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ODREĐIVANJE SADRŽAJA OLOVA U SPANAĆU PRIMENOM POTENCIOMETRIJSKE STRIPING ANALIZE (PSA)
DETERMINATION OF THE LEAD CONTENT IN SPINACH BY UTILIZATION OF THE POTENTIOMETRIC STRIPPING ANALYSIS (PSA)

Lj. Babincev[1], Lj. Rajkovic[2]

[1]*Faculty of Technical Sciences, Kosovska Mitrovica*
[2]*Faculty of Technology-Metallurgy, Beograd*

Izvod

Usvajanje elemenata u biljkama je regulisano nizom faktora koji zavise od same biljke i od uslova životne sredine. Glavni faktor koji kontroliše sadržaj minerala u biljkama jeste genetski potencijal za ishranu različitim mineralima. Ispoljava se u nejednakoj osetljivostii i sposobnosti za akumuliranjem pojedinih elemenata. Specifičnost vrste i genotipova u odnosu na mineralnu ishranu proističe iz njihove prilagođenosti određenim ekološkim uslovima, specifičnosti metabolizma, morfološke i anatomske građe. Sposobnost biljke da usvoji mikroelemente zavisi od morfologije korena koji ima tri važne funkcije: vezivanje biljke za podlogu, usvajanje i transport materije i vode, sinteza fitohormona i drugih organskih jedinjenja. Pored uloge u usvajanju elemenata iz zemljišta koren je i svojevrstan filter. Esencijalni elementi vrlo lako prolaze korensku barijeru, lako prolaze u nadzemne delove biljaka i mobilni su kroz njih, dok se toksični elementi zadržavaju, u koliko nisu prisutni u prevelikim koncentracijama. Poznato je da epidermis korena predstavlja barijeru za usvajanje olova. Cink je sa karakteristično velikom mobilnošću kroz biljku i slabim zadržavanjem u korenu. Toksičan i u fiziološkom smislu, biljci nepotreban, kadmijum se vrlo lako kreće ka nadzemnim delovima. Kako je olovo najslabije mobilno u nadzemne delove biljke, sobzirom na njegovu veliku toksičnost ako uđe u lanac ishrane, u ovom radu je određivan sadržaj olova u listu spanaća. Određivanje sadržaja olova vršeno je Potenciometriskom striping analizom (PSA). Potenciometrijska striping analiza (PSA) je elektroanalitička veoma osetljiva metoda za određivanje tragova mnogih teških metala. Ovom određivanju prethodila su ispitivanja granica osetljivosti i opsega linearnosti.

Ključne reči: *Potenciometrijska striping analiza, olovo, biljke, spanać*

Abstract

Absorption of elements by plants is regulated with a series of factors that depend on the plant in issue and conditions of living environment. The main factor that controls the mineral content in plants is a genetic potential for sustenance with different minerals. It is being exhibited with unequal sensitivity and ability for accumulation of specific elements. Particularity of species

and genotypes with regard the mineral sustenance originates from their adjustment to specific ecological conditions, particularity related to metabolism, morphological and anatomic structure. A plant's ability to absorb microelements depends on morphology of its root, which has three major functions: connecting the plant to its surface, absorption and transport of substance and water, synthesis of phytohormones and other organic compounds. In addition to its role in inclusion of elements from the ground, the root doubles as a fairly good filter. Essential elements pass through the root barrier easily, straightforwardly advancing to the upper parts of a plant while still being mobile, whereas toxic elements are withheld, unless their presence is in ample concentrations. It is known that the root epidermis represents a barrier for lead absorption. Zinc has a distinctively large mobility through the plant and minor accretion in the root. While toxic and physiologically unnecessary for any plant, cadmium advances rapidly towards the upper parts. Since lead has the worst mobility towards the upper parts of the plant, taking into consideration its high toxicity levels if it becomes the food chain constituent, this report elaborates determination of the lead content in spinach leaves. This determination of the lead content was conducted by utilization of the Potentiometric Stripping Analysis (PSA).The Potentiometric Stripping Analysis (PSA) is an electro-analytic highly sensitive method for determining the content of many heavy metals. Examining of sensitivity threshold and linearity range was conducted prior to this measurement.

