



Report of Summer Student Program

**STUDY OF MAJOR AND TRACE ELEMENTS
IN MOSS SAMPLES FROM GEORGIA USING NEUTRON
ACTIVATION ANALYSIS**

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Introduction

Biomonitoring of atmospheric heavy metal deposition using pleurocarpous mosses is a well-established technique in Europe. Since 2000, the European biomonitoring program has been included in the UNECE ICP-Vegetation international cooperation program. From 2014 the international moss monitoring program „Heavy Metals in Mosses” has been coordinated by the Joint Institute for Nuclear Research [1]. This program was applied for the first time in Georgia in the frame of Shota Rustaveli National Science Foundation grant AR/198/9-240/14 (28.04.2015-28.04.2017) “Investigation of atmospheric deposition of heavy metals in Georgia using moss biomonitoring and physical analytical techniques”. The possibilities of biomonitoring technique for geophysical conditions of Georgia were preliminary investigated in 2014 [2]. The results of this investigation show that Georgian industries and agricultural sector provide considerable anthropogenic impact on the environment of the Caucasus. The use of moss biomonitoring technique and NAA is a first attempt to study heavy metal atmospheric deposition in Georgia, a country different relief and climate. To provide more information about the state of the atmosphere pollution, 36 moss samples of *Hylocomium splendens*, *Hypnum cupressiforme* and *Pleurozium schreberi* were collected in different parts of Georgia during the summer of 2015. The present work was carried out in the Sector of Neutron Activation Analysis and Applied Research FLNP JINR as the development of longtime collaborative investigations in the field of biotechnology carried out with I. Javakhishvili Tbilisi State University, E. Andronikashvili Institute of Physics. In moss samples of Georgia 2015 collection total of 37 elements were determined by instrumental neutron activation analysis at the IBR-2 reactor of FLNP JINR in frame of JINR Summer student Program (15.09 – 15.11) 2015 Dubna, Russia.

Principles of Neutron Activation Analysis

Neutron activation analysis (NAA) was first developed by Hungarian chemists G. Hevesy and H. Levi in 1936 [3]. Afterwards this method was developing and potentials were explored. NAA is capable of detecting many elements at extremely low concentrations. The sensitivity, reliability and simplicity of NAA made it widespread. It finds use in environmental monitoring, foodstuff monitoring, chemistry, medicine, geology, geoecology, archaeology, forensic science, est. Today this method is even used as reference for other analysis methods [4].

Neutron activation analysis (NAA) is a method for determination of elements based on measuring the radioactivity, which appears after converting stable atoms into radioactive isotopes by bombarding the material with a neutron flow. These isotopes have unstable nuclear configuration and emit gamma rays, which can be quantitatively identified by gamma-ray detectors. Thus, the basic essentials required to carry out an analysis of samples by NAA are a source of neutrons, gamma-ray detectors and a detailed knowledge of the reactions that occur when neutrons interact with target nuclei.

Nuclear reactions

Neutrons do not carry a charge and react with atomic nuclei by scattering or by absorption, without electrostatic repulsion. When a neutron is absorbed by the target nucleus, a highly energetic state is produced and the resulting nucleus immediately loses the excess energy by emission of a gamma ray, a proton or an alpha particle. The gamma rays, protons and alpha particles produced during these reactions are all emitted spontaneously and therefore they are only detected if they are monitored during the activation process.

The nature of the nuclear reaction and the activation product depends on the energy of the neutron. According to their energy there are three types of neutrons:

1. Thermal neutrons with the most probable energy 0.025 eV. They are in thermal equilibrium with the atoms of the moderator;
2. Epithermal neutrons are neutrons of intermediate energies, which are in the process of slowing down by collisions with the nuclei of the moderator. Their energies range from 0,5 eV to 1 MeV;
3. Fast neutrons energy distribution ranges from 1 MeV to 15 MeV.

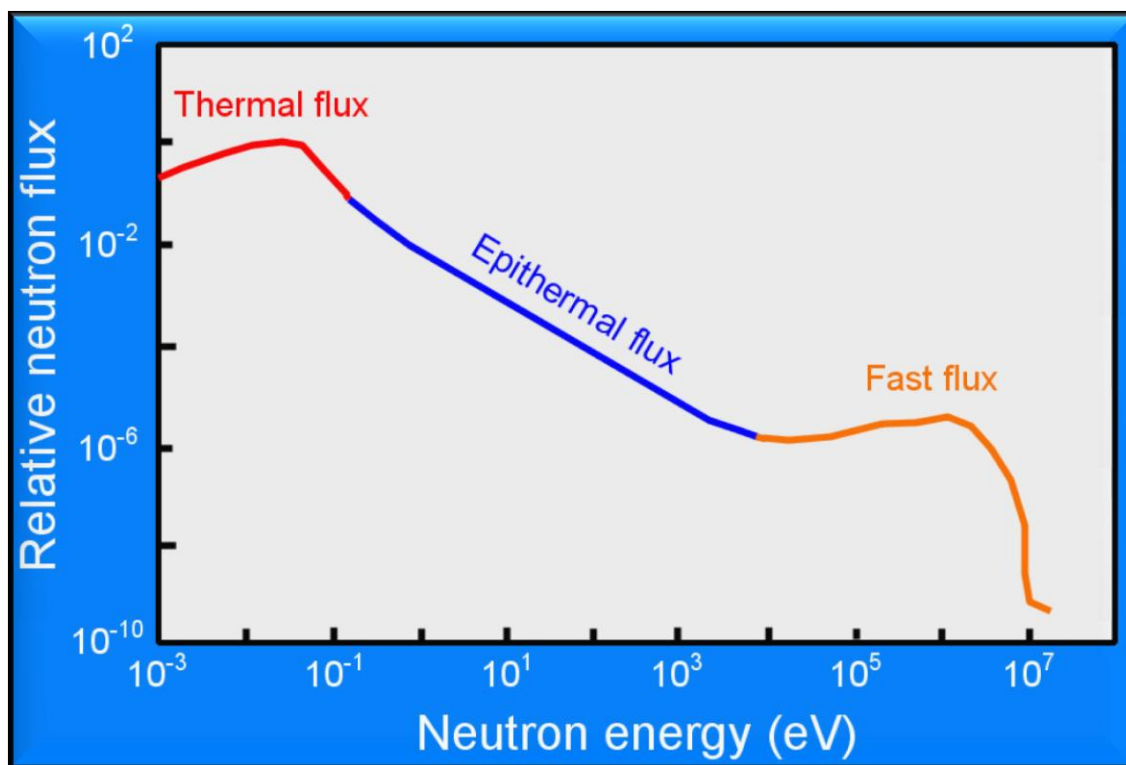


Fig.1 Schematic representation of the neutron flux spectrum in a nuclear reactor.

