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Selenium and Trace Element Distribution in *Astragalus* Plants: Developing a Differential Pulse Polarographic Method for Their Determination

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Astragalus plants have a wide range of applications in pharmaceuticals (gum tragacanth), as thickening agents in foods, and may have applications in controlling cancer cells. They are used as feed for animals and they are indicator plants for selenium. Because of their use in health-related areas it is very important to determine their selenium and trace element content with high accuracy.

A new differential pulse polarographic method was established for trace element determination (10 elements) and their distribution in these plants. The *Astragalus* plants investigated in this work are *Astragalus microcephalus* and *Astragalus lusitanicus*, which grow almost everywhere. Their roots, stems, and leaves were analyzed separately. Since some *Astragalus* plants are known to accumulate selenium, the most emphasis was given to its determination.

The *Astragalus* plants were wet digested and their DPP polarograms were taken in various media. In pH 2 acetate buffer, Se, Mo, Cd, Pb, Cr, Zn, and As peaks, and in pH 4 acetate buffer, Cu, Se, Mo, As, and Zn peaks could be separated and determined. In the presence of EDTA at pH 4, Cu, Ti, Se, and As peaks, and at pH 6, Fe, Cu, Ti, and As peaks could be separated and determined. Thus, by adjusting the pH and medium, it was possible to determine 10 trace elements in the same solution. While in *Astragalus microcephalus* plants the Se content was 183 ± 15 μg/g, another plant, *Elymus* (*Gramineae*), which was taken from the same soil had no selenium, which indicates that selenium is accumulated in this kind of astragalus plant. On the other hand, no selenium was found in *Astragalus lusitanicus* plants. The types of *Astragalus* plants that accumulate selenium to the greatest degree are known to grow in Canada, USA, Russia, Afghanistan, Spain, and Turkey.

According to the results, most of the elements are accumulated in the roots, but selenium was also distributed in the stems and leaves. Although large quantities of Cr were present in the roots, it was under the detection level in the stems and leaves. On the other hand, there was a level of iron present in both the roots and leaves. The proposed method is simple, fast, and cheap, does not require any preconcentration or separation procedure, and can be safely used with many biological materials.

Key Words: Trace elements, tragacanth gum, determination, *Astragalus* plants, differential pulse polarography

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Introduction

The accumulation of selenium by certain plants has been known for some time and has been the subject of considerable research. According to Rosenfeld and Beath[1] there are 24 species and varieties of plant that grow in seleniferous soils, and for which selenium is an essential nutrient. Such plants have been designated as indicator plants; among them are more than 20 types of *Astragalus*, *Macranthera*, *Haplopappus*, and *Stanleya*. The types of *Astragalus* plants that accumulate selenium to the greatest degree are *adsurgens* (44 mg/kg) and *bisulcatus* (43 mg/kg) in Canada and USA, *asper* (52 mg/kg), *demetrii* (47 mg/kg), and *galegiformis* (41 mg/kg) in Russia, *incanus* (53 mg/kg) in Spain, and *tephrosioides* (61 mg/kg) in Afghanistan.[2] There are 450 *Astragalus* types, and, while some of them do not grow in selenium-deficient soils, some do.

Some plants are known to accumulate selenium; one study[3] found that selenium was accumulated in the following plants in the following concentrations: *Brassica juncea*, 138 mg/kg; *Astragalus praleongus*, 517 mg/kg; onion, 96-140 mg/kg. The selenium content of garlic was determined by cathodic stripping voltammetry,[4] which varied from 36 to 485 ng/g, according to the soil in which it was grown. *Medicago sativa* collected from 3 regions was analyzed[5] for its selenium content by using differential pulse cathodic stripping voltammetry and selenium was found in the plants (0.78 mg/kg) from only 1 region.

Tragacanth gum is obtained from some *Astragalus* plants. It is the most viscous of the known natural water soluble gums and is an excellent emulsifying agent with good stability in response to heat, acidity, and age. It has numerous applications in pharmaceuticals, as a thickening agent in foods, and may have applications in controlling cancer cells. The best grades are obtained from the roots of some species; *Astragalus microcephalus*, *Astragalus gummifer*, and *Astragalus echinacea* have been cited as the principle sources of tragacanth gum.