***Key words**: Potentiometric Stripping Analysis, lead, plants, spinach.*

1. INTRODUCTION

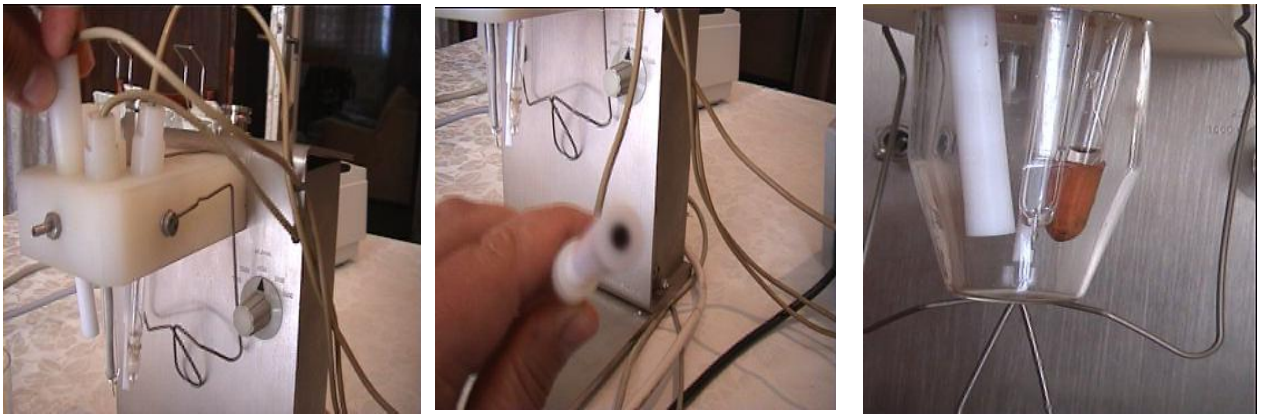
Contamination of living environment leads to accretion of detrimental matters within plants. Plants have a powerful system in the matter flow, they take up minerals dissolved in water in their cells and a plant is unable to defend from redundant excess matters in surrounding environment during its development. Besides that, plants have a very important role in circularization of heavy metals in nature. For the most part, heavy metals enter the food chain through the plants. Many of them are essential for development of plants (Cu, Zn, Mn, Fe, Co, Mo) while there are others with severe toxic effect on plants if present in large concentrations (Cd, Pb, Ni) [1-3]. Accumulation of heavy metals in soil and plants is often a consequence of both natural and anthropogenic processes. The most apparent anthropogenic resources of pollution with heavy metals are excavation mines, foundries, metal processing industries, towns' solid and liquid waste landfills, means of transport etc. The reason for sampling and analysis of plants is based on an assessment of risk that they will enter the food chain and became hazardous to health of human population. Considering the broad usage of spinach in consumption, we have followed the idea to conduct a quantitative analysis of one microelement in spinach samples at immediate disposal. Absorption of microelements with nutrients affects activity of enzymes, which are essential for development and preservation of human beings. In this particular case, the goal of our research was to determine the content of lead in spinach by utilization of the potentiometric stripping analysis (PSA).

PSA method represents a very susceptible and straightforward electro-analytic volt-ampere metering technique for determining the traces of heavy metal ions. It is based on monitoring of

mutual conditionality of electricity and voltage in electro-chemical redox reactions. It is conducted in three consecutive phases. The first phase is the pre-electrolyses and it is conducted with intensive stirring of solvate, during which the ions of examined ionic type are stored/collected on/within the operational electrode. After the assuaging of solvate, stored matters are dissolved (oxidized) and returned to solvate. This phase is called the oxidation or stripping and it is monitored with a conditionality curve of the potential of oxidation (qualitative characteristic) in relation to the time of oxidation (quantitative characteristic) at a constant electricity level [4]. PSA method can determine very low concentrations of analyzed ionic types, of 10^{-6}-10^{-10} mol/dm$^{3.}$