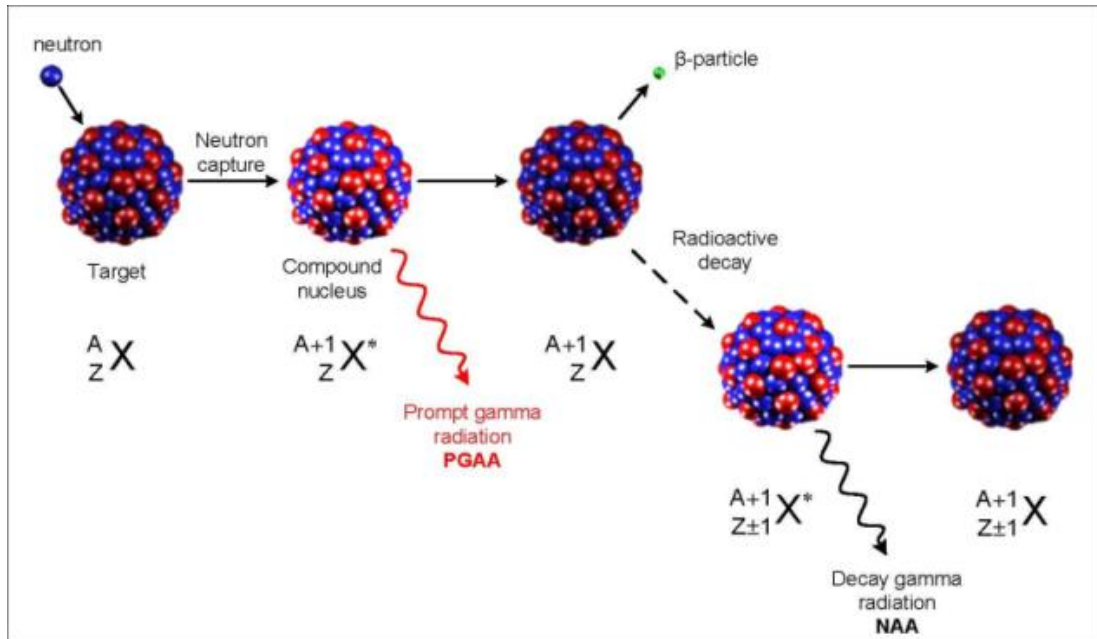
Fast neutrons induce different reactions. Collisions between fast neutrons and nuclei occur with such force that alpha particles or protons are ejected. The loss of protons results in the formation of new elements with lower atomic numbers.

Type	Nuclear equation	Model	Change in mass/atomic numbers
Alpha decay	${}^A_Z X \rightarrow {}^4_2 \text{He} + {}^{A-4}_{Z-2} Y$		A: decrease by 4 Z: decrease by 2
Positron emission	${}^A_Z X \rightarrow {}^0_{+1} e + {}^{A}_{Z-1} Y$		A: unchanged Z: decrease by 1

These fast neutrons are too energetic for neutron activation. Only a low-energy collision is required. In order to make them suitable, the highly energetic neutrons are passed through a moderator, consisting of water, paraffin, or graphite. Fast neutrons are slowed by colliding with atoms of the moderator and become low energy, slow, or thermal neutrons.

The main reaction occurring with thermal neutrons is (n, γ) reaction. The process of neutron capture adds 8 MeV of energy. In this case the collision of the thermal neutron with the target nucleus forms the highly energetic level of the product nucleus. The newly formed isotope has the same atomic number but mass number is greater by one.

The excitation energy within the compound nucleus is unfavorable and the compound nucleus will almost instantaneously de-excite into a more stable configuration by emission of a gamma ray which is called a prompt gamma, since it is emitted immediately after irradiation.



In most cases this more stable configuration gives a radioactive nucleus. The newly formed radioactive nucleus now decays by the emission of beta particles and gamma rays. The gamma rays have characteristic energies, which establish the presence of particular elements. This decay process is at much slower rate than the initial de-excitation and depends on unique half-life of the radioactive nucleus.

Cross Sections

Atomic nuclei occupy only very small space within matter and neutrons can penetrate thousands of atomic layers before they hit an atomic nucleus. The concept of a cross section is used to characterize the probability that a particular nuclear reaction will take place. In other words it is effective area for collision. The cross section is expressed in units of area, usually in barn 10^{-24} cm^2 , which is approximately the size of an atomic nucleus. Some nuclei, however, have cross sections much larger than their nucleus. This can be explained by long-range forces, which influence the neutron even if it passes the nucleus at a large distance. Some nuclei of rare Earth elements have cross sections of more than 1000 b for slow neutrons. Nuclear events involve either scattering or absorption processes. Thus, the total cross section is expressed as the sum of absorption cross section and scattering cross section:

$$\sigma_{\text{tot}} = \sigma_{\text{abs}} + \sigma_{\text{s}}$$

Cross sections of general processes can be further subdivided into the reaction cross sections according to the fate of the compound nucleus, for example:

$$\sigma_{\text{abs}} = \sigma(n,\gamma) + \sigma(n,p) + \sigma(n,\alpha) + \sigma(n,2n) + \sigma(n,n)$$

The cross section and the neutron fluence rate are neutron energy dependent. Reactions of the (n,γ) type have the highest cross section (typically in the order of 0.1–100 b) for thermal neutrons, whereas the other reactions $((n,p), (n,\alpha), (n,n'), (n,2n))$ mainly occur with fast neutrons at cross sections 2 or 3 orders of magnitude lower. In several cases, nuclear reactions result in the conversion of a stable nucleus into another stable nucleus. The cross section for thermal neutrons is often inversely proportional to the neutron velocity; in the epithermal region, the neutron cross section can be very high for the neutrons of a discrete energy and the neutron cross section versus neutron energy relationship shows ‘resonance peaks’.

Irradiation Facilities, Sample Preparation and Analysis

Procedures for NAA

Main procedures of neutron activation analysis are carried out according to the scheme shown in Fig.2

