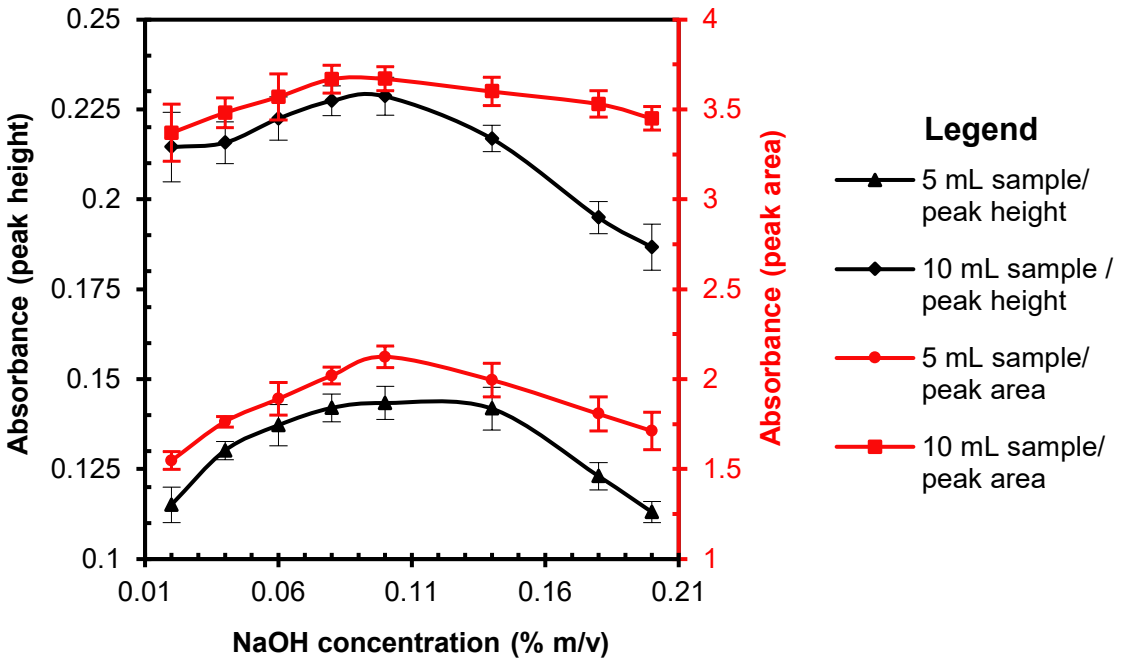

Fig. S3. Effect of NaOH concentration used for stabilization of 1.5% (m/v) $NaBH_4$ on the Se response from aliquot volumes of 5 mL and 10 mL standard containing 5 µg L^{-1} Se(IV) in 3% (v/v) HCl in peak height and peak area measurement of transient absorption signal. Volume of 1.5% $NaBH_4$ solution stabilized in 0.02–0.2% NaOH: 4 mL (15 s pumping time). Error bars correspond to RSD for five successive measurements.

Influence of the volume of $NaBH_4$ solution

The volume of 1.5% $NaBH_4$ solution in 0.1% NaOH as derivatization reagent added to 5 or 10 mL standard aliquot containing 5 µg L^{-1} Se(IV) in 3% (v/v) HCl was controlled by the pumping time *via* software programming of the HG-HR-CS-QFAAS instrument. Results are presented in Fig. S4. According to Fig. S4, the absorption signal increased with an increase in reducing reagent volume up to 4 ml and the peak became higher and narrower. Therefore, a volume of 4 mL reagent was found optimal for Se determination.

