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Letters to Editor

Determination of Heavy Metals in Samples of Moss by DPV

I. Baranowska, K. Srogi

Department of Analytical and General Chemistry, Silesian University of Technology,
M. Strzody 7, 44-100 Gliwice, Poland

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Abstract

The heavy metals cadmium, nickel, lead, molybdenum and zinc were determined in 19 samples of moss taken from 19 points situated in the Gliwice and Sanok areas. Samples (segregated, cleaned and powdered) were digested by the wet method in the microwave mineralizer. Differential pulse anodic stripping voltammetry (DPASV) was used for determination of Cd, Ni, Mo, Pb and Zn. Oriental tobacco (CTA-ATL-1) as the certified reference material was applied in those investigations.

Keywords: heavy metals, microwave mineralization, differential pulse voltammetry, bioindicators

Introduction

Development of widely understandable ecoanalytics and tightening demands of environmental protection have put forward new requirements for analytical methods and systems of analytical control. Methods applying biological materials in analysis of complex environmental matrix are used as well in classic biomonitoring as in bioanalytics.

The ability of accumulating xenobiotics by bioindicators has been applied for years in monitoring procedure.

To assess the degree of pollution of the environment by xenobiotics moss is frequently applied as an accumulative indicator [1-2].

A number of features classify moss as a good bioindicator: it does not have a cuticle and epidermis so that its leaves can easily let heavy metal ions in; there is a lack of roots and tissues conducting mineral salts; it can collect heavy metal ions from rainfall and dry deposition; a concentration of heavy metals in mosses is a function of deposition of heavy metals in the air [3-4].

On the basis of literature data it was found that moss is very useful in the assessment of deposition of heavy metals in the environment [5-6]. To obtain reliable results an application of certified reference materials (CRM) is recommended. The basic function of CRM is to ensureaccuracy and coherence of analytical determination [7]. They are also used for the calibration and testing of analytical procedures [8]. The important factor determining credibility of the whole procedure of metal analysis in moss is the stage of collecting samples and homogeneity of biological material.

It is known that the concentration of metals in moss depends on type and age of moss and the anatomical part taken for analysis as well as the place where the sample is collected [9-10]. Microwave mineralization is applied for the digestion of plant material and keeping metals without losses in the solution [11].

Experimental

Apparatus and Reagents

Determination of heavy metals was carried out using inversion pulse voltammetry using EKO-TRYBO POLAROGRAPH (Praha, R. Czech) equipped with the hanging mercury electrode and silver-silver chloride as a reference electrode. For pulverization and homogenization of material agate mill Restesch GmbH was used. Mineralization in close system was carried out in a microwave set - Milestone 1200 ML MEGA (Italy).

H_2O_2 30%; ammonium buffer 1.0 M, pH=10.0; acetate buffer 1.0M pH=4.7 and pH=3.5; dimethylglyoxime 1% solution in ethanol, 8-hydroxyquinoline -0.1% in $CC1_4$; tin(II) chloride-10% in hydrochloric acid; $K_2S_2O_8$ 4% water solution.

All reagents were of analytical grade (POCH Gliwice, Poland).

Nitric acid sp.pure, 68% (Zaklady Azotowe Tarnow-Moscice).

Chloric acid 70% (Veb Laborcheme Apold-Germany).

The level of analyzed metal ions in used acids was checked.

Working solutions of investigated ions (Pb, Zn, Cd, Ni and Mo) were prepared by dilution of standard solutions 1 mg mr^1 (Backer).

Certified reference material (CRM): oriental tobacco (CTA-OTL-1, Poland).

Collecting and Preparing Samples

Moss samples were collected in the area of Gliwice and Sanok. *Orthodicranum montanum,* living in numerous places in a forrested part of Sanok and *Hypnum cupressiforme fo. filiforme* living almost in the whole area under investigation both in Gliwice and in Sanok were used for the experiments. Also, moss occurring sparsely in the area of Sanok and Gliwice like *Plagithenium loetum, Polytrichum formosum, Entodon schreberi* were analyzed.