2. EXPERIMENTAL PART

For determining the threshold of sensitivity, linearity and applicability of PSA method for determining contents of lead, a standard lead solvate of pura purity (″suprapur″) was used, as well as supplementary HCl electrolyte with the role to adjust the pH value of environment and suitable conductivity. Other chemicals were of analytic purity (″proanalysis″). All accrued solvates were stored in poly-ethylene bottles. Equipment for PSA was assembled on the Department for instrumental methods of the Faculty of Technology in Novi Sad in cooperation with RO ELU Leskovac and it comprises the M1 stripping analyzer, with a microcomputer and a processing unit which utilizes three electrodes in the pre-electrolyses phase. A thin-layered mercury electrode was used as a working electrode. A referential electrode was Ag/AgCl, and supplementary was a platinum wire immersed in glass tube [5]. All electrodes and mixing unit were set up in rigid part of mixing unit which provides mechanical stability and compactness of the entire system. Determining of ions of lead in water by PSA method was preceded by formation of mercury films on electrode of glassy carbonate, as an inert base. Mercury film was formed from standard mercury(II)-nitrate solvate prepared of elementary mercury(II)-nitrate solvate with content of mercury(II)-nitrate in amount of 1000 mg/dm$^{3.}$ Volume of added standard was 2 cm^3, and concentration of lead in solvate for storing was 100 mg/dm^3. Mercury(II)-ions were stored under constant electricity level of -48.90 μA and for a period of time of 240 s. Once formed operational electrode was utilized in the same series of samples until the occurrence of non-reproductive results [6-9].Determining of lead content was conducted on the basis of electrolytic depositing on negative operational electrode under the potential of depositing of Pb(II)-ions of –0,999 V, in accordance with theoretical and practical experiences and ending potential of oxidation of -0,150 V, with pH value of solvate of 1,6 i.e. content of supplementary electrolyte of 50 μdm^3. The potential of oxidation of lead ranged from 390 to 410 mV.



Picture 1. Parts of PSA equipment

The samples which were analyzed with PSA method must be in dissolved state. Such state was preceded by collecting and preparing of spinach samples for analyses. Spinach was sampled from Kosovska Mitrovica green market. Five samples were taken in specific mode, successively, each Saturday in timely period of four weeks in the month of February from same producers. Sampled spinach was grown in neighboring villages of Kosovska Mitrovica, more accurately in Vučitrn municipality villages on Priština-Kosovska Mitrovica main road. Taking of samples of vegetable leafs was conducted in the midst of vegetation. The most trustworthy analysis is leafing[10], wherein completely formed leafs are sampled in amounts from 35-55. After sampling, preparation of plant material for analysis is conducted, starting from washing and all the way to accomplishing the aspired results. Washing of plant material is done with tap water, then with distilled water and lastly with de-ionized water. Thereafter, plant material is first dried on airy space for several days first and then milled and absolutely dried on 105°C until becoming a permanent mass. Burning of dried plant material was conducted on 500°C in a measured porcelain dish overnight. After cooling, the received ashes are measured together with the porcelain dish and the quantity of ashes was derived from the difference in masses of empty porcelain dish and dish with ashes. In the following procedure, 1 g of ashes is measured precisely and quantitatively transferred into an appropriate Erlenmeyer flask. Transferred ashes are first treated with water and then 5 cm^3 of concentrated HNO_3 are added. Resulting solvate is carefully steamed in a sand-bathroom on moderate temperature until it becomes a nearly dry matter. Then several drops of concentrated HCl are added and steamed until it becomes a nearly dry matter again. After steaming, the resulting white mass is then dissolved with 5 cm^3 2 % of HCl, transferred into a normal dish of 100 ml and liquefied until the leveling line [11]. Final measurements were conducted with the M1 stripping analyzer.

3. RESULTS AND DISCUSSION

Determining of ions of metal in all tests with standard solvates by utilization of PSA method is characterized by significant dissipation of measuring results. The most frequent reason for dissipation of results are: inadequate cleanliness of used dishes and utensils, improper storage of standard solvates and age of solvate, cleanliness of air in the laboratory, filter paper, inaccurate calibration of laboratory dishes etc. Due to the said reasons, the statistic processing of results was conducted, q-test, i.e. the averaged value $\overline{X}$ was calculated, expressed in μg/dm^{3,} the value of standard differentiation, *S*, expressed in μg, as a measure of precision of achieved results, the variation coefficient K_v in %, as a measure of re-productivity and percentage error *Er*, %, in order to have enhanced insight and analyzing of the achieved results. It has been experimentally concluded that the lowest lead content which is detectable by utilization of the PSA method is 2,25 μg/dm^3 with determination error of 5,78 %, while the maximum lead content is 2700 μg/dm^3 with determination error -10 %. The linearity of signal response of PSA method for determination of lead content is for 22,00-2190,00 μg/dm^3 interval content. The optimal range for determining lead, in terms of accuracy, is from 22,00 to 890,00 μg/dm^3, Table 1.

