CHEMISTRY VS ECOLOGY

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> Dedicated to the memory of my father Grigore Duca and my mentor Alexei Sytchiov

> > "La vie – c'est un procédé chimique" Antoine-Laurent Lavoisier

"Chemistry versus Ecology" – this report title has not been chosen by chance. An average human and even sometimes a scientist is sure that Chemistry and Ecology are opposite to each other. But is it true indeed? To answer this deep question, first of all, the detailed definitions are to be given.

What is Chemistry? It is the science about chemical reactions, but also about the composition, structure and chemical properties of the substance. The aphorism given above proves chemistry to be a fundamentally important science, which studies the matter surrounding us. However, along with the very significant progressive constituent (new technologies and methods) chemistry has a strong negative impact that is manifested through the destruction of the environment by pollution. In addition, the fight between Chemistry and Ecology is considered inevitable, because



any chemical substance, when its maximum admissible concentration is reached, can become a matter of serious environmental concern. In this case, a question appears: how this problem is to be faced?

Ecology is the main scientific area dealing with the problem mentioned above. Everyone knows that it is ecology that detects and warns about the environmental problems. But what does Ecology mean? The definition is the following: ecology is a science, which studies the influence of living organisms to each other and their natural environment (by Ernst Haeckel). Ecology emerged as a distinct discipline at the turn of the 20th century; the relationship between a living organism and its environment had been explained in 1843 by German chemist Justus von Liebig, taking as an example the relations "plant-soil". However, the notion "ecology" is much older, originating from the era of ancient thinkers. The first "ecologist" has been considered Aristotle or perhaps his student, Theophrastus (the latter has described the interrelationships between animals and their environment as early as in the 4th century B.C.).

Nowadays, when practically all the scientific fields overlap with each other, many interdisciplinary areas of ecology appeared. It should be noted that the current difficult ecological situation caused the appearance of the following areas: geographical ecology, physics of the environment, mathematical ecology, genetic ecology, spiritual ecology and many others. Why do so many sciences contact with ecology? Probably because ecology examines the most important phenomenon - the interaction between living organisms and the environment they live in, this notion meaning to an even greater extent healthy lifestyle, a form of friendly relations, a process of purification, the actions, which are opposite to pollution. As soon as these problems can have different impacts, the more complex approach is needed.

Coming back to the topic of the apparent opposition of chemical and ecological studies, the attention should be focused on the sciences which appeared on their border. The most important of them are:

- Ecological Chemistry that studies processes influencing formation of environmental chemical composition, adequate for the biological value of habitation.
- Chemical Ecology that is concerned with the study of chemical substances involved in the interactions of different living organisms.
- Environmental Chemistry that studies chemical and biochemical phenomena that occur in the environment.
- Green Chemistry that promotes the development of the products and processes that minimize the use and generation of hazardous chemicals.

Ecological chemistry is developing quite intensively in recent years at the scientific and educational level. The worldwide known Universities and even colleges had set up the departments to educate the young experts in Ecological Chemistry. Many institutions in the USA, Canada, Germany, Sweden, Norway, Poland, Russia, Kazakhstan, Ukraine, Belarus, China, South Africa and many other countries are based on chemical knowledge as the main scientific priority in solving countries' environmental problems.

A prodigious scientific activity in ecological chemistry has starter about three decades ago. Then, in the 80s of the past century, on the border between chemistry and ecology a new direction called ecological chemistry has emerged. It came to reveal the chemical aspect of environment interaction with living organisms. The ecological chemistry has found supporters and promoters in different countries. This fact led us to organize in 1985 the First International Seminar on ecological chemistry and Chemical Technology of Moldova State University, being the first one in Moldova specialized in ecological chemistry and environmental protection, which began to prepare doctoral students in the Environmental Protection and Rational Use of Natural Resources areas.

The first textbook on ecological chemistry was edited in 1994 and subsequently translated in three languages. In 1991, the Research Centre of Applied and Ecological Chemistry (RCAEC) was established, comprising four scientific laboratories. The research performed at the department, at the RCAEC's laboratories, was dealing with the chemistry and technology of industrial processes and treatment of water and wastes and was crowned with important elaborations, such as:

- Oxidation theory of water chemical self-purification process and of seasonal dynamics of natural waters redox-state;
- Theory of nitrogen compounds transformations with the support-immobilized microorganisms;
- Theory of the redox-state of the environment;
- Methods of physical-chemical and biochemical treatment of solid and liquid wastes;
- Inhibition methods of N-nitrosamines formation using reductons;
- Mechanisms of catalytic and photochemical oxidation of organic compounds with oxygen, hydrogen peroxide and free radicals.