Among *Astragalus* plants, only 150 have thorns, and animals use them as feed during the winter when fresh grass is not available. Selenium is a nutritional requirement for animals; selenium-deficient feed causes white muscle disease, which results in paralysis. This is observed mostly in lambs between 1 week and 4 months of age. In the early stages of this disease there is weakness in the legs. Eventually the lamb cannot stand and dies in about 2-10 days. Thus, it is important that their food contains selenium. In areas where this disease is observed in animals the soil is selenium deficient; however, it is also known that the ingestion of seleniferous plants by cattle has given rise to the well-known toxic conditions of blind staggers and alkali disease.[6]

For the determination of trace elements in biological samples electrochemical methods have rarely been used, perhaps due to the necessity of possessing electrochemical know-how and knowledge of automation. Nonetheless, the high sensitivity and reproducibility of these techniques, combined with inexpensive instrumentation make them eminently suited for this task. Thus, trace elements in biological materials can safely be analyzed using electroanalytical methods.[4,5,7–9] There is no need for pre-concentration or extraction techniques, in which loss and contamination of materials are possible.

The purpose of this work was to investigate the selenium accumulation in *Astragalus* plants grown in the same soil and to establish a simple differential pulse polarographic (DPP) method for the determination of as many trace elements as possible. To the best of our knowledge this is first investigation of trace elements in such plants.

Experimental

Apparatus

A polarographic analyzer system (PAR 174 A) equipped with a mercury drop timer was used. The natural drop life of the mercury electrode was in the range of 2-3 s (2.4 mg/s). A Kalousek electrolytic cell with a reference saturated calomel electrode (SCE) separated by a liquid junction was used in the 3-electrode configuration. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X-Y recorder with a drop life of 1 s, a scan rate of 5 mV s^{-1}, and a pulse amplitude of 50 mV.

Reagents

All chemicals used were of analytical reagent grade (Merck, Darmstadt) and triply distilled water was used in the preparation of their solutions at all stages of analysis. Dilute solutions were prepared before every use from their stock solutions (0.1 M) to prevent the solution from ageing. The mercury (Analar) used was obtained from BDH Chemicals Ltd., Poole, UK.

Preparation of the sample

Astragalus microcephalus samples were collected from Kars in eastern Turkey, a high plateau about 2000 m a.s.l. This plant is known to grow at altitudes of 850-3000 m.[10] It is found in several provinces in Turkey as well as in Canada, Russia, Iran, and Caucasia.[1,11] The stems of the plant have thorns, the roots were more than 80 cm deep in the soil, and the diameter near the ground was about 8 cm. After washing and drying the roots they were cut into small pieces and blended. The stems and the leaves of the plants were also separated and then cut into small pieces.

The second *Astragalus* plant studied, *Astragalus lusitanicus*, was collected from Muğla in southwestern Turkey. One additional plant, *Elymus* (*Graminea*), which grew in the same soil as *Astragalus microcephalus* was also studied.

Digestion of samples

Samples were dried in an oven until constant weight was obtained. Some (10 g) dried sample and 20 mL of HNO_3:$HClO_4$ (1:1) were transferred into a 100-mL long-necked (30 cm) glass flask. The samples were kept in the acid mixture overnight with a glass funnel covering the mouth of the flask. Then, the flask was heated over a flame by turning the flask until nitrogen oxide fumes were completely given off. HNO_3 was added when the sample in the flask became dark brown, due to the possibility of explosion. The addition of about 5 mL of HNO_3 was necessary when the sample was cooled. Digestion was completed with the appearance of white perchloric acid fumes when approximately 1.0 mL of solution remained and the sample was clear and colorless. The evaporation of acids was completed in 3-6 h, since based on our previous work,[12] when the digestion period was longer there was loss of elements. The total acid consumption was about 10 mL of $HClO_4$ and 55 mL of HNO_3. Finally, 2.0 mL of HCl acid was added and heated for about 4-20 min to convert all Se(VI) to Se(IV). This step is important since Se(VI) is not electroactive and thus must be reduced to Se(IV), which is electroactive. The digested sample was cooled to room temperature, we rinsed the funnel into the flask with water, and then the contents were transferred into a 10.0-mL calibrated flask,

completing the volume with triply distilled water. This sample was kept in a Teflon bottle in a refrigerator. The same amount of acids, after evaporation, had no impurity peak when a polarogram was recorded under the same conditions.