Table 1. Sensitivity of PSA for determining of microcontent of lead

Element	μg/dm^3		S (μg)	Kv(%)	Er(%)
	X_s	$\overline{X}$			
Pb	2,25	2,38	0,30	12,60	5,78
	8,95	9,35	1,15	12,30	4,47
	22,48	24,98	2,52	11,34	1,12
	890,76	880,58	99,15	11,26	-1,14
	2700,00	2430,00	964,71	39,70	-10,00

Spinach is one most valuable vegetable in terms of nutritive values. Spinach, lat. Spinacia oleracea, is green leafy vegetable that belongs to Chenopodiaceae family. It favors moderate climates and it is available throughout the year. 13 Different flavonoids with antioxidative and potentially anticancerogenous effect were identified in examinations of spinach. According to a study from 2004, published in September, in Journal of Nutrition, carotenoid neoxanthin, present in spinach, fights against prostate cancer in two ways: by preventing multiplication of cancer cells and by stimulating their self-destruction. Besides that, spinach is a remarkable resource of vitamin C and beta carotene, major antioxidants with important role in limiting the amount of detrimental free radicals in body and consequences that free radicals leave behind. Another important role in human body is prevention of cholesterol oxidation and consequently, protection of cardio vascular health. It represents a major resource of pholates as well as vitamins B, K, Fe etc. Due to massive nutritive value of this plant species, and considering the fact that it is grown in large quantities in the aforementioned area, burdened with mining and industrial compounds, we have examined the lead

content in it. The results of this microelement's examinations from directly offered samples are shown in Table 2.

Measured lead content in spinach samples is compared with critical concentration of metal for a grown plant. The average critical and toxic concentrations of lead for spinach (comprised on the basis of date from literature) [10], is10 and 20 μg/g of DM (dry matter).

The lead content in spinach grown in surrounding villages of Kosovska Mitrovica, on private households in Prilužje, Plemetina, Babin most, Vučitrnska Banjska and Banjska Slatina, i.e. villages situated in Vučitrn municipality, located on Priština-Kosovska Mitrovica road, exceeds the critical concentration on almost all locations. The content of this microelement in the aforesaid plants in a period of one month did not change significantly which was expected because in fully or almost fully developed leafs the content of elements does not change significantly in a period of time during vegetation, Picture 2.