The 20th anniversary of the department's formation is marked this year. During its growth and development, the department staff has been making the Chemistry students think "ecologically". Actually, among the brightest realizations of the department is the significant number of students and publications. More than 20 monographs and manuals, 11 didactic materials for theoretical courses, 15 handbooks for laboratory works and approximately 500 scientific articles in the field of ecological chemistry and environmental protection were published; 130 patents for inventions were obtained. The titles of the main scientific monographs are highly suggestive, regarding the complexity and actuality of the problems discussed. They are the following: "Environmental redox-processes" (2001), "Ecological Audit"(2002), "Combustion technologies and reducing the air emissions" (2002), "Natural waters' pollution and self-purification processes" (2002), "Environmental issues in power engineering" (2003), "Ecologically pure wine industry" (2004), "The hydrochemistry of small rivers" (2004), "Environmental economy" (2005), "Wastes management" (2006), "The fundamentals of winery wastes treatment" (2007), "Ecological chemistry of nitrates, nitrites and N-nitrosamines" (2009), "ENOXIL – an ecological preparation for plant protection" (2010), "Secondary wine products" (2011).

Only the professional staff is working at the department (28 persons), including academician - 1, professors - 6, associate professors - 8, superior lecturers - 8, assistant lecturers - 5. The number of students is the following: license students (courses I, II, III) – 234, master students – 69, doctoral students – 8. 120 foreign students graduated from the department during the period of its activity as well. It should be mentioned that in 2008-2009 academic year the specialty entitled *Ecological chemistry and environmental protection* (master and doctorate studies) at the Department of Industrial and Ecological Chemistry of the State University of Moldova has been introduced.

Thus, the department had played an important role in the development of ecological chemistry and environmental protection in the Republic of Moldova and reached a very honorable position in this scientific area.

The educational aspect of Ecological Chemistry in Moldova is represented not only by the State University of Moldova (SUM), but also by the University of the Academy of Sciences of Moldova, "A. Russo" State University, Bălți and Tiraspol State University, where not only the special courses are delivered, but the research laboratories and entire research centers work as well.

Nevertheless, the fundamental research in the ecological chemistry area is carried out mainly in two major institutions – the Research Center Applied and Ecological Chemistry of SUM and the Institute of Chemistry of ASM. There are 6 main research directions:

- 1. Ecological Chemistry of Water
- 2. Ecological Chemistry of Atmosphere
- 3. Ecological Chemistry of Soil
- 4. Ecological Chemistry of Wastes
- 5. Ecological Chemistry and Renewable Energy

6. Ecological Chemistry and Healthy Lifestyle.

1. Ecological Chemistry of Water

A bright example of Ecological Chemistry promoting is the state program entitled "Water Quality Management and Research", where the chemistry and ecological condition of water sources have been studied under the several research projects.

Starting with the fundamental research in this direction, we will pass to the project entitled "SELF PURIFICATION OF SURFACE WATER" supervised by dr. Viorica Gladchi that deals with the Catalytic redox processes in natural water. Analyzing the chemical composition of water, we can conclude that it includes the substances featuring the required properties to enable the running of redox-catalytic processes: oxidative equivalents (O_2 , H_2O_2 , etc..), reductants (DH_2 and catalysts ($M^{(n-1)+}/M^{n+}$).

The reducers are among the participants in the redox process in water: autochthonous substances - resulted from metabolism and decomposition of hydrobionts; allochthonous substances - substances that penetrate the aquatic environment together with atmospheric precipitates or wastewater. Copper and iron are metals having the great importance for redox transformations and can be found in water in catalytic concentrations.

The transformations of nitrogen compounds were studied by Prof. Maria Gonta under the project "NITRATES, NITRITES AND NITROSOAMINES". The scheme of oxidation and reduction of nitrites and nitrosoamines in water, food and living organisms was elaborated. The mechanism of nitrites reduction in the presence of various antioxidants, that caused the decrease in oncology maladies at people of different age was proposed. This research was carried out in cooperation with the Nebraska Cancer Center (dr. Irina Stepanov, USA).

Under the project "PSEUDO JAHN-TELLER INSTABILITY OF HIGH- SYMMETRY PROTONATED WATER CLUSTERS" leaded by m.c. Ion Geru, the new experimental data on the different energy of $H_{2n+1}O_n^+$ systems decay, and those referring to the spatial dimensions of non-homogenates in the water hydrogen bonds' network were obtained. This fundamental research has a prospective application, especially in water purification from heavy metals. The brightest result of this project is that the 65th water anomaly has been found – it consists in the diffusion indices increase in the case of Ca²⁺ ions concentration increase.