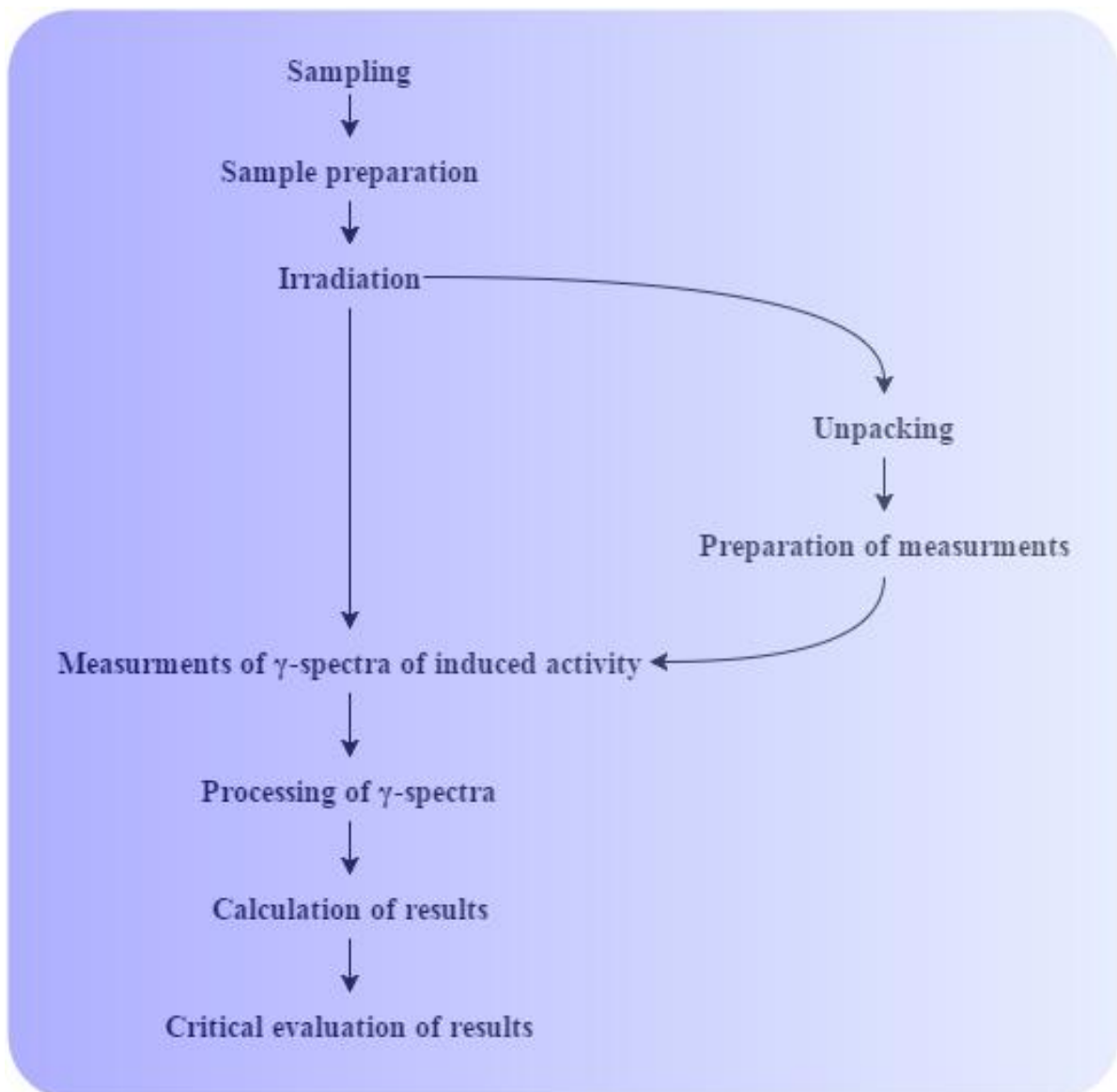


Fig.2 Schema of procedures for NAA

Study Area and Sampling

As earlier investigations showed that only pleurocarpous mosses should be sampled to study atmospheric deposition of major and trace elements [5][6][7][8].

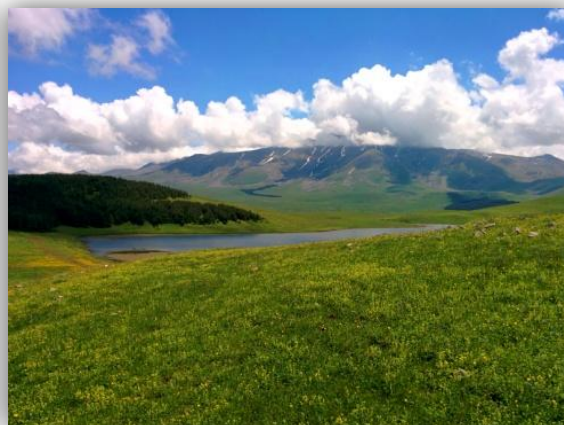
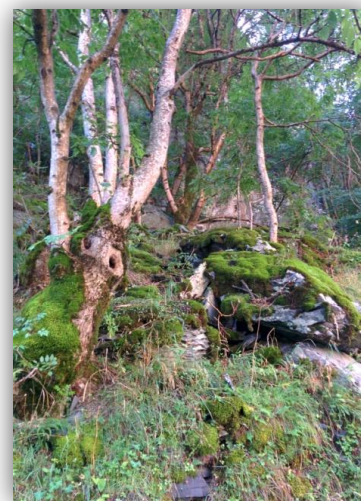


The mosses, especially the carpet-forming species have well-developed surface to obtain most of nutrients directly from dry and wet deposition. The growth segment of mosses can be easily identified. There is little uptake of metals from the substrate, despite the fact that rhizoids externally look like roots, they don't have such function as adsorption, they only anchor the organism to a substrate. Thus, these

plants are relatively independent of the substrate on which they grow and they can provide direct information about atmospheric heavy metal deposition over a certain period of time. Moreover they have a high capacity to retain many trace elements what makes sampling and chemical analysis more robust and some of moss species are able to be tolerant to even a high pollution level.

Therefore, three moss species: *Hylocomium splendens*, *Pleurozium schreberi* and *Hypnum cupressiforme* were chosen for this study in Georgia.

A total of 36 moss samples of *Hylocomium splendens*-13, *Pleurozium schreberi*-8 and *Hypnum cupressiforme*-15, growing on organic top-soils in forests, covering foothills, subalpine and alpine belts and other natural environments, have been collected during the summer months of 2015. The vertical altitudes of samples range from 161 m to 2052 m. 17 samples were taken in the South Georgia - Meskheti. Here in Meskheti deciduous forests generally reach 1500 meters above the sea. In the range of 1500-2200 meters they are replaced with coniferous forests and above 2200 meters coniferous forests give way to alpine grasslands. The climate in this part of Georgia is mild, moderately humid. Annual precipitation is approximately 500 - 650 mm. 8 samples were collected in Eastern Georgia, the majority of which are from Khevsureti. The climate in this mountain region is cold and temperate. The rainfall here averages 1012 mm. 9 samples were taken in the northwestern part of Georgia – Svaneti. The landscape of this region is dominated by mountains and deep gorges, which separate them. The zone which extends from 800 meters to roughly about 2500 meters above sea level consists of mixed and



coniferous forests. Alpine meadows lie above 2450 – 3200 meters. Average temperatures and precipitation vary considerably with elevation. Annual precipitation ranges between 1000 and 3200 mm.

The sampling was carried out according to the standard procedure described in The ICP Vegetation - Moss survey protocol [9]. The sampling sites were located at least 300 m from main roads and populated areas, and at least 100 m away from smaller roads or single houses. The sampling points were situated 3 m away from tree canopy. For each sampling site 5 to 10 sub-samples were taken within 50 x 50m area and combined to one collective sample. Descriptions of all sites have been recorded for future use together with geographical coordinates determined by GPS and time of the sampling. Each sampling site was photographed and archived. The sampling map is shown in Figure 3.

