

Załącznik 3 – Annex 3

SUMMARY OF PROFESSIONAL ACCOMPLISHMENTS

Dr inż. Wojciech Józef Przybyłowicz

**„Rozwój i wybrane zastosowania skaningowej mikrowiązki
protonowej w biologii i geologii”**

**„The development of scanning proton microprobe and
selected applications in biology and geology”**

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Dr inż. Wojciech Józef Przybyłowicz

1. Name and Surname: Wojciech Józef Przybyłowicz

2. Date and place of birth: 24 December 1952, Stary Sącz

3. Diplomas / academic degrees :

- Master of Science, Engineer, discipline: Geophysics, specializing in Nuclear Geophysics; 1976; Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology, Cracow.

M.Sc. thesis: „Determination of uranium in loose materials by fission track method”; Supervisor: Prof. Jerzy Niewodniczański

- Doctor of Philosophy in Technical Sciences; 1985; Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology, Cracow.

Ph.D. thesis: „Application of selected physical methods in studies of the geochemistry of serpentinite rocks from the Lower Silesia”; Supervisor: Prof. Witold Żabiński

4. Employment History:

January 1994 – present: Senior Research Scientist, Materials Research Department, iThemba Laboratory for Accelerator Based Sciences, Somerset West, South Africa (Former names of the institute and research group: until 1999 - National Accelerator Centre, Van de Graaff Group; from 1999 until 2010 - Materials Research Group; in 2001 the National Accelerator Centre became iThemba LABS)

January 1992 – December 1993: Visiting Research Fellow, Schonland Research Centre for Nuclear Sciences, University of the Witwatersrand, Johannesburg, South Africa

January 1986 – July 1987: Post-Doctoral Fellow, McMaster University, Department of Geology, Hamilton, Ontario, Canada

From January 1992 until present: Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Cracow, Poland

1. October 1985 – present: Adjunct Professor, Faculty of Physics and Applied Computer Science, AGH University of Science and Technology, Cracow

2. October 1979 – September 1985: Senior Assistant, Institute of Nuclear Physics and Techniques, The Academy of Mining and Metallurgy, Cracow

3. October 1977 – September 1979 – Assistant, Institute of Nuclear Physics and Techniques, The Academy of Mining and Metallurgy, Cracow

4. October 1976 – September 1977: Junior Assistant, Institute of Nuclear Physics and Techniques, The Academy of Mining and Metallurgy, Cracow

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5. Scientific achievement as grounds for the habilitation procedure

Title:

„The development of scanning proton microprobe and selected applications in biology and geology”

(a) I present a series of thirteen scientific publications as grounds for the habilitation procedure. This series include the following scientific articles:

- [H1] **W.J. Przybyłowicz**, J. Mesjasz-Przybyłowicz, C.A. Pineda, C.L. Churms, K.A. Springhorn and V.M. Prozesky. Biological applications of the NAC nuclear microprobe. X-ray Spectrometry 28 (1999) 237-243. (IF 1.381)
- [H2] **W.J. Przybyłowicz**, J. Mesjasz-Przybyłowicz, C.A. Pineda, C.L. Churms, C.G. Ryan, V.M. Prozesky, R. Frei, J.P. Slabbert, J. Padayachee and W.U. Reimold. Elemental mapping using proton-induced x-rays. X-ray Spectrometry 30 (2001) 156-163. (IF 1.414)
- [H3] D. Budka, J. Mesjasz-Przybyłowicz, G. Tylko, **W.J. Przybyłowicz**. Freeze-substitution methods for Ni localization and quantitative analysis in *Berkheya coddii* leaves by means of PIXE. Nuclear Instruments and Methods in Physics Research B 231 (2005) 338-344. (IF 1.414)
- [H4] Grzegorz Tylko, Jolanta Mesjasz-Przybyłowicz and **Wojciech J. Przybyłowicz**. X-ray microanalysis of biological material in the frozen-hydrated state by PIXE. Microscopy Research and Technique 70 (2007) 55-68. DOI 10.1002/jemt.20387. (IF 1.644)
- [H5] Y.D. Wang, J. Mesjasz-Przybyłowicz, G. Tylko, A.D. Barnabas, **W.J. Przybyłowicz**. Micro-PIXE analyses of frozen-hydrated semi-thick biological sections. Nuclear Instruments and Methods in Physics Research B 306 (2013), pp. 134-139. <http://dx.doi.org/10.1016/j.nimb.2012.12.051>. (IF 1.266)
- [H6] **W.J. Przybyłowicz**, C.A. Pineda, V.M. Prozesky and J. Mesjasz-Przybyłowicz. Investigation of Ni hyperaccumulation by true elemental imaging. Nuclear Instruments and Methods in Physics Research B104 (1995) 176-181. (IF 1.193)
- [H7] K. Vogel-Mikuš, M. Regvar, J. Mesjasz-Przybyłowicz, **W.J. Przybyłowicz**, J. Simčič, P. Pelicon, M. Budnar. Spatial distribution of Cd in leaves of metal hyperaccumulating *Thlaspi praecox* using micro-PIXE. New Phytologist 179 (2008) 712–721. doi: [10.1111/j.1469-8137.2008.02519.x](https://doi.org/10.1111/j.1469-8137.2008.02519.x). (IF 5.178)
- [H8] **W. J. Przybyłowicz**, J. Mesjasz Przybyłowicz, P. Migula, E. Głowacka, M. Nakonieczny, M. Augustyniak. Functional analysis of metals distribution in organs of the beetle *Chrysolina pardalina* exposed to excess of nickel by Micro-PIXE. Nuclear Instruments and Methods in Physics Research B 210 (2003) 343-348. (IF 1.041)
- [H9] J. Mesjasz-Przybyłowicz and **W.J. Przybyłowicz**. Micro-PIXE in plant sciences: present status and perspectives. Nuclear Instruments and Methods in Physics Research B189 (2002) 470-481. (IF 1.158)

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- [H10] **W.J. Przybyłowicz**, V.M. Prozesky and F.M. Meyer. True elemental imaging of pyrites from Witwatersrand reefs. *Nuclear Instruments and Methods in Physics Research B104* (1995) 450-455. (IF 1.193)
- [H11] R. Frei, V.M. Prozesky and **W.J. Przybyłowicz**. Studies of progressive leaching in single mineral Pb-Pb dating. *Nuclear Instruments and Methods in Physics Research B 130* (1997) 676-681. (IF 1.016)
- [H12] A. Agangi, A. Hofmann, **W. Przybyłowicz**. Trace element zoning of sulfides and quartz at Sheba and Fairview gold mines: Clues to Mesoarchean mineralisation in the Barberton Greenstone Belt, South Africa, *Ore Geology Reviews* 56 (2014) 94-114. <http://dx.doi.org/10.1016/j.oregeorev.2013.08.016>. (IF 2.417)
- [H13] S. Fuchs, **W.J. Przybyłowicz**, A.E. Williams-Jones. Elemental imaging of organic matter and associated metals in ore deposits using micro-PIXE and micro-EBS. *Nuclear Instruments and Methods in Physics Research B 318* (2014) 157-162. <http://dx.doi.org/10.1016/j.nimb.2013.06.055>. (IF 1.266.)

