Sequential and Single Step Extraction Procedures Used for Fractionation of Selenium in Soil Samples

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The sequential extraction and single step extraction procedures were used to assess potentially bioavailable selenium from Slovak soils. The used sequential extraction allows to differentiate five forms of selenium bound in soils (exchangeable fraction and fraction bound to carbonates, fraction bound to manganese-iron oxides, fraction bound to organic matter, fraction bound to humic compounds, fraction bound to sulfides) and residual fraction. The single step extraction of soil with 0.1 mol dm^{-3} KH_2PO_4 + K_2HPO_4 (pH 7.0; P-buffer) releases different forms of selenium: soluble, adsorbed, ligand-exchangeable, and plant protein-bound. Application of the P-buffer to soils resulted in a low recovery of selenium (3—12 %) when compared to the total selenium content of the respective soils. The total selenium was determined after total decomposition using HNO_3 and HF acids. The determination of selenium in the individual fractions and total selenium was performed by flow injection hydride generation atomic absorption spectrometry (FI-HGAAS).

Interest in selenium concentrations in the environment and in foodstuffs stems from the dual role of selenium as an essential nutrient at low concentrations and as a toxic substance at higher levels of concentrations. The amount of selenium in the food chain and thus in human body is a result of selenium soil amount, which varies greatly throughout the world. For this reason soil is one of the most important parts of the environment where the determination of selenium is necessary. However, quantification of the total selenium in soils gives no information about the chemical species or the soil fraction with which it is associated and, therefore, provides little information about the availability to plants. The separation of various chemical forms is therefore very important. The chemical forms of selenium and their solubility depend mainly on the redox potential and the pH of the soil [1]. Other factors which influence the species of selenium present and its bioavailability include organic matter content, iron oxide levels, and clay type and content. Selenium in soils can exist in various forms: soluble, exchangeable, bound to organic matter, sulfides, carbonates, oxides, *etc.*

It is important to be able to assess how much selenium, irrespective of chemical form, is available for plant uptake. An alternative approach is represented by sequential extraction. In studies of trace metals, *Tessier et al.* [2] discussed the utility of using sequential extraction procedures for identifying their origin, occurrence, movement, and availability. A few studies on selenium fractionation in sediments and/or soil samples have been reported in recent literature [3—10].

In the present work modified sequential extraction procedure [11] originally designed for fractionation of the toxic elements in sediments and by *Žemberyová et al.* [12] applied for fractionation of copper and nickel in soils was used for fractionation of selenium in two Slovak soils. Single step extraction with 0.1 mol dm^{-3} KH_2PO_4 + K_2HPO_4 (pH 7.0; P-buffer) was chosen from literature [13] for assessing the amount of potentially bioavailable selenium in six soils from various parts of Slovakia. The quantification of total selenium and selenium in extracts was performed by flow injection hydride generation atomic absorption spectrometry (FI-HGAAS).

EXPERIMENTAL

The reagents used were of anal. grade. Concentrated mineral acids (HCl, HF, HNO_3), NaOH, $NaBH_4$, and selenium stock solution (SeO_2, 1000 mg dm^{-3}) were from Merck, Darmstadt, Germany. KH_2PO_4, K_2HPO_4, concentrated CH_3COOH, and $NH_2OH.HCl$ were from Lachema Brno, Czech Republic.

The standard reference material of sediment GBW 07309 Chinese stream sediment was from Promochem, USA.

The selenium standard solutions (0.5—8 μg dm^{-3})

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were prepared daily independently of each other by separate dilution of the same standard solution in deionized water (Water Pro PS, Labconco, Kansas City, USA).

The soil samples were from surroundings of Slovak towns: Trnava (TT1, TT2, and TT3, the first, the second, and the third horizons, respectively), Zvolen (ZV1), Prievidza (PD1), Liptovský Mikuláš (LM1), Galanta (GA1), Nitra (NR1), and Dunajská Streda (DS1). They were obtained from Soil Science and Conservation Institute (Slovak Republic). Samples were air dried, sieved (0.2 mm) and ground in agate mortar.

