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The Heavy Metals Monitoring in some Food Supplements

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Abstract

The paper proposes the heavy metals monitoring in some food supplements: gemoderivates in glycerolhydroalcohol and cold pressed plants (*Vaccinium myrtillus L., Rubus idaeus* L.) and forest fruit oils (*Hippophae rhamnoides L., Ribes nigrum L.*). The heavy metals concentrations (Cr, Zn, Pb, Ni, Cd, Fe, Mn, Al, Co, As, Sn) have been determinate by AA spectrometry and some of them by electrochemical method: cyclic voltammetry.

Keywords: heavy metals, AA spectrometry, cyclic voltammetry, food supplements

1. Introduction

Nature provides us with its most awesome remedies. Every tree species bears in its sap, in its buds and its green twigs a sum of substances and energies, which are quite diverse and with a healing power that few imagine. A number of local forest fruits and plants were tested. Several extracts were prepared for „draining" therapy from buds, shoots, twigs, bark from young branches, inner bark from the roots, sap and seeds. Draining is a term specifically used in homeopathy, synonimous with the concept of purification (detoxification) which precedes any natural treatment. Extracts from the buds and twigs of various plants have similar effects on the human body as synthesis hormones, but much gentler (curing, while not perturbing other functions) [1,2]. Food supplements pertaining to gemotherapy appear as glycerolhydroalcoholic extracts, dilluted 1:10, obtained from fresh vegetal tissue in full growth stage. These contain embrionary tissue which focuses all of the plant's genetic information, and is richer in vitamins, oligoelements, minerals, nucleic acids or growth factors than the plant it self. Food supplements extracted from cold pressed forest berries and buds (sea buckthorne, blackberry, blueberry, raspberry, strawberrys) are extremely important because they keep all of the beneficial compounds (notably the natural antioxidants) in much larger concetrations and are meant for (post-)convalescence states, vitamin deficiencies, etc.

The paper studies the food supplements obtained from black currant buds (Ribes nigrum L.), billberry twigs (Vaccinium myrtillus L.), raspberry twigs gemoderivates (Rubus idaeus L.), sea buckthorn oil (Hofigal, Parapharm company) and sea buckthorn mash Hippophae rhamnoides L.(Cluj).

The black currant bud extract stimulates the corticoadrenal into the secretion of hormones with anti-inflammatory and antialergic properties, activates the catabolism of urea, uric acid and cholesterol, stimulates the protidic metabolism and eozinophilia and reduces VSH. The billberry twig extract is recommended in the treatment of diabetes,

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dysentery, enterocolytes, visual disorders, gingivitis and states of capillary fragility. The raspberry twig extract Gemoderivate is recommended for women's endocrine disorders, ovary cysts and female sterility.

Fruits of sea buckthorns have been subject of many researches due to their energizing, equilibrating, antitumoral, antioxidant, analgesic, anti-inflammatory, anti-asthmatic, antibacterial,etc. effects. They contain all the vitamins needed by the human body: A, E, F, D (unstable in acidic media), C, K, P, entire complex of B vitamins (unstable in alkaline media) [2,3].

2. Materials and method

2.1. Samples preparations.

Oil products have been weighed and treated by concentrated nitric acid (67%, Merck, heavy metals free). Samples digestion has been achieved in a 1000W MWS-2 – Berghof type microwave oven using a three-step program: T_1=160ºC, t_1=15 min., P_1=40-60% from total power, T_2=210ºC, t_2=15 min., P_2=60-80%, T_3=210ºC →100ºC, t_3=15 min., P_3=0%.Thus resulted solutions have been completed with ultrapure water (RO System Operating Barnstead apparatus) to equal volumes in 25 ml calibrated flasks.

The food supplements from black currant buds, billberry twigs and raspberry twigs could not be processed using the above method due to the glycerine content. Samples were subjected to calcinate at 1000°C in Nabertherm HTC 03/14 – Germany oven for 2 hours. The heating speed was 5.5°C/min. The calcinate residue was reprocessed with 2 ml HNO_3 67%.

2.2. Methods of analysis.

Heavy metals in food supplements have been studied by AA spectroscopy. The AA spectroscopy is carried out on a novAA 400 G atomic absorption spectrometer, with graphite furnace, with WinAAS 3.17 software for evaluation, control and result presentation, a so-called cookbook, for every element, and a HS 55-1 hydride generator [4].

Calibration curves have been plotted using standard solutions of metals in search. The samples undergo an acidic digestion with high purity, metal free 65% HNO_3 using the MWS-2- Berghof-Germany mineralization and digestion system.

