



JOINT INSTITUTE FOR NUCLEAR RESEARCH
Frank Laboratory of Neutron Physics

FINAL REPORT ON THE SUMMER STUDENT PROGRAM

**Application of modern physico-chemical methods of
analysis (AAS, INAA) to determine the accumulative
ability of sorghum bicolor**

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Abstract

The metal accumulation capacity of Sorghum bicolor sorts Sucro and Biomas was determined using modern physicochemical methods of analysis: atomic absorption spectroscopy (AAS) and neutron activation analysis (NAA). Accumulation experiments were conducted using the soils collected in the sanitary protection zones of metallurgical enterprises and near the central highway of the city of Tula with geochemical anomalies caused by a complex pollution with heavy metals (HM).

High concentrations of Pb and Cd have been determined in the root system of sorghum bicolor Sucro growth on the soils collected near Tulachermet and Kosogorsk Metallurgical Plant (17.14 and 18.01 mg/kg for Pb and 1.48 and 2.09 mg/kg for Cd). Sorghum bicolor Biomas growth on the soils collected near the Kosogorsk Metallurgical Plant showed the high concentration of Pb (16.60 mg/kg). Both studied sorts are promising for research of bioaccumulative capability with the subsequent recommendation of their introduction to urban ecosystems with the purposes of phytoremediation.

Introduction

Literature review

Throughout its history until today, the Tula region remains one of the most industrially and agriculturally developed regions in Russia. The Tula region is characterized by a crisis ecological situation. Intensive industrial development has led to many undesirable consequences - pollution of air, water and soil, disruption of water regime. The natural environment of the region carries a huge anthropogenic load as a result of the activities of 473 enterprises of various economic complexes: machine-building, chemical, metallurgical, fuel and energy, mining, road-transport, agricultural, etc. (Yermakova E.V., 2002). Each year, about 165,000 tons of harmful substances enters to the atmosphere of the Tula region. The largest share of these emissions (90%) is accounted for by industrial enterprises. Stationary sources of air pollution are mainly located in Tula: metallurgy (Tulachermet, Kosogorsky Metallurgical Works), engineering and defense industry (KBP, Tulamashzavod, Tula Weapons Plant) (http://www.dishisvobodno.ru/eco_tula_obl.html)

Pollutants released into the air are transported by air masses and accumulate over time in the soil. Industrial emissions are not the only source of pollution. A significant share in soil pollution is made by motor transport emissions, violation of mineral extraction technology, unbalanced use of mineral fertilizers, herbicides and pesticides on agricultural fields, excessive recreational impact on urban soils, which leads to a deterioration in water-physical properties and loss of fertility, making a significant contribution to soil pollution. As a result, soils experiencing intense anthropogenic impact cease to fulfill their ecological functions. One of the most important indicators of anthropogenic pollution of soils is the accumulation of heavy metals. The available data on soil pollution in the region are scarce and indicate on high pollution of the soils of the Suvorov, Shchekino, Novomoskovsk and Lenin's regions of Tula, which correlates with the development of industry. However, there is no information on the content of HM in different ecosystems, both naturally (forest, steppe, meadow) and man-made (urban environment, deposits, etc.). It is not possible to reliably estimate the intensity of accumulation of HM by different types of soils and ecosystems, as well as to identify the role of the anthropogenic factor in this process. (Arlyapov V.A., Volkova E.M., 2015). In this regard, it is necessary to introduce methods for soil bioremediation.

Plants are natural biofilters, absorbing various compounds from the air and water environment, soil solution and from the surface of soil particles. One of the factors affecting plants is the absorption of heavy metals, which is often an indicator of anthropogenic impact. At the same time, the accumulation of heavy metals in plants can be considered as an indicator of the state of the environment (Volkova E.M., Gorelova S.V., 2012)

One of the most effective methods for removal of heavy metals from soils is phytoremediation. The restoration of the environment using plants gain interest due to the possibilities of this technology opens up when cleaning contaminated areas. Over the past ten years, phytoremediation has become very popular, due to its low cost. Since in the process of phytoremediation only the energy of the sun is used, this technology is much cheaper than technologies such as excavation, washing and burning of the soil. Since phytoremediation is used directly in the area of pollution contributes to reducing costs and reducing the contact of the contaminated substrate with people and the environment (Bryuzgina A.L., 2013)

To determine the accumulative ability of sorghum bicolor and oilseeds sunflower grown on soils contaminated with heavy metals, modern physical and chemical methods of analysis are

used. The most effective are considered atomic absorption spectrometry (AAS) and instrumental neutron activation analysis (INAA).