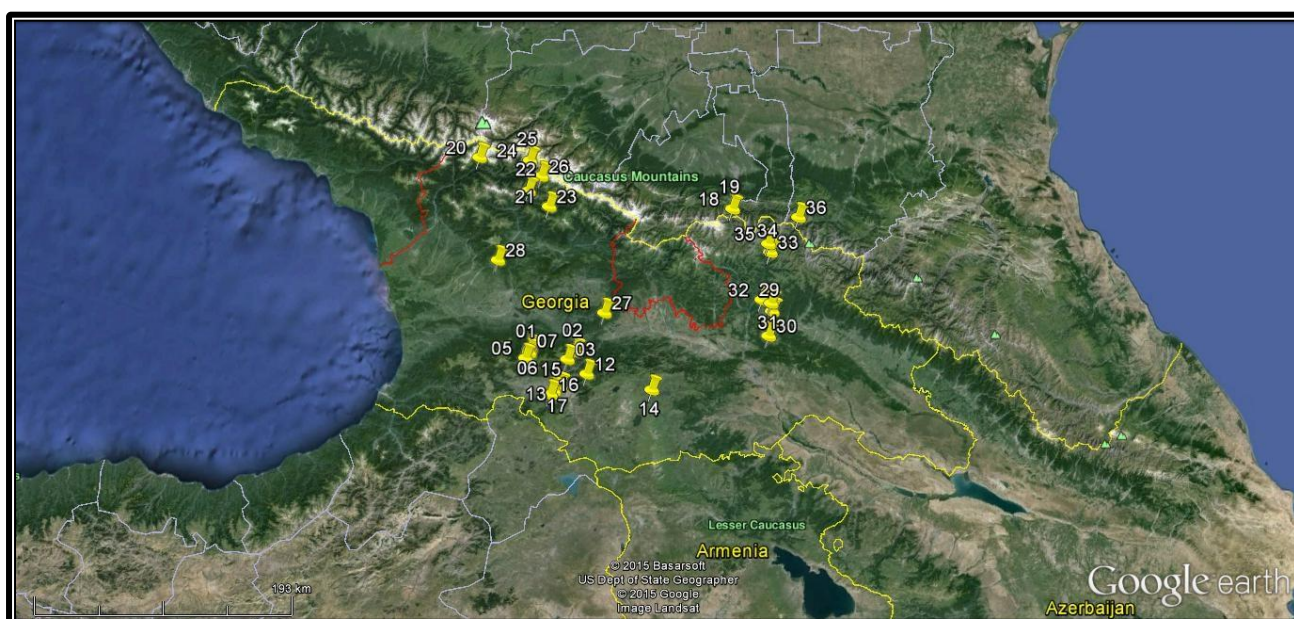
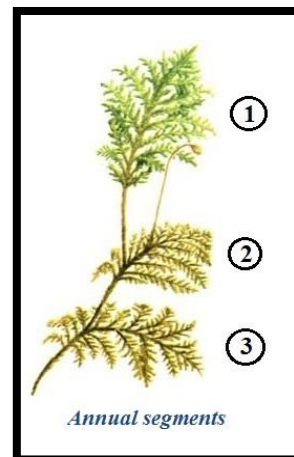


Fig.3 The sampling map

Sample Preparation

It must be remarked that instrumental neutron activation analysis (INAA) is non-destructive method. In contrast to the techniques such as atomic absorption (AA or AAS) or inductively coupled plasma-atomic emission spectroscopy (ICPAES) the samples don't request any chemical treatment, neither prior, nor after the activation. There is no fundamental necessity to convert solid material into a solution prior.

The preparation of 36 moss samples was performed in the chemical laboratory. In the case of instrumental determination, the preparation of samples involves only the preparation of representative ones. Each sample was cleaned from extraneous materials like soil particles, leaves, needles, etc. To prevent contamination of the material, plastic tweezers and disposable polyethylene gloves were used during all handling of the samples.





Only the green and green-brown shoots from the last three years growth were taken for analysis. After cleaning about 0.3 g of mosses were pelletized in press-forms. Then the samples were precisely weighed. Moss samples for short-term irradiation were heat-sealed in polyethylene foil bags, while the samples for long-term irradiation were packed in aluminum cups.

Irradiation Facilities and Analysis

Next step was performed in the experimental setup called REGATA for INAA at the reactor IBR- 2.

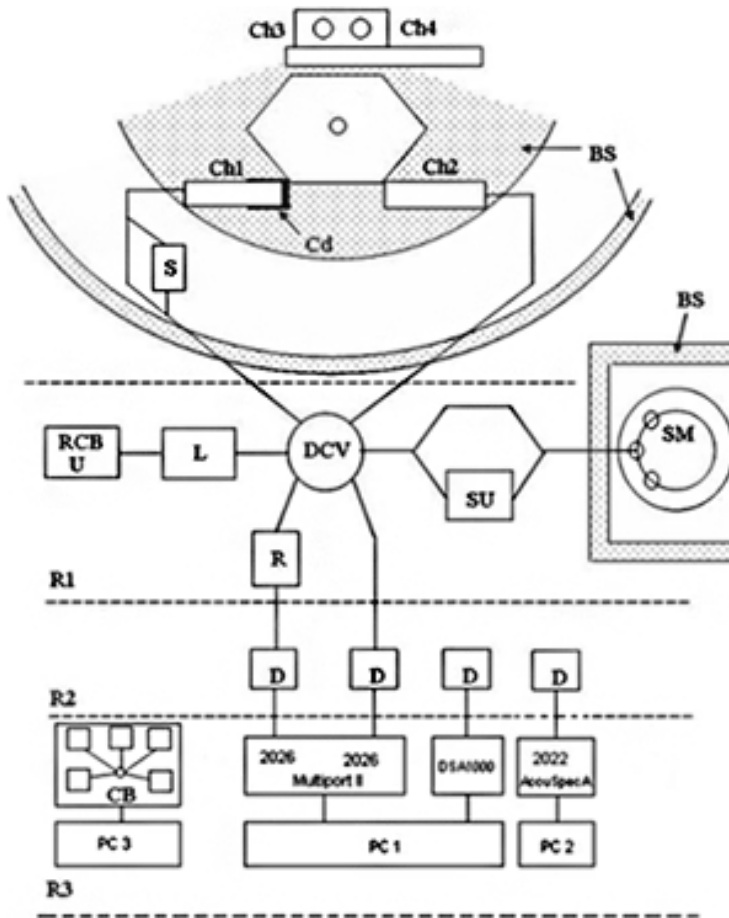


Fig.4 The scheme of the REGATA experimental setup :
 Ch1–Ch4—irradiation channels, S—intermediate storage, DCV—directional control valves, L—loading unit, RCB—radiochemical glove-cell, U—unloading unit, SU – separate unit, SM—storage magazine, R—repacking unit, BS—biological shield, D—

The IBR-2 is a pulsed fast reactor with PuO₂ fuel elements. The uniqueness of the reactor is in its reactivity modulator. It is a complex mechanical system, which consists of two rotating parts - a main movable reflector (OPO, at 1500 rpm) and an auxiliary movable reflector (DPO, at 300 rpm) that create reactivity pulses. These reflectors rotate in opposite directions. Only every fifth pulse is positive. The exact moment when both reflectors approach the reactor core, intense pulse of neutron flux is produced: $\sim 10^{16}$ n/cm²/s, with a power of 1850 MW in pulse.