Polarographic determination

About 10.0 mL of electrolyte in the polarographic cell was de-aerated by a stream of nitrogen gas (99.99%) for about 10 min. Polarograms were recorded mostly by scanning the potential from 0.0 to –1.5 V (depending on pH) at a scan rate of 2-5 mV s^{-1}. If addition of EDTA was needed, then 5.0 mL of buffer, 3.0 mL of 0.3 M EDTA, and 2.0 mL of water were used as the electrolyte. The peak potentials of Se(IV), Ti(IV), As(III), As(V), Fe(III), Cu(II), Pb(II), Cr(III), Cd(II), Mo(VI), and Zn(II), which are commonly found in biological materials, were determined at pH values of 2, 4, 6, and 8, in acetate buffer, and in the presence and absence of EDTA. The polarogram of the digested sample was recorded with various electrolyte and pH values. The trace elements in the digested sample were determined by standard additions.

Procedure

For simultaneous determination the digested sample is added into the polarographic cell containing pH 2 acetate buffer and Se, Mo, Pb, Cd, Cr, Zn, and As are determined by standard additions; then pH is increased to 5-6, EDTA is added, and Cu, Fe, and Ti are determined. Thus, it is possible to determine 10 trace elements in 1 sample.

For validation of these results the same experiment can be repeated at pH 4, and Cu, Se, Mo, As, and Zn peaks can be obtained, the determination of these ions can be made, and results can be compared.

Results and Discussion

The polarograms of roots, stems, and leaves of digested *Astragalus microcephalus* samples were separately recorded so that the distribution of trace elements in the plants (especially selenium) could be observed. Trace element determinations were performed in various media so that the overlapping peaks could be separated. For the qualitative and quantitative validation of ions, the elements have to be observed and determined under different conditions. The same procedure was applied to *Astragalus lusitanicus* and *Elymus* (*Gramineae*) samples for comparison. At each condition the elements commonly found in the biological samples were added to the polarographic cell in the presence of plant sample and their peak potentials were recorded. The standard addition method was used for the determination of trace elements.

Astragalus microcephalus plant

Roots of *Astragalus microcephalus*

There were 4 root samples (A, B, C, D) for each different digestion time, which ranged from 3 to 6 h, in order to observe the effect of digestion time. The evaporation periods for the acid mixture and HCl during digestion are given in Table 1. For each of them polarograms were recorded under different electrolyte conditions. Some polarograms are given as examples.

Table 1. Digestion times for roots, stems, and leaves of *Astragalus microcephalus*.

	Root				Stem		Leaves
	A	B	C	D			
Evaporation time (h), (each 10 g, dried)	6	3	4.5	3	3	7	3
Time for HCl evaporation (min)	10	20	6	4	40	-	5

The polarogram of sample A (root) taken at pH 2 in acetic acid is given in Figure 1. As can be seen there are peaks at about –0.27 V (b), –0.54 V (c), –0.67 V (d), –0.9 V (e), and at –1.05 V (f). At 0 V the current (a) may belong to both Fe and Cu, as they overlap. According to our preliminary studies, the first peak at –0.27 V belongs to Mo, the peak at –0.54 V to Se(IV), the peak at –0.67 V to As, the peak at –0.9 V to Cr, and the last one at –1.05 V to Zn. Their presence was confirmed by standard additions and by the similarities of their quantities determined in different electrolytes.

