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NUCLEAR AND RELATED ANALYTICAL METHODS APPLIED IN BIOLOGY: PIXE AND ICP-AES

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Abstract. *With a particular Nuclear Analytical Method (NAM) we can make research activities on biology: trace element distribution and metabolism, effects and functions; nutrition and micronutrient deficiency; toxicology, epidemiological studies. Prominent features of NAMs are sensitivity, selectivity, multielement determination and linearity of the calibration function covering a concentration range of several orders of magnitude. In this article we present two analytical methods Particle Induced X-Ray Excitation (PIXE) and Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) and their applications in trace elements analysis on Basella Plants and on Downer cow syndrome.*

Keywords: PIXE and ICP-AES methods, tropical plant, veterinary medicine

1. Introduction

Nuclear analytical methods (NAMs) are based on techniques in which use is made of the properties of the nucleus (like activation analysis), or a combination of nuclear and electronic properties (like X-ray fluorescence spectrometry and PIXE). The working principle of nuclear analytical methods (NAMs) is not influenced by the chemical bond. Consequently, they are independent counterparts to the well-known chemical procedures. NAMs obey fundamental laws or can be described and understood thoroughly. This qualifies them as candidates for reference methods**.** Although following similar nuclear reaction schemes, they comprise bulk analyzing capability (neutron and photon activation analysis) as well as detection power in surface near regions of solids (ion beam techniques). The researches based on advanced methods using particle beams and electromagnetic radiations have been imposed by the solving of some necessities with interdiscipinary character aroused during the economic-social development. Many fields like the biology and environment, use for solving different problems, the results of researches obtained by a series of methods of analysis and techniques of high and ultra-high sensibility, including profile methods [1][2]. Many fields like the biology and environment, use for solving different problems, the results of researches obtained by a series of methods of analysis and

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techniques of high and ultra-high sensibility, including profile methods [1, 2].

At the same time VUT have good partnership agreement for using experimental facilities with Institute for Nuclear Physics and Nuclear Engeneering Horia Hulubei from Bucharest and Special Steinless Steel Factory from Targoviste. These methods use particle beams (charged particle beams, neutrons) and also electromagnetic radiations.

2. Experimental methods

Spectrometric techniques are based on the data delivered by characteristic energy transfers between radiations and matter. They provide information about the nature and concentration of elements in either crystallized or non-crystallized materials.

To some degree, they provide additional information about chemical bonds. Energy transfers during interaction with high-energy radiations can be expressed in two complementary ways: alteration of matter and alteration of radiation, the total energy being preserved. This results in the following complementary effects which are exploitable for spectrometry:

- Excitation-de-excitation processes of atomic core levels leading to characteristic secondary emission.

- Characteristic absorption processes.

Those two types of effects provide data which are theoretically identical and can both be used for analytical purposes, leading, respectively, to two groups of techniques:

- secondary emission spectrometry, which is including X-ray fluorescence analysis (XRF) and Charged Particle Induced X_Rays Emission (PIXE).

- absorption spectrometry (AAS) and emission spectrometry (ICP-AES).

The data provided by any spectrometric methods are expressed in terms of a spectrum processing depends on the basic physical effect.

Qualitative analysis requires us to identify a given element by means of one or several characteristic emission lines or absorption lines. Identifying of the major elements is mostly straightforward. Analysing the minor elements or the trace elements is determined by the sensitivity of the method; it may vary over a large range, depending on the physical process involved, on the atomic number and on the instrumental characteristics.

The main parameter in qualitative analysis is the detection limit of elements. For a given analytical method, the detection limit depends on the element to be analyzed and on the specimen which contain it. In emission spectrometry, a given

element a is determined by the detector counting when the spectrometer is adjusted on a characteristic line of the element. In absorption spectrometry, the energy range of counting of the spectrum is not so well defined. In any case the significant measurement is the signal-to-noise ratio.

The aim of quantitative analysis methods is to determine the mass fraction of any detectable elements in a specimen. For a given element, the intensity of a characteristic emission line on the characteristic absorption effect should be measured. The intensity measurement provided by a spectrometer depends on three kinds of parameters: physical parameters characterising the nature and the concentration of the element to be analysed; physical parameters due to any other elements in the specimen (matrix effects) and instrumental parameters.