The facility REGATA consists of four channels for irradiation (Ch1-Ch4), the pneumatic transport system (PTS) and three gamma-spectrometers located at three special rooms on the ground floor of the reactor IBR-2 (Fig.4). The channels Ch1, Ch2 are cooled by air, while the channels Ch3, Ch4 are cooled by water. Air-cooled channels (Ch1 and Ch2) are connected to the pneumatic system REGATA, which provides the transportation of the containers by compressed air (at 3-6 atm. pressure).

Table 1 Main characteristics of the irradiation channels at 1.5 MW

Irradiation site	Neutron flux density (n/cm ² s) 10 ¹²			T ⁰ C	Channel diam., mm	Channel length, mm
	Thermal	Resonance	Fast			
Ch1	Cd-coated	3.31	4.32	70	28	260
Ch2	1.23	2.96	4.1	60	28	260
Ch3	Gd-coated	7.5	7.7	30-40	30	400
Ch4	4.2	7.6	7.7	30-40	30	400

Transport containers are in two types: polyethylene for short-term irradiation and aluminum container for long-term irradiation (Fig.5). In polyethylene containers samples can be irradiated up to 30 minutes, in aluminum for over 4 hours. The internal volume of the polyethylene container is equal to approximately 4 cm³, and the volume of the aluminum container is 1.5 times larger.



Fig.5 Transport containers for irradiation

PTS has loading (L) and unloading (U) units to load and to extract containers from the system. All devices of the pneumatic system are equipped with photosensors for indication of the container position in the system and for correct operation of all mechanisms. The polyethylene containers are transported to irradiation site

for 7-20 s, the aluminum containers flight faster - (3-7) s. Acoustic detectors placed behind the first ring of a biological shield, allow one to determine the time of a container arrival and departure accurately. The knock of the capsule at the bottom of the channel forms the last clearing pulse tracked by acoustic detectors. After this pulse, the irradiation timer starts counting the time of irradiation.

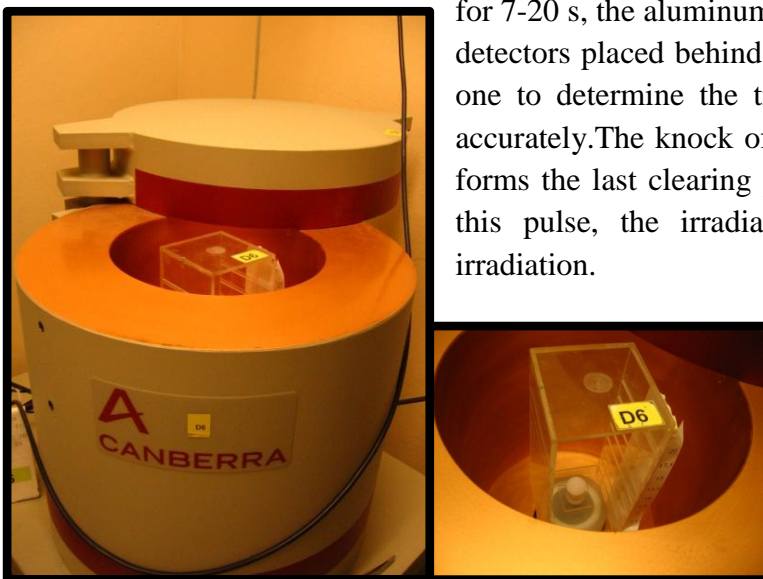


Fig.6 High-purity Germanium Detector

The time of irradiation depends upon the species of interest. A number of elements have more than one isotope which can be activated by neutrons. Each activation product has its own cross-section, isotopic abundance and decay scheme. To optimize the

procedure, an appropriate nuclear reaction must be selected.

In nuclear reactor the neutron flux cannot be freely changed, but there are some possibilities to choose. The effective cross-section of a nucleus depends on the energy of the bombarding particles.

On IBR2 the irradiation channels Ch1 and Ch2 are the same, but Ch1 has a Cadmium thermal neutron filter to reduce the thermal neutron fraction in the irradiation facility, thus, increase the average neutron energy. This channel is used to determine elements associated with long-lived radionuclides (Na, Sc, Cr, Fe, Co, Ni, Zn, As, Se, Rb, Mo, Sb, Cs, W, Th, U). Usually samples are irradiated for 100 hours in the cadmium-screened Ch1, while to determine short-lived radionuclides (Cl, Ca, V, Mn) samples are irradiated for 3 minutes in the Ch2.

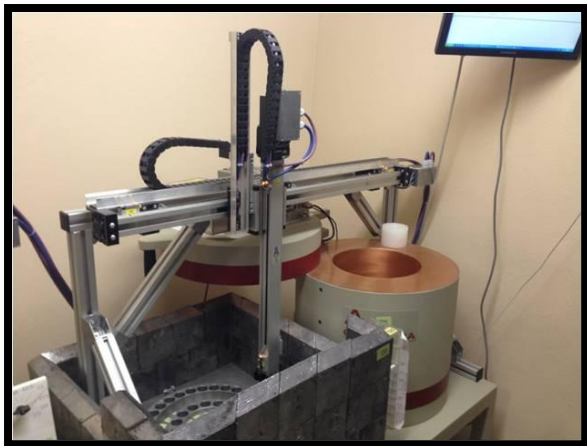


Fig.7 Sample changer.

Automation system for measurement of gamma-ray spectra of induced activity is used to conduct analysis of large sets of samples, to process large amounts of data, to increase productivity of analysis, to improve quality of results, to decrease the number of human errors, and to minimize human involvement in routine long-term measurements of spectra [10]. The system consist of three high-purity Ge detectors (Fig. 6), three sample changers and control software. Each sample changer has 45 slots for containers with samples (Fig.8). All the information required for processing of the spectra is read automatically from the NAA database. The program automatically finds the necessary energy and efficiency calibration files for the selected position of a sample above the detector and records the calibration in the spectra. The special spring-pressed grab is used to capture the container, to move to the detector and to hold above it in one of the three fixed height positions during the measurement of the spectrum. The program automatically finds the necessary energy and efficiency calibration files for the selected position of a sample and records the calibration in the spectra. After completion of the measurement, all information is automatically saved in NAA database and the container is returned to the same location of the disk which then rotates for selection of the next sample.



Fig.8 Disk with 45 slots for containers

Personal computer controls the pneumatic transport system and amplitude spectrometers. All units of gamma-spectrometers and counting electronics are made in CAMAC at JINR. The gamma ray spectra from samples are evaluated in order to find out energies and intensities of the γ lines, to identify the radionuclides, to calculate theirs decay rates, the last one is used as the basis for determining the amount of elements present in the sample. The software for peak search, peak fitting and nuclide identification routines is developed at FLNP JINR [11].

Results

The preliminary results show that in some samples a remarkable concentration of the light and heavy crust elements is observed. The most obvious explanation of this phenomenon is that the content of mineral soil particles is physically captured on the moss surface, which may be released by wind erosion, or other pollutant.