Some of soil properties for TT1 and ZV1 are listed in Table 2. These soils were used for sequential extraction and single step extraction. Another soil samples were used only for single step extraction.

The determination of selenium in soil and soil extracts was made by a Perkin—Elmer model 1100 B (Norwalk, Connecticut, USA) atomic absorption spectrometer equipped with flow injection hydride system FIAS-200 with automated sampler AS-90 and an electrically heated quartz tube. Argon was used as the purge gas. A KS 125 basic IKA-shaker (IKA Labortechnik, Germany) and a K70D centrifuge (MLW, Germany) were used for extraction procedures and centrifugation. Autoclave vessels (JZD Zahnašovice, Czech Republic) were used for decomposition of soil samples. A drying box KBCG (Premed, Poland), pH-meter PHM 64 (Radiometer Copenhagen, Germany), and analytical balances Sartorius 1702 (Germany) were used for another analytical procedures.

Instrumental parameters and FI-HGAAS program are given in Table 1.

Table 1. Instrumental Parameters and FI-HGAAS Program

FI-HGAAS	
Wavelength	196.0 nm
Slit	2.0 nm
Quartz tube temperature	900 °C
Integration time	15 s
Light source	Hollow cathode lamp (made by Perkin—Elmer)
Lamp current	18 mA
Argon flow rate	60 cm^3 min^{-1}
Sample volume	0.50 cm^3
Carrier solution	3 vol. % HCl
Reducing agent	0.2 % $NaBH_4$ in 0.05 % NaOH

Reduction of Se(VI) to Se(IV)

7 cm^3 of the sample (decomposed or extracted) were pipetted into a polyethylene flask. Then 5 cm^3 of concentrated HCl were added and mixed thoroughly. The flask was covered and placed to a drying box for 30 min at 95 °C. Then the flask was cooled to room temperature and used for the determination of selenium in the same day.

Total Decomposition for Determination of Selenium by FI-HGAAS

1.00 g of the soil sample was weighed into a teflon autoclave vessel and 10 cm^3 of an acid mixture of concentrated HF and concentrated HNO_3 (1 : 1) were added. The sample was destroyed at 160 °C in a drying box for 6 h. Then the sample was evaporated to dryness on a sand bath. After the addition of 5 cm^3 of 10 vol. % HCl the sample was evaporated for a while. Then the solution was made up to 50 cm^3 volume with deionized water, filtered (Whatman 42) to the polyethylene flask and refrigerated (4 °C) until selenium analysis. Total decomposition was replicated three times for every sample.

Total Decomposition for Determination of Selenium by Cathodic Stripping Voltammetry (CSV) [14]

0.3—0.4 g of the sample was put into a teflon crucible and an acid mixture of concentrated H_2SO_4 and concentrated HNO_3 (1:1) was added. The crucible was closed and the mixture was heated on a sand bath at 110 to 115 °C for at least 6 h until a feebly yellow solution resulted. If the digestion was incomplete the procedure was repeated. Then the mixture was carefully evaporated until dense fumes of SO_3 appeared. Total decomposition was replicated three times for every sample.

Sequential Extraction Procedure

Step one (solution A: selenium present in ion-exchange form and bound to carbonates). 2.00 g of the soil sample was weighed into a teflon vessel. 40 cm^3 of ammonium acetate (1 mol dm^{-3} adjusted to pH 5.0 with acetic acid) was added and the vessel was shaken for 6 h at ambient temperature on a mechanical shaker (500 min^{-1}). After centrifugation (3000 min^{-1}) for 20 min the solution was decanted and the residue washed with 2 cm^3 of deionized water. The washings were added to the solution and diluted to 50 cm^3. The solution was filtered (Whatman 42) to the polyethylene flask and refrigerated (4 °C) until selenium analysis.

Step two (solution B: selenium present in the reductive phase bound to manganese-iron oxides). 40 cm^3 of 1 mol dm^{-3} hydroxylammonium chloride and 25 vol. % acetic acid (1:1) solution was added to the residue of step one. Extraction and separation procedures were as described in step one. The solution and washings were diluted to 50 cm^3.