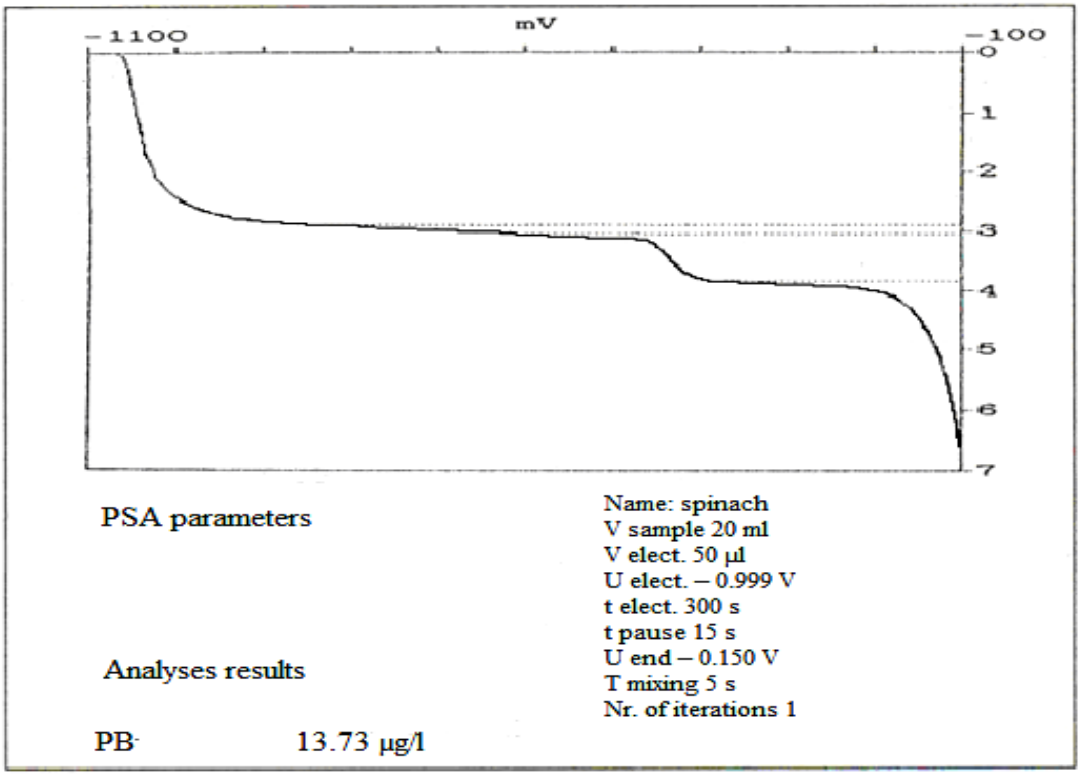
Picture 2. Sample of spinach leafs

This is not the case with younger leafs which grow intensively or with old leafs from which some elements are rapidly transferred into younger organs or into organs for depositing of reserve matters [1-3]. The lead content is the largest in spinach leafs grown in Vučitrnska Banjska locality. It is slightly smaller in Banjska Slatina, while the spinach from Plemetina could be referred to as the healthiest in terms of the lead content. Considering the fact that critical concentrations are always 10% lower in comparison to those which are required for the best outputs [2]. Graphical PSA plot presentation of one of the conducted measurements is shown in Picture 3.

Table 2. Content of Pb in spinach samples

Locations where grown	$\overline{X}_{Pb\ (\mu g/g\ SM)}$			
	7.02.2009.	14.02.2009	21.02.2009	28.02.2009.
A_1^*	14,87	14,34	14,56	15,38
A_2^*	11,77	12,54	12,19	12,46
A_3^*	15,02	15,43	15,26	15,72
A_4^*	18,69	18,88	19,06	19,46
A_5^*	16,89	17,79	18,33	17,64
Critical concentration in spinach Pb (µg/g SM)			10	
Toxic concentration in spinach Pb (µg/g SM)			20	

* *A_1-Prilužje, A_2-Plemetinae, A_3-Babin most, A_4-Vučitrnska Banjska, A_5-Banjska Slatina*