The concentrations of determined elements are shown in table 2. The accuracy varies from 1% to 15%, but in iodine it is approximately 30% that can be explained by low concentration of this element in the samples.

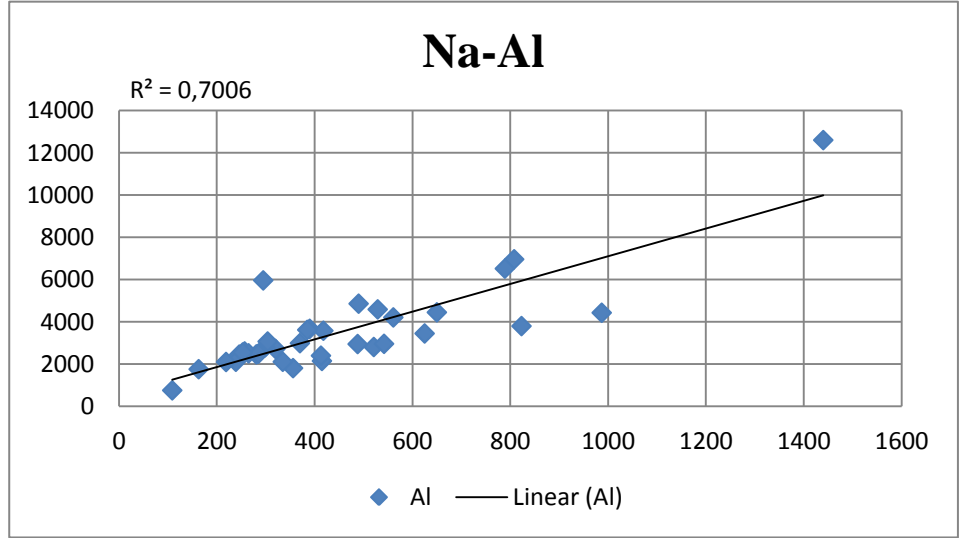
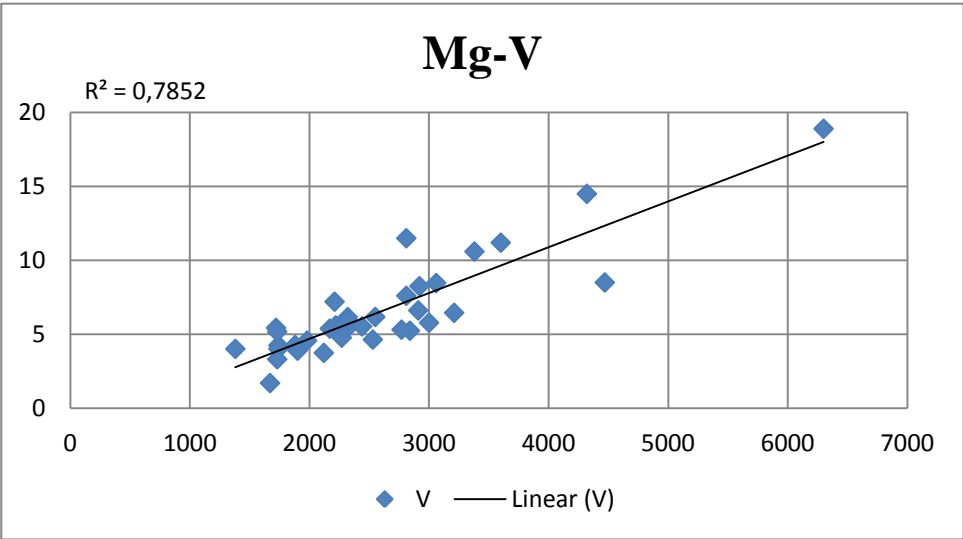
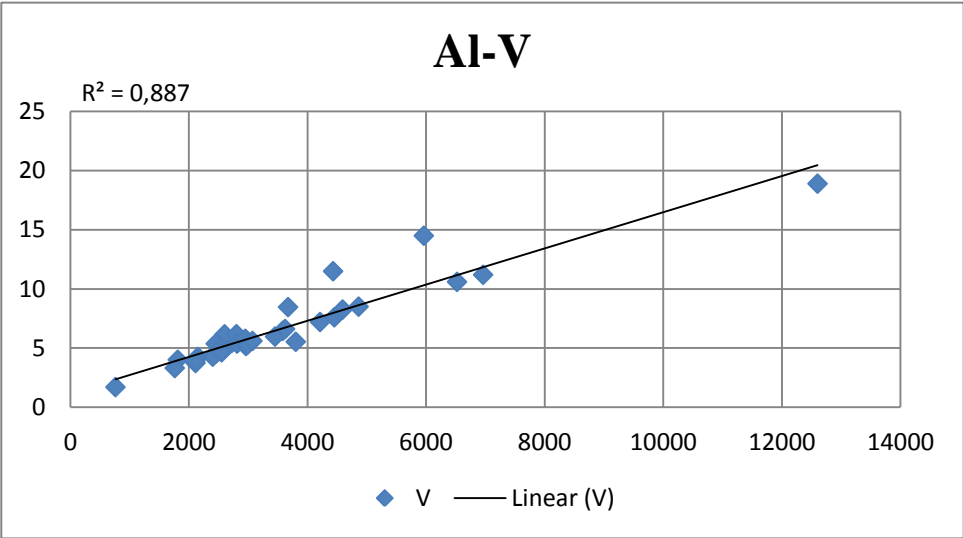
Table 2 Coordinates and element concentrations (mg/kg) of each sample.