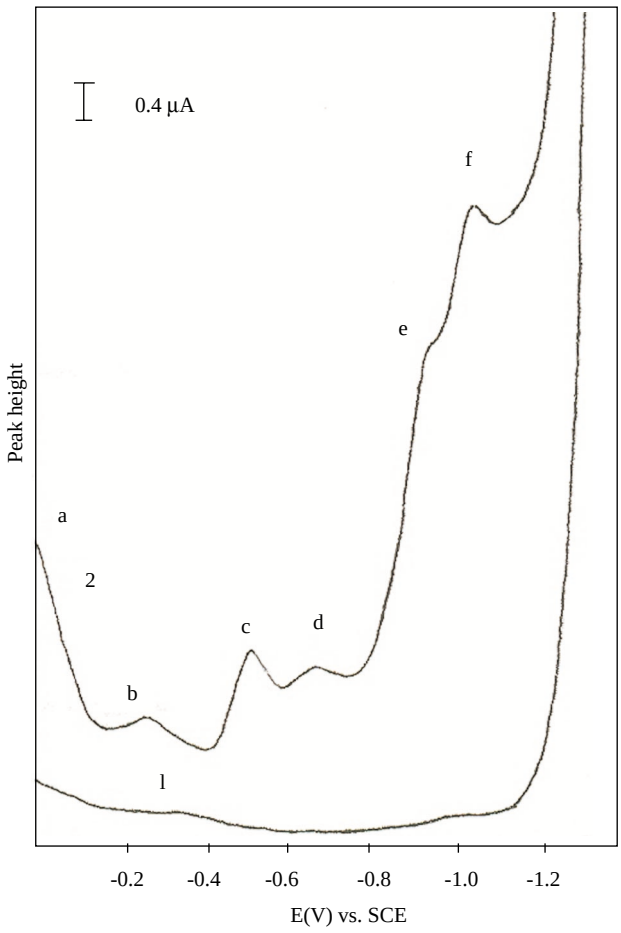

Figure 1. DPP polarogram of digested root sample A. (1) 10 mL of acetate buffer, pH 2; (2) 1 + 0.1 mL of sample.

A polarogram for sample B (root) is given in Figure 2, which was recoded in pH 4 acetate buffer. The peaks were observed at –0.1 V (b, Cu), –0.3 V (c, Mo), –0.70 V (d, Se), –0.8 V (e, As), and –1.0 V (f, Zn). Copper could not be observed at pH 2 since its reduction was about 0 V at pH 2; however, with increasing

pH its reduction shifted to more negative potentials, as expected. The presence of the mentioned ions was confirmed by standard additions and by recording polarograms under different conditions.

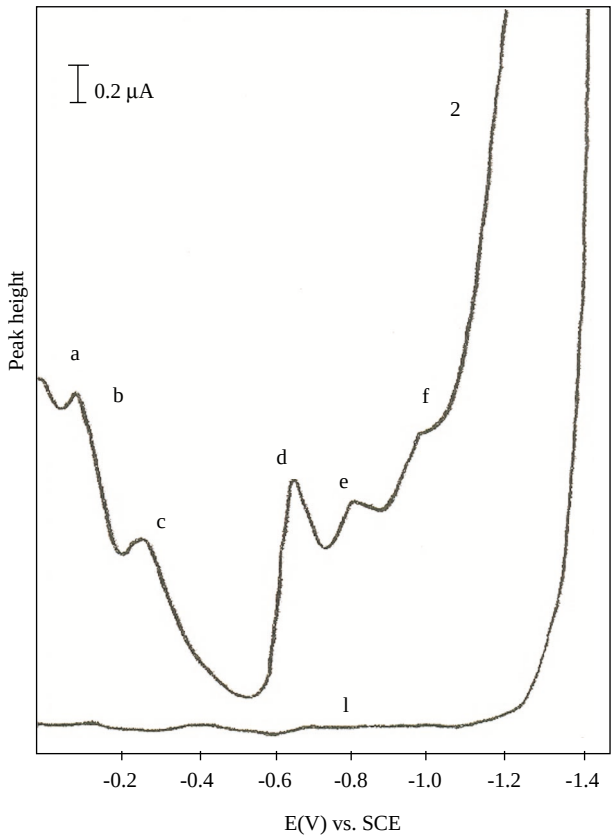

Figure 2. DPP polarogram of digested root sample B. (1) 10 mL of acetate buffer, pH 4; (2) 1+ 0.1 mL of sample.

Another polarogram recorded at pH 4 in EDTA for root B is shown in Figure 3. There were peaks at –0.14 V (a, Cu), –0.33 V (b, Ti), –0.68 V (c, Se), and the last peak at –0.80 V (d, As). Because of the complexing effect of EDTA, the copper peak shifted from –0.1 V to more negative potentials, but Fe still could not be observed. According to our preliminary work we know that Ti can be observed in this medium.