"SYNTHESIS AND STUDY OF PHOTOSENSIBILIZATORS ON THE BASE OF FURANE-CARBOXILIC ACID, FUROCEN-PORFIRINE, QUINOLINE-PIRIDINE AND IMIDAZOLINE DERIVATIVES" – the project, conducted by acad. Constantin Turtă is aimed to elaborate the new synthesis methods of substances mentioned above and also to obtain and to make a deep physical-chemical analysis of Fe- and Ti-nanoparticles. This work is a spin off in the new photocatalytical method to obtain hydrogen from water.

"THERMODYNAMIC ANALYSIS OF ALUMINUM HETEROGENEOUS SPECIATION IN NATURAL WATERS", supervised by the Institute of Chemistry ASM, dr. hab. Igor Povar, has the following results: the new type of diagrams based on graphical and computerized methods, which quantitatively describe the distribution of soluble and insoluble, inorganic and organic, monomeric and polymeric aluminum species in heterogeneous aquatic systems was elaborated. The factors influencing the distribution of soluble and insoluble aluminum species in aquatic systems were also analyzed.

"MONITORING OF PHOSPHORUS FORMS IN NATURAL WATERS FOR THE ENTIRELY SYSTEM WATER-PARTICULATE MATERIALS-BOTTOM", the project supervised by the Institute of Chemistry ASM, dr. hab. Vasile Rusu, reached the following goals. Water samples have been collected from 46 springs in Ialoveni region. The physical-chemical composition of water has been determined, which served as basis for the estimation of the water quality. 7% (3 springs) of water are in compliance with drinking water status, 35% (16 springs) of water has the hardness between 7 and 10 mg/dm³ (acceptable for consumption), 5 of those 16 springs are polluted with nitrates. 58% (27 springs) contain hard water and 37% (17 springs) have shown a mineralization higher than 1000 mg/dm³.

"THE ROLE OF THE DNIESTER RIVER'S TRIBUTARIES IN THE FORMATION OF ITS CHEMICAL COMPOSITION AND THE SPRINGS OF DNIESTER BASIN AS NATURAL ECOSYSTEMS AND DRINKING WATER SOURCES" – the project, directed by the Ministry of Ecology and the State University of Moldova (dr. Elena Bunduchi, dr. Nelly Goreaceva), has as the main purpose to determine the sources of toxic chemical substances flow in Dniester river and to elaborate several protective measures (including the most dangerous areas in the list of those protected by State). The water quality of Dniester was estimated by oxygen content, which confirmed the permanent pollution of water. The class of the water quality degraded from I and II – very clean water (portions of the streams near Dubasari up to the capture point, located upstream the mouth of Byc river) to class III – moderately polluted water (downstream the mouth of Byc river and upstream the mouth of Botna river) and class IV – polluted water - downstream the mouth of Botna river.

The project entitled "THE HYDRAULIC BALANCE AND BIOGEOCHEMISTRY OF GROUNDWATER IN THE RURAL AREAS OF THE REPUBLIC OF MOLDOVA", headed by the Academy of Sciences of Moldova, the Institute of Geology and Seismology, dr. Constantin Moraru, works on the elaboration of the model hydrogeochemical map of groundwater in the Central-Eastern zone and on obtaining the standard data on the groundwater quality. The project "GEOINFORMATIONAL SUPPORT FOR HYDROLOGIC RISK ESTIMATION IN PRUT RIVER BASIN", implemented by the Institute of Ecology and Geography of the Academy of Sciences of Moldova, dr. Nicolae Boboc (Institute of Ecology and Geography) and dr. Viorel Bostan (UTM), aims to create a cartographic database in digital format and to use software (HEC-RAS, HEC-GeoRAS, ArcHydro) for hydrologic risk estimation.

The biodiversity study is represented by the project entitled "BIODIVESITY AND ECOLOGICAL SUCCESSIVES OF AQUATIC ECOSYSTEMS OF PRUT RIVER'S INFERIOR SECTOR" leaded by Prof. Elena Zubkov. The important qualitative and quantitative indices of aquatic ecosystems will be obtained, and also the antropic influence on biocoenosis functioning will be evaluated. The investigations referring to the role of some chemicals and nutrients on the production-destruction processes in Prut river will be done.