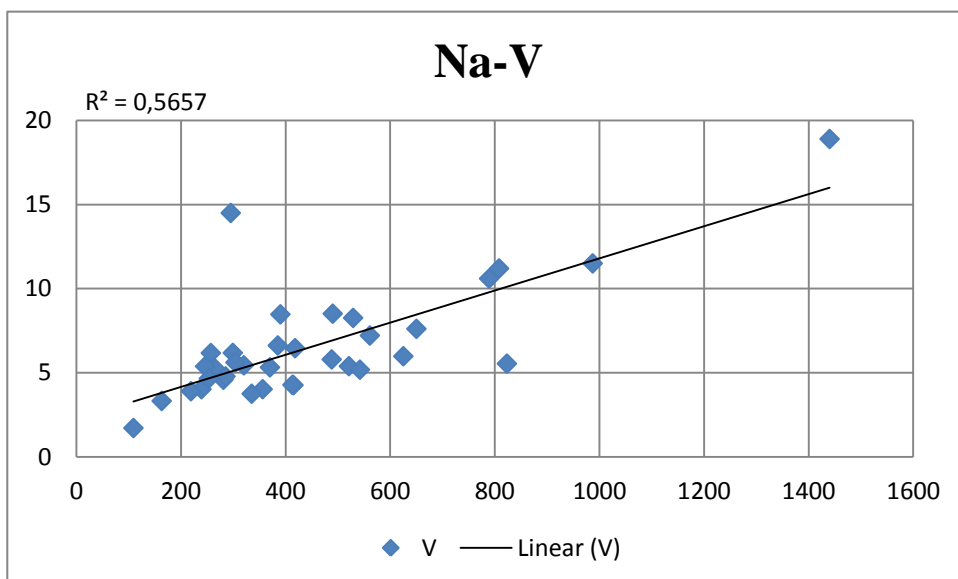
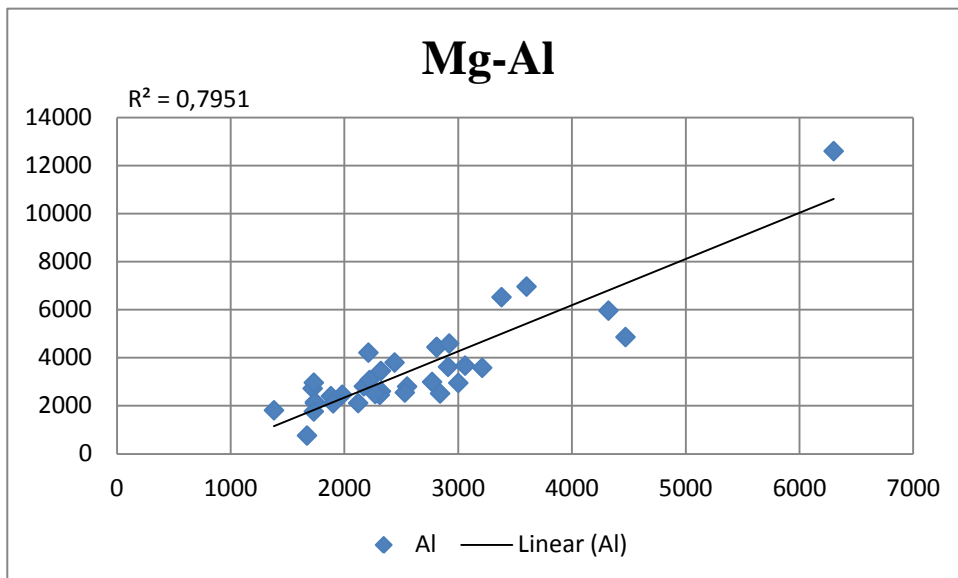
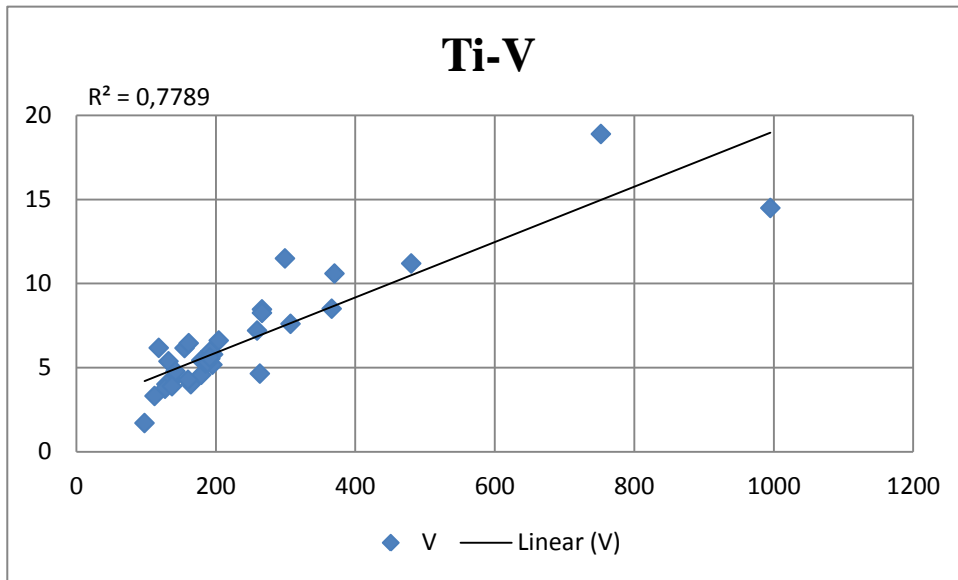
Sample	Latitude	Longitude	Altitude	Na	Mg	Al	Cl	Ca	Ti	V	Mn	I
1-01	41°47'35.70"N	42°50'36.70"E	1661	390	3060	3670	110	7020	266	8,47	70,8	0,916
1-02	41°48'6.70"N	43°14'25.40"E	1082	320	1720	2720	237	6110	179	5,44	62	1,38
1-03	41°45'13.80"N	43°10'30.30"E	1041	415	1740	2150	325	4880	137	4,26	89,1	0,961
1-04	41°45'13.80"N	43°10'30.30"E	1041	987	2810	4430	305	5660	299	11,5	111	0,686
1-05	41°45'29.40"N	42°48'42.20"E	1603	281	1980	2460	129	6540	179	4,58	108	0,898
1-06	41°49'0.50"N	42°51'4.90"E	1977	257	2320	2600	181	5370	155	6,17	186	1,63
1-07	41°48'17.60"N	42°50'45.30"E	1815	109	1670	759	158	5050	97,6	1,71	21,7	0,577
1-08	41°49'18.20"N	42°51'3.80"E	2052	299	2550	2800	204	6720	118	6,18	171	2,14
1-09	41°49'18.20"N	42°51'3.80"E	2052	265	2840	2510	244	5930	185	5,25	113	1,39
1-10	41°49'0.50"N	42°51'4.90"E	1977	246	2310	2450	149	5170	132	5,38	99,3	1,63
1-11	41°48'6.70"N	43°14'25.40"E	1082	239	1740	2110	122	5460	164	4,02	92,3	1,39
1-12	41°39'35.70"N	43°20'31.30"E	1885	285	2270	2490	113	5660	142	4,78	229	1,82
1-13	41°34'36.90"N	43°6'43.30"E	1341	521	2170	2810	333	5880	184	5,39	55,7	1,2
1-14	41°33'37.80"N	43°54'8.10"E	1783	650	2810	4450	328	10900	307	7,61	92,5	1,4
1-15	41°32'55.20"N	43°3'31.00"E	1220	356	1380	1810	57,3	8170	129	4,02	43,7	0,959
1-16	41°31'33.70"N	43°1'52.00"E	1305	823	2440	3800	118	6100	192	5,54	115	1,21
1-17	41°34'36.90"N	43°6'43.30"E	1341	488	3000	2950	184	8330	196	5,79	78,7	1,41
1-18	42°43'17.10"N	44°36'36.10"E	1648	413	1880	2400	99,5	4890	160	4,28	76,5	1,15
1-19	42°43'29.60"N	44°36'19.00"E	1700	542	1730	2960	133	6660	195	5,18	49,1	1,31
1-21	42°44'7.60"N	43°0'3.10"E	2487	335	2120	2110	295	5170	127	3,75	110	0,766
1-22	42°50'1.80"N	42°48'58.90"E	930	1440	6300	12600	251	10200	752	18,9	350	1,53
1-23	42°44'7.60"N	43°0'3.10"E	2487	808	3600	6960	118	7860	480	11,2	197	1,05
1-24	43°1'52.40"N	42°49'37.50"E	1920	529	2920	4590	182	5560	266	8,26	246	1,03
1-25	43°1'52.40"N	42°49'37.50"E	1920	418	3210	3580	101	5460	161	6,46	393	1,56
1-26	42°55'49.50"N	42°55'44.50"E	1764	789	3380	6520	121	6520	370	10,6	96,7	0,745
1-27	42°3'12.20"N	43°29'31.10"E	980	625	2320	3450	210	6070	192	5,98	195	2,79
1-28	42°23'9.10"N	42°33'32.50"E	161	295	4320	5960	183	8880	995	14,5	118	2,04
1-29	42°3'37.10"N	44°56'42.60"E	1300	253	2530	2550	83,5	8040	263	4,65	124	1,18
1-30	41°54'9.20"N	44°54'45.10"E	1430	370	2770	2990	99,6	6940	188	5,32	142	1,69
1-31	42°6'43.10"N	44°56'10.20"E	1173	561	2210	4210	136	6400	259	7,21	86,4	2,57
1-32	42°8'5.50"N	44°51'24.40"E	1355	490	4470	4860	90,6	9070	366	8,51	525	1,44
1-33	42°26'24.90"N	44°56'7.30"E	1228	163	1730	1760	164	9230	112	3,32	74,2	0,954
1-34	42°26'24.90"N	44°56'7.30"E	1228	219	1900	2100	116	8880	137	3,9	79,4	1,27
1-35	42°29'40.60"N	44°55'9.70"E	1340	385	2910	3620	150	9250	204	6,62	158	1,52
1-36	42°39'47.30"N	45°11'11.11"E	1378	304	2220	3070	74,9	7480	191	5,61	47,3	1,18