Picture 3. Graphical PSA plot presentation of one of the conducted measurements

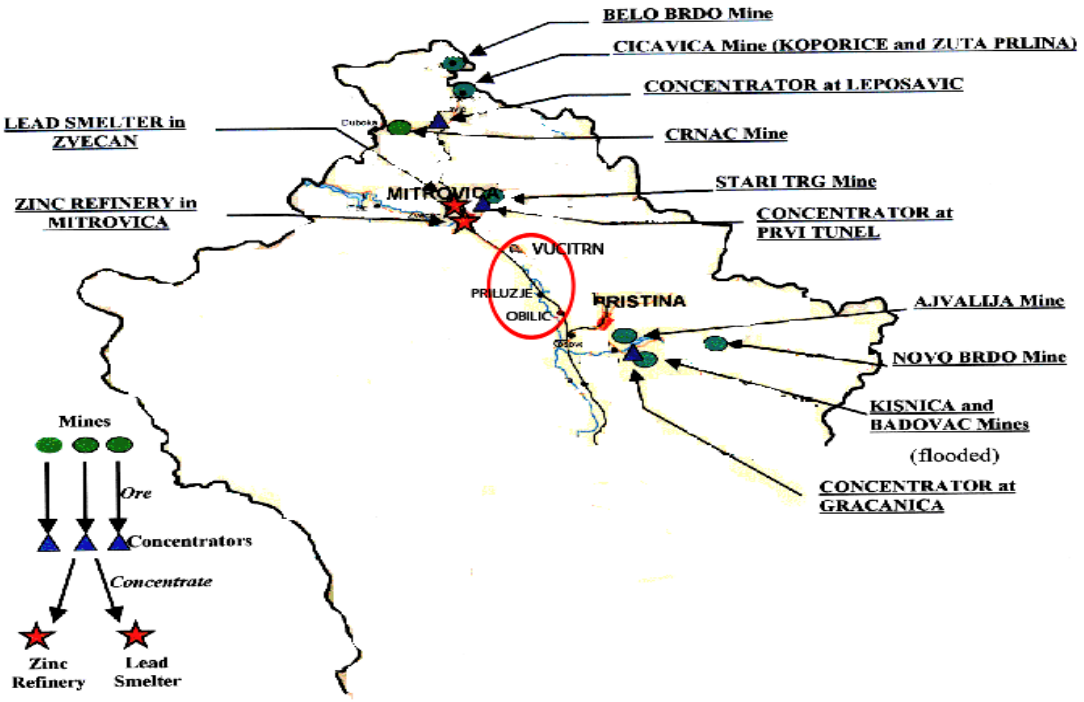
Since this is a specific cumulative poison this sample ought not to be used as food. Reliability of these data was confirmed by recording of samples by the atomic absorption spectrophotometry (AAS) on atomic absorber Perkin-Elmer 370 A (Wellesley, Massachutesetts). Results of this recording are shown in Table 3, [12]. This plant species has an exceptional need for N, P, Mn, Cu, Mo. It is less sensitive to Zn, but very tolerant to Pb. Origin of lead content in this plant is not clearly defined, assumingly adopted from external environment. Since the sampled spinach was grown in neighboring villages of Kosovska Mitrovica, on private agricultural land of Prilužje, Plemetina, Babin most, Vučitrnska Banjska and Banjska Slatina, i.e. villages situated in

Vučitrn municipality, on Kosovska Mitrovica-Pristina main road, Picture 3., more precisely in closest vicinity of Obilić power plant,

Table 3. Pb content in spinach samples determined by AAS

Location where grown	$\overline{X}$ Pb (µg/g SM)			
	7.02.2009.	14.02.2009	21.02.2009	28.02.2009.
A_1^*	15,5	15,0	15,2	16,1
A_2^*	12,3	13,0	12,8	13,1
A_3^*	15,7	17,0	16,0	16,4
A_4^*	19,5	19,7	21,2	21,6
A_5^*	17,7	18,6	19,2	18,9
Critical concentration in spinach Pb (µg/g SM)			10	
Toxic concentration in spinach Pb (µg/g SM)			20	

and ash deposits, factory for production of paints and lacquers, factory for production of galvanized sheets-Vučitrn, mining sites Novo Brdo, Ajvalija, Kišnica and Badovac etc.



Picture 3. Geographical position of areas where the analyzed samples were grown

Adoption of microelements from the surrounding environment depends on concentration and mutual interaction of ions, land, soil drought, osmotic potential of soil solvate, soil micro flora, relative humidity of air, temperature, light, agro-technical measures and insufficient agro-technical awareness of producers. In this particular case, it is impossible to exclude a possibility of irrigation of fat soil with water from Sitnica River, which drains the entire area burdened with mining and industrial compounds, or regional traffic road which is particularly used on part from Priština to

Kosovska Mitrovica as the source of this element in soil can also be atmospheric pollutants from motor vehicles.

4. CONCLUSION

It has been experimentally concluded that the linearity response of the PSA method for interval of content ranges from 22,00 to 2190,00 μg/dm^3 of lead. The accuracy of this method is within limits of ± 2 % in this interval. For smaller content in the interval in range from 10 to 20 μg/dm^3 response exceeds the limit of accuracy, i.e. the determination error is slightly bigger than 4%. For even smaller contents, the determination error exceeds the value of 5%.

The PSA method can be used for determining the content of lead in spinach leaves with the determination error of approximately 4,5 %. The lead content in spinach samples, sampled from Mitrovica green market in the month of February, does not exceed the toxicity level. Increased content in relation to critical concentration does not have an effect on full development of this plant but it is a reasonable argument for precautionary measures by local population as well as a necessity for further monitoring of content of heavy metals in this specie, extremely important in food.

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