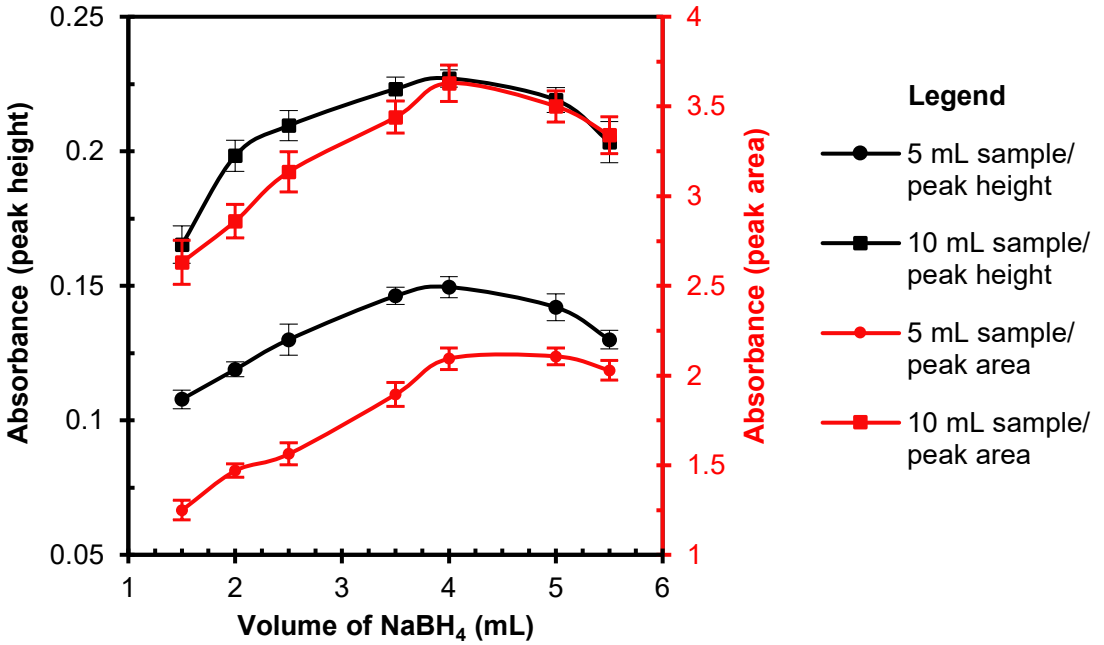

Fig. S4. Optimization of the volume of 1.5% (m/v) $NaBH_4$ solution in 0.1% (m/v) NaOH *via* pumping time (5–20 s) on the Se response from aliquot volumes of 5 mL and 10 mL standard containing 5 µg L^{-1} Se(IV) in 3% (v/v) HCl in peak height and peak area measurement of transient absorption signal. Error bars correspond to RSD for five successive measurements.

Inter-day reproducibility of calibration curve and LOD

The results obtained in different days for the calibration curve using Se(IV) standard solutions up to 10 µg L^{-1} and LODs in liquid and solid samples in peak height and peak area measurement of absorption are presented in Table S3.

Table S3. Inter-day reproducibility of the parameters of the calibration curves, LOD and LOQ of Se by HG-HR-CS-QFAAS in consecutive days using peak height and peak area measurement

Number of days	Sample volume (mL)	Signal measurement	Calibration curve parameters			Standard deviation of the blank	LOD		LOQ	
			Intercept	Slope (L μg^{-1})	R^2		Liquid ($\mu g\ L^{-1}$)	Solid (mg kg^{-1})[a]	Liquid ($\mu g\ L^{-1}$)[b]	Solid (mg kg^{-1})[b]
9	5	Peak height	0.0034	0.0308	0.9994	0.0009	0.119	0.059	0.356	0.178
	5	Peak height	0.0005	0.0290	0.9994	0.0012	0.126	0.063	0.377	0.188
	5	Peak height	0.0039	0.0306	0.9982	0.0019	0.120	0.060	0.359	0.179
	5	Peak height	-0.0008	0.0307	0.9988	0.0014	0.119	0.059	0.357	0.178
	5	Peak height	0.0039	0.0277	0.9991	0.0023	0.132	0.066	0.396	0.198
	5	Peak height	-0.0031	0.0281	0.9995	0.0033	0.130	0.065	0.389	0.194
	5	Peak height	0.0091	0.0289	0.9988	0.0026	0.126	0.063	0.379	0.189
	5	Peak height	-0.0017	0.0285	0.9981	0.0025	0.146	0.073	0.438	0.219
	5	Peak height	-0.0014	0.0315	0.9981	0.0026	0.116	0.058	0.348	0.174
3	10	Peak height	0.0048	0.0477	0.9985	0.0029	0.084	0.042	0.253	0.126
	10	Peak height	0.0050	0.0483	0.9983	0.0028	0.068	0.034	0.203	0.102
	10	Peak height	0.0038	0.0466	0.9995	0.0025	0.078	0.039	0.233	0.117
3	5	Peak area	0.0903	0.4135	0.9986	0.0468	0.121	0.061	0.363	0.182
	5	Peak area	0.0710	0.5113	0.9977	0.0465	0.134	0.067	0.402	0.201
	5	Peak area	0.0667	0.4357	0.9989	0.0405	0.126	0.063	0.378	0.189
3	10	Peak area	0.102	0.8058	0.9979	0.0528	0.070	0.035	0.21	0.105
	10	Peak area	0.1074	0.7858	0.9992	0.0549	0.073	0.037	0.219	0.110
	10	Peak area	0.1124	0.7546	0.9985	0.0548	0.069	0.035	0.207	0.104