b) Description of the achievement as grounds for the habilitation procedure

Introduction

The nuclear microprobe is a relatively complex instrument utilizing ion beams of few MeV energy (typically 2-3 MeV) generated by small particle accelerators (Watt and Grime, 1987). Protons and alpha particles are the most often used types of ions; therefore the facility is also often referred to as a "proton microprobe". High versatility of nuclear/proton microprobes, especially simultaneous use of few complementary analytical methods, resulted in applications in a wide range of scientific disciplines. The methods can be divided among those requiring high intensity ion currents, of the order of 100 – 1000 pA, and those where very low currents are necessary, down to fA or even single ions. The first category comprises particle induced x-ray emission (PIXE), nuclear reaction analysis (NRA) including particle induced gamma-ray emission (PIGE), Rutherford backscattering spectrometry (RBS) and elastic backscattering spectrometry (EBS or BS). Low ion currents are necessary in secondary electron imaging (SEI), ion beam induced luminescence (IBIL) or in the analysis of charge induced by ion beams (ion beam induced charge - IBIC). Minimal currents or only single ions are utilized in the scanning transmission ion microscopy (STIM) and in the studies of radiation effects caused by ions and modification of materials by ion beams (single ion machining, single event upsets, single ion irradiation) (Watt, 1997). Most often the instrument is equipped with a scanning system allowing to raster an ion beam over the specimen surface; hence the instrument is referred to as a "scanning nuclear microprobe" or a "scanning proton microprobe".

In contrast to some other, commercially available instruments such as electron microscopes or mass spectrometers, which are sold together with the computer programs and detailed instruction manuals, in scanning nuclear microprobes only the main components, such as object and collimator slits, quadrupole lenses for beam focusing and scanning systems, are commercially available. The whole instrument is individually assembled and there is a large variety in selection of an experimental chamber, a method of charge integration and normalization of results. Each research team has to decide which ion beam techniques would be used in a routine manner, what would be the number and type of detectors and what would be an acquisition system of such a facility. Acquisition systems are very often built as a single, unique combination of hardware and software, tailored to the

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requirements of a particular instrument and the type of operation. The necessity of such individual approach creates an opportunity for constant development and improvement of techniques, known in their basic form for some time. Among about 60 nuclear microprobes worldwide none is truly identical. The commissioning of each new microprobe requires intensive work of a team of scientists and technicians. Similarly, applications of the ion beam analysis methods in various science disciplines require cooperation of an interdisciplinary team. Therefore the results of such cooperations are mostly presented in multidisciplinary publications with many co-authors. The role of a physicist in such cases is not only the development of appropriate methodology and selection of experimental conditions, but also supervision and control of the interpretation of results by experts in other science disciplines. Such role requires in-depth understanding of a research problem that has to be solved with a use of physical methods.

In the course of my scientific career I had twice an opportunity of developing “from scratch” the methods utilising focused ion beams, and next using them in various applications related to materials engineering, geology, biology and environmental studies. The first time I participated in the final phase of development and commissioning of a new scanning proton microprobe at the Schonland Research Centre for Nuclear Sciences, University of the Witwatersrand, Johannesburg, in South Africa. Next I have been working on methodology and applications of a new scanning nuclear microprobe at the National Accelerator Centre, Somerset West, also in South Africa.

Publications selected as grounds for the habilitation procedure describe the development of methodology and analytical capabilities in the second research centre, with emphasis on analyses of biological materials, as well as selected applications of this methodology in biology and geology.

The development of methodology and analytical capabilities

Biological applications of proton microprobes draw attention from the times when the first facilities have become operational in the seventies (Horowitz *et al.* 1976). However, only few laboratories were able to offer to the biologists something more than single test results without practical significance for the development of this field of science. Potential success requires long-term, systematic cooperation between ion beam analysts and biologists. Moreover, the key issue is proper preparation of biological material, in order to ensure proper representation of the distribution of elements in the measured samples, identical to the elemental distribution in a living material from which they were obtained. The laboratory at the National Accelerator Centre, Somerset West, South Africa (now: iThemba Laboratory for Accelerator Based Sciences) is one of the research centres where permanent development of methodology and technical capabilities has led to many successful collaborations on research projects relevant to biology and environmental studies. A series of selected publications illustrates the development of analytical capabilities in this laboratory, with my leading participation. The scanning nuclear microprobe has been commissioned in 1991 and systematically improved in subsequent years. Since 1994 until present I have been working on the development of analytical capabilities of this facility. The microprobe utilises ion beams generated by the 6 MV single-ended Van de Graaff accelerator (CN model from High Voltage Engineering USA). Magnetic quadrupoles (OM150 lens system), slits and the scanning system as well as the experimental chamber, were manufactured by the Oxford Microbeams Ltd. The main features of the facility have been described by Prozesky *et al.* (1995). Data are registered using the XSYS general purpose data acquisition system (Indiana Univ. 1985), most often in the “event-by-event” mode. Data analysis is performed using subsequent versions of GeoPIXE software (Ryan, 2000). With regard to the PIXE method, this software uses an advanced method of quantitative mapping of elemental distribution, the *Dynamic Analysis* (Ryan and Jamieson, 1993). Elemental maps are generated using all x-ray lines forming K, L or M group of lines of a particular element. The interferences between them