3. Particle Induced X-ray Emission (PIXE)

Among the methods designed for the determination of the very low chemical concentrations, the Particle Induced X-ray Emission (PIXE)[4] method is the best one specially for measuring the elements. This method is based on the fact that the bombardment of the sample with a charged particle beam causes the ionization of the atomic inner shells followed by a subsequent of the characteristic X-rays. When the X rays spectrum is detected by high resolution detector, the well-known Z-dependence of the X rays energies, as well as the intensities of the individual X rays line, allow a straight forward determination of the target element. The detection limit of this method is very good because [3][4]:

i) intense fluxes of exciting radiation are available,

ii) the X rays production yields for particles with energies in the MeV range are large and

iii) the background associated with the exciting radiation is rather low. The sample preparation technique does not require a very special chemical preparation which may cause some losses in concentration or some contamination. A quantitative determination of an element content in a sample by PIXE method can be done both by absolute or relative measurements.

The target samples for PIXE are doped with standard solution (1:1) of Yttrium (standard for spectrum normalization and systematic error elimination) consisting of 130 mg/l of Yttrium (prepared from Y_2O_3) in deionised water. Measurements of target elements are made using a 3.0 MeV proton beam extracted from the TANDEM Van de Graaff accelerator and passes through a collimator (3×4 mm) before reaching the target. X-ray spectra were measured with a spectrometric chain having a Ge hyperpure detector (100 mm^2×7 mm) with a 160 eV resolution at 6.4 KeV of Ka line of iron. The detection limit is in the range (1-10 ppm).

4. ICP-AES (Induced Coupled Plasma)

The ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) spectrometer used by us are a Baird ICP2070 -Sequential Plasma Spectrometer from Special Stainless Steel Factory from Târgoviște, which consists of a sample introduction system, a plasma torch, a plasma power supply and an optical measurement system .The sample must be introduced into a plasma in a form that can be effectively vaporized and atomized (small droplets of solution, small particles of vapour).

The plasma torch confines the plasma to a diameter of about 18 mm. Atoms and ions produced in the plasma are excited and emit light. The intensity of light emitted at wavelengths characteristic of the particular elements of interest is measured and related to the concentration of each element from samples. Baird ICP2070 -Sequential Plasma Spectrometer use as a plasma gas Argon and the plasma is sustained in a quartz torch and the plasma is generated using a radiofrequency generator at 40.68 MHz. Temperatures of 5000-9000 K have been measured in the plasma. The detection systems used are a sequential monochromator with a wavelengths range (160-800) nm. The optical emission spectra are made using a personal computer. The detection limit is in the range (0.1-10 μg/L).

5. Experimental Results

5.1. The Basella Plant.

These quantitative methods were applicable to elemental analysis of Basella plants, cultivated in Variety Testing Centre and in Green Houses of Târgoviște.

Basella plant belong to Basellaceae family is a tropical plant used as a vegetable.

Table 1. Basella Alba L leaves samples

Samples	*Green mass* [g]	*Dried mass* [g]
P1	5	0.2966
P1’	5	0.3080
P2	5	0.4412
P2’	5	0.4133
P3	5	0.4317
P3’	5	0.4174
P4	5	0.4458
P4’	5	0.4511

One of the problems of actual foods department is the variety about the vegetables with a great nourishment value. Is the case of Basella plant like the native spinach (*Spinacea oleracea)*. The mineral elements concentrations give information about the nourishment value of Basella plant leaves. We used PIXE and ICP analysis methods to determine the elemental composition of Basella leaves cultivated in Variety Testing Centre (VTC) and green houses of Târgoviște for a given level of soil fertility. PIXE was applied on Basella Alba L leaves, which are listed in table 1.

In the experiment we used young Basella Alba Leaves sorted in four groups: Basella Alba - green house (P1, P1'); Basella Alba - VTC (P2, P2'); Basella Alba - VTC cultivated in soil treated with an artificial fertiliser using N (P3, P3'); Basella Alba - VTC cultivated in soil treated with natural fertiliser (P4, P4').