[a] LOD – limit of detection in solid was calculated for 0.5 g digested sample, made up to 50 mL and diluted 1:5; [b] LOQ –limit of quantification was considered as 3xLOD

Comparative values for LOD and LOQ in HG-HR-CS-QFAAS and other spectrometric methods

Comparative values for LOD and LOQ in HG-HR-CS-QFAAS and other methods for Se determination in several matrices using different sample preparation procedures are given in Table S4. Data in Table S4 indicate that LOD and LOQ in a method depend not only on the spectral detector but also on the pathway used to separate Se species from the matrix, namely hydride generation with/without, in-or out-atomizer preconcentration of gaseous species, separation and preconcentration of Se species by liquid-liquid micro-extraction or solid phase micro-extraction. Sensitivity for HG-HR-CS-QFAAS in batch system falls within the range covered by the other methods given for comparison in Table S4. The limit of detection in the HG-HR-CS-QFAAS was better than those reported for Se in soil by HG-GFAAS and HG-QFAAS using classical hydride generation with $NaBH_4$ without preconcentration and detection by conventional line-source atomic absorption, or UV photo-induced derivatization in formic acid coupled with in-atomizer trapping and detection by HG-GFAAS or AFS. Our LOD was even better than that in ICP-MS with solid phase micro- extraction without derivatization and no dynamic reaction cell or direct solid sample analysis such as EDXRF. On the other hand, LOD was poorer than in methods using solid phase micro-extraction and detection by GFAAS/HR-CS-GFAAS, those based on detection by HG-AFS, HG-QFAAS after hydride trapping on a gold amalgamator, UV photo-induced derivatization and QFAAS detection, or direct ICP-MS analysis with dynamic reaction cell. Unlike UV-PVG derivatization in formic acid, which requires addition of hydrogen in the Ar stream for Se hydride atomization, the classical derivatization with $NaBH_4$ provides the needed hydrogen. In the same time, non-spectral matrix interferences are lower in classical derivatization.

Table S4. Limits of detection and quantification of Se by HG-HR-CS-QFAAS (batch system) obtained in this study and reported in other methods, with or without derivatization

Method[a]	Matrix	Sample preparation	LOD	LOQ	Reference[b]
HG-HR-CS-QFAAS	Food of animal and vegetal origin, dietary supplements and soil.	Derivatization with $NaBH_4$ without preconcentration	0.062±0.004 mg kg^{-1}	0.188±0.011 mg kg^{-1}	This paper
	Water		0.125±0.007 µg L^{-1}	0.377±0.021 µg L^{-1}	This paper
GFAAS	Edible mushroom	Preconcentration by eutectic solvent micro-extraction	0.32 µg L^{-1}		3
GFAAS	Food	Preconcentration by liquid-liquid micro-extraction	0.00461 µg L^{-1}	0.0154 µg L^{-1}	4
GFAAS	Food and water	Preconcentration by solid phase micro-extraction	0.00606 µg L^{-1}	0.02 µg L^{-1}	5
GFAAS	Blood	Preconcentration by eutectic solvent micro-extraction	0.015 µg L^{-1}		6
HR-CS-GFAAS	Food	Preconcentration by solid phase micro-extraction	0.01 µg L^{-1}		32
HR-CS-GFAAS	Soil	Direct analysis on solid powder	30 µg kg^{-1}	100 µg kg^{-1}	33
HG-GFAAS	Soil	Derivatization and in-atomizer trapping	6 µg L^{-1}	20 µg L^{-1}	31
HG-QFAAS	Water	Derivatization with $NaBH_4$ and trapping on a gold amalgamator	0.013 µg L^{-1} 0.007 µg L^{-1}		14 15
HG-QFAAS	Water	Derivatization with $NaBH_4$ without trapping	0.23 µg L^{-1}		15
UV-PVG-QFAAS	Dietary supplements	UV photochemical vapor generation with HCOOH	0.04 µg L^{-1}	0.134 µg L^{-1}	2
UV-PVG-GFAAS	Coconut water	UV photochemical vapor generation with HCOOH and in-atomizer trapping	0.65 µg L^{-1}	2.2 µg L^{-1}	11
HG-AFS	*Astragalus mongholicus*	Derivatization with KBH_4	0.01µg L^{-1}		16
UV-LED-PVG-AFS	Mineral water	UV photochemical vapor generation with HCOOH	0.21 µg L^{-1} Se(IV) 0.28 µg L^{-1} Se(VI)		18
MSPE-ICP-MS	Environmental water	Preconcentration by solid phase micro-extraction	5.3 µg L^{-1}		20
DRC-ICP-MS	Total blood	Direct liquid sampling on whole blood	0.015 µg L^{-1}		21
EDXRF	Plants	Direct analysis on solid powder	3600 µg kg^{-1}		30

