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INVERSION-VOLTAMPEROMETRIC DETERMINATION OF HEAVY METALS IN MEDICINAL PLANT SAMPLES

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Abstract. *Increasing interest in the use of medicinal plants or their mixtures (herbal teas) in the prevention and treatment of diseases requires confirmation of the safety of the plants used. The reason is the heavy metals they contain can enter the human body together with the useful substances. The purpose of this paper is to determine the content of some heavy metals in medicinal plants produced by AltaiVita, Barnaul, Russia; KIMA, Barnaul, Russia, and PharmaCvet, Moscow, Russia by inversion voltammetry. The content of heavy metals we determined in 10 samples of medicinal plants from producer "AltaiVita". We preliminarily determined the moisture content of the examined raw material. The Cd content was 0.16-0.50 μg/kg, Pb - 0.33-0.85 μg/kg, Zn - 0.010-0.043 μg/kg, Cu - 10.1-55.9 mg/kg, that does not exceed maximum allowable content by standards of State Pharmacopoeia for medical plants and SanPiN 2.3.2.1078-01 for dietary supplements ("dried tea"). According to the standard content in plants (normal, average and toxic content of copper) exceeded in almost all samples (except for Cinquefoil white). We compared the content of heavy metals in samples of medicinal plants from AltaiVita, KIMA, and PharmaCvet. The content of Cd, Pb, Zn in KIMA and PharmaCvet samples was an order of magnitude or higher than the content of these metals in AltaiVita samples; the content of Cu in AltaiVita samples was comparable or 2 times lower than in KIMA and PharmaCvet ones.*

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Introduction

Many types of medicinal plants are used as analgesics, antipyretics, anti-inflammatory agents. Worldwide, herbal teas are used as prevention of a number of diseases, as tea is one of the most common drinks consumed by humans [1]. At the same time, along with useful substances, heavy metals contained in plants can enter the human body [2].

The relevance of the study is due to the increasing interest in the use of medicinal plants or their mixtures (herbal teas) in the prevention and treatment of diseases.

The purpose of the study is to determine the content of some heavy metals in medicinal plants from the producers of "AltaiVita" by inversion voltammetry.

We chose 10 samples of medicinal plants from the manufacturer "AltaiVita", Barnaul, Russia. In order to compare the content of heavy metals in the samples of medicinal plants, we tested 3 samples of the producer "PharmaCvet", Moscow, Russia and 2 samples of the producer "KIMA", Barnaul, Russia.

It is known the formation of the chemical composition of plants occurs under the simultaneous influence of a large number of environmental factors, but soil composition plays a particularly important role. For example, according to the Altai Federal Scientific Center of Agrobiotechnology, the soil cover on the territory of Altai Krai is composed of more than thirty types of soil [3]. Depending on the source of pollution there are noticeable differences in the distribution profile of heavy metals in the soil [4]. Regardless of the source of heavy metal pollution in an area, an increase in their levels in the soil almost always leads to an increase in the concentration of toxic ones in plants. [5-7]. Anthropogenic impact affects not only medicinal plants, but also humans who use infusions derived from these plants [8, 9].

Zinc is accumulated in the most vitamin-rich parts of plants [10]. In the absence or deficiency of zinc, the biosynthesis of vitamins and growth agents, auxins, is impaired. In large quantities, zinc can be a carcinogen; it belongs to the first class of danger along with arsenic, beryllium, cadmium, mercury, selenium and lead [9].

Copper is involved in regulating the water balance of plants, has high biochemical properties, accumulates effectively, forming complex compounds [11], but, like zinc, can be toxic. Copper belongs to the second class of hazard. Natural levels of lead in plants from uncontaminated areas range from 0.1-10.0 mg/kg dry weight with an average concentration of 2 mg/kg. Excess lead suppresses respiration, photosynthesis and reduces the intake of zinc, calcium, phosphorus and sulphur [12].

The normal content of cadmium in plants is 0.05-0.2 mg/kg air-dry weight. Cadmium is not an essential element for plant life, but the metal is actively absorbed by plants. As a chemical analogue of zinc, cadmium can replace it in the enzymatic system required for glucose phospholysis and the accompanying process of carbohydrate formation and decomposition [13].

Morphological variation caused by heavy metals can appear as flower polymorphism caused by lead, zinc or molybdenum, disruption and changes in leaf pigmentation caused by zinc, copper, etc. [14].

Plants capable of accumulating large amounts of heavy metals in their above-ground organs, many times exceeding their concentrations in the soil, are called accumulator plants [15]. They have formed mechanisms of resistance to heavy metals in the process of evolution. The presence of indicator plants, in which the amount of metal in the plant increases with the increase in the concentration of the element in the soil, can be used to indicate an increase in the content of certain elements in their nutrient environment [16-18]. The modification of some plants, including agricultural waste, with various substances [19], e.g. linseed fibre with L-arginine [20], allows the development of biosorbents for the treatment of heavy metals.