"ASSESSMENT OF CHILDREN MORBIDITY IN PRUT ZONE BY HYDRICALLY TRANSFERED DISEASES AND ELABORATION OF PREVENTION MEASURES" – in the framework of the project of the Ministry of Health, National Center of Public Health, dr. hab. Grigore Friptuleac, the current state of Prut river was analyzed and the concrete indices of water's chemical composition and pollution degree, dependent on the seasons, were obtained.

Water treatment is one of the main branches of ecological chemistry of water. This area is represented by a sound number of research projects. They are as follows:

"EFFICIENT USE OF AUTOCHTHONOUS ACTIVE CARBON IN THE WATER TREATMENT PLANT OF "APĂ-CANAL CHIŞINĂU", headed by the Institute of Chemistry of Academy of Sciences of Moldova, dr. hab. Tudor Lupaşcu, aimed to determine the physical-chemical properties of autochthonous active carbon, to make a clear classification of the adsorbents in accordance with their adsorption parameters, to find those pollutants that should be eliminated and to establish the optimal methods for their removal.

"TEXTILE WASTEWATER TREATMENT FROM TEXTILE COLORANTS" (supervisors: the Ministry of Ecology, State University of Moldova, dr. hab. Maria Gonța) aimed to elaborate the new efficient methods of reducing the concentration of textile colorants in the presence of cationic and anionic surfactants using the combination of coagulants and oxidizers for water treatment.

"ANAEROBIC TREATMENT OF THE DISTILLERY AND WINERY WASTEWATER WITH OBTAINING OF BIOGAS AND USEFUL PRODUCTS" – in the framework of this project, directed by the Ministry of Ecology, State University of Moldova, dr. Victor Covaliov, the new technical solutions for water treatment have been elaborated, the new additives for stimulation of anaerobic fermentation and methanogenesis of bio-active substances, obtained from agro-industrial wastes were tested; a new method of methanogenic synthesis of vitamin B_{12} in the contents of sludge resulted from the anaerobic wastewater treatment; a new method of biomethane concentration increase in biogas have been proposed.

The project "PHYSICAL-CHEMICAL TREATMENT AND CONDITIONING OF NATURAL WATERS", supervised by dr. Olga Covaliova, has the following development purpose – to elaborate the cheaper treatment technologies; to reach efficient water treatment; to ensure the reuse of treated water. A new method of underground waters treatment with the removal of hydrogen sulfide has been elaborated. The combined method and integrated reactor have been developed for the degradation of benzothiazoles and their mineralization to simple molecules, such as water, carbon dioxide, etc. It was found that under the UV-irradiation, photo-degradation of benzothiazoles runs efficiently, but rather slow. Therefore, it was proposed to combine photo- and biodegradation methods, which is an energy-saving and efficient method.

The results of the project, headed by dr. Oleg Bogdevici, entitled "WATER QUALITY CONTROL AND MONITORING" are the validation of analytical methods for the determination of priority monitoring substances, in accordance with the Water Framework Directive; the determination of the spectrum of toxic organic pollutants from potential pollution sources at urban and rural areas of Republic of Moldova; the creation of the database of groundwater potable sources - more than 3000 hydrogeological boreholes were included.

2. Ecological Chemistry of the Atmosphere

Even if this is not the first priority area of ecological research in Moldova, some research have been carried out in this field. For example, the theoretical study, carried out by dr. Natalia Gorincioi "RADICAL REACTIONS IN THE ATMOSPHERE", had to examine the diversity of hydroxyl radical reactions in the atmosphere, the legitimacies of photochemical smog and PAN compounds formation, both of them being severe irritants to eyes, and having phytotoxic effect. The presence of the strongly reduced carbon (CH₄) in a strong high-energy oxidant (stratosphere: O₃, O₂, hv) has been determined; consequently, methane at high altitudes increases the concentrations of hydroxyl and water radicals. The ways of PAN (peroxyacetylnitrates) decomposition were also studied (reverse process, photolysis, thermal degradation, reaction with OH-radicals).

The project entitled "EXCESSIVE POLLUTION OF THE ENVIRONMENT WITH HEAVY METALS – LEAD, CADMIUM AND SULPHATES", directed by Prof. Alexandru Teleuța, aimed to investigate the negative impact of increased lead content in the atmosphere. It has been established that heavily polluted air with SO, (0,3-0,5 mg/m³)

(in regions – Rezina, Râbnița, Căușeni Tighina, Tiraspol and Cahul) increases the withering process of such tree species as oak, willow and pine - *Tilia microphyla* and *Pinus nigra*. The species of lichen sensitive to pollution - *Usnea hirta, Peltygera canina, Ramalina fraxinea* have disappeared from these areas.