Objective of the study: use of modern physico-chemical methods of analysis (AAS, INAA) to determine the accumulative ability of sorghum bicolor.

Tasks:

1. Soils sampling
2. Performing experiments with varieties of sorghum and sunflower on soils contaminated with heavy metals
2. Sample preparation for AAS and INAA
3. Samples analysis using AAS
4. The results interpretation

Relevance of the topic:

Atomic absorption spectrometry (AAS) is based on measuring the absorption of resonant radiation by free atoms in the gas phase in a relatively short time. Having arisen more than half a century ago, the AAS reached a very widespread analysis of the elemental composition of a substance in analytical practice. AAS is distinguished by high selectivity, sensitivity, express. Also, the advantage of AAS is its versatility with respect to the most diverse objects of analysis, as well as the possibility of using it both for determining traces, and reliable and accurate determination of the main components in samples of complex compositions. (S.N. Chegrintsev, 2014)

Neutron activation analysis (NAA) is one of the most advanced analytical methods widely used in geology, biology, medicine, ecology and materials science, both for scientific research and for applied purposes. The development of the instrumental neutron activation analysis method (INAA) and the expansion of its application is largely related to the development of reactor technology.

Neutron activation analysis on reactor neutrons is characterized, above all, by the stability of the analysis conditions and the possibility of conducting mass measurements during large-scale studies. At the same time, they provide a high neutron flux density and the possibility of their use in the entire energy spectrum (thermal, resonant and fast), as a result of which they significantly improve the selectivity, sensitivity and accuracy of NAA.

NAA is a non-destructive method of analysis, when it is carried out there is no need for chemical dissolution of the sample, which allows to avoid losses and ensure high accuracy of the analysis. The method makes it possible to determine the concentration of 40-45 elements simultaneously, has high sensitivity, selectivity and performance. (Dmitriev A.Yu., 2015)

As part of international research programs in Sector of Neutron Activation Analysis and Applied Research, FLNP, JINR is performed analysis of a large number of samples, which requires marking, storing and accounting for the analyzed samples, measuring and processing spectra, as well as systematization of the analysis results. (Frontasyeva M.V., 2016)

Sampling

Sample preparation

To determine the accumulation of HM by sorghum bicolor variety Sucro and Biomas the soils were collected in the sanitary protection zones of metallurgical enterprises and near the central highway of the city of Tula. Materials used for sampling: garden tools, paper, running and distilled water, paper envelopes, a pencil.

Plants were carefully removed from the pots without damaging the root system. Roots were separated from the soil. The root system and shoots were washed with running water, then twice with distilled water and dry thoroughly. After underground and above-ground parts of the plants were separated from each other, put in envelopes corresponding to their options study and signed (the name of the plant and the organ, the sampling point, the date). Then samples were fried in the oven at 40 ° C to constant weight. In total 16 samples were obtained for analysis.

Work with the Database

Information about samples: sample number, sample type, longitude and latitude, collection point, designated elements, note , was introduces in the Samples_Info-2.0.1 and the client information files (Figures 1,2).

Information about the client should contain: country, zip code, city, street, house, organization, address, gender, initials, full name, country code, telephone, email. mail, note. (Figure 2.) After the samples are entered into the database, a batch of samples is assigned an individual number, for example, Ru - 47-19-56-d.