To determine the concentration of heavy metals in medicinal plants, physico-chemical methods of analysis (inversion voltammetry; atomic absorption spectroscopy; mass spectrometry) are used [21]. In this study we used the method of inversion voltammetry which is characterized by high sensitivity and expressiveness [22, 23].

Main body

We measured on a TA-Lab voltammetric analyser complete with IBM compatible computer. We used a mercury-film electrode, a silver chloride electrode, a reference electrode, and an auxiliary electrode as an indicator electrode. For sample decomposition we used a programmable dual-chamber PDP-Lab oven.

As objects of the study we chose 10 samples of medicinal plants of the manufacturer "AltaiVita" Barnaul: chamomile (lat. *Flores Chamomilla officinalis*), thyme (lat. *Thymus serpyllum*), spearmint (lat. *Mentha piperíta*), milfoil (lat. *Achillea millefolium*), marjoram (lat. *Origanum vulgare L.*), chinquefoil (lat. *Empetrum nigrum L.*), marsh cinquefoil (lat. *Comarum palustre*), white cinquefoil (lat. *Potentilla alba*), Willow-herb (lat. *Chamaenerion*), Saint-Mary-thistle (lat. *Silybum marianum L.*).

In order to compare the content of heavy metals in samples of medicinal plants, 3 samples of producer "PharmaCvet", Moscow were tested: Willow-herb (lat. *Chamaenerion*), thyme (lat. *Thymus serpyllum*), chamomile (lat. *Flores Chamomilla officinalis*) and 2 samples of producer "KIMA", Barnaul: marjoram (lat. *Origanum vulgare L.*), peppermint (lat. *Mentha piperíta*).

The samples were stored dried in paper packages, each species stored separately from the other. Each package had the date and the information about the place of collection. The shelf life of flowers, leaves, roots, rhizomes, and bark is 2-3 years, fruits and berries – 3-4 years [13]. After opening, the samples were stored in a glass container with a tightly closed lid. All the samples of medicinal plants above had a current shelf life. Before the determination of heavy metals (copper, zinc, lead, and cadmium), the moisture content of the plant material was determined.

Determining moisture

The moisture determination method is based on the determination of the loss in weight due to hygroscopic moisture and volatile substances when drying raw materials to an absolutely dry state [24].

We preground a sample of dried plant material in a mortar and placed it in a pre-dried blotter to constant weight. Drying of 1 g of medicinal plant material was conducted in open beakers in a desiccator at 105 °C until weight constancy [25].

The moisture content (X) of the raw material in per cent is calculated according to the formula:

$$X = (m_1 - m_2)100/m_3,$$

where m_1 is the weight of the sample bag before drying, g; m_2 is the weight of the sample bag after drying, g; m_3 is the weight of the sample before drying, g.

Tables 1-3 show the results of moisture determination in medicinal plants samples from different manufacturers.

Table 1. Results of moisture determination in medicinal plants samples produced by "AltaiVita"

Sample No.	Sample name	Weight (pre-drying weight), g	Weight (weight after drying), g	Moisture, %
1	Marjoram	1.0041	0.9518	5.21
2	Spearmint	1.0039	0.9357	6.85
3	Thyme	1.0002	0.9428	5.20
4	Chamomile	1.0015	0.9552	5.35
5	Willow-herb	1.0087	0.9360	5.59
6	Crowberry	0.9982	0.9532	4.51
7	Saint-Mary-thistle	1.0031	0.9636	3.94
8	Marsh cinquefoil	1.0027	0.9562	4.64
9	Cinquefoil white	1.0091	0.9459	6.26
10	Milfoil	1.0031	0.9410	5.74

Table 1 shows that the moisture content corresponds to the norm, with Saint-Mary-thistle having the lowest moisture content at 3.94% and Peppermint having the highest one at 6.85%.

Table 2. Moisture determination in 2 samples of medicinal plants from the manufacturer "KIMA" Moscow

Sample No.	Sample name	Weightm(pre-drying weight), g	Weight (weight after drying), g	Moisture, %
1	Marjoram	0.9826	0.9167	6.71
2	Spearmint	1.0311	0.9477	8.09

Table 3. Moisture determination in 3 samples of medicinal plants from the manufacturer "PharmaCvet" Barnaul

Sample No.	Sample name	Weight (pre-drying weight), g	Weight (weight after drying), g	Moisture, %
1	Thyme	1.0459	0.9742	6.95
2	Chamomile	1.0840	1.0180	6.09
3	Willow-herb	1.0234	0.9552	6.66

Tables 2, 3 show the normal moisture content.