The voltammogrames are obtained using PGZ 402 Voltalab 80, with VoltaMaster 4, version 7 software. Platinum electrodes (S_{work}=7.85 mm^2, S_{aux}=50 mm^2, standard electrode SCE), and an 50 cm^3 BEC/EDI X51V001 electrochemical cell, from Radiometer Copenhagen are part of the Voltalab system. Calibration curves have been plotted using standard solutions for some metals in search: $Fe(NO_3)_2$ and Sn Cl_2 [5-9].

3. Results and Discussions

Three food supplements used in homeopathic medicine were studied, as well as three concentrated sea buckthorn based food supplements. The heavy metals presence in the studied samples can be noticed in Table 1 and Table 2. Some of these metals, in small amounts are useful to the human body: Mn, Co, Fe, Cu, Ni, Se. Higher concentrations of heavy metals in Gemoderivates 1 DH were registered, but these concentrations are related to calcination residues at 1000°C. Tin plays no known natural biological role in humans, and possible health effect of the tin are a subject of dispute. Tin itself is not toxic, but most tin salts are.The Sn registered concentrations are higher than we expected in all cases.

The classes of enzymes that have Mn cofactors are very broad and include such classes as oxidoreductase, transferases, hydrolases, lyases, isomerases, ligases, lectins and integrins. The reverse transcriptases of many retroviruses (though not lentiviruses such as HIV) contain manganese. The best known manganese-containing polypeptides may be arginase, the diphtheria toxin, and Mn-containing superoxide dismutase (Mn-SOD).

Cobalt in small amounts is essential to many living organisms, including humans. Having 0.13-0.30 mg/Kg of cobalt in soil markedly improves the health of grazing animals. Co is a central component of the vitamin cobalamin, or vitamin B_{12}.

Fe is essential for nearly all known organisms. In cell, iron is generally stored in the centre of metalloproteins, because "free" iron – which binds non-specifically to many cellular components – can catalyze production of toxic free radicals.Copper is an essential trace nutrient to all high plants and animals. In animals, including humans, it is found primarily in the bloodstream, as a co-factor in various enzymes, and in copper-based pigments

Table 1. Heavy metals concentrations

No.	Sample	Concentration, ppm					
		Cr	**Zn**	**Pb**	**Al**	**Cd**	**Sn**
1.	Gemoderivate 1 DH blueberry buds (*Ribes nigrum*)*	112.16	14.91	2.67	89.6	0.008	62.99
2.	Gemoderivate 1 DH bilberry twigs (*Vaccinium myrtillus*)*	2.90	9.97	4.58	94.46	**	59.93
3.	Gemoderivate 1 DH raspberry twigs (*Rubus idaeus*)*	2.57	22.98	7.43	63.71	**	46.25
4.	Sea buckthorn oil, (*Hippophae rhamnoides L.*, Hofigal product)	**	1.21	0.27	1.33	0.007	21.18
5.	Mashed Sea buckthorns (mashed *Hippophae rhamnoides L.*, Cluj product)	**	5.77	0.92	2.42	0.025	28.31
6.	Sea buckthorn oil, (*Hippophae rhamnoides L.*, Parapharm product)	**	1.027	0.54	2.07	0.032	35.60

* glycerolhydroalcoholic extract 1:10; ** under limit detection

Table 2. Microelements metals concentrations

No.	Sample	Concentration, ppm					
		Mn	**Se**	**Co**	**Fe**	**Cu**	**Ni**
1.	Gemoderivate 1 DH blueberry buds (*Ribes nigrum*)*	12.32	**	3.31	1240.8	**	30.67
2.	Gemoderivate 1 DH bilberry twigs (*Vaccinium myrtillus*)*	4.65	**	0.38	408.8	**	2.17
3.	Gemoderivate 1 DH raspberry twigs (*Rubus idaeus*)*	22.95	**	2.29	330.6	1.94	10.94
4.	Sea buckthorn oil, (*Hippophae rhamnoides L.*, Hofigal product)	0.02	**	**	4.51	**	**
5.	Mashed Sea buckthorns (mashed *Hippophae rhamnoides L.*, Cluj product)	0.55	**	**	21.11	**	1.74
6.	Sea buckthorn oil, (*Hippophae rhamnoides L.*, Parapharm product)	0.03	**	**	13.08	0.11	**

* glycerolhydroalcohol extract 1:10; ** under limit detection

Table 3. Sn concentrations in samples

No.	Sample	E_{SCE} , V	I_{peak}, mA/cm^2	Conc., mg/L
1.	Gemoderivate 1 DH blueberry buds (*Ribes nigrum*)*	1.375	0.5056	5.9395
2.	Gemoderivate 1 DH bilberry twigs (*Vaccinium myrtillus*)*		0.1434	3.4745
3.	Gemoderivate 1 DH raspberry twigs (*Rubus idaeus*)*		0.1404	3.4541
4.	Sea buckthorn oil, (*Hippophae rhamnoides L.*, Hofigal product)		0.1456	3.3206
5.	Mashed Sea buckthorns (mashed *Hippophae rhamnoides L.*, Cluj product)		0.1191	3.1485
6.	Sea buckthorn oil, (*Hippophae rhamnoides L.*, Parapharm product)		0.1037	3.0485