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and additional peaks such as pile-up 'sum' peaks or escape peaks are eliminated. Continuum x-ray background is also subtracted. Maps are quantitative, expressed in concentration units (mg kg^{-1} or wt. %). National Accelerator Centre was the second laboratory in the world where this mapping method has been integrated with the data acquisition system (Ryan *et al.*, 1995). This advanced method of mapping has many advantages, especially when dealing with very complex PIXE spectra, with many overlapping x-ray lines. A detailed procedure of quantitative analyses of thick samples has been developed and described by van Achterbergh *et al.* (1995) and applied in the study of geochemistry of eclogites. I had a task of extending the procedure of quantitative elemental microanalysis for intermediate and thin samples, in particular biological materials. For intermediate samples it required the development of a method of analysis of composition and thickness of the matrix, with emphasis on biological matrix. A typical approach is to use an additional method, STIM or BS. In STIM it is only possible to analyse the areal density, and the matrix composition has to be assumed *a priori*. Most often in this case an average elemental composition of a biological matrix is assumed, known from measurements performed using other methods. STIM measurement is very quick, but simultaneous use of PIXE and STIM in the so-called "on-axis STIM" necessary for the measurement of areal density (a detector measuring the energy of particles having passed through a measured material is positioned at 0° angle in relation to the primary beam) is not possible due to too high differences between beam currents required for effective use of these methods. STIM tests showed additional difficulties, specific to our laboratory. Due to relatively high energy instabilities of the proton beam generated by an outdated accelerator, setting up of adequate parameters of measurements was very time-consuming, which did not allow for more routine analyses. Using BS method allows for determination of areal density and the concentrations of main components of a biological matrix – a direct measurement of carbon, nitrogen and oxygen, and indirectly hydrogen. It has to be stressed that the determination of hydrogen content is only possible under the assumption that hydrogen is the only element "complementing" the obtained matrix composition up to 100%, and it requires an absolute and accurate measurement of charge deposited by the charged particles in the measured material. This is because changes in the value of charge and the contribution of hydrogen in the matrix have the same influence on the R(BS) spectra. An additional problem in analysis of biological materials is variation of the matrix composition depending on the tissue and organ as well as variation of areal density in micro- and macroareas, caused mainly by variations in water content in the structures. These problems were encountered for the first time in our laboratory, because earlier the applications of R(BS) method were restricted to analyses of relatively simple systems of thin metallic layers in studies related to materials science where, from methodical point of view, measurements of elemental ratios rather than absolute concentrations, were sufficient and it was possible to use experimental conditions fulfilling the Rutherford criterion. The simulations of RBS spectra obtained under such conditions are easy to perform. For thin and intermediate targets of biological materials using alpha particles proved impossible due to significant damage of specimens during measurements, and often total destruction of organic substrate (formvar) on which they were mounted. In addition to much smaller damage of the analysed material by proton beam, much deeper material penetration by protons is also beneficial, making possible the determination of areal density in a much broader range than it is possible for alpha particles or heavier ions. It should be stressed that the BS method (for protons this terminology ought to be used, because the Rutherford criterion is not met for typically used energies of ion beams) can analyse materials of any thickness. For thick samples one cannot obtain the areal density, but such information is not necessary, and the information on matrix composition is still possible. However, proper analysis of BS spectra requires accurate cross-sections; in this case cross-sections for elastic scattering of protons of energies up to at least 3 MeV, the most often used energy in micro-PIXE measurements. In 1993 the cross-sections for carbon, oxygen and silicon have been published for proton energy range 1.0 – 3.5 MeV and scattering angles 110° , 150° i 170° (Amirikas *et al.*, 1993). Since the annular detector used in measurements of

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BS spectra was positioned at an average scattering angle of 176° , I adopted the published cross-sections for 170° in the RUMP software (Doolittle, 1986) used for simulations of (R)BS spectra and I have tested them performing measurements of standards for all three elements. The results of simulations were satisfactory, therefore these very standards were used at our laboratory until the improved cross-sections were published by Gurbich for carbon and oxygen (Gurbich, 1997; Gurbich, 1998), and next nitrogen (Gurbich, 2008). A parametrization method of cross-sections' determination used by him allowed calculations for any selected scattering angle. In addition, calculations of cross-sections for those, and next for some other elements, were made accessible to all interested users thanks to the installation of his software package (Sigmacalc) at the unrestricted internet site (Gurbich, 2007). Until the cross-sections for nitrogen have been calculated (Gurbich, 2008) and next made accessible, this element was not included in my simulations of BS spectra of biological materials. It was the consequence of my tests proving that the omission of nitrogen in a biological matrix leads to much smaller errors than the use of incorrect cross-sections for elastic proton scattering on this element. Another possible and often used approach was an approximation of a biological matrix by the composition of cellulose ($C_6H_{10}O_5$). It has been proved earlier that the error of such an approximation for analyses of elements in the $22 < Z < 56$ and 1 - 4 MeV proton energy, does not exceed 10% (Pineda and Peisach, 1998).

Publication (Przybyłowicz *et al.*, 1999a-H1) is a summary of the developed methodology of analysis of biological materials, with my leading contribution. In addition to the already discussed application of the elastic backscattering in matrix effect correction in PIXE, the instrumental and data acquisition details have also been reported therein. In addition, I paid attention to possible problems associated with the use of non-optimal measurement conditions, including a possibility of partial loss of the analysed material and loss of some elements due to the usage of too high currents of proton beam.

In addition to the description of methodology adopted for analyses of typical biological materials, modifications performed for analysis of the lightest elements available for PIXE method - sodium and magnesium - have also been described. A more detailed description of the experimental conditions proposed for the analysis of these elements is given in (Przybyłowicz *et al.*, 1999b), with discussion of the pros and cons of measurements with significantly reduced energy of protons - down to 1.5 MeV and 0.6 MeV - in comparison with the most often used energy of 3 MeV.

A lot of attention was paid to the proper preparation of the tissues, to preserve the structure of live material and to retain kinetic processes occurring in tissues. For this purpose, very rapid freezing of the material in liquid propane was applied (isopentane was initially used) cooled with liquid nitrogen, and next slow freeze-drying (the drying cycle of the frozen material is usually few days long). The details of this method of preparation are given in (Przybyłowicz *et al.*, 1999a-H1).

Using quantitative elemental mapping by micro-PIXE in many research projects in the field of geology, biology and environmental studies allowed me to develop optimal methods of data interpretation. I discuss the possibilities and limitations of this method, illustrating them with examples from my own measurement results obtained in the realisation of a number of research projects, in (Przybyłowicz *et al.*, 2001-H2). Compared here is a simple qualitative elemental mapping method based on a defined "energy gates" in the energy ranges corresponding to the selected x-ray lines of an element, and much more advanced, quantitative mapping utilising the *Dynamic Analysis* method as well as other methods of quantitative analysis based on "event-by-event" data storage.

An important limitation of the *Dynamic Analysis* method is the assumption of uniform matrix - composition of major elements and thickness of the sample - in the whole scanned area. In some cases of analyses of the freeze-dried organic material this assumption is not fulfilled. Good example of

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this problem are analyses of the leaf epidermis (Przybyłowicz *et al.*, 1995a-H6), where the freeze-dried epidermis material showed significant local changes of areal density, from 0.4 to 5 mg/cm². For this reason, the maps of epidermis could not be regarded as fully quantitative. Quantitative results from maps were in such cases verified on the basis of point analyses, calculated with the use of individual areal densities obtained from BS method. However, this approach is time consuming and it may be better to prepare the material in a way that ensures a constant thickness of the matrix. It is one of the reasons that a lot of attention has been given to other, apart of freeze-drying, methods of preparation of soft biological tissues.