Samples were collected for 15 days. Selected green fragments of moss were prepared for investigation according to the procedure typical for such samples described in literature.

Specification of collecting stands is in Table 1.

Moss living on beech bark *(Fagus silvatica)* up to an elevation of 1.3 m was taken for analysis. Trunk of beech was divided into two zones: (I) 0-0.40 m; (II) 0.40-1.30 m. The aim of the work was to establish the net of points for collecting samples for analysis. Because of the lack of living moss in some parts of towns it was impossible to achieve uniform distribution of points for collecting samples. The assumption of collecting moss in points situated in a distance of 70 m away from the communication routes of the towns was realized. 19 points were established for sample collecting 6 of them for -*Orthodicranum montanum,* 3 for *Hypnum cupressiforme fo. filiforme,* 2 for *Pohlianutans,* 3 for *Plagithenium loetum,* 2 for *Entodon schreberi,* 2 for *Polytrichum formosum* and 1 for *Pleurozium schreberi.*

Collected samples of moss were thoroughly segregated and cleaned (from particles of soil, needles, leaves, and bark). Cleaned samples were placed on a filter paper and dried in the air and in a dryer at 100°C to a constant mass. Next, samples of moss were pulverized for homogenization in agate mill for 10 minutes. Obtained powder was weighed on an analytical balance with accuracy of 0.0001 g. In the case of plant material containing hard particles proper homogenization is important. For establishing the optimum time of grinding periods 10, 15, 20 and 25 min. were tested. To achieve full homogenization and good analytical results for O.lg sample 10 min. of grinding is sufficient.

Table 1. Specification of place, date and the kind of moss collected for analysis.

No	Date	Location	Kind of moss	Zone of the tree
1	14.05.98	green area of Sanok	*Entodon schreberi*	I
2	17.05.98	Chopin Park in Gliwice	*Hypnum cupressiforme fo. filiforme*	I
3	17.05.98	Gliwicko – Zabrski Forest	*Orthodicranum montanum*	II
4	17.05.98	Bytom – Piekary Śl. – Steel Plant	*Plagiothecium loetum*	I
5	14.05.98	afforested area of Dąbrówka Mała	*Orthodicranum montanum*	I
6	14.05.98	Sanocki Park in city centrum	*Pohlianutans*	II
7	16.05.98	near the road A4, in the wood Maciejów	*Orthodicranum montanum*	I
8	19.05.98	street I A.W.P. 10 in Sanok	*Pleurozium schreberi*	I
9	17.05.98	Chrobry Park in Gliwice	*Plagiothecium loetum*	I
10	18.05.98	heritage park in Sanok	*Polytrichum formosum*	II
11	18.05.98	Łabędzki Forest	*Orthodicranum montanum*	I
12	19.05.98	street 3–ego Maja 3 in Sanok	*Orthodicranum montanum*	I
13	19.05.98	street Posada in Sanok	*Pohlianutans*	II
14	21.05.98	sport's aerodrome in Sanok	*Plagiothecium loetum*	I
15	22.05.98	near the road A4, in the wood Maciejów	*Hypnum cupressiforme fo. filiforme*	I
16	22.05.98	Chopin Park in Gliwice	*Entodon schreberi*	I
17	29.05.98	Bytom – Piekary Śl. – Steel Plant	*Orthodicranum montanum*	I
18	22.05.98	Gliwicko – Zabrski Park	*Polytrichum formosum*	I
19	29.05.98	heritage park in Sanok	*Hypnum cupressiforme fo. filiforme*	I