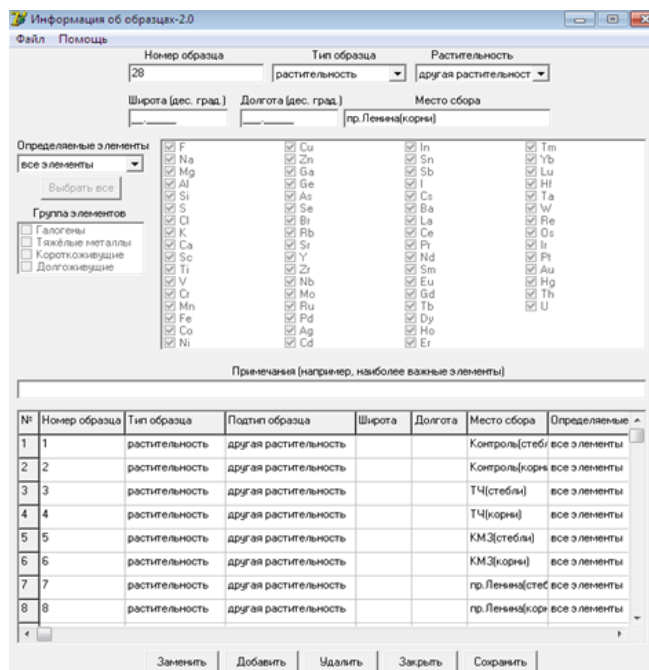


Figure 1. Samples_Info-2.0.1

Figure 2. Client_Info-1.3

Various analytical methods can be used to determine heavy metals, but previous research has shown that the following methods are most suitable for this purpose:

- 1) AAS (atomic absorption spectroscopy)
- 2) ICP-ES / MS (mass spectroscopy with inductively coupled plasma emission)
- 3) INAA (instrumental neutron activation analysis).

Atomic Absorption Spectroscopy (AAS)

Conducting AAS

Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in a gaseous state. Atomic absorption spectroscopy was first used as an analytical technique and the fundamental principles were established in the second half of the nineteenth century by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff. (University of Heidelberg, Germany).

AAS principle

Atomic absorption spectroscopy is based on the principle that when a beam of electromagnetic radiation is transmitted through a substance, the radiation can either be absorbed or transmitted, depending on the wavelength of the radiation. Absorption of radiation will lead to an increase in the energy of the molecule. The energy produced by the molecule is directly proportional to the wavelength of the radiation. Increasing the energy of a molecule leads to electronic excitations, where electrons transfer to higher energy levels. The specific wavelength that a given molecule can absorb depends on changes in vibrational, rotational, or electronic states.

Flameless atomizers

In AAS, flame is the most common means of atomizing a sample.

However, despite the simplicity of the definitions and good metrological characteristics, the flame as an atomizer does not always satisfy the requirements for the definition of traces; not enough high sensitivity, the need for more economical consumption of samples, the occurrence of side reactions and small, approx. 0.001 s, time of stay.

Particles in the analytical zone were the impetus for the development of electrothermal atomizers (ETA) as an alternative to the flame.

Sample preparation

Used equipment:

1. Homogenizer (FRITSCH pulverisette)
2. Mineralizer (MARS6).

Precautions must be taken to avoid contamination of the samples, both on the lab tables and on smoke particles. Work with materials should be carried out on clean laboratory paper, glass or polyethylene. Be sure to use talc-free polyethylene gloves. When working with samples it is prohibited to use metal tools.

Plant material samples are first ground in a homogenizer (FRITSCH pulverisette) until a homogeneous mass is formed. Grinding samples occurs at 400 rpm, you can also set the time and repetition. For homogenization, an agate glass with six agate balls is used. The agate glass is placed in a homogenizer and fix it. Then the samples are packaged in sachets and signed. Then they are weighed on an analytical balance of 0.2 grams, the mass is recorded with an accuracy of four decimal places. Samples poured into teflon glasses. 3 ml of 70% HNO₃ (conc) and 2 ml of 30% H₂O₂ are added. It is necessary to carry out a primary reaction in the open air so that the organic matter is dissolved, and CO₂ is evaporated. Then the mixture is stirred in a glass with a glass rod, closed and put in a mineralizer (MARS6). The samples are mineralized according to a special program:

Stages of mineralization

1. The first stage takes 10-15 minutes, the device picks up the temperature and pressure.
2. The second stage lasting 10 minutes, at a given temperature and pressure is the decomposition of organic substances.
3. The third stage of cooling, comes at a temperature of 160 ° C, cooling goes to 70 ° C.