Step three (solution C: selenium bound to organic

matter, exchanging for acid-base effect at pH 1.0). 10 cm^3 of 0.1 mol dm^{-3} HCl was added to the residue from step two. Extraction and separation procedures were as described in step one. The extract and washings were diluted to 25 cm^3.

Step four (solution D: selenium bound to humic compounds). The remaining solid phase was kept in contact with 10 cm^3 of 0.5 mol dm^{-3} NaOH, extracted and separated as described in step one. The solution and washings were evaporated on a sand bath to a small volume (1—2 cm^3), then digested with 4 cm^3 of concentrated HNO_3 and 2 cm^3 of concentrated HF in a teflon autoclave vessel at elevated pressure in a drying box, as it is described by total decomposition for determination of selenium by FI-HGAAS. The acid solution was diluted to 25 cm^3, filtered (Whatman 42) to the polyethylene flask and refrigerated (4 °C) until selenium analysis.

Step five (solution E: selenium bound to sulfides). To the residue from step four 10 cm^3 of 8 mol dm^{-3} HNO_3 was added. The teflon vessel was covered with a teflon cover and the content was digested in a water bath with occasional manual shaking for 3 h at 85 °C. After separation of the solution and washings the solution was diluted to 25 cm^3, filtered (Whatman 42) to the polyethylene flask and refrigerated (4 °C) until selenium analysis.

Step six (solution F: selenium in residue). Residue from step five was decomposed using 10 cm^3 of an acid mixture of concentrated HF and concentrated HNO_3 (1:1) as it is described in total decomposition for determination of selenium by FI-HGAAS.

Each treatment was replicated three times.

Single Step Extraction with 0.1 mol dm^{-3} KH_2PO_4+ K_2HPO_4 (pH 7.0; P-buffer)

5.00 g of the soil sample was weighed into a teflon vessel. 25 cm^3 of 0.1 mol dm^{-3} KH_2PO_4 + K_2HPO_4 (pH 7.0) was added and the vessel was shaken for 2 h at ambient temperature on a mechanical shaker (300 min^{-1}). After centrifugation (3000 min^{-1}) for 15 min the solution was decanted, filtered (Whatman 42) to the polyethylene flask and refrigerated (4 °C) until selenium analysis. Single step extraction procedure was replicated two times.

RESULTS AND DISCUSSION

A common problem in selenium hydride determination is that various cations as well as anions interfere with the hydride formation in the analyte solution. Many authors have reported that high final acid concentration, especially that of hydrochloric acid (up to 6 mol dm^{-3}), is effective for the reduction or even elimination of interferences [15]. Although in our case nitric acid was used in sample preparation and rather high concentrations of metal ions were present, the combination of the standard addition method, appropriate dilution of the sample, and final HCl concentration (5 mol dm^{-3}) successfully reduced their influences. The detection limits (determined on the basis of the three times measured standard deviation of the blank sample) and precisions (the relative standard deviations) of the total determination, sequential extraction and single step extraction are described in the following text. Evaluation by the standard addition method was made using the CHEMSTAT statistical software (Version 1.20; TriloByte; 1991).

Until now different techniques were used for soil decomposition. We used total decomposition with a mixture of HNO_3 and HF acids in autoclave vessels under elevated pressure. Digestion with included HF was conducted in sealed vessels since SeF_4 and SeF_6 are volatile. Using these conditions there were observed no selenium losses during the total decomposition. Accuracy of the used decomposition was checked by analyzing of the standard reference material of sediment GBW 07309 Chinese stream sediment. The mean value ± SD was (165 ± 21) ppb (n = 5), while certified value ± SD was (160 ± 30) ppb (recovery 103 %). Total selenium in soils used for sequential extraction was determined by the presented method and by cathodic stripping voltammetry (CSV) [14]. The results obtained with the soils TT1 and ZV1 (Table 2) by the two methods agreed with an acceptable precision (Table 3). This means that FI-HGAAS offers good precision and accuracy in the determination of selenium as well as CSV. All these observations indicated that we can use the results of total selenium content as 100 % for comparison of recovery of the used sequential extraction procedure. For comparison of recovery of the used single step extraction total selenium in soils determined by CSV was used. Detection limit at the experimental conditions applied for total determination was 5.0 ppb. Precision for total determination was less than 5 %.