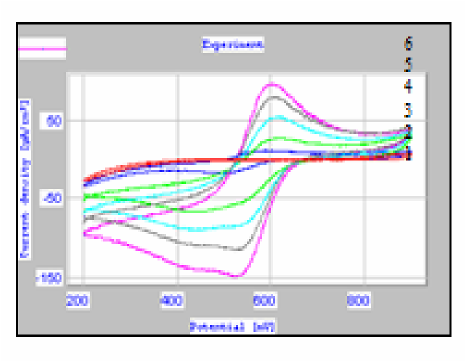

Figure 1. Cyclic voltamogramms for equilibrium $Fe^{3+} + e^{-} \rightarrow Fe^{2+}$

1 – support electrolyte HNO3 0.1 M; 2 – c=25.64 mg/L; 3 – c=50.00 mg/L;4 – c=95.24 mg/L; 5 – c=136.36 mg/L; 6 – c=173.91 mg/L;

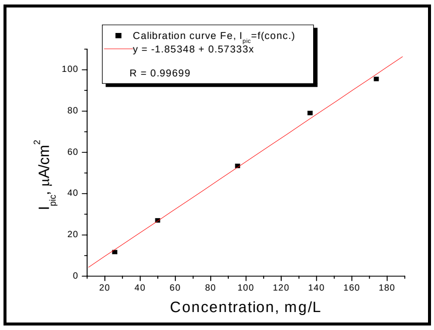

Figure 2. Calibration curve for iron concentration determination in food supplements, I_{peak}=f(conc.), E_{SCE} = 608 mV

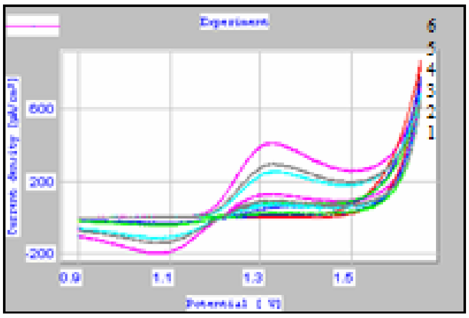

Figure 3. Cyclic voltamogramms for equilibrium $Sn^{4+} + 2e^{-} \rightarrow Sn^{2+}$

1 – support electrolyte HNO3 0.1 M; 2 - c=6.8333 mg/L; 3 - c=13.5257 mg/L; 4 - c=20.0816 mg/L; 5 - c=26.5050 mg/L; 6 – c=32.8000 mg/L

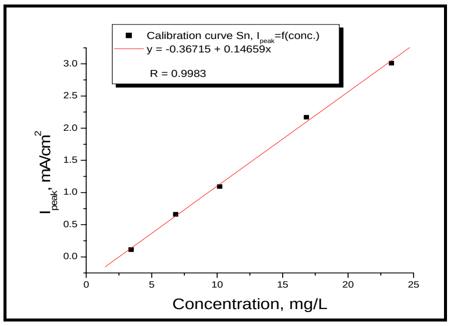

Figure 4. Calibration curve for tin concentration determination in food supplements, I_{peak}=f(conc.), E_{ESC} = 1.375 V

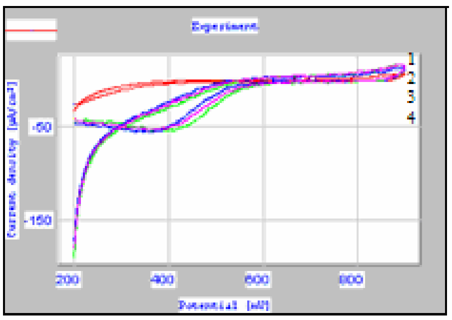

Figure 5. Fe concentration in calcinated food supplements Hofigal, E_{SCE}=608 mV

1- electrolyte support, 2- gemoderivate 1 DH blueberry buds, 3- gemoderivate 1 DH billberry twigs, 4- gemoderivate 1 DH raspberry twigs

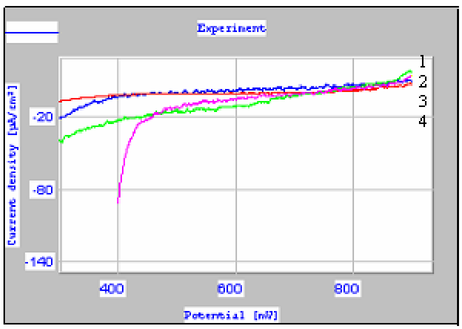

Figure 6. Fe concentration in concentrated food supplements based on sea buckthorn fruits, E_{SCE}=608 mV