Then open the containers and wait until they cool to room temperature. The contents of the teflon glasses are filtered through a red tape filter 3 times, transferred into 50 ml volumetric flasks and brought to the mark with distilled water.

AAS technique

Device used for AAS:

Thermoanalytical spectrometer Thermo Scientific iCE 3000.

The spectrometer provides unsurpassed metrological characteristics, and provides a number of advantages over instruments from other manufacturers:

- profitability (gas consumption is 1.5-2 times lower);
- an efficient spraying system can significantly reduce sample consumption;
- Stockdale's double-beam optical design provides high sensitivity and stability;
- improved burner design eliminates the problem of ash deposits on the gap;
- fast change of atomizers;
- automatic optimization and tuning of optics;

- video surveillance system for the processes occurring inside the cuvette of an electrothermal atomizer;

- compact and light weight.

<http://www.bvr.by/kscms/uploads/editor/file/ice3000.pdf>

The method of determining the elements of the AAS method:

1. Turn on the device, turn on the cooling unit and wait 30 minutes to heat the graphite furnace to 3000 ° C. In a graphite furnace, there is a plate of graphite, in order to increase the combustion of graphite, it is blown by a stream of inert argon gas.

2. Then the cuvette must be rinsed and blown 3 times and burned. The device is fully automated, all commands are executed on the computer using the Thermo SOLAAR program. The sample number, weight, and dilution should be entered into the computer.

3. To measure each element has its own lamp, (in the maintenance journal of the lamps they look at how long it takes to warm up a particular lamp).

4. Then prepare the standard (used standards of the company Fluka with a known concentration of 1 g/l for each element). The standard is diluted 10 times, getting 100 mg/l, then 100 times getting 1 mg/l and another 100 times getting 100 µg/l. The final dilution is the initial solution (head standard), according to which the calibration graph will be constructed. The calibration graph is constructed in the coordinates “Optical density” and “Concentration of the element being determined in the reference solution”.

5. After sample preparation, an aliquot is taken from the liquid sample into small Teflon containers and placed in the working part of the spectrometer. Also, a dilution solution (distilled water) and a calibration form (deionized water) are placed in the working section. After that, determine the optical density in the analysis of the identified samples according to the calibration graph.

6. Get a table with the content of elements (the device gives the results taking into account the dilutions).

Neutron Activation Analysis (NAA)

Physical basis of neutron activation analysis

Neutron activation analysis (NAA) - activation of elements during neutron irradiation is one of the leading methods of activation analysis.

NAA allows determining the following range of elements: Ag, Al, As, Au, B, Br, Ca, Cd, Ce, Cl, Cr, Cs, Cu, Dy, Eu, Fe, Ga, Hf, Hg, I, Ir, K, Li, Mg, Mn, Zn, Mo, Na, Ni, Np, Os, Pt, P, Pu, Rb, Sb, Se, Sn, Ta, Te, Th, W, V, U. At the same time, the analysis error in most practical cases range from 1 to 10 percent, and the detection limit is from hundreds of nanograms (100×10^{-9}) to picogram units (1×10^{-12}). (Kuznetsov R.A., Atomizdat, M., 1967), (Aleksandrovsky V.B., 1988)

The advantages of neutron activation analysis over other methods of chemical analysis of environmental samples

Characteristic indicators of analytical methods that have special priority in chemical analysis in general and in the analysis of environmental objects in particular are:

1. The lower limit of the determined contents or the detection limit (at present the required level is $n \times 10^{-5}$ %).

2. Error, correctness (at the level of a few units of percent).
3. Selectivity.
4. Minimum (of the order of $n \times 10^{-3}$ g) sample mass (a particularly important indicator in the analysis of unique samples).
5. Instrumentalization and relatively simple possibility of automating the stages of analysis and the method as a whole.