[a] GFAAS – graphite furnace atomic absorption spectrometry; HR-CS-GFAAS – high-resolution continuum source graphite furnace atomic absorption spectrometry; HG-GFAAS – hydride generation graphite furnace atomic absorption spectrometry¸ UV-PVG-QFAAS – UV-photochemical vapor generation quartz furnace atomic absorption spectrometry; UV-PVG-GFAAS – UV-photochemical vapor generation graphite furnace atomic absorption spectrometry; HG-AFS – hydride generation atomic fluorescence spectrometry; UV-LED-PVG-AFS – UV-LED photochemical vapor generation atomic fluorescence spectrometry; MSPE-ICP-MS – Magnetic solid phase micro-extraction inductively coupled plasma mass spectrometry; DRC-ICP-MS – dynamic reaction cell inductively coupled plasma mass spectrometry; EDXRF – energy dispersive X-ray fluorescence spectrometry

[b] References in this table are those indicated in TEC paper

Concentration of concomitants in the analyzed samples

The concentration of concomitants determined by ICP-OES using SPECTRO CIROS^{CCD} spectrometer (Spectro, Kleve, Gemany) in solution are presented in Table S5. It was found that mineral matrices had no negative effects on selenium hydride generation, recovery and sensitivity of the HG-HR-CS-QFAAS method.

Table S5. Mineral matrix in solution of digested samples analyzed for Se determination by HG-HR-CS-QFAAS in batch system