For PIXE measurements the target sample have been prepared in the following manner: the washed leaves were simply air-dried at a temperature of 500 in a clean box preventing further contamination. The dried leaves were grained and after powdering a layer of the samples material were deposited on hostaphan foils. We identified and determined the following elements: P, Ca, Mg, K, Fe, Mn, Zn. (*see table 2*)

Table 2. The concentration of elements in Basella Alba L leaves samples, mg/100g

Samples	P	Ca	Mg	K	Fe	Mn	Zn
P1	67.40	294.31	147.02	230.04	4.80	1.50	1.32
P1'	67.54	293.27	146.81	231.82	4.72	1.37	1.25
P2	82.40	381.37	158.20	228.32	5.20	1.72	1.24
P2'	82.78	389.33	161.25	225.20	4.98	1.80	1.33
P3	88.20	316.38	152.72	202.20	4.92	1.62	0.98
P3'	89.12	318.02	150.25	200.82	4.80	1.58	0.87
P4	94.02	351.22	156.12	227.51	5.12	1.25	1.12
P4'	94.50	364.71	156.82	225.72	5.02	1.25	1.02

The concentration of elements: Ca, Mg, K is often an essential requirement in agro alimentary domain because theirs presence in a big concentration is beneficial and give a remarkable nourishment value of Basella plants.

Samples in the ICP experiment, was young *Basella Alba L* leaves plant grown in different conditions at Variety Testing Centre (VTC) of Târgoviște, Romania. Six groups of *Basella Alba L* were measured as shown in table 3. The leaves of the plants were collected of approximately in some position in two successively months. The washing leaves were simply air-dried at a temperature of 1050 ºC in

a clean box preventing further contamination. The dried leaves were grained and after powdering, 2.00 g powder leaves have been digested in 40 ml acid nitric. After a set aside in fume cupboard overnight they obtained liquid was gently boiled (without major loss of volume). For a good digestion 3 ml acid perchloric have been added and 2-3 ml water after cooling. The cooled solution was diluted with water at 250 ml solution and nebulized into plasma.

Table 3. The samples groups of *Basella Alba L* measured by *ICP* method

Sample	*Growth fertilizer conditions*
B1(VTC)-July	-
B2(VTC) –July	Natural fertiliser (excrements)
B3(VTC) –July	NPK fertiliser
B4(VTC)-August	-
B5(VTC) –August	Natural fertiliser (excrements)
B6(VTC) -August	NPK fertilizer

Experimental results obtained by ICP and PIXE methods are presented in table 4.

Table 4. Mg and Fe concentration obtained by ICP method and PIXE method

Elements	B1(VTC) July	B2(VTC) July	B3(VTC) July	B4(VTC) August	B5(VTC) August	B6(VTC) August
Mg	22.65	18.33	16.14	21.00	20.72	18.75
Fe(ICP)	2.53	1.33	0.75	2.87	1.87	0.45
Fe(PIXE)	2.28	1.25	0.64	2.33	1.53	0.15

5.2.In Veterinary Medicine.

We have investigated by PIXE and ICP methods the microelementals of blood serum samples collected from healthy and ill cows (Downer cow syndrome (DCS)). Until today the origin of DCS is uncertain [5, 6]. At the beginning, we assume some connections among the diminution of some nutritive elements from food, the activity of some enzymes and the origin of DCS [7-9]. The samples were collected from cows at some animal farms on the neighbourhood of Târgoviște city. For Ca and P content of the analysed samples, we used PIXE based on the internal standard method. The ICP analysis method was used for Mg content determination. The determinations of phosphatase alkaline enzyme of analysed blood serum samples were made by a spectrometric analysis using Bessey-Lowry method. In studying diseases, a significant variation in Ca, P, Mg and enzyme contents was observed. Those changes correlate with age, season of alimentation and type of disease.

A typical X-ray spectrum from a blood serum sample collected from a DCS cow is shown in figure 2.