When comparing the samples of medicinal plants from different manufacturers presented in Table 2 and 3, we determined the moisture content of samples of thyme, chamomile, and willow-herb from manufacturer "PharmaCvet" and samples of marjoram and peppermint from manufacturer "KIMA" are slightly higher than that of the same samples from manufacturer "AltaiVita".

We performed the sample preparation for heavy metal determination as follows. We put pre-cut and weighed on analytical scales 1.000 g of medicinal plants into clean quartz beakers with a capacity of (20-25) cm^3. Then we treated it with concentrated nitric acid (1-2) cm^3, evaporated on an electric stove at 130 °C to one third of the original volume, avoiding splashing. We added 1.0 cm^3 of concentrated nitric acid and 0.5 cm^3 of 30% hydrogen peroxide solution dropwise to each beaker and evaporated to dryness, gradually raising the temperature from 150 °C to 350 °C, until no more fumes were emitted. We then ashed the samples in an oven at 450 °C for 20 minutes. The operation of adding concentrated nitric acid with hydrogen peroxide and incubation was repeated until homogeneous white, grey or reddish ashes (without black carbon inclusions) were obtained [23].

We dissolved the resulting precipitate in 1.00 cm^3 of concentrated formic acid, topped up with bi-distilled water to 10.0 cm^3 and transferred to penicillin vials.

To ensure the purity of the reagents used, a "blank sample" was prepared, similar to the sample preparation of the analysed object, but containing no analytical sample.

Determination of heavy metals (cadmium, lead, copper, and zinc) by inverse voltammetry

We analysed according to MU 31-04/04 "Determination of zinc, cadmium and copper in foodstuffs" [26]. In our study we used basic solutions containing 100.0 mg/dm^3 of cadmium, lead, copper and zinc prepared from state standard samples with certified concentrations of elements of 1.00 mg/cm^3 (1000 mg/dm^3).

Due to the significant differences in the content of the metals to be determined, the samples were analysed from a single solution according to the following scheme: Cd and Pb concentrations were determined first, followed by Cu concentrations and then Zn concentrations.

In each beaker we added an aliquot of the sample with a volume of (0.5-1.0) cm^3. We set the preparation time to 30 s, sample parameter values are following: sample type - solid with mineralisation; dimension - mg/kg; sample mass 1 g; mineralisation volume - volume obtained after dissolving ash 10 cm^3; aliquot volume - (0.5-1.0 cm^3).

We recorded the voltammetry of samples and samples with the addition of a certified mixture. The analysis of the "blank sample" is conducted in the same way.

The results of the heavy metal content of the medicinal plant samples including the conversion to dry matter are shown in Tables 4 and 5.

Table 4. Content of heavy metals in samples of medicinal plants from producer "AltaiVita"

Sample No.	Sample name	Cd mkg/kg	Pb mkg/kg	Cu mg/kg	Zn mkg/kg
1	Marjoram	0.16±0.02	0.85±0.05	21.7±0.8	0.017±0.006
2	Spearmint	0.29±0.02	0.33±0.02	22.7±0.9	0.027±0.005
3	Thyme	0.16±0.03	0.43±0.03	37.9±2.7	0.027±0.003
4	Chamomile	0.34±0.02	0.38±0.02	20.4±0.5	0.020±0.005
5	Willow-herb	0.65±0.04	0.45±0.03	18.2±0.8	0.034±0.002
6	Crowberry	0.38±0.06	0.57±0.03	26.2±1.1	0.012±0.002
7	Saint-Mary-thistle	0.19±0.02	0.58±0.05	56±7	0.011±0.003
8	Marsh cinquefoil	0.29±0.03	0.76±0.03	57±5	0.043±0.003
9	Cinquefoil white	0.27±0.03	0.68±0.02	10.1±0.5	0.038±0.002
10	Milfoil	0.50±0.02	0.81±0.06	39 ±3	0.010±0.003
Rationing	Dried teas, mg/kg	1.0	6.0	–	–
	State Pharmacopoeia 3rd edition, mg/kg	1.0	6.0	–	–
	Normal, mg/kg	0-0.5	2-14	6-15	25-250
	Toxic, mg/kg	>100	–	>20	>400

The results above show Cd content was 0.16-0.50 μg/kg, Pb was 0.33-0.85 μg/kg, Zn was 0.010-0.043 μg/kg, and Cu was 10.1-55.9 mg/kg. The highest cadmium content was 0.65 μg/kg in a sample of willow-herb, while the lowest was 0.16 μg/kg in marjoram. The highest lead content was in the marjoram sample 0.85 μg/kg, and the lowest was in the peppermint sample 0.33 μg/kg. The copper content is the highest in the marsh cinquefoil sample at 55.9 μg/kg, and is the lowest in the white cinquefoil sample at 10.1 μg/kg. The zinc content is higher in the marsh cinquefoil sample at 0.043 μg/kg, while the milfoil sample is 0.010 μg/kg.