Although the procedures for the preparation of biological materials are in general well known from electron microscopy (Echlin, 1992), it is always important to know their limitations in application to a particular type of material. Particularly important was the information about the pros and cons of selected procedures for replacing water at low temperatures by certain organic solvents (freeze substitution and low temperature embedding). These procedures could potentially be used as an alternative to freeze-drying. Our research group has conducted extensive comparative study using eight procedures with selected organic solvents at low temperature, for the most interesting plant material – leaves of *Berkheya coddii*, nickel hyperaccumulator from South Africa. The obtained results have been summarized in the publication (Budka *et al.*, 2005-H3). The biopsies were punched from the leaves, air was excluded with the use of hexadecane, and the material was frozen using the high-pressure freezer and transferred to the various freeze-substitution media (acetone, methanol, diethyl ether, tetrahydrofuran) and embedded in resin. Dimethylglyoxime (DMG) was added to substitution media as a precipitation agent of Ni, in order to reduce movements of this element during water substitution process. The obtained results suggest caution in using the tested procedures and selected resins. For only one of them – using diethyl ether – the obtained nickel distribution was the same as for the control results – material prepared by air-drying, freeze-drying or analysed in the frozen-hydrated state. For all remaining procedures the results were incorrect. Sometimes the obtained Ni distribution was quite the opposite of the distribution in the controls (case of tetrahydrofuran). The reported study draws attention on big risk of false results for water substitution with organic solvents, even at low substitution temperatures.

Rapidly frozen hydrated (to a temperature of 100K) biological material retains the same distribution and concentration of ions in the body, as it was at the time of arresting life processes. Therefore, microanalysis without any further processing of this material is most desirable. Avoidance of freeze-drying step has an additional advantage, namely the specimens sectioned with a cryo-ultramicrotome or cryo-microtome retain an identical areal density in the whole analysed area, in contrast to the freeze-dried material, wherein there are significant, often dramatic, local differences of areal density. This makes it possible to use the same matrix in each pixel of elemental maps recorded, without necessity of corrections for local changes, which greatly simplifies and accelerates the interpretation of results. Measurements of hydrated tissue are possible by using the electron microscopes equipped with commercially available systems enabling transport of the frozen material into the measuring chamber (cryo-transfers) and then analyzing them in frozen state. However, the excitation of characteristic x-ray using electrons, which takes place in a scanning electron microscope with a semiconductor detector (SEM-EDS - Scanning Electron Microscope with Energy Dispersive Spectrometry) does not allow for the analysis of trace elements due to too low sensitivity of the method. The sensitivity of micro-PIXE is on average at least two orders of magnitude higher, which makes it potentially a much better method for analyses of materials in frozen-hydrated state. In 2004, I proposed a modification of our proton microbeam facility aimed at quantitative analysis of the distribution of elements in soft biological tissues in the frozen-hydrated state.

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The only earlier attempts made in this respect in the world was the use of 2 MeV proton beam for elemental microanalyses of rat eye and kidney tissue kept at 243 K and at atmospheric pressure (Horowitz *et al.*, 1976) and, much later, analysis of onion tissue cooled with liquid nitrogen gas blown onto the sample for the maintenance of specimen temperature of 100 K, also at atmospheric pressure (Sakai *et al.*, 2005). I created a research team that in 2005-2006 conducted an adaptation of our proton microprobe enabling low-temperature measurements of soft biological tissues in a vacuum, and next performed measurements of selected plant and animal tissue (Tylko *et al.*, 2007a-H4; 2007b). These were the first documented quantitative analyses of this type in the world. A commercially available cryotransfer system from a scanning electron microscope has been modified, and coupled to the experimental chamber of the nuclear microprobe. Dr Grzegorz Tylko (postdoctoral fellow) acted as the prime contractor in the research team. Much attention was paid to the behavior of the biological material before and during the measurements. Pressure vapour changes of water, oxygen, nitrogen and carbon compounds (CO, CO₂, CH_n, CH_n-R) were monitored during the transfer of frozen specimen to the cold stage and when keeping it in the frozen state in the experimental chamber before, and next during analysis, during scanning with proton beam with varying scanning speeds and for different values of beam current. The correctness of the whole process of standardless analysis has been tested using biological standards (gelatine solution doped with heavier elements) both in frozen-hydrated state and when frozen and next freeze-dried. The concentrations of elements obtained from PIXE measurements were in good agreement with the concentrations calculated from the weighted masses of elements added to the gelatin. Observations of maps for the doped heavier elements confirmed their homogeneous distribution in the gelatine matrix, an important proof of the correctness of preparation of these standards. Similarly, the matrix composition results from BS method were consistent with the composition calculated from weighted masses. The BS results were later additionally confirmed by electron microanalysis and Monte Carlo simulations (Tylko *et al.*, 2010). The analyses of selected plant and animal material in the frozen-hydrate state and next freeze-dried, showed good consistency of elemental distribution.

The analyses were first restricted to thick samples, for technical reasons (Tylko *et al.*, 2007a-H4; Tylko *et al.*, 2007b), but later modifications of the experimental setup and further tests made possible analyses of semi-thick and thin specimens as well (Wang *et al.*, 2013). The achievements of our team have been recognized in the world and are being followed (Vavpetič *et al.*, 2013). There is no doubt that the analysis of biological tissues in a frozen-hydrated state will be more often used in the future (Lombi *et al.*, 2011).

Selected applications in biology

Among the wide range of applications of proton microbeam in analysis of biological materials, one can distinguish a cycle of publications related to studies of hyperaccumulation – an unusual plant response to metalliferous soils, manifested by uptake of metals from soil and their accumulation in plant shoots, up to concentrations highly exceeding the toxicity levels. Study of the transport of nickel in the different parts of *Senecio coronatus*, one of the African hyperaccumulators, was only the second publication where micro-PIXE has been used to study this phenomenon (Mesjasz-Przybyłowicz *et al.*, 1994), after an early work of the Harvard group on *Haumaniastrum robertii*, a cobalt hyperaccumulator growing in Zaire (Morrison *et al.*, 1981). Studies related to nickel hyperaccumulation started during my employment at the Schonland Research Centre for Nuclear Sciences, University of the Witwatersrand, Johannesburg, South Africa. In addition to measurement of the average concentrations of nickel and many other elements in all main tissues of *Senecio coronatus*, an assessment of their distribution in different tissues was also performed (Mesjasz-Przybyłowicz *et al.*, 1994). Mapping of the elements described in this paper was carried out qualitatively, due to limitations of the data acquisition system at the Schonland Research Centre that