Value	Element concentration (mg L^{-1})																
	Soil																
	Na	K	Mg	Ca	Al	Fe	Cr	Mn	Co	Ni	Cu	Zn	Sr	Ba	Cd	P	S
Min.	0.1	0.1	0.4	0.1	0.2	0.1	0.01	0.001	0.005	0.01	0.002	0.002	0.003	0.01	0.003	0.1	0.01
Max.	6.7	14.2	45.1	11.3	20.4	15.6	0.4	0.15	0.04	0.09	0.39	2.0	1.31	0.71	0.05	5.7	1.9
Mean	1.7	3.6	9.4	2.4	6.1	3.6	0.1	0.03	0.02	0.04	0.05	0.2	0.13	0.17	0.02	1.4	0.5
St. dev.	2.0	4.6	14.7	3.6	6.8	5.1	0.1	0.05	0.01	0.03	0.13	0.7	0.37	0.24	0.02	1.7	0.6
	Onion																
Min.	0.02	0.1	0.004	0.008	0.003	0.001	<LOD	0.001	0.03	0.02	0.003	0.001	0.003	0.002	0.01	0.1	0.03
Max.	4.4	147.7	6.9	13.2	0.3	0.4	<LOD	0.2	0.05	0.12	0.22	1.5	0.4	0.03	0.08	10.6	11.8
Mean	0.6	10.5	0.6	1.2	0.6	0.04	<LOD	0.05	0.04	0.07	0.07	0.1	0.08	0.004	0.03	1.9	1.6
St. dev.	1.3	29.3	1.5	2.9	0.07	0.01	<LOD	0.01	0.01	0.07	0.08	0.3	0.01	0.01	0.03	3.1	3.0
	Meat (fish, pork and chicken) and liver																
Min.	1.6	4.6	0.7	0.6	0.02	0.03	0.004	0.004	0.005	0.01	0.05	0.2	0.01	0.002	0.01	0.1	0.1
Max.	11.3	37.0	4.0	139.0	0.2	0.1	0.3	0.23	0.08	0.13	0.8	2.1	0.17	0.03	0.22	1.0	0.9
Mean	6.0	18.2	2.2	24.2	0.1	0.2	0.07	0.07	0.02	0.06	0.2	0.7	0.06	0.02	0.06	0.4	0.4
St. Dev.	3.1	10.8	1.1	43.0	0.06	0.3	0.09	0.09	0.02	0.04	0.2	0.5	0.05	0.01	0.07	0.4	0.3
	Dietary supplements																
Min.	0.8	2.0	1.6	2.2	0.03	0.1	0.02	0.004	0.03	0.2	0.01	0.1	0.5	0.001	0.002	1.5	
Max.	3.2	8.0	24.8	64.0	0.16	0.3	0.05	0.16	0.13	1.3	0.08	2.4	4.8	0.008	0.013	16.	
Mean	1.9	4.7	11.8	22.6	0.09	0.2	0.03	0.05	0.10	0.8	0.04	0.7	2.5	0.004	0.007	6.7	
St. Dev.	1.2	3.0	8.6	19.5	0.05	0.1	0.01	0.05	0.04	0.4	0.04	0.7	1.6	0.003	0.004	6.3	
	Water																
Min.	1.8	0.4	0.6	2.3	8.5	14.9	2.3	2.5	0.02	3.9	4.9	4.92	16.4	2.9	0.5	1.0	16.7
Max.	4.5	0.8	4.4	14.7	41.8	178.0	9.6	38.0	1.9	20.1	387.0	119.4	94.2	23.1	4.9	5.1	80.3
Mean	3.0	0.6	1.9	6.6	20.2	67.1	5.5	14.4	0.7	11.3	132.7	35.4	43.4	9.8	1.6	3.0	56.7
St. Dev.	1.3	0.2	2.2	7.1	18.2	68.6	3.3	14.8	0.9	8.1	220.4	48.1	44.0	11.5	1.9	2.9	27.7

Se content in test samples

Table S6. Concentration of Se in fish muscle, pork and chicken meat and liver

Sample	Concentration, mean ± CI[a] (mg kg^{-1})	RSD (%)
Tuna	1.44 ± 0.13	7.3
Tilapia	0.16 ± 0.02	10.1
Carp	0.18 ± 0.01	4.5
Hake	0.80 ± 0.10	10.1
Cod	0.45 ± 0.06	10.7
Nile perch	0.58 ± 0.07	9.7
Trout	0.08 ± 0.01	10.1
Herring	0.15 ± 0.02	10.7
Chicken meat	0.30 ± 0.03	8.1
Chicken meat	0.22 ± 0.03	11.0
Chicken meat	0.39 ± 0.03	6.2
Chicken liver	1.26 ± 0.16	10.2
Pork muscle	0.33 ± 0.04	9.8
Pork liver	0.28 ± 0.03	8.6

[a] – Confidence interval (n = 5, 95% confidence level)

Table S7. Concentration of Se in dietary supplements

Formulation	Declared value (μg/tablet)	Found value		
		Mean ± CI[a] (μg/tablet)	Recovery, mean ± CI[a](%)	RSD (%)
S1	45	45.2 ± 4.4	100 ± 10	7.8
S2	100	100.6 ± 7.1	101 ± 7	5.7
S3	20	18.5 ± 1.6	93 ± 9	7.0
S4	100	110.6 ± 6.1	111 ± 6	4.4
S5	23.3	24.7 ± 1.3	106 ± 5	4.2
S6	10	8.8 ± 1.3	88 ± 15	11.9
S7	55	55.4 ± 7.8	101 ± 14	11.3
Pooled recovery (%)			100 ± 9	

[a] – Confidence interval (n = 5, 95% confidence level)

Table S8. Concentration of Se in green onion non-/biofortified with selenate applied to soil in different concentrations (n = 5 parallel measurements)