3. Ecological chemistry of soil

Soil is a very important natural value of Moldova. The food safety, export of agricultural products, welfare and ecological situation of the country depend mainly on the condition and production capacity of the soil. The current state of the soil layer is unsatisfactory (50% of agricultural land) and even critical (about 10%). The applied research "SOIL'S CONDITION AND PROTECTION", conducted by acad. Andrei Ursu, acad. Serafim Andrieş and Prof. Boris Boincean aims to keep the fertile soil layer and to minimize the surface and ground water pollution with nutrients and plant protection substances. The set of measures implemented by the "Nicolae Dimo" Institute against soil erosion phenomena, ensures the soil loss reduction below the allowable 5 t / ha limit. The economic effect is 100-650 euro / ha. The application of phytoameliorators (4 years cultivation of the perennial herbs on leached chernozem and the ordinary vetch with green fertilizers) led to the increasing of the reserves of nitrogen and organic substances in soil, partial remediation of soil characteristics (structure, favorable agronomic aggregates' hydrostability, reducing the bulk density), significant increase of agricultural production. Experimentally it was established that the application of the environmentally friendly agricultural practices lead to the remedy of soils' biological properties by the increase of the invertebrates' biomass and the number of species, increase of microbial biomass, intensification of enzymatic activity, improvement of biodiversity level.

4. Ecological chemistry of wastes

The wastes include all the objects or substances which the holder discards, intends to discard, or is legally obliged to discard, i.e. all the unwanted or useless material. Wastes may be classified according to their origin, their properties, how hazardous they are, and how they may be sorted, recovered or treated.

If we talk about the autochthonous wastes, the grape seeds, as well as the other secondary products of wine industry should be firstly considered. Such industrial wastes contain the increased tannins amounts, which represent a series of natural compounds with polyphenolic structure. The interest towards these compounds has appeared due to their antioxidant properties, i.e. these compounds trap the free radicals, thus protecting the human organism from various diseases, such as cancer and cerebral ictus. The majority of enotannins are soluble in ethylic alcohol and are insoluble in water. This circumstance hindrances the utilization of enotannins in various fields, including pharmaceutics. The specialists of Ecological Chemistry Laboratory of the Institute of Chemistry, directed by prof. Tudor Lupascu, have elaborated and patented a procedure for hydrosolubilization of enotannins. Using the method of mass spectrometry, it was found that the procedure consists in breaking of catechol polymers in enotannins. As the result of this procedure, a new product was obtained, formed from monomers, dimers, trimers of catechol, polydentate carboxylic acids, peroxidic compounds etc. Microbiological, pharmacological and toxicological studies, performed under the project "NEW PREPARATIONS FROM GRAPE SEEDS FOR MEDICINE, VETERINARY AND AGRICULTURE" and "EVALUATION OF ACTIVITY OF MEDICINAL AND AGRICULTURAL PREPARATIONS OBTAINED ON THE BASIS OF THE BIOLOGICALLY ACTIVE COMPOUND ENOXIL IN CLINICAL AND FIELD TRIALS" in the frames of the State Program "PROCESSING AND UTILIZATION OF WINERY WASTES AND OBTAINING OF NEW COMPOUNDS" directed by acad. Gh. Duca, demonstrated that Enoxil is not toxic and possesses the significant antifungal and antibacterial properties. On the basis of the active compound Enoxil, the preparations Enoxil-M and Enoxil-A were elaborated and tested under the clinical and field conditions. The field tests showed that the preparation Enoxil-A increases the resistance of agricultural cultures by 60% towards the action of root rot and of vine - by two folds, as well as towards grey rot. Clinical trials of the medicinal preparations Enoxil-M produced at "Farmaco" enterprise demonstrated a positive effect in the treatment of diseases induced by fungi and bacteria, of plagues provoked by thermal and surgical lesions. The results indicated above refer to the State Program, which have been realized during the period 2004-2008 and had a very important objective of efficient management of the wastes originated from wine industry and consequently from the autochthonous raw material. In the framework of this State Program, certain studies had been carried out:

"ANAEROBIC TREATMENT OF WINERY WASTEWATERS WITH BIOGAS OBTAINING AS AN ALTERNATIVE SOURCE OF HEAT AND ELECTRIC ENERGY" headed by dr. V. Covaliov;