Table 5. Comparative content of heavy metals in samples of medicinal plants from different producers

Sample No.	Sample name	Produser	Cd mkg/kg	Pb mkg/kg	Cu mg/kg	Zn mkg/kg
1	Marjoram	KIMA	3.44±0.02	5.59±0.05	23.5±0.7	0.43±0.04
		"AltaiVita"	0.16±0.02	0.85±0.05	21.7±0.8	0.017±0.006
2	Spearmint	KIMA	3.15±0.02	3.15±0.03	35.3±2.3	0.17±0.03
		"AltaiVita"	0.29±0.02	0.33±0.02	22.7±0.9	0.027±0.005
3	Thyme	PharmaCvet	5.96±0.04	22.3±0.02	40.1±1.5	0.12±0.03
		"AltaiVita"	0.16±0.03	0.43±0.03	37.9±2.7	0.027±0.003
4	Chamomile	PharmaCvet	3.47±0.03	3.01±0.02	57±5	0.10±0.02
		"AltaiVita"	0.34±0.02	0.38±0.02	20.4±0.5	0.020±0.005
5	Willow-herb	PharmaCvet	13.4±0.2	7.47±0.06	32±7	0.65±0.05
		"AltaiVita"	0.65±0.04	0.45±0.03	18.2±0.79	0.034±0.002
Rationing		Dried teas, mg/kg	1.0	6.0	–	–
		State Pharmacopoeia 3rd edition, mg/kg	1.0	6.0	–	–
		Normal, mg/kg	0-0.5	2-14	6-15	25-250
		Toxic, mg/kg	>100	–	>20	>400

The comparative analysis shows that the content of the heavy metals Cd, Pb, Zn is lower in the samples of producer "AltaiVita" than in the samples of producers "PharmaCvet" and "KIMA", and the content of Cu is comparable or 2 times lower.

The significant difference in cadmium content was observed for samples of marjoram and peppermint from producer "KIMA", 21.5 and 10.9 times, respectively; zinc 25.3 and 6.3 times; lead 6.6 and 9.5 times and copper 1.1 and 1.6 times, respectively. In samples of thyme, chamomile and willow-herb from producers "AltaiVita" and "PharmaCvet" was observed a significant increase of cadmium concentrations by 37.2; 9.3; 20.6 times; lead by 37.3; 7.9; 20.6 times; copper by 1.1; 2.8; 1.8; zinc by 4.4; 5; 19 times, respectively.

The highest content of Cd = 13.4 μg/kg, Zn = 0.65 μg/kg was found in willow-herb (producer PharmaCvet) and Pb = 22.3 μg/kg in thyme (producer PharmaCvet).

The content of Cd, Pb, Zn does not exceed the maximum allowable content according to the norms of the State Pharmacopoeia and SanPiN 11-63 RB 98.

The Cu content of medicinal plants exceeds the maximum allowable metal content according to the state pharmacopoeia.

Sample No. 9 ("AltaiVita") - cinquefoil white is in the normal Cu content interval and does not exceed the toxic concentration.

Sample No. 5 ("AltaiVita") - willow-herb exceeds the interval of normal Cu content, but does not exceed the toxic concentration.

The other samples exceeded the content of heavy metals, which is due to the possible unfavourable territorial location of the medicinal plant collection site, near highways and industrial areas, leading to an excessive accumulation of heavy metals.

Conclusions

The content of heavy metals was determined in 10 samples of medicinal plants from producer "AltaiVita". The content of Cd was 0.16-0.50 μg/kg, Pb was 0.33-0.85 μg/kg, Zn was 0.010-0.043 μg/kg, Cu was 10.1-55.9 mg/kg, which does not exceed the maximum allowable content according to the standards of State Pharmacopeia for medicinal plants [27] and SanPiN 2.3.2.1078-01 for dietary supplements (dry teas) [28]. According to plant standards (normal, average and toxic) [29], copper content was exceeded in almost all samples (except cinquefoil white).

We compared the content of heavy metals in samples of medicinal plants from producer "AltaiVita" with samples from producers "KIMA" and "PharmaCvet". The content of Cd, Pb, Zn in "KIMA" and "PharmaCvet" samples was an order of magnitude or higher than the content of these metals in "AltaiVita" samples; the content of Cu in "AltaiVita" samples was comparable or 2 times lower than in "KIMA" and "PharmaCvet" ones.

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