Sample	Se in soil (mg kg^{-1})		Se in onion (mg kg^{-1})				
	Set 1	Set 2	*Allium ampeloprasum*	*Allium schoenoprasum*	*Allium cepa*	*Allium fistulosum*	*Allium senescens*
Not fortified	0.71 ± 0.08	0.62 ± 0.08	0.30 ± 0.04	<LOD	0.94 ± 0.13	<LOD	1.01 ± 0.08
Biofortified level 1	9.0 ± 0.5	5.4 ± 0.4	43.5 ± 2.1	47.1 ± 2.8	20.6 ± 2.3	28.5 ± 3.0	191 ± 13
Biofortified level 2	33.6 ± 1.9	24.3 ± 1.7	429 ± 38	767 ± 38	425 ± 26	270 ± 22	1073 ± 87
Biofortified level 3	123 ± 10	133 ± 11	1147 ± 84	2760 ± 253	1990 ± 110	1376 ± 79	3040 ± 200

Table S9. Concentration of Se in water samples fortified with selenate

Water sample	Concentration of HCl in sample (% v/v)	Se in original sample, Mean ± CI[a] (µg L^{-1})	Added amount of Se (µg L^{-1})	Recovered amount of Se, mean ± CI[a] (µg L^{-1})	Recovery, Mean ± CI[a] (%)	RSD (%)
Tap	3	< LOD	1	1.06 ± 0.16	106 ± 15	12.2
Tap	3	< LOD	5	5.16 ± 0.32	105 ± 6	5.0
Tap	6	< LOD	1	1.02 ± 0.09	102 ± 9	7.1
Tap	6	< LOD	5	5.24 ± 0.28	105 ± 5	4.3
Non-carbonated	3	< LOD	1	1.02 ± 0.14	102 ± 14	11.1
Non-carbonated	3	< LOD	5	5.04 ± 0.53	101 ± 11	8.5
Non-carbonated	6	< LOD	1	1.20 ± 0.21	120 ± 18	14.1
Non-carbonated	6	< LOD	5	5.16 ± 0.20	105 ± 4	3.1
River	3	< LOD	1	1.02 ± 0.15	102 ± 15	11.8
River	3	< LOD	5	4.80 ± 0.27	96 ± 6	4.5
River	6	< LOD	1	1.10 ± 0.18	110 ± 16	13.2
River	6	< LOD	5	4.91 ± 0.24	98 ± 5	3.9
Pooled recovery (%)					104 ± 11	

[a] – Confidence interval (n = 5, 95% confidence level)

Daily intake of Se in adults and teenagers *via* foods and dietary supplements analyzed in this study

Fig. S5 presents the daily intake of Se in adults and teenagers *via* foods and dietary supplements analyzed in this study in comparison with AI level established by EFSA (70 µg/day, or 1 µg/day/kg body weight)[13]. Estimate was made for a serving of 150 g fish, chicken and pork meat, 80 g liver, 30 g green onion and one tablet of dietary supplement. An average water content of 80% for meat and 90% for green onion was used to express the results in wet samples. Of biofortified onion samples, only those corresponding to the first fortification level were retained, excepting *Allium senescens* variety, exhibited a high bioaccumulation factor for Se. Non-biofortified onion samples were considered those containing Se above LOD.