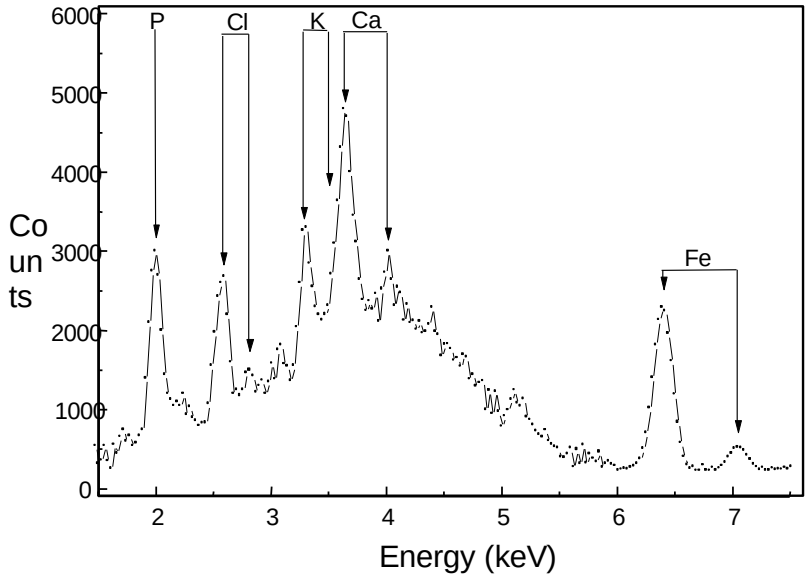

Fig. 1. PIXE spectrum of blood serum sample of DCS cow.

At the same time for trace element determinations, we use the ICP method. The reason for that choice is the impossibility of measuring the content of Mg with PIXE because of the absorption of the X-rays in the windows of the chamber and the detector. On the other hand, we cannot use only the ICP because for that type of measurements the quantity of blood serum needed is proportional to the number of the analysing elements.

Phosphatase alkaline enzyme (Pa) is implicated in bone growth process and its activity determines the balance between the content on Ca and P in blood (for a normal organism, the ratio Ca/P is among 1 and 2, for rachitic organisms is greater than 3, and for osteopourotic ones is less than 1). Magnesium is an activator of the enzyme.

To study the metabolic changes produced by phosphatase alkaline enzyme, we have collected blood serum from some cows with interest in medical reasons and to make determinations of P, Ca and Mg contents using both, PIXE and ICP methods.

The reference values for contents of Ca, P, Mg and phosphatase alkaline enzyme are represented in table 5. Concrete dates obtained from healthy and DCS cows at the beginning and at the end of the winter of 1998-1999 years are presented in tables 6, 7 and 8.

Table 5. Reference values for contents of Ca, P, Mg and Pa in blood serum of cows

	Ca (mg/dl)	P (mg/dl)	Mg (mg/dl)	Phosphatase alkaline (I.U./l)
Reference value	8.0-11.0	5.0-7.2	2.1-2.8	10.0-36.0

Table 6. Contents on Ca, P, Mg and Pa of blood serum samples collected during December 1998 from healthy cattle (cows with number 3,5,7,8,9,10 are on the first two weeks of lactation and 1, 2, 4, 6 are on the last two weeks of pregnancy)

Cattle number	Ca mg/dl	P mg/dl	Mg mg/dl	Pa I.U./*l*
1.	9.9	5.4	2.4	9.50
2.	11.8	6.6	2.5	8.82
3.	8.3	6.5	2.4	12.98
4.	12.2	6.0	2.8	12.74
5.	9.7	7.1	2.3	18.86
6.	9.9	6.3	2.4	16.66
7.	9.1	6.4	2.5	12.98
8.	9.2	8.1	2.7	12.25
9.	10.8	7.7	2.9	11.76
10.	9.3	8.1	3.1	10.04
average	10.0±9.4%	6.8± 10.9%	2.6±8.4%	12.66 ± 14.4%

Table 7. Contents on Ca, P, Mg and Pa of blood serum samples collected during march 1999 from healthy cattle (cows with number 2, 4, 5, 7, 8, 9 are on the first two weeks of lactation and 1, 3, 6, 10 are on the last two weeks of pregnancy)