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only allowed mapping with the use of 'energy gates'. It has been found, inter alia, a substantial enrichment of nickel in the leaf epidermis. High sensitivity of the method allowed tracking the distribution of many other elements, and it has been found that the epidermis was also enriched in Cu, Fe as well as Zn and Mn (Mesjasz-Przybyłowicz *et al.*, 1994). This publication drew the attention of plant physiologists on the possibility of micro-PIXE method and was followed by many studies related to hyperaccumulation of nickel and other elements. Studies were continued at the National Accelerator Centre, Somerset West, South Africa (the present name of the institute is: iThemba Laboratory for Accelerator Based Sciences). In the next publication of nickel hyperaccumulation in *Senecio coronatus* a quantitative method of elemental mapping - *Dynamic Analysis* – has been used (Przybyłowicz *et al.*, 1995a-H6). An assumption of constant thickness and matrix composition in the scanned areas was met for most tissues, but the freeze-dried epidermis material showed significant local variations of areal density, between 0.4 and 5 mg/cm². For this reason, the maps of the epidermis could not be considered as fully quantitative. The quantitative results for maps were in such cases verified on the basis of point analyses calculated using individual areal densities obtained from BS method. High sensitivity of micro-PIXE is of particular importance in studies of non-hyperaccumulating genotypes. In studies on comparison of the hyperaccumulating and non-accumulating genotype of *Senecio coronatus* a clear difference in the behavior of nickel and other elements, both with respect to their concentration and distribution, has been found (Mesjasz-Przybyłowicz *et al.*, 1997; Mesjasz-Przybyłowicz *et al.*, 2007). Elemental analyzes were carried out in parallel with cytological studies. Large anomalies identified on the maps of elemental distribution helped in the discovery of some specialized cells' structures in the roots of this plant (Mesjasz-Przybyłowicz *et al.*, 2007). It should be noted that the most typical distribution of a hyperaccumulated element, well documented for *Senecio coronatus*, is not a feature of each and every hyperaccumulating plant. For *Berkheya coddii*, another nickel-hyperaccumulating plant growing in South Africa, the highest nickel concentrations were found not in the leaf epidermis, but in the vascular bundles and the mesophyll (Mesjasz-Przybyłowicz and Przybyłowicz, 2011).

Micro-PIXE and micro-BS were also used in studies related to mycorrhiza, a phenomenon of symbiotic association between a fungus and roots or other organs of vascular plants. Such symbiosis is beneficial to both species, by the mutual exchange of nutrients. Mycorrhizal symbiosis in hyperaccumulators was discovered relatively recently in all species occurring in South Africa (Turnau and Mesjasz-Przybyłowicz, 2003). Soon after the micro-PIXE method was used for quantitative assessment of the influence of mycorrhizal fungi on the concentration and distribution of elements in the roots of hyperaccumulator *Berkheya coddii*. There was a significantly higher concentration of P, Ca, Zn and Cu in the mycorrhizal roots in comparison with the roots where there was no mycorrhiza. In addition, the distribution of P, K, Cl, Zn and Ni has been changed as a result of the impact of mycorrhiza (Orłowska *et al.*, 2008; 2013).

Analysis of cadmium distribution in plants hyperaccumulating this element is much more difficult because, due to its higher toxicity in comparison with other elements, the concentration limit defining hyperaccumulation is 0.01% dry weight, an order of magnitude less than for other hyperaccumulators. Moreover, the use of L lines for analysis constitutes a major challenge for the programs used for fitting spectra, due to overlap of these lines with the K lines of potassium, an element present in plants at much higher concentrations than cadmium.

This interesting problem has been discussed in the publication dealing with the distribution of elements in the leaves of *Thlaspi praecox*, the hyperaccumulator of zinc and cadmium (Vogel-Mikuš *et al.*, 2008-H7). Comparison of the results of cadmium mapping by *Dynamic Analysis* method for K and L series of x-ray lines used separately leads to the conclusion that even this advanced mapping method

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sometimes yields false results for the L series. Only cadmium maps based on K lines give reliable distribution of this element.

Applications of the proton microprobe in studies of hyperaccumulation were not restricted to plants. Discovery of insects feeding exclusively on leaves of hyperaccumulating plant *Berkheya coddii* was a big surprise (Mesjasz-Przybyłowicz and Przybyłowicz, 2001) and initiated intensive studies aimed at understanding the adaptation mechanism of these insects to such unusual, potentially toxic, food. Elemental mapping by micro-PIXE and micro-BS of internal organs of the insects from *Chrysolina pardalina* species (now the species name has been changed to *Chrysolina clathrata*) has been utilised to test the ability of these insects to the environment very strongly enriched in nickel and to know, qualitatively and quantitatively, the relationship between nickel and other elements in physiologically relevant structures of these insects (Przybyłowicz *et al.*, 2003-H8). It has been proven that the Malpighian tubules are responsible for the elimination of the metal from the hemolymph (Przybyłowicz *et al.*, 2003-H8; 2005).

Publication (Mesjasz-Przybyłowicz and Przybyłowicz, 2002-H9) summarizes the role of micro-PIXE method in the study of basic processes in the field of plant physiology. The current knowledge on the mechanisms of transport of elements in plants, the consequences of their deficiency or excess, uptake of nutrients and their functions in the metabolism of plants and other related issues, is still mainly based on the results of analysis by chemical methods that only provide data on the concentrations of elements and neglect the information on their distribution in the microregions.

On the other hand, advanced research on the transport of elements at the cellular level is carried out mostly using electron microscopes with very good spatial resolution, but the electron excitation of characteristic x-rays does not provide sufficient sensitivity for the analysis of trace elements. Micro-PIXE is a method that allows bridging the gap between these two approaches, as illustrated by examples of applications coming from my own studies obtained in collaboration with botanists and specialists in the field of plant physiology. The paper discusses strengths and weaknesses of the method compared with other available methods of microanalysis. In particular, attention was paid to the correct methods of material preparation, the complementarity of ion analysis techniques used in combination with micro-PIXE, measurement geometries, data acquisition, the advantages of quantitative mapping of elemental distribution and the conditions necessary for the complete quantitative elemental analysis.

Selected applications in geology

Using advanced method of elemental mapping has resulted in a series of publications in which the study of the distribution of elements in a quantitative manner was a key element. Few articles addressed the issues related to the geochemistry of gold deposits in South Africa, mainly from Witwatersrand reefs. The first publication was related to the mechanism of electrochemical deposition of gold on the surfaces of the pyrite grains from these reefs. In one of the geological models explaining the occurrence of gold, attention is drawn to the oscillatory presence of zones on the surface of pyrite grains, enriched and depleted in arsenic. It is believed that they generate a mixed conductivity, the n-type conductivity in zones depleted in As, and p-type conductivity in zones enriched in As, as well as the deposition of gold on the cathode (Möller, 1993; Möller and Kersten, 1994). Indeed, for the studied pyrites I obtained a convincing picture of the spatial relationship between arsenic and gold on their surfaces (Meyer *et al.*, 1994; Przybyłowicz *et al.*, 1995-H10).