The prevalence of NAA methods, especially using reactor neutrons, is associated with a number of advantages. Among them are the main ones:

- Affordable way to get high neutron fluxes
- Simple procedure for conducting sample irradiation and analysis.
- High selectivity of determination at low detection limits of elements in low mass (and quite often) of an unbreakable sample. (Pilipenko A.T., Pyatnitsky I.V., 1988)

Sample preparation for irradiation

1. Preparation of samples for irradiation is carried out in a specially equipped chemical laboratory using spatulas and tweezers made of polymeric materials in order to avoid the entry of foreign contaminants. In most cases, samples are ground, homogenized and dried at a certain temperature to constant weight. For each type of sample material has its own rules of sample preparation. Plant samples are weighed on an analytical balance, the sample mass is 0.3 g. Then the samples are placed in special molds. Forms with a sample are placed under a press, where they under pressure take the form of tablets, then are re-weighed on an analytical balance with an accuracy of four decimal places and are packed twice in plastic bags and signed according to the sample number.

2. The prepared sample is placed in containers for irradiation. The materials used for them are polyethylene and aluminum. The internal volume of the container is about 5 cm^3 with an outer diameter of 26 mm. Aluminum containers are similar in size to polyethylene, but they have a larger useful volume. Any of these materials has a low absorption cross section for thermal neutrons and is resistant to radiation, although each material has its own characteristics (Nazarov V.M., Frontasyeva M.V., 1982)

Irradiation process at the IBR-2M reactor

Work on the irradiation of the studied samples in the Laboratory of Neutron Physics the JINR Frank Institute is held at the IBR-2 high-flow pulsed fast reactor on the REGATA pneumotransport unit (PTU). Samples analysis will be performed in the period October-December 2019.

Actual results

Table 1- Accumulative ability of the studied plants in relation to lead and cadmium

Experimental variant	Sampling point	Plant part	Pb	Cd
			concentration	
			mg / kg	mg / kg
Sorghum bicolor Sucro	Control	sprout	0,87	0,14
		root	2,65	0,45
	Tulachermet	sprout	0,70	0,17
		root	17,14	1,48
	Kosogorsk Metallurgical Plant	sprout	1,24	0,18
		root	18,01	2,09
	Lenin Avenue	sprout	0,44	0,09
		root	8,48	0,33
Sorghum bicolor Biomas	Control	sprout	0,66	0,13
		root	7,17	0,37
	Tulachermet	sprout	1,82	0,05
		root	3,55	0,21
	Kosogorsk Metallurgical Plant	sprout	1,32	0,06
		root	16,60	0,49
	Lenin Avenue	sprout	0,49	0,11
		root	9,10	0,34

- 1) The obtained data on the content of Pb and Cd in the studied plants of sorghum bicolor Sucro and Biomas grown on soils show high concentration of HM concentration. According to the data obtained, the Pb content in the shoots of Sorghum bicolor Sucro varies from 0.44 to 1.24 mg/kg and in the roots from 2.65 to 18.01 mg / kg with the highest value for soils collected near KMP, Tulachermet and the Lenin Avenue. At the sampling points Tulachermet and Lenin Avenue, the significant accumulation of Pb was noticed, while the content of Cd was on the level of control. According to the data, the Cd content in the shoots of Sorghum bicolor Sucro ranges from 0.09 to 0.18 mg/kg and in the roots from 0.33 to 2.09 mg/kg, with the highest values for Tulachermet and KMP. High accumulation of Pb and Cd in the root system was observed for the sampling points of Tulachermet and KMP.
- 2) According to the data obtained, the Pb content in the shoots of Sorghum bicolor Biomas ranges from 0.49 to 1.82 mg / kg with the highest value for Tulachermet and in the root system from 3.55 to 16.60 mg / kg with the maximum value for KMP. The content of Cd in the shoots ranges from 0.05 to 0.13 mg / kg, in the root system from 0.21 to 0.49 mg / kg. It is suggested that, roots of Sorghum bicolor Sucro and Biomas are a barrier preventing penetration of lead and cadmium into the aboveground organs.

- 3) As part of the summer program, we conducted AAS samples for cadmium and lead, prepared samples for NAA. NAA results will be in October when the IBR-2M is launched.