A polarogram recorded at pH 6 in EDTA for root A is given in Figure 4. There were peaks at –0.1 V (a), –0.33 V (b), –0.70 V (c), and –1.1 V (d). The first 2 peaks belong to Fe and Cu ions, respectively. This is the best medium for the determination of copper and ferric ion peaks. Because of the complexing effect of EDTA the peaks are shifted to more negative potentials and can thus be separated. In the same medium the selenium peak appears at –0.70 V, but it is too small for its accurate determination.

Stem and leaves of *Astragalus microcephalus*

The polarograms of digested stem samples had peaks similar to root samples. At pH 2 there was a Mo peak at –0.22 V, a selenium peak at –0.52 V, and an arsenic peak at –0.63 V. However, one sample was digested without using HCl as the last step for the reduction of selenate to selenite. In this polarogram no selenium peak was observed as expected, but, instead a small peak at –0.37 V appeared, which belongs to lead. This lead peak could not be observed in samples when HCl was used during digestion. According to our previous work[12] it was shown that when HCl was used during digestion the recovery for lead was quite low.

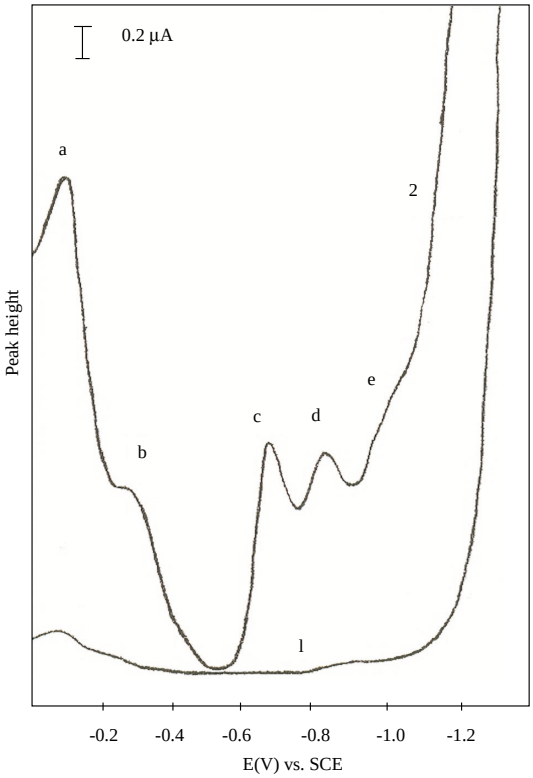

Figure 3. DPP polarogram of digested root sample B. (1) 5 mL of acetate buffer + 3 mL of EDTA + 2 mL of water (pH 4); (2) 1 + 0.1 mL of sample.

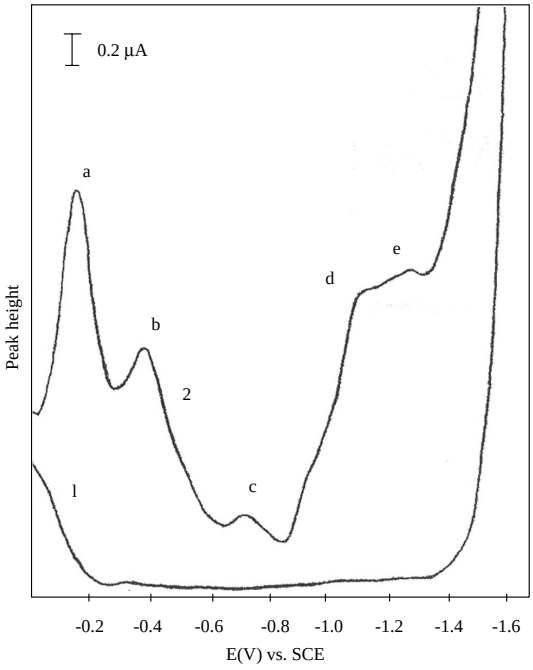

Figure 4. DPP polarogram of digested root sample A. (1) 5 mL of acetate buffer + 3 mL of EDTA, + 2 mL of water (pH 6); (2) 1 + 0.1 mL of sample.