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Elemental mapping of pyrites and pyrrhotites, the main sulfide minerals from the Witwatersrand basin, has been continued and constituted an important source of their classification, often revealing details impossible to observe using conventional petrographic methods (Foya *et al.*, 1999; Reimold *et al.*, 1999; 2004). Pyrites from other ore deposits have also been studied. Micro-PIXE elemental maps allowed identification of two generations of pyrites from the Spitskop ore deposit, Saldania Belt, South Africa (Belcher *et al.*, 2004). Also in this case As maps gave evidence of the existence of zones of alternating high and low concentrations of this element, and the presence of fine-grained gold at the boundaries between As-rich and As-poor bands is consistent with the model electrochemical deposition (Belcher *et al.*, 2004). Elemental mapping of pyrites from unusual pyrite-bearing carbonate sediments in Zimbabwe (Bulawayo-Bubi Greenstone Belt) allowed identification of specific geochemical features for each generation of pyrites (Xenophontos *et al.*, 1999; 2001).

An interesting application of micro-PIXE method was a study of gold solubility in synthetically created magma, at low concentration level of this element. These studies are important to understand the mechanism of the formation of gold deposits. In this case the most important elements, apart of gold, were sulphur and chlorine. Mapping the distribution of other elements (Si, K, Fe, Zn) was also very important because of the coexistence of the melt and crystalline phases. Precise selection of coordinates for point analyses, aimed at determination of gold concentration, was therefore a critical issue. Experimental conditions used for point analyses allowed obtaining a limit of detection of this element at the level of 2 ppm. The results indicate that sulfur has better property of gold complexation as compared with chlorine (Stevens *et al.*, 2004).

Micro-PIXE was also used in a studies related to the isotopic dating of rocks, namely studies of progressive leaching in single mineral Pb/Pb dating. This procedure has been proposed for dating with the use of rock-forming minerals (Frei and Kamber, 1995). In contrast to rock dating using accessory phases, age data from rock-forming silicates permit a more direct link between important structural, petrographical, and petrological observations and time and, therefore, provide better constraints on models of geological processes. An extensive methodical study aimed at thorough understanding of the stepwise leaching mechanism was carried out on a single crystal of gem quality titanite. Micro-PIXE elemental maps allowed observation of the migration of major and trace elements during the leaching process (Frei *et al.*, 1997a). The most important was to follow the behavior of elements directly used in dating with progressive leaching - uranium, thorium and lead. In addition, the concentrations of these elements obtained from a selected area in the "unleached" area of titanite were used to calculate the age using the method proposed by Montel (Montel *et al.*, 1994). The obtained age was in very good agreement with the result obtained by the independent isotopic method (Frei *et al.*, 1997b-H11).

In spite of continuous development of other methods of elemental microanalysis, using the micro-PIXE method remains an important part of a comprehensive research related to the geochemistry of gold deposits. In a recent work on the origin of gold in the Barberton Greenston Belt (South Africa), elemental mapping of sulfide minerals (pyrites, arsenopyrites, chalcopyrites) allowed us to identify several formation stages of these minerals, and the zonal distribution of trace elements provided information on the processes leading to the creation of this deposit. The results of elemental mapping performed with a modern electron microscope equipped with two spectrometers - wavelength dispersive spectrometer (WDS) and energy dispersive spectrometer (EDS) - were complemented by the quantitative elemental maps obtained with our micro-PIXE system (Agangi *et al.*, 2014-H12). Analysis in a wider energy range and the ability to analyze more elements, based on the series of K and L x-ray lines, in comparison with the capabilities of the electron microscope, allowed for a more complete analysis of minerals, and therefore greatly facilitated the interpretation of results. Publication by Fuchs (Fuchs *et al.*, 2014-H13) is another example showing the advantages of the use

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of micro-PIXE method in comprehensive studies related to the genesis of gold deposits, this time present together with organic matter, and other elements of strategic importance (mainly uranium but also thorium and the rare earth elements), from the Au-U-bearing Carbon Leader Reef of the Witwatersrand in South Africa. The analysed bituminous material has complex elemental composition, of a very high spatial diversity. In addition, the occurrence of mineral inclusions of small size, of an entirely different chemical composition relative to the organic matter, has been found. Quantitative elemental mapping of the whole scanned areas was therefore not possible. The appropriate methodological approach in such case is to identify areas of the same or similar chemical composition and analysis of PIXE spectra from these areas. In the case of organic matter maps of light elements - carbon and oxygen - obtained using the EBS method, can be very helpful in the interpretation of results. For the reported case they need to be interpreted with caution, because highly variable amounts of heavy elements (U, Au, Pb) made fitting of the EBS spectra very difficult and the obtained atomic ratios of light elements (C, O and indirectly H) were possibly distorted by their presence. However, additional EBS qualitative maps of C and O, obtained from the same areas as micro-PIXE elemental maps, were helpful in the interpretation of results (Fuchs *et al.*, 2014-H13).

5. Discussion of other scientific activities

5.1. Period before obtaining the Ph.D. degree

I started my research activity when I was a student at the Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology in Cracow. My first publication on the determination of uranium in loess by detecting fission fragments of uranium-235 (Przybyłowicz, 1975) came out when I was a student of the third year of Geophysics, specializing in Nuclear Geophysics. I continued the topic determining uranium in bulk materials in the framework of my M.Sc. thesis. My tutor and next thesis supervisor was prof. dr hab. Jerzy Niewodniczański from the Institute of Nuclear Physics and Techniques.

In 1976 I defended my Master of Science thesis „Determination of uranium in loose materials by fission track method” and next I became employed in the Department of Nuclear Geophysics, the Institute of Nuclear Physics and Techniques of the Academy of Mining and Metallurgy in Cracow. In 1976-1985 I participated in scientific research conducted at the Department, exploring some analytical methods available at the Institute. During this period I co-authored two publications and one patent related to the integrated instrumental analysis of soft brown coals (Chruściel *et al.*, 1983; Kreft *et al.*, 1984; Chruściel *et al.*, 1986), two publications on the composition of light isotopes in two geological deposits (Wydrza and Machów) (Pawlikowski and Przybyłowicz, 1979; 1980), and I also started characterisation of serpentinite rocks from Lower Silesia (Poland) with the use of instrumental neutron activation analysis and fission track method (Przybyłowicz, 1986; Janczyszyn and Przybyłowicz, 1987). The first publications on the applications of proton beam in mineralogy and geology also date from this period. Initially I was using collimated beams of protons, generated in the C-48 cyclotron at the Institute of Nuclear Physics in Cracow (now the Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences). These studies focused on the selected type of rocks - serpentinites from Lower Silesia. The use of PIXE and PIGE methods in geology was at that time a pioneer application on a Polish scale and constituted part of my doctoral dissertation (Przybyłowicz *et al.*, 1986a; 1986b).