Cattle number	Ca mg/dl	P mg/dl	Mg mg/dl	Pa I.U./l
1.	9.7	6.5	2.3	25.42
2.	8.2	5.9	3.1	15.19
3.	9.8	7.5	2.3	17.15
4.	9.1	6.6	2.5	19.55
5.	9.2	7.0	2.3	11.27
6.	10.8	5.5	2.6	13.23
7.	10.1	7.0	3.2	12.00
8.	9.3	6.5	2.9	18.57
9.	9.5	7.0	2.7	14.55
10.	11.6	8.1	3.5	19.75
average	9.7±7.0%	6.7±8.2%	2.7±10.0%	16.66±17.0%

Table 8. Contents on Ca, P, Mg and Pa of blood serum samples collected during december 1998 - march 1999 from DCS cattle (cows with number 2,5,6,7,9,10 are on the first two weeks of lactation after parturition and 1,3,4,8,11,12 are on the last two weeks of pregnancy)

Cattle number	Ca mg/dl	P mg/dl	Mg mg/dl	P a. I.U./l
1.	7.0	3.9	2.0	63.01
2.	7.5	3.5	2.2	88.12
3.	7.0	3.7	2.0	65.91
4.	7.1	4.4	2.3	64.05
5.	6.9	4.6	3.5	45.50
6.	5.7	3.8	2.4	57.50
7.	9.7	5.5	2.3	40.43
8.	6.8	3.9	2.3	67.51
9.	6.0	4.5	2.3	56.01
10.	6.5	4.6	2.5	45.03
11.	8.2	5.3	2.6	57.44
12.	7.5	5.2	2.0	41.07
Average	7.2±9.9%	4.4±12.0%	2.4±10.8%	57.63±17.5%

Conclusions

The results obtained by ICP method for iron concentration in Basella plants, cultivated in Variety Testing Centre and in Green Houses of Targoviste, are in good concordance with the results for iron concentration obtained by PIXE method (presented in table 4), so the both method PIXE and ICP are complementary methods for elemental analysis (figure 2)

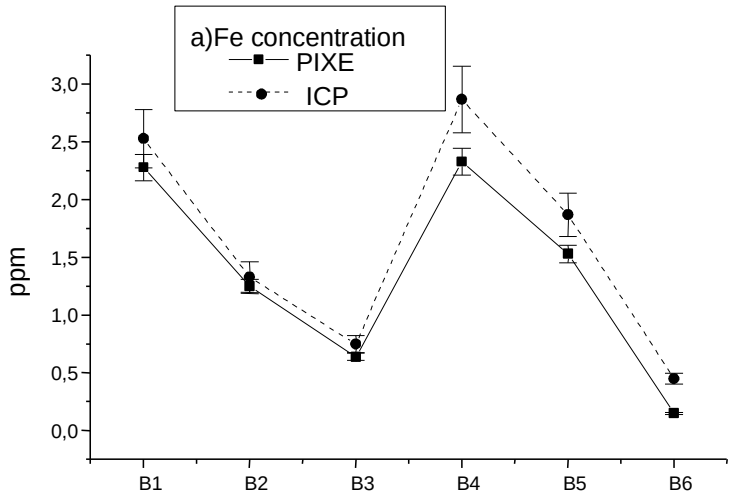

Fig. 2. Fe concentration in Basella Alba L leaves obtained by PIXE and ICP methods:complementary methods.

In the case of NRAM's applied on the Downer cow syndrome we can see from the results presented before, that the mineral and enzymatic parameters in the serum have values in the normal limits for the healthy cows even during the winter, when the mineral content of drying food (hay) is very poor. On the other hand, we can observe important decreases under the normal limits of the amount of Ca, P and Mg in the blood serum of DCS cows. It is easy to see the tendency of the DCS animals to have hypocalcemia and hypophosphatemia. Those perturbations can be the causes of the installations of the ill cows in the lypostatic and decubital attitudes.

The enzymatic level is an extremely sensitive parameter in DCS and can serve as a diagnostic parameter. As the effect of decreases of Ca, P and Mg amount in the blood serum of DCS cows, we observe an increase of the amount of some enzymes like CPK, TGO and Pa. That secondary effect is a very grave phenomenon from the clinical point of view because simultaneous increase of the level of all specified enzymes is in most cases irremediable.

The results obtained in this research work are a preliminary step in order to see the primary causes of the DCS. Those causes can be extremely various and their correct determination is the basic plan of the therapeutically act.

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