References

1. Sharma R. K., Agrawal M. Biological effects of heavy metals: An overview // J. Environ. Biol. 2005. V. 26, N 3/4. P. 1–13.
2. Авцын А.П. Микроэлементозы человека / А.П. Авцын, А.А. Жаворонков, М.А. Риш, Л.С.Строчкова // - М.: Медицина. 1991. - 496 С.
3. Большаков В.А. Загрязнение почв и растительности тяжёлыми металлами / В.А Большаков, Н.Я.Гальпер, Г.А. Клименко, Т.И. Лыткина, Е.В. Башта // М., 1978, 52 с.. Sharma R. K., Agrawal M. Biological effects of heavy metals: An overview // J. Environ. Biol. 2005. V. 26, N 3/4. P. 1–13. Salt D.E, Blaylock M., Kumar N.P. e.a. Phytoremediation: A novel strategy for removal of toxic metals from environment using plants. Biotechnology, 1995, 13:468-474.
4. Ермакова, Е.В. Изучение атмосферных выпадения Тяжелых металлов и других элементов на территории Тульской области с помощью метода мхов-биомониторов / М.В. Фронтасьева, Э. Стейнес // журнал «Экология», Издательский отдел Объединенного института ядерных исследований, Дубна, ОИЯИ,- 2002.
http://www.dishisvobodno.ru/eco_tula_obl.html
5. Арляпов В.А., Волкова Е.М., Нечаева И.А., Скворцова Л.С. Содержание тяжелых металлов в почве как индикатор антропогенного загрязнения Тульской области //Известия Тульского государственного университета Естественные науки. 2015. Вып. 4. С. 194–204.
6. Волкова Е.М., Горелова С.В., Музафаров Е.Н. Биомониторинг антропогенного загрязнения Тульской области на основе анализа накопления тяжелых металлов в торфяных залежах болот // Известия Тульского государственного университета. Естественные науки. 2012. Вып. 2. С. 253–263.
7. Брюзгина А.Л. ФИТОРЕМЕДИАЦИЯ, КАК СПОСОБ ОЧИСТКИ ПОЧВ, ЗАГРЯЗНЕННЫХ CU, NI, ZN // Научное сообщество студентов XXI столетия. ЕСТЕСТВЕННЫЕ НАУКИ: сб. ст. по мат. XIII междунар. студ. науч.-практ. конф. № 13, 2013г.
8. Чегринцев С.Н. Атомно-абсорбционный анализ: методические указания к выполнению лабораторных работ по курсу «Физико-химические методы анализа» для студентов IV курса, обучающихся по направлению 240501 «Химическая технология материалов современной энергетики» / С.Н. Чегринцев. Томский политехнический университет. – Томск: Изд-во Томского политехнического университета, 2014. – 44 с.
9. Дмитриев, А.Ю. Разработка автоматизированного комплекса для массового многоэлементного нейтронно-активационного анализа на реакторе ИБР-2 ДНФ ОИЯИ // диссертация на соискание ученой степени кандидата физико-математических наук. – Дубна, 2015. – с.1.

10. Фронтасьева, М.В. Тяжелые металлы азот и стойкие органические загрязнители (СОЗ) в европейских мхах: одновременный сбор мхов в 2015 г. / Пособие по мониторингу. Издательский отдел Объединенного института ядерных исследований 141980, г.Дубна , Московская обл., ул. Жолио-Кюри, 6, -2016, -с.1-2.
11. <http://www.bvr.by/kscms/uploads/editor/file/ice3000.pdf>
12. Кузнецов Р. А. Активационный анализ, Атомиздат М., 1967, стр. 27.
13. Физико-химические методы анализа/Под ред. В.Б. Александровского. – Л.: Химия, 1988
14. Пилипенко А.Т., Пятницкий И.В. Аналитическая химия. Т. 1,2. – М.: Химия,1988
15. Назаров В.М., Фронтасьева М.В.. Перспективы использования ИБР-2 для аналитических целей. Ядерно-физические методы анализа в контроле окружающей среды, Труды II Всесоюзного совещания Рига, 20-22 апреля 1982 г.
16. Ananiev V.D. et al. Full power start-up of the IBR-2 reactor and the first physical experiments at its beams. *Atomnaya Energiya*, Vol. 57, No. 4, 1984, p. 227-234 (in Russian).

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