Zones: I – 0-0.40 m of the tree; II – 0.40-1.30 m of the tree

Mineralization

0.1 g of pulverised samples of moss, weighed with the accuracy of 0.0001 g were placed in Teflon vessels suitable for mineralization under pressure in a microwave oven. Then to each vessel 4 ml of concentrated HNO_3 sp. pure and 1 ml of H_2O_2 were introduced. The following parameters were performed for the standard program I of the oven: (1) 2 min., 252 W; (2) 2 min., without heating, (3) 6 in., 250 W, (4) 5 min., 400 W, (5) 5 min., 650 W; (6) 10 min., ventilation. Samples after mineralization were transferred quantitatively to the 25 ml measuring flasks and filled to the mark with distilled water. Obtained solutions were dark yellow and contained small amounts of precipitation. Because of this conditions for microwave mineralization were changed for some samples of moss. Digestion was carried out in solution containing 4 ml HNO_3 and 2 ml H_2O_2. Increasing of current power was proposed for the (1) and (4) stage of mineralization as well as extension of the time of particular steps. The following steps of digestion procedure (program II) were worked out by the authors: (1) 6 min., 400 W, (2) 2 min., without heating; (3) 7 min., 250 W; (4) 6 min., 550 W, (5) 6 min., 650 W, and (6) ventilation 10 min. Samples after mineralization were transferred quantitatively to the 25 ml measuring flasks and filled with water to the mark. Changing the conditions of microwave digestion: volume of used solutions, time and microwave irradiation energy resulted in obtaining clear light yellow solutions.

Electrochemical Determination of Zn, Cd, Ni, Mo and Pb

Analysis of metals was carried out by the voltammetric method using differential pulse polarography (DPASV). Before each measurement electrolytes were bubbled 10 min. with nitrogen. Analysis of Cd, Zn and Pb: 1.0 ml of solutions obtained after mineralization was placed in the 10 ml measuring flask and 9.0 ml of acetate buffer pH=4.7 were introduced and the samples were filled with water to the mark. Measurements were taken by the triple standard addition method. 20 µl of standard solution containing mentioned above elements of concentration 0.02 mg/ml were added to 10 ml of tested sample.

In the case of Mo analysis 0.2 M acetate buffer of pH=3.5 was applied. [12]. 1.0 ml of sample was placed into a 10 ml measuring flask then 8 ml of buffer solution were added followed by the addition of 10 µl of 8-hydroksyquinoline of concentration 0.2 mmol/1 and 20 µl 0.05 mol/1 of $SnCl_2$ solution as a reducing agent. Solutions were filled to the mark with distilled water. Polarographic curve was recorded and next the subsequent 10 µl portions of standard molybdenum solutions 0.005 mg/ml were added and polarographic curves were recorded each time.

Determination of nickel was done in the ammonium buffer pH=10.0 [13]. Dimethylglyoxime was applied as a ligand and 4% solution of $K_2S_2O_8$ was used for the oxidation of Ni(II) ions to Ni(IV). To 1.0 ml of sample in 10 ml measuring flask 5 ml of 0.1 mol/1 H_2Dm solution, 30 ml of $K_2S_2O_8$ and 8.0 ml of NH_3/NH_4CI buffer pH=10.0 were introduced and filled to the mark. For masking of Fe ions 1M Na_2HPO_4 was used. Ni content in the sample was found by the standard addition method introducing subsequently 20 µl portions of 0.02 mg/ml standard Ni solution to 10 ml of the tested sample.

To work out the analytical procedure and instrument calibration the CRM - oriental tobacco, and model solutions of examined ions were applied.

The conditions for the determination of Zn, Cd and Pb in the acetate buffer by DPV method on the basis of analysis of CRM are given below: pH= 4.5 ± 0.3; concentration time: 30-60 sec; break 10-15 sec. Peaks for Zn, Cd and Pb of half-wave potential $E_{1/2}$: -0.98, -0.58, -0.38V, respectively, were recorded against Ag/AgCl as a reference, electrode. In the case of nickel determination in ammonium medium the peak of $E_{1/2}$ -0.1 IV was recorded against Ag/AgCl. In 0.2 M acetate buffer with the addition of Sn(II)Cl molybdenum gives a good shaped voltammetric peak at $E_{1/2}$ -0.25 V after concentration during 60 sec. Investigations of aforementioned elements were carried out at sensitivity 0.1 nA, in the range of 0 to 1.0 µA and rate of potential change: 20 mVs^{-1}. For calculation of the content of analyzed ions in examined systems and reference materials, algorithm of standard software of EKO-TRYBO POLAROGRAPH was applied.