The peaks in the polarograms of the leaves recorded at pH 2 were quite small. There was a Mo peak at –0.3 V, a selenium peak at –0.53 V, and an arsenic peak at –0.68 V.

Astragalus lusitanicus and Elymus (Gramineae) plants

Astragalus lusitanicus and *Elymus* (*Gramineae*) samples were digested according to the procedures described for *Astragalus microcephalus*. The same trace element procedure was applied and peaks for iron, zinc, titanium, and chromium were observed and determined.

Determination of trace elements in *Astragalus* plants

Selenium

Selenium in *Astragalus microcephalus* was determined in different electrolyte solutions. As can be observed from Figures 1 and 2, at pH 2 selenium had a peak at about

–0.54 V and at pH 4 it was at –0.65 V; both were well shaped. A selenium peak can be observed in EDTA solutions as well; at pH 4 it is at –0.67 V and at pH 6 it is at –0.70 V. Thus, it was possible to determine selenium content in various electrolytes and a comparison of results could be made. At higher pH values the peak is small. The determination of selenium at pH 2 with standard additions is given in Figure 5. In root samples A, B, and C the average selenium content was 183 ± 15 mg/kg, and in sample D it was 143 ± 10 mg/kg. The reason for this difference in sample D may have been the HCl vaporization time. In A, B, and C samples HCl evaporated in 6-20 min, but in sample D this time was 4 min. According to our former work[12] we found that if the HCl evaporation time was < 5 min the time for reduction of Se(VI) with HCl was not sufficient.

The evaporation time of acids had no effect on the result of selenium; as can be seen with A, B, and C samples, with changing evaporation times the results were nearly the same.

The selenium content in stems was 55 mg/kg and in leaves 63 μg/g, indicating that the selenium is mostly collected in roots. No selenium peak was observed in the samples of *Astragalus lusitanicus* or *Elymus* (*Gramineae*), which were taken from the same soil (Tables 2 and 3). The validation of selenium was made with 2 different methods, cathodic stripping[4] and differential pulse stripping voltammetry[5], and good consistency was observed between the results.

Copper and ferric ion

As can be seen from the polarograms recorded, with various electrolytes the iron peak was clearly observed and could be separated from copper in pH 6 EDTA (Figure 4). Thus, both iron and copper ions could be determined in this medium. A polarogram for the determination of copper is given in Figure 6 as an example. As can be seen, the peak for iron appears at about –0.1 V and it was possible to determine iron from this peak. Copper had a peak in pH 4 EDTA as well and its determination could be made in 2 media. For A, B, and D root samples (*Astragalus microcephalus*) the average copper content was 102 ± 5 mg/kg, and iron content of root samples A and B was 377 ± 28 mg/kg (iron content in leaves was also quite high, 257 mg/kg).

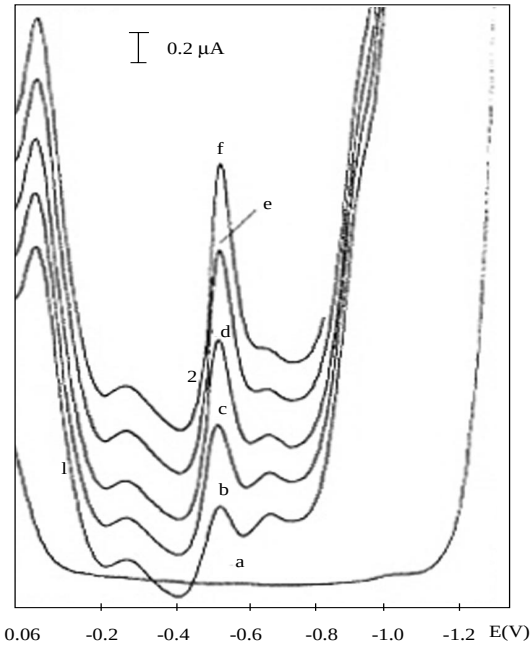

Figure 5. Determination of Se(IV) in root by standard additions. (a) 10 mL of acetate buffer, pH 2; (b) a + 0.1 mL of sample; (c) b + 0.1 mL × 10^{-3} M Se(IV); (d) c + 0.1 mL × 10^{-3} M Se(IV); (e) d + 0.1 mL × 10^{-3} M Se(IV); (f) e + 0.1 mL × 10^{-3} M Se(IV).