The used sequential extraction procedure provides information on the amount of selenium associated with different soil fraction (exchangeable frac-

Table 2. Some of Soil Properties

Soil	Locations of sample collection	Sampling depth/cm	Soil type	pH (H_2O)	w(Humus)/%
TT1	Surroundings of Trnava	5—15	Calcaro-haplic chernozem	5.93	3.00
ZV1	Surroundings of Zvolen	10—20	Stagno-gleyic luvisol	6.54	2.83

Table 3. Results of Total Selenium Content Determined by the FI-HGAAS and CSV Methods

Soil	Total Se content/FI-HGAAS (Mean value ± SD)/ppb	Total Se content/CSV* (Mean value ± SD)/ppb
TT1	486 ± 15	520 ± 30
ZV1	245 ± 8	220 ± 10

*Published in [14].

tion and fraction bound to carbonates, fraction bound to manganese-iron oxides, fraction bound to organic matter, fraction bound to humic compounds, fraction bound to sulfides, and residual fraction). Fraction distribution (D) of selenium was calculated as: $D = (F \cdot 100)/S$, where F and S correspond to the amount of selenium in the given fraction (ppb) and the sum of all fractions (ppb), respectively. The sum of all fractions (470 ppb and 234 ppb) was very close to the total soil content (486 ppb and 245 ppb). Thus, we were able to recover the high percent of the total soil selenium (97 % and 95 %) by the fractional partitioning technique. The fraction recovery (R) of selenium was calculated as: $R = (S \cdot 100)/T$, where S and T correspond to the sum of all fractions and total soil selenium level determined by mixed acid decomposition, respectively. All these observations indicated that there was good precision in the fractional partitioning technique results (Table 4). Selenium content in various extract solutions of soil samples is shown in Table 5.

Application of extraction methods to selective removal of selenium is complicated by the fact that selenium may exist in more than one oxidation state (selenate(VI), selenite(IV), elemental selenium(0), and selenide(-II)), each of which has a unique behaviour. Inorganic selenide may exist as insoluble metal selenides (*e.g.* iron selenide) or may be found in ferroselite ($Fe^{II}Se_2$), a pyrite analogue. Since biological detritus can form a portion of soil, organic selenide species (selenoamino acids in proteins) may also be associated with soils as potentially adsorptive species, selenite and selenate may be found in phases such as carbonate, iron oxides, and manganese oxides. Further, a portion of soil selenium may exist in the elemental state, depending on the ambient redox conditions. Overall, the type of soil association falls into two categories, adsorbed selenite and selenate and covalently bound selenide (inorganic and organic forms). Elemental selenium might also be classified as covalently bound [3].

The selenium availability to plants is influenced by several soil factors. It is positively correlated with pH, salinity, and content of $CaCO_3$ in soils. In acidic, clay soils and soils containing high organic matter, selenium is present as selenides and selenium sulfides. The species are slightly soluble in water and therefore not available to plants. In well-drained neutral soils selenium is present preferably in the form of selenites. However, the presence of iron species reduces its availability to plants. In alkaline and well-oxidized soil selenium occurs as selenates. These species are highly mobile and available to plants [15].

The sequential extraction procedure was applied for selenium fractionation in two types of Slovak soils: calcaro-haplic chernozem from surroundings of Trnava (TT1) and stagno-gleyic luvisol from surroundings of Zvolen (ZV1), pH 5.93 and 6.54, respectively. The histograms (Fig. 1) indicate that selenium in soil samples TT1 and ZV1 was similarly distributed. We can say that soil TT1 is weakly acid and ZV1 neutral. As it was mentioned above in these soils selenium is present preferably in the form of selenites, not very soluble and less mobile than Se(VI) and therefore less available for plant uptake.