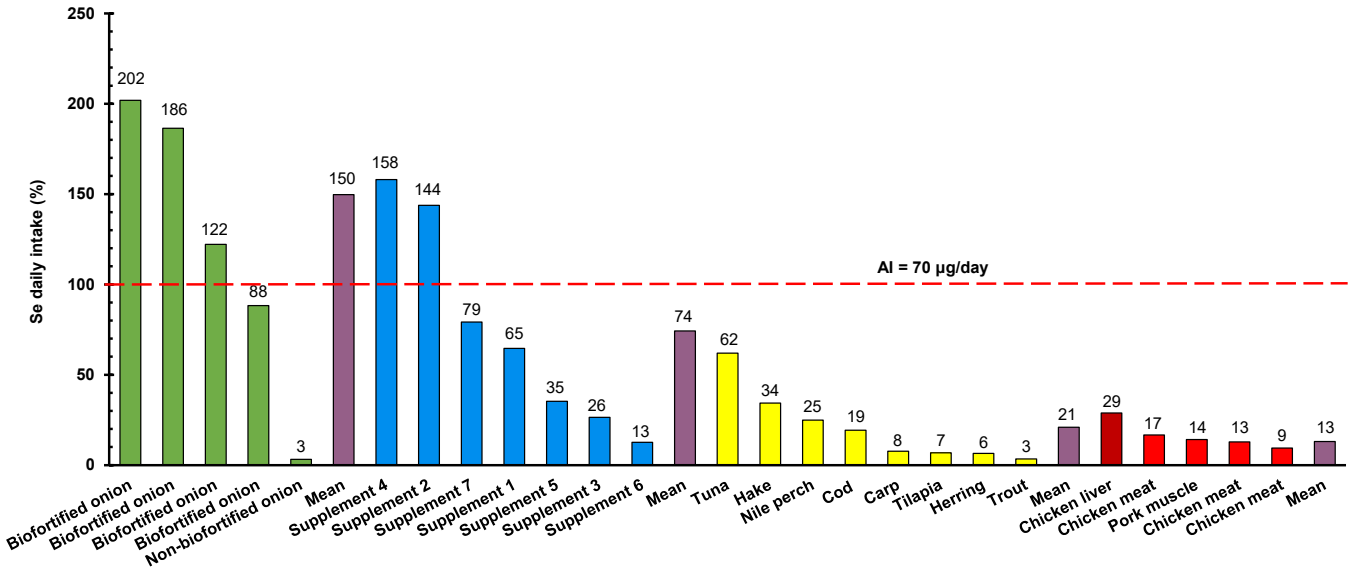

Fig. S5. Average Se intake from a serving of 150 g fish, chicken and pork meat, 80 g liver, 30 g green onion (first biofortification level, except *Allium senescens*) and one tablet of dietary supplement. Reference AI established by EFSA: 70 µg/day[13].

According to Regulation (EU) No. 1169/2011, the value to be taken into account when deciding a significant contribution of minerals should be 15% of the Daily Reference Intakes for a serving.[14] As shown in Fig. S5, fish, chicken and pork meat and liver represent rich sources of Se. From our data, the daily intake of Se *via* fish muscle consumption was in the range 21 ± 16% of AI (95% confidence level) depending on variety and growth regime, with higher values for wild ocean fish (62% tuna, 34% hake and 20% cod) and lower for farmed fish (8% carp and 4% trout). Results were similar to those reported by Ullah *et al*.[3] who mentioned ocean fish like shark, tuna and cod as rich sources of Se in human diet. Contribution from chicken and pork meat was found to be 13 ± 4%, while that coming from liver of around 30% of AI. Unfortified onion grown on soil considered marginal relative to Se level (0.124–0.142 mg kg^{-1} wet soil) according to the classification of Zhang *et al*.[18] (deficient < 0.125 mg kg^{-1} Se, marginal 0.125–0.175 mg kg^{-1} Se) emphasized the lowest contribution, in the range 3.2 ± 4.2%. Selenium deficiency may be overcome by dietary supplements consumption covering the 74 ± 57% or consumption of biofortified vegetables, such as onion. The biofortified onion grown on soil enriched with 5–9 mg kg^{-1} Se in dry soil (1–1.8 mg kg^{-1} wet soil) was found to provide 150 ± 50% AI per 30 g serving. Although the AI value of 70 µg/kg was exceeded in the case of some dietary supplements and biofortified onion, the upper limits of 250 and 300 µg/day Se for adolescents and adults set by EFSA were not reached.[13] Biofortified *Allium senescens* variety with extreme Se content (191 ± 13)–(3040 ± 200) mg kg^{-1} was excluded from this evaluation because its consumption may result in exceeding the daily adequate intake, posing risk to health. The reason of high Se concentrations in this onion variety could be the high accumulation factor from soil enriched with elevated amounts of selenate. Consumption of biofortified onion grown on soil enriched with selenate would be an alternative to the common synthetic dietary supplements. However, a particular attention should be paid on selenate amount applied on soil, which influences the level of biofortification and further Se intake *via* onion consumption to avoid crossing the border between beneficial and toxic effect. On the other hand, the level of Se fortification in soil and vegetables requires a careful monitoring, while final products should be accompanied by quality certificates.

Author contributions

Lucia Chirita: Investigation, Methodology. **Eniko Covaci**: Formal analysis, Visualization. **Augustin Mot**: Data curation, Resources. **Michaela Ponta**: Validation, Writing – original draft. **Alexandra Gandea**: Investigation. **Tiberiu Frentiu**: Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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