Results and Discussion

Biological materials like moss are highly inhomogeneous. They contain numerous organic compounds of different steps of stability and impurities of sparingly soluble mineral components. Incomplete mineralization of samples during microwave digestion makes analyte transfer difficult for the solution. On the other hand, residues of organic matrix influence electrochemical measurements.

In the presented work the investigation of mineralization conditions for the determination of analytes in CRM, oriental tobacco were carried out. Interference caused by incomplete decomposition of organic matrix digested according to program I (HNO_3/H_2O_2 (4:1)) was observed in analysis of investigated metal ions in moss samples. Similar incorrectness was observed while using increased amounts of H_2O_2 (30%). Badly shaped and unrepeatable peaks were recorded and the results of cadmium, zinc, nickel and lead in oriental tobacco (CRM) occurred to be out of the range of the confidence interval given for CRM by producers. Thus, the additional stage of microwave digestion was applied. The amount of H_2O_2 (30%) was enlarged and the time of mineralization was extended. Additional irradiation with UV light (10 min.) was applied after mineralization.

In Table 2 the results of the determination of metals in oriental tobacco (CRM) mineralized according to the I and II program are shown. In program II the mixture of HNO_3 + H_2O_2 (4:2) was used. Because the results of determination of chosen metals in samples mineralized according to program II were found in the range of confidence interval of Certified Reference Material, the samples of moss were mineralized according to this program.

Table 2. Results of the determination of metals in oriental tobacco mineralized according to the program I and II, n=5.

Program	cadmium	nickel	lead	zinc
mineralization	mg kg^{-1}			
I	1.71	5.58	5.91	54.0
II	1.18	6.22	4.36	48.7
certificate value	1.12±0.12	6.32±0.65	4.91±0.80	49.9±2.4

Statistical evaluation of results using Student's t-test does not reveal statistical essential differences between the results of metals' determination in oriental tobacco ($p > 0.05$), mineralized according to program II, (n=5).

Based on the experiments and measurements on CRM the optimum conditions for the determination of investigated ions in analyzed samples were established.

In Table 3 statistical distribution of the results of Cd, Pb, Zn and Ni determination in electrolytes given above with application of CRM and model solutions (RM) for calibration are gathered. Given results are mean values calculated from n=6 measurements.

Table 3. Statistical evaluation of results of zinc nickel cadmium and lead determination in moss sample *Orthodicranum montanum* obtained by DPV using oriental tobacco (CRM) and model solutions (RM) for calibratio. All conentrations given in mgkg^{-1}.

Element		Mean	Standard deviation	Confidence interval	Real Value
zinc	CRM	267.8	1.01	2.9	267.8 ± 2.9
	RM	269.1	5.49	16.0	269.1 ± 16.0
cadmium	CRM	5.1	0.81	2.3	5.1 ± 2.3
	RM	5.8	1.31	3.7	5.8 ± 3.7
nickel	CRM	19.4	1.41	4.0	19.4 ± 4.0
	RM	19.9	3.84	11.2	19.9 ± 11.2
lead	CRM	164.2	0.83	2.4	164.2 ± 2.4
	RM	167.5	5.47	15.9	167.5 ± 15.9

The presented table shows the difference in results of metal determination in moss samples with application of CRM and model solutions for calibration of apparatus parameters and analytical procedure.

The procedure worked out on the basis of CRM gives more precise results of what is proved by values of standard deviation and confidence interval.