Aqueous and exchangeable fractions are available for leaching and plant uptake, whereas the acid extractable fraction may be conditionally available [9].

Table 4. Recovery of the Used Sequential Extraction Procedure

Soil	Total Se content/FI-HGAAS (Mean value ± SD)/ppb	Sum of all fractions/FI-HGAAS Mean value/ppb	Recovery/%
TT1	486 ± 15	470	97
ZV1	245 ± 8	234	95

Table 5. Selenium Content in Various Extract Solutions of Soil Samples

Soil	Se content in extract solutions, w(mean value ± SD)/ppb					
	A	B	C	D	E	F
TT1	3.4 ± 0.5	< D.L.	< D.L.	249 ± 16	83 ± 8	135 ± 8
ZV1	< D.L.	< D.L.	< D.L.	128 ± 14	49 ± 5	57 ± 7

D.L. = detection limit.

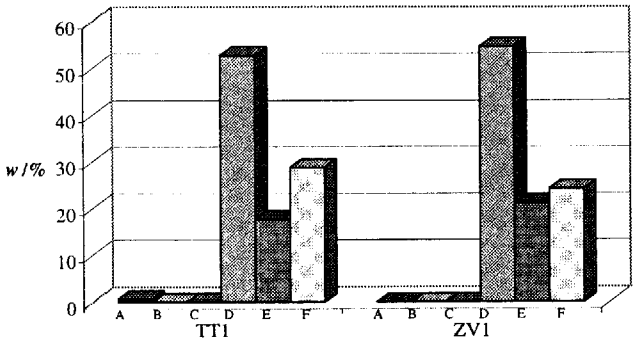

Fig. 1. Histograms of selenium fractions (in %) in extract solutions A—F.

In our case fraction A (1 mol dm^{-3} ammonium acetate at pH 5.0 used for extraction) represents potentially bioavailable selenium. Fraction recovery of total selenium is too low (less than 1 %).

In acid and neutral soils selenium is usually present as Se(IV) complexes of iron oxides and oxyhydroxides and has low solubility. Hence in these soils selenium is largely unavailable to plants [7]. For effective and selective dissolution of amorphous iron oxides hydroxylammonium chloride can be used [6]. Selenium content in fraction B (bound to manganese-iron oxides) was under detection limit, so it seems that no selenium is associated with amorphous iron oxides or there is a possibility that selenium adsorbed on the amorphous iron oxides is released during reductive dissolution of the iron oxides and readsorbed by the remaining solid phase [5].

Acid hydrolysis is a common way to release metals and nonmetals from the organic structure. Nevertheless, its action on Se-bearing soil organic materials has not been adequately investigated [6]. Selenium bound to organic matter can be represented by selenoamino acids, organoselenium compounds derived from decomposing plant tissues or inorganic selenium incorporated into the organic fraction abiotically or by microbiological activity. Another "organic" selenium can be associated with the humic compounds which can be subdivided into humic acids, fulvic acids, and humin. In this sequential extraction scheme it is possible to distinguish the content of selenium bound to organic matter (fraction C, 0.1 mol dm^{-3} HCl used for extraction) and bound to humic compounds (fraction D, 0.5 mol dm^{-3} NaOH used for extraction). Amount of total selenium in fraction C was under detection limit, so we found no selenium bound to organic matter which can be released by 0.1 mol dm^{-3} HCl.

Selenium in soil samples was dominated in fraction D (bound to humic compounds) which accounted for over 50 %. Selenium bound to humic and fulvic acids is unavailable to selenium uptake.

As it was mentioned above, in acidic clay soils and soils with high content of organic matter, selenium is present as selenides and selenium sulfides [15]. The species are slightly soluble in water and therefore not available to plants. 8 mol dm^{-3} HNO_3 is a strong oxidizing medium and is effective in dissolving various sulfide minerals. Because of the similar ionic radii of sulfide ion and selenide ion, selenium readily substitutes for sulfur ion sulfide minerals. The content of selenium in sulfide minerals varies widely from specimen to specimen. Pyrite and other sulfide minerals, rich in selenium substitution can be decomposed by the rather drastic oxidative acid dissolution [6]. Selenium bound to sulfides is represented in our scheme by solution E. Percentage recovery of total selenium in this solution is about 18 % and 21 %.