1- electrolyte support, 2- sea buckthorn oil Hofigal, 3- mash sea buckthorn Cluj, 4- sea buckthorn oil Pharapharm

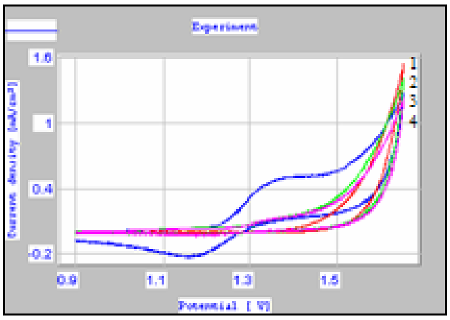

Figure 7. Sn concentration in calcinated food supplements Hofigal, E_{SCE}=1.375 V

1- electrolyte support, 2- gemoderivate 1 DH blueberry buds, 3- gemoderivate 1 DH billberry twigs, 4- gemoderivate 1 DH raspberry twigs

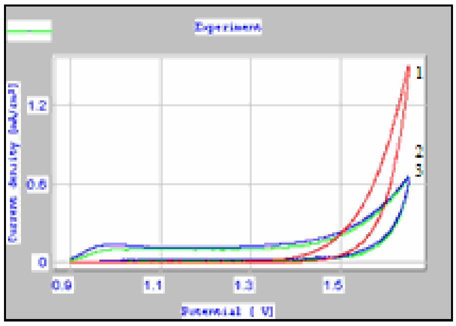

Figure 8. Sn concentration in concentrated food supplements based on sea buckthorn fruits, E_{SCE}=1.375 V

1- electrolyte support, 2- sea buckthorn oil Hofigal, 3- mash sea buckthorn Cluj

Nickel plays numerous roles in the biology of microorganisms and plants. In fact urease (an enzyme which assists in the hydrolysis of urea) contains nickel. The NiFe-hydrogenases contain Ni in addition to iron-sulfur clusters. A nickel-tetrapyrrole coenzyme is present in the methyl coenzyme M reductase which powers methanogenic archaea, a group of single-celled microorganisms.

Although it is toxic in large doses, selenium is an essential micronutrient for animals. It is a component of the unusual amino acids selenocysteine and selenomethionine. In humans, selenium is a trace element nutrient which functions as cofactor for reduction of antioxidant enzymes such as glutathione peroxidases and certain forms of thioredoxin reductase found in animals and some plants [2,3].

In order to determine the concentration through cyclic voltammetry, etalon curves have been traced for the heavy metals with high concentrations (Figures 1-4). Regarding to Sn etalon voltammograms, the higher values for current density of peaks are observed at the same recording rate (50 mV/s) as Fe etalon voltammograms.

The electrochemical method has only been applied for the higher concentrations of metals Fe and Sn (Fig.5-8).Fe concentrations on calcinate food supplements Hofigal (E_{SCE}=608 mV) were 10.9840 mg/L (Ipeak = 4.444 µA/cm2) in billberry, 9.6550 mg/L (Ipeak = 3.682 µA/cm2) in blueberry and 9.093 mg/L (Ipeak = 3.3600 µA/cm2) in raspberry food supplements. In concentrated food supplements based on sea buckthorn fruits, only Fe from Hofigal oil can be detected by electrochemical method. His concentration is 9.7544 mg/L (Ipeak = 3.739 µA/cm2).

The rest of curves are situated under base line. The values of Sn concentrations in food supplements using electrochemical method are presented in Table 3.

4. Conclusions

Forest fruits and plants can concentrate large amounts of heavy metals. Their concentrations depend on both the soil composition and climatically conditions, as well as structure and the "absorbability and storage" possibility of fruits and plants. It has been registered to high values only for Sn concentrations [10, 11] by AA spectroscopy (µg/L).

In cases of calcinated samples can appear much more errors due to manually process of resolve a very smal amount of residuu in HNO_3 (0.0015-0.0030 g) because in gemoderivates the ratios glycerolhydroalcoholic:extract are 1:10. The Cr, Zn, Pb, Al, Cd values concentrations are under legal limits [10] except for concentrations of Cr in Gemoderivate 1 DH blueberry buds and Pb in Gemoderivate 1 DH raspberry twigs.

The present study showed two analytical methods for heavy metals concentrations determination: AA spectrometry and cyclic voltammetry i = f(E).

The electrochemical method is practical, faster and easier, but is unsuitable for heavy metal monitoring in food supplements. The registered values were about 100 times smaller for Fe and 10 times smaller for Sn.

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