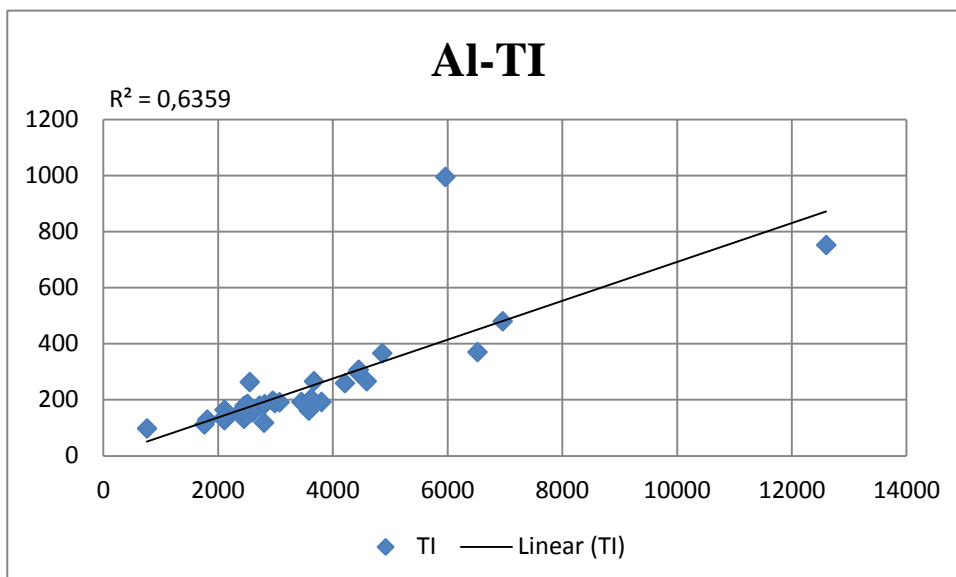
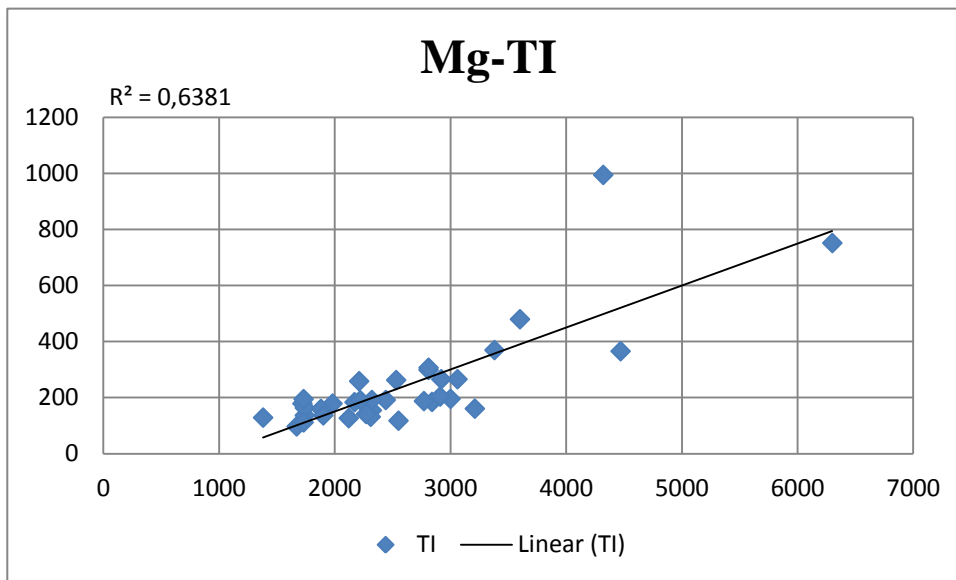
Data were subjected to simple statistical analysis in order to explore the possible associations existing between different variables (Table 3). The correlation matrix shows that most metals are well-correlated. The most noticeable positive correlations are between Al and V ($r=0.94$); Mg and V ($r=0.89$); Ti and Al ($r=0.89$); Ti and V ($r=0.88$). There are also significant correlations between Na and Al ($r=0.84$); Mg and Ti ($r=0.80$); Al and Ti ($r=0.80$); Na and V ($r=0.75$).

Table 3 A correlation matrix for the elements.

	Na	Mg	Al	Cl	Ca	Ti	V	Mn	I
Na	1,00								
Mg	0,63	1,00							
Al	0,84	0,89	1,00						
Cl	0,28	0,07	0,12	1,00					
Ca	0,24	0,49	0,45	-0,06	1,00				
Ti	0,50	0,80	0,80	0,09	0,48	1,00			
V	0,75	0,89	0,94	0,18	0,41	0,88	1,00		
Mn	0,32	0,66	0,48	-0,12	0,18	0,30	0,43	1,00	
I	0,00	0,16	0,13	-0,04	0,09	0,17	0,14	0,27	1,00







Conclusion

The results obtained in this report are only a part of the study of atmospheric deposition of major and trace elements in Georgia based on moss analysis. All results of NAA of the moss samples in this investigation will be delivered in February 2016 at The 29th Task Force Meeting of the UNECE ICP Vegetation to be held in Dubna.

Such kind of studies is very important from the environmental and social point of views. They serve for assessing air pollution in Georgia and close one more “white spot” in the map of Europe. My involvement in this study is very important to me, not only as a biologist, but as a citizen.

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