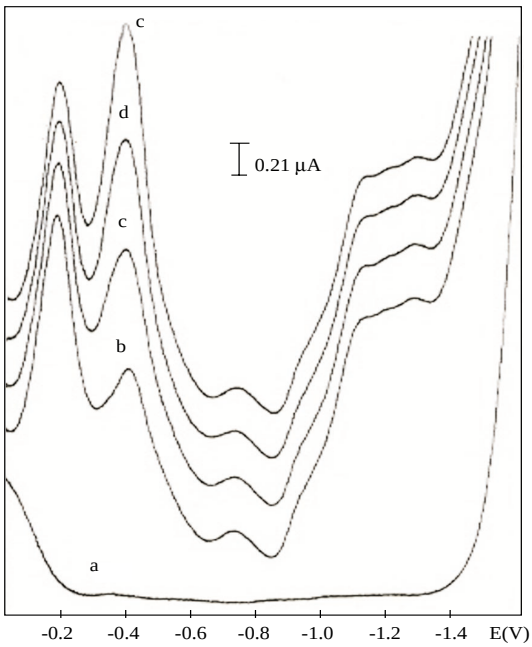

Figure 6. Determination of Cu(II) in roots by standard additions. (a) 5 mL of acetate buffer (pH 6) + 3 mL × 0.3 M EDTA + 2 mL of water; (b) a + 0.1 mL of sample; (c) b + 0.1 mL × 10^{-3} M Cu(II); (d) c + 0.1 mL × 10^{-3} M Cu(II); (e) d + 0.1 mL × 10^{-3} M Cu(II).

The similarities of iron and copper quantities in A, B, and D samples indicate that there is no effect of the evaporation times of acids. In roots of *Astragalus lusitanicus* the iron content was

336 ± 5 mg/kg, and in stems and leaves together it was 209 ± 10 mg/kg; however, no peak for copper was observed.

In *Elymus* (*Gramineae*) the iron content was 558 mg/kg, which is higher than both of the *Astragalus* plants, but here too no copper peak was observed.

Chromium

Chromium could be best observed in pH 2 acetate buffer with a peak at –0.9 V (Figure 1). The polarogram had to be recorded at low sensitivities because of the large chromium peak. Chromium content was nearly the same for both A and B root samples at pH 2, the average being 695 ± 20 mg/kg. In a polarogram of leaf sample no peak for chromium was observed, indicating that most chromium is accumulated in roots. *Elymus* (*Gramineae*) grown in the same soil contained only 50 mg/kg chromium.

Arsenic

As can be seen (Figures 1-3), in *Astragalus microcephalus* the peak of arsenic observed at pH 2 was –0.63 V, at pH 4 it was –0.8 V, and in pH 4 EDTA it was –0.82 V. Arsenic was determined in 3 of these media and similar results were obtained. In root sample B at pH 2 the arsenic peak was 111 ± 5 mg/kg, at pH 4 it was 115 ± 5 mg/kg, and in pH 4 EDTA it was 110 ± 5 mg/kg. No arsenic peak was observed in samples of *Astragalus lusitanicus* or *Elymus* (*Gramineae*).

Zinc

Zinc was determined at pH 2 with a peak at –0.95 V. In root samples of *Astragalus microcephalus* the zinc content was about 80 mg/kg, in stem and leaf samples it was only

7 μg/g. In roots of *Astragalus lusitanicus* zinc content was 15 ± 1 mg/kg, in stem and leaf sample it was 23 ± 1 mg/kg, and in *Elymus* (*Gramineae*) it was 25 ± 2 mg/kg.

Titanium

Titanium was best observed in EDTA at pH 4, with a peak at –0.33 V. Although titanium has peaks at pH 2 and 4 of about –0.80 V, this medium was not preferred since they were too small for accurate determination. The titanium content in root samples B and D of *Astragalus microcephalus* (from Kars) was 62 ± 4 μg/g. In *Astragalus lusitanicus* (from Muğla) titanium content was 22 ± 10 mg/kg, and in *Elymus* (*Gramineae*) (from Kars) it was 69 mg/kg. As can be seen, both plants that were grown in the same soil (in Kars) had similar amounts of titanium.