Fraction F represents selenium in residue. Mixed-acid solution including HF is the strongest liquid chemical reagent to destruct the silicate structure. Although selenium is not considered to be situated in the silicate lattice, its compounds may occur in accessory minerals imbedded within the silicate matrices or tiny particles containing selenium may be occluded by siliceous materials. Selenium in this final fraction is highly resistant as far as its impact on the environment is concerned [6]. In our case percentage recovery of total selenium in residual fraction was about 29 % and 24 %.

Detection limits of the used procedure varied from 1.2 ppb up to 5.5 ppb depending on the solution used for extraction. Precision for determination of selenium in extract solutions was between 5 % and 11 %.

The extraction of soil with P-buffer at pH 7.0 releases different forms of selenium: soluble, adsorbed, ligand-exchangeable, and plant protein-bound (if present in nondecomposed seleniferrous plant material in soil) [13]. The 0.1 mol dm^{-3} KH_2PO_4 + K_2HPO_4 (pH 7.0; P-buffer) was developed for extraction of soluble and ligand-exchangeable selenium to avoid the low pH value (4.8) of the KH_2PO_4, which has been shown to introduce selenium redistribution errors in soil extractions [13]. Application of the P-buffer to soils results in a low recovery (3—12 %) when compared to the total selenium content of the respective soils (Table 6). It is due to acid and neutral pH Slovak soils (the most of Slovak soils are acid or neutral, only 20 % are alkaline). It is important to say again that in acid soils selenium is found mainly in the form of selenite, not very soluble and not very available for plant uptake, while in alkaline soils, selenite becomes oxidized and then selenate is produced, which is much more soluble and easily available for plant uptake. The detection limit at the experimental conditions applied was 1.2 ppb. The precision for determination of selenium in P-buffer extract solution was between 3 % and 8 %.

If we compare results of selenium amount from fraction A (1 mol dm^{-3} ammonium acetate at pH 5.0 used for extraction) and from single step extraction, we can see that using P-buffer extraction we are able to obtain more selenium in extract solution. It can be

Table 6. Results of the Single Step Extraction with 0.1 mol dm^{-3} KH_2PO_4 + K_2HPO_4 (pH 7.0; P-buffer)

Soil	Sampling depth/cm	Se content in extract Mean value/ppb	Total Se content (Mean value ± SD)/ppb*	Recovery/%
LM1	10—20	30	260 ± 10	12
GA1	5—15	21	280 ± 20	8
NR1	10—35	20	310 ± 30	6
DS1	10—30	38	760 ± 40	5
ZV1	10—20	7	220 ± 10	3
TT1	5—15	41	520 ± 30	8
TT2	30—40	14	240 ± 30	6
TT3	70—80	11	90 ± 4	12

*Published in [14].

due to the fact that the used P-buffer is more suitable for assessing potentially bioavailable selenium for plant uptake than solution used in step A or at pH 7.0 humic and fulvic acids are partially soluble and in P-buffer extract solution a part of selenium amount from solution D is present.

To avoid matrix interferences, method of standard addition was used in all cases.

CONCLUSION

The sequential extraction procedure has provided information on the amount of selenium associated with different soil fraction (exchangeable and bound to carbonates, bound to manganese-iron oxides, bound to organic matter, bound to humic compounds, bound to sulfides, and residual). The sum of the selenium amounts in the fractions was in good agreement (97 % and 95 %) with the total selenium amounts found by total decomposition. We compared selenium amount in solution A from the used sequential extraction procedure and in solution from the single step extraction procedure. We can consider selenium in these solutions as potentially bioavailable. Fraction recovery of total selenium in solution A was less than 1 %. Fraction recovery of total selenium in P-buffer extract solution was between 3—12 %. It is too low. The main reason is that the most of Slovak soils are acid and neutral.

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