In 1985 I defended my doctoral thesis entitled “Application of selected physical methods in studies of the geochemistry of serpentinite rocks from the Lower Silesia” at the Faculty of Geology, Geophysics and Environmental Protection, AGH University of Science and Technology, Cracow. The thesis supervisor was prof. dr hab. Witold Żabiński.

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5.2. Period after obtaining the Ph.D. degree

In October 1985 I got appointed as adjunct professor in the Institute of Nuclear Physics and Techniques of the Academy of Mining and Metallurgy in Cracow.

In the period from January 1986 to July 1987 I was on a scientific internship (Post Doctoral Fellow) at the Department of Geology of McMaster University in Hamilton, Canada, as a fellow of the International Atomic Energy Agency. My research objective was isotopic dating of calcites using the uranium-series method, in the research team of Professor Henry Schwarcz (Przybylowicz *et al.*, 1991). I also became interested in accelerator mass spectrometry, developed by the IsoTrace laboratory at the University of Toronto, and I participated in the dating experiments conducted using iodine-123 isotope.

Upon return to Poland I again became interested in the PIXE applications in geology. It was obvious that the use of a focused proton beam (micro-PIXE) gives much wider analytical capabilities compared to a collimated beam, especially when combined with the observations and analyzes of the same micro-areas using an electron microscope, electron microprobe, and also SRIXE (Synchrotron Radiation Induced X-ray Emission). Since this technique was not available in Poland, I initiated contact and cooperation with the group of Dr. Kurt Traxel from the Max Planck Institut für Kernphysik in Heidelberg (by then in the Federal Republic of Germany) and I conducted the measurements there. Among my first studies with the use of the proton microprobe are the analyses of carbonate minerals and black shales from the copper ore deposits of Lower Silesia, attractive due to the presence of noble metals of high economic importance. The published work had both methodical and applicative aspects (Przybyłowicz *et al.*, 1990a i 1990b). In addition, upon my initiative, tests of SRIXE method have been carried out for selected geological materials using synchrotron microbeam in Daresbury (Daresbury Synchrotron Radiation Source) (Przybylowicz *et al.*, 1992). Analyses of geological materials at that beam line were then carried out for the first time; its main intended purpose was biomedical studies. Full use of the results would not have been possible without the intensive cooperation with geologists from the Faculty of Geological Prospecting, Academy of Mining and Metallurgy in Cracow – prof. dr hab. Henryk Kucha, Dr Adam Piestrzyński and Dr Piotr Wyszomirski (I give the name of the faculty and academic titles from the period of collaboration and publication of results).

In 1992 I have been invited to participate in the final stage of commissioning of the scanning proton microprobe in the Schonland Research Centre for Nuclear Sciences, University of the Witwatersrand, Johannesburg, in South Africa. I accepted this invitation and treated as an important scientific challenge and an opportunity to gain an experience, potentially useful in Poland in the future, because at that time no operational nuclear microprobe existed in Poland. This facility has been built on one of the beamlines of the 6 MV tandem Van de Graaff accelerator (EN model), and the setup made possible the use of ion beams generated by a second accelerator – 2.5 MV single-ended Van de Graaff accelerator (Watterson *et al.*, 1993). The aim of this interesting combination was on one hand to use high ion currents from the small single-ended Van de Graaff, mainly for PIXE analysis, and on the other hand protons of higher energies and heavier ions generated by the tandem. The main components of the instrument – quadrupole lenses (OM150 system), slits and scanning system, were manufactured by the Oxford Microbeams Ltd., while the experimental chamber has been built and assembled locally, as well as the multi-parameter data acquisition system (Watterson *et al.*, 1993).

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My main task was testing the system and development of the methodology of quantitative PIXE analyses, and next its application in selected research projects related to geology, archaeology and materials engineering. Due to the relatively low intensity of ion currents achievable by the tandem, the size of focused proton beam was about 5 micrometers for the currents of the order of 500 pA. Such performance was far from potential capabilities of the used quadrupole lens system, nevertheless adequate to complete the first research projects. Currents achievable with the use of single-ended Van de Graaff were higher by up to one order of magnitude but, due to constant technical problems experienced during exploitation of this accelerator, its use never went beyond the testing phase. In addition to the WIMPS data acquisition system, I also tested and next used the OMDAQ acquisition system (Grime and Dawson, 1995). In quantitative PIXE analysis I decided to use the same approach as the one used by the research team in Guelph, Canada (Maxwell *et al.*, 1988), which is a compromise between the use of synthetic standards and exclusive use of fundamental parameters to determine matrix effects. My calculations of the so-called *instrumental constant H* were done by using simple single-element standards (thick and thin specimens) and international reference standards (powdered rock standards fabricated by fusing them into thick glass targets, synthetic glasses doped with known concentrations of selected elements and some steel standards). After initial testing of the WITSHEX software (Lipworth *et al.*, 1990) I decided to use the GUPIX software (Maxwell *et al.*, 1988) for PIXE analyses. In my opinion the most important advantage of this software is usage of the best available data bases of physical parameters and their constant verification by its authors. Background removal is done by a mathematical filtering technique using the "top-hat" convolution filter, and the process is fully automatic. Another very important feature of GUPIX is advanced treatment of PIXE spectra of multi-layered targets, often encountered e.g. in materials science studies. I have performed many tests of PIXE methodology using all available international reference standards. In addition, I initiated comparative measurements of geological materials previously analysed at the renowned „Australian CSIRO Heavy Ion Analytical Facility" in Sydney – garnets, ilmenites and clinopyroxenes. Comparisons were successful; there were no systematic differences in the results obtained in both centers.

Analyses of elemental impurities in archaeological materials from the Rooiberg tin field, the only Iron Age tin mining and smelting site in South Africa, were among the first applications of the scanning proton microprobe (Przybyłowicz *et al.*, 1993a). Micro-PIXE scans revealed interesting microstructures of iron and copper. Microanalyses of carbonado – polycrystalline pea-sized and nut-sized aggregates of black diamond that probably formed during the explosion of supernova star – have shown the distribution of iron and manganese, associated with the matrix binding the diamond crystals (Przybyłowicz *et al.*, 1993a). Aluminium, chromium, nickel and copper were also detected. Another micro-PIXE application in geology was the analysis of mineral inclusions in diamonds. Geochemistry of major and trace elements in these inclusions provides information on the conditions of crystallization of diamonds, thus allows predicting potential sites of occurrence of diamonds. The use of so-called "geothermometer nickel" allows the calculation of the equilibrium temperature of the diamond based on nickel content in such a microscopic piece of the mineral embedded in a diamond. Measurements of nickel concentration in the olivine inclusions embedded in a diamond and the temperature calculated on this basis showed very good agreement (within the measurement error) with the results of other methods (Sellschop *et al.*, 1993). Analysis of corrosion pits in the cemented tungsten carbide samples (WC-Co) is in turn a good example of superiority of the scanning proton microprobe over the more popular scanning electron microscope with EDX attachment, in studies of materials. PIXE microanalysis identified the impurities of sulphur, iron and molybdenum in the corroded areas. None of these elements have been identified using an electron microscope, because their concentrations were too low (Przybyłowicz *et al.*, 1993b).