Molybdenum and lead

Molybdenum could be observed both at pH 2 and 4, at about –0.25 V. In root samples A, B, and D, 90 ± 15 mg/kg of Mo(VI) was found. Lead could be determined only when the sample was digested with the acid mix of HNO_3 and $HClO_4$. In this case HCl was not used for the reduction of selenate. The polarogram recorded at pH 2 had a peak at –0.38 V, which belonged to lead. In *Astragalus microcephalus* stem samples lead content was about 14 ± 2 mg/kg.

All of the results obtained for element quantities in *Astragalus microcephalus* are summarized in Table 2, and the results for *Astragalus lusitanicus* and *Elymus* (*Gramineae*) are given in Table 3.

Table 2. Trace elements in roots, stems, and leaves of *Astragalus microcephalus*.[a]

Astragalus microcephalus	Se(IV) mg/kg	Cu(II) mg/kg	Fe(III) mg/kg	Zn(II) mg/kg	Mo(VI) mg/kg	Ti(IV) mg/kg	Cr(III) mg/kg	As(III) mg/kg	Pb(II) mg/kg	Cd(II) mg/kg
Root	183 ± 20	102 ± 5	377 ± 28	80 ± 15	90 ± 15	62 ± 4	695 ± 20	112 ± 3	ND	ND
Stem	55 ± 1	ND	-	7	-	-	-	-	14 ± 2	ND
Leaves	63 ± 3	-	257	-	-	-	ND	-	ND	ND

ND: not detected

[a]mg/kg $\times\pm$ t $\times$ s/n$^{1/2}$, t: confidence interval, 90% (n = 4)

Table 3. Trace element quantities in *Astragalus lusitanicus* and *Elymus* (*Gramineae*).[a]

Elements	Se(IV) mg/kg	Cu(II) mg/kg	Fe(III) mg/kg	Zn(II) mg/kg	Ti(IV) mg/kg	Cr(III) mg/kg
(*Astragalus lusitanicus*) Root	ND	ND	336 ± 5	15 ± 1	22 ± 10	-
(*Astragalus lusitanicus*) Stem and leaves	ND	-	209 ± 10	23 ± 1	-	-
Elymus (*Gramineae*)	ND	ND	558	25 ± 2	69	50

ND: not detected

[a] mg/kg, $\times\pm$ t $\times$ s/n$^{1/2}$, t: confidence interval, 90% (n = 4)

Conclusions

Astragalus plants are important because of their use in pharmaceuticals, as thickening agent in foods, as feed for animals, and in controlling cancer cells. They are also used as indicator plants for selenium. Thus, it is very important to know their trace element content.

Trace elements (Cu, Fe, Cd, Se, Ti, Mo, As, Zn, Pb, and Cr) in *Astragalus* plants can be determined simultaneously with a simple and cheap instrumental technique (DPP) from one digested sample solution, without any time consuming extraction or preconcentration procedures. Selenium, molybdenum, lead, cadmium, chromium, zinc, and arsenic were separated and determined from one polarogram recorded in pH 2 acetate buffer. Copper, selenium, molybdenum, arsenic, and zinc were separated and determined in pH 4 acetate buffer, and copper, titanium, selenium, and arsenic in pH 4 EDTA solution, thus enabling determination of element quantities at different conditions. Iron and copper can be separated best in pH 6 EDTA. Determining the element quantities in various media and using different methods have confirmed the accuracy of the results. The evaporation time of acids had no effect on the element contents; however, the HCl evaporation time had an effect on the quantity of selenite. When the HCl evaporation time was $<$ 5 min, the time for the reduction of selenate to selenite was not sufficient.

According to the results most of the elements are accumulated in roots, but selenium was also distributed in stems and leaves. Although a large amount of Cr was present in roots, it was below the detection level in stems and leaves. Iron, on the other hand, had high levels in both root and leaves. It can be concluded that *Astragalus microcephalus* accumulated selenium from soil, while *Elymus* (*Gramineae*) did not, even though they were grown in the same soil.

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