Investigations carried out in the area of Gliwice and Sanok revealed that mosses such as *Hypnum cupressiforme fo. filiforme* and *Ortodicranum montanum* are useful as good bioindicators for environmental pollution by heavy metals.

These taxons were chosen because of their abundance on the examined area as well in the industrial zone as in the clean, not polluted area, which enabled comparison of metal content in these regions.

Contents of particular ions in examined samples are gathered in Table 4.

Table 4. Average contents of zinc, cadmium, nickel, molybdenum and lead in examined samples of moss determined with application of CRM (mg kg^{-1}).

No	zinc	cadmium	nickel	molybdenum	lead
1	110.8	3.4	22.2	0.9	26.5
2	337.1	2.6	32.1	1.1	268.5
3	614.8	5.7	45.1	0.9	236.4
4	1004.7	27.8	62.8	3.5	726.5
5	267.8	5.1	19.4	1.1	164.2
6	432.1	4.3	23.2	2.2	290.3
7	518.8	5.9	56.1	2.7	337.1
8	362.3	4.6	36.6	0.5	123.1
9	607.3	12.5	58.7	0.8	115.3
10	201.1	2.2	17.2	2.5	254.1
11	253.2	3.9	21.4	3.8	224.1
12	527.7	10.5	36.4	5.1	328.7
13	625.5	32.5	59.7	1.1	457.8
14	325.7	6.4	18.1	2.6	308.7
15	723.7	77.8	61.1	3.1	625.6
16	425.6	21.5	42.5	2.2	212.3
17	1147.1	25.6	58.8	4.3	528.7
18	695.4	6.2	42.2	1.7	299.7
19	110.3	2.1	12.2	2.2	156.7

In the majority of samples low contents of molybdenum and high contents of zinc were found. In the area of the steel plant near the road from Bytom to Piekary SI., where the influence of industrial and communication factors play a role the higher contents of all metals were found in samples of moss. High concentrations of Cd, Zn, Mo, Ni and Pb are also observed in samples originating from town development in the vicinity of routes leaving Gliwice. As it could be predicted, much lower concentrations of these metals were found in plant samples collected from green areas of Gliwice, located in Labedzki Forest and in Chopin Park. Investigations on accumulation of heavy metals in samples of one species of mosses *Hypnum cupressiforme fo. filiforme* originating from one place gave reproducible results. Contents of metals in samples of various moss species collected from the same place revealed variability due to the different ability for cumulating metals by moss exposed to the same circumstances.

In Table 5 the results of metal determination in various moss species collected from the same places are gathered.

Taking the sum of average contents of heavy metals in moss as a criterion for assessment of polluted areas it can be found that the greatest amount of metals occurred in a sample of *Pohlianutans* moss collected from the

Table 5. Average amounts of chosen metals in various moss species collected from the same place. All concentrations given in mg kg-[1].

Location	Kind of moss	Ni	Zn	Mo	Pb	Cd
Bytom-Piekary Śl. Steel Plant	*Plagithecium loetum*	62.8	1004.7	3.5	726.5	27.8
	Orthodicranu montanum	58.8	1147.1	4.3	528.7	25.6
Forest Chopin in Gliwice	*Entodon schreberi*	32.1	337.1	1.1	268.5	2.6
	Hypnum cupressiforme fo. filiforme	42.5	425.6	2.2	212.3	21.5
Heritage Park in Sanok	*Polytrichum formosum*	17.2	201.1	2.5	245.1	2.2
	Hypnum cupressiforme fo. filiforme	12.2	110.3	2.2	156.7	2.1

forrested area of Sanok, from a region less subjected to pollution. It is difficult, however, to state unambiguously weather it is the effect of pollution of the specific accumulative feature of mentioned moss species.

Carried out investigations of the contents of heavy metals in all samples gave new data on the contamination of the environment in the towns of Silesian and Carpatian regions, taking mosses as bioindicators.

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