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In addition, I began studies related to nickel transport in the tissues of the plant *Senecio coronatus*, the hyperaccumulator of this element (Mesjasz-Przybyłowicz *et al.*, 1994). The results obtained during my two-year stay in the Schonland Research Centre for Nuclear Sciences were published in a total of six publications indexed in the *Web of Science*, two refereed conference proceedings and were reported at the 22 conference presentations.

From January 1994 until present time I am employed as a Senior Research Scientist at iThemba Laboratory for Accelerator Based Sciences, Somerset West, South Africa (the previous name of the institute: National Accelerator Centre), dealing with the development of methodology and applications of the nuclear microprobe. Over the years, I participated in numerous scientific collaborations with local and foreign universities or research centers; many of them have been established at my initiative. The results of selected few collaborations in the field of biology and geology have been presented in a series of publications submitted as the basis for the habilitation procedure. The results of many other collaborations have been discussed in other publications, the full list is given in a separate annex. I was the initiator and South African coordinator of six international research projects, mostly focused on methodology for microanalysis of biological materials using the nuclear microprobe. They were cooperation with Physikalisches Institut der Universität Heidelberg and the Max-Planck-Institut für Kernphysik (MPI) in Heidelberg (Germany) during the 1997-2000 period; with Centre d'Etudes Nucléaires de Bordeaux-Gradignan (CENBG), Bordeaux (France) during the 1998-2000 period; twice with the Department of Nuclear Physics, Lund Institute of Technology, Lund, Sweden in 2000-2001 and 2009-2013. I participated twice in international scientific cooperation projects initiated by the International Atomic Energy Agency, as the coordinator and the main contractor from South Africa, in 2005-2008 and 2009-2012. In addition to this I was eight times a member of the team participating in official international collaborations. The most fruitful collaborations include two with Polish scientific institutions, with a team of prof. Katarzyna Turnau of the Department of Botany of the Jagiellonian University (three times in 2000-2006) and a team of prof. Paweł Migula from the Department of Animal Physiology and Ecotoxicology, Faculty of Biology and Environmental Protection, University of Silesia in Katowice (three times, in 2001-2006). In addition, I participated in collaboration with the Laboratoire Sols et Environnement INPL-ENSAIA/INRA in Nancy (France) in 2009-2011. I am currently a member of the team participating in an international scientific cooperation project coordinated by the International Atomic Energy Agency on the use of synchrotron radiation in environmental research and studies of materials.

In addition to the undoubted success of our research team, and a reputation achieved in the field of applications of the scanning proton microprobe in biology and environmental studies, also noteworthy are research projects in the field of geology, where micro-PIXE and occasionally micro-BS is typically used as a supplement to other types of microscopy (mainly optical and scanning electron microscopy) or microanalysis (with the use of WDS or EDS x-ray spectrometry coupled to an electron microscope, SIMS or LA-ICP-MS methods), and in the field of materials engineering.

The results of my research during employment at iThemba LABS have been published in 100 publications and 5 refereed conference proceedings indexed in the *Web of Science* database, 2 refereed but not indexed publications, 72 refereed but not indexed conference proceedings, 19 non-refereed conference proceedings and 301 conference presentations including 17 invited plenary talks and lectures at the international conferences. I was the official supervisor or external supervisor of 8 completed M.Sc. and 3 completed Ph.D. theses. However, most often the applications of nuclear microprobe constituted a part rather than the entire thesis. In such cases I was not an official supervisor, but I participated in the research projects and trained students in the theory and practice of ion beam analysis (mainly PIXE and RBS-BS methods) and I controlled the correctness of the

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interpretation of the obtained results and their presentation in the theses and publications. Such training was undertaken for 31 doctoral students, 26 graduate and three undergraduate students.

In addition to the development of the methodology related to the nuclear microprobe and applications, I am interested in supplementary methods, in particular those enabling micro-imaging of the chemical forms of elements (XANES / EXAFS) and giving information about organic compounds. Since 2009, I participate in experiments with the use of synchrotron radiation. So far, EXAFS measurements to study the chemical forms of nickel in plants hyperaccumulating this element were performed at the "SOLEIL" synchrotron in France (SAMBA beamline) and recently also the European Synchrotron Radiation Facility (ESRF) in Grenoble. Measurements to obtain information about organic compounds in these plants by infrared microspectroscopy were conducted on the SMIS beam line of the "SOLEIL" synchrotron. In both cases, the preliminary results have already been presented at conferences, and publications are in preparation.

In summary, the results of my scientific research have been published in 115 research articles indexed in the *Web of Science* database, 10 publications in journals outside the list of the Journal Citation Reports (JCR), 77 refereed conference proceedings, and were shown at 354 conference presentations. In addition, I was twice a co-author, upon invitation, of chapters on the PIXE method. My research outputs have so far been cited 1242 times in 932 publications (this number excludes self-citations). Their cumulative *impact factor* is 155.6. The Hirsch index (h-index) is 22.

Seventeen times I was giving invited talks at international scientific conferences. Six times I served as coordinator of international research collaborations, and I was eight times a member of research teams pursuing international cooperation within the framework of bilateral agreements between the governments. I refereed manuscripts for 16 journals of international scope. I am member of the Editorial Board of "X-Ray Spectrometry" journal (Wiley Interscience) and a member of the „International Advisory Committee for the PIXE (Particle Induced X-ray Emission) Conferences". In addition, I was twice a guest editor of the special issues of scientific journals – the special issue of the "Nuclear Instruments and Methods in Physics Research B", volume B158 (1999) – proceedings of the „6th International Conference on Nuclear Microprobe Technology and Applications (ICNMTA)", and the special issue of "X-Ray Spectrometry" volume 33(3): May-June 2004: "X-ray techniques in South Africa". I am the main organizer of the conference "International Conference on Particle Induced X-ray Emission" that will take place in 2015 in Stellenbosch, South Africa (jointly with Dr. Carlos Pineda-Vargas) and the editor of the planned special issue of the "Nuclear Instruments and Methods in Physics Research B", dedicated to this conference.

A detailed list of my research and teaching achievements as well as organizational activities is presented in a separate annex.

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