"NEW TECHNOLOGIES OF NEW REDUCTONS OBTAINING FROM THE WINERY WASTES", directed by dr. hab. M. Gonța;

"NEW MEDICINAL, VETERINARY AND AGRICULTURAL PREPARATIONS ON THE BASIS ON THE GRAPE SEEDS AS RAW MATERIAL", headed by dr. hab. T.Lupaşcu;

"ELABORATION AND TRANSFER OF OILS OBTAINING TECHNOLOGY FROM THE WINERY WASTES", supervised by dr. E. Iorga;

"ELABORATION OF THE TECHNOLOGICAL COMPLEX OF CONCENTRATED ANTHOCYANINES PRODUCTION FROM GRAPES", headed by dr. C. Bodean;

"ELABORATION OF TECHNOLOGICAL REGULATIONS FOR TARTARIC ACID AND ITS DERIVATIVES OBTAINING USING THE AUTOCHTHONOUS RAW MATERIAL", directed by dr. hab. M. Gonța and dr. A.Mereuța;

"MEDICINAL AND AGRICULTURAL PREPARATIONS OBTAINED ON THE BASIS OF BIOLOGICALLY ACTIVE SUBSTANCE ENOXIL UNDER THE FIELD CONDITIONS", directed by dr. hab. T. Lupaşcu;

"ELABORATION OF GRAPE SEEDS TREATMENT BIOTECHNOLOGY FOR OBTAINING OF OILS WITH HIGHLY DETERMINED QUALITY PROPERTIES", headed by dr. V. Ţuţuc;

"THE USE OF THE ALCOHOL INDUSTRY WASTES", supervised by dr P. Parasca;

"PHOTOCATALYTICAL TREATMENT OF THE DISTILLERY GRAINS FOR INTENSIFICATION OF BIOGAS GENERATION AND NATURAL FERTILIZERS' PRODUCTION", headed by dr. V. Covaliov;

"PILOT PLANT AND TECHNOLOGICAL REGIMES FOR THERMAL NEUTRALISATION OF TOXIC SEDIMENTS CONTAINING PRUSSIAN BLUE", headed by dr. E. Obadă.

If we consider the wastes classified as noxious and hazardous, much attention should be paid to "NITRATES, NITRITES AND N-NITROSOAMINES STUDY". This is the title of the project, headed by the State University of Moldova, dr. hab. Maria Gonța, which studied the chemical risk and the influence of these chemical substances, belonging to the group of dangerous ones, on the environmental conditions. The NO_2 reduction mechanism in the presence of inhibitors and the secondary aliphatic amines nitrosation mechanism have been proposed. It was shown that the reductants interact in a reactionary system with the formed species (at higher pH, N_2O_3 is dominant), thus resulting in the reduction of nitrosating agents concentration. The ways of people exposure to NNA were revealed, including the exogenous (eating, smoking, cosmetic products, polluted air, water, industrial products) and even endogenous one (nitrosation of precursors in the gastrointestinal system).

It is to be mentioned that even if the economic costs of wastes managing are high, the money can often be saved by the efficient wastes monitoring and management, including the fees collection, modifying of vehicles, and, especially, public education. The latter is popularizing by State University of Moldova and other Universities of the country as well.

5. Renewable energy

For the Republic of Moldova, a country which doesn't possess such energy sources as oil and natural gas, the "RENEWABLE SOURCES OF ENERGY STUDY" (the project, supervised by the Institute of Power Engineering of the Academy of Sciences of Moldova, dr. hab. Postolache Gh.) has become a very actual research. In the framework of the project, it was established that for Moldova the following types of renewable energy sources are convenient: biomass energy, solar thermal energy, solar photoelectric energy, wind energy, hydropower. The technical potential of these types of renewable energy sources were considered, as well as the volume of energy production substituted by the renewable energy sources and the technical potential of main types of renewable energy (million tons of conventional fuel). The main applied results of the project are the following:

- two biogas plants constructed in collaboration with Dutch scientists for livestock and poultry production (Colonita);
- Installation for the use of methane from household wastes (Tintareni);
- Oil production plant from rapeseed;
- Photoelectric cell production plant that produces traditional membrane cells (Orhei);
- In 2009 the Academy of Sciences of Moldova has launched a testing ground on renewable energy sources (State Program).
- Boiler installations for straw incineration (Stefan-Voda). In order to obtain the heat energy from biomass (pressed straw), Moldagrotehnica JSC has launched the production of boilers, licensed by ALCON (capacity ranges from 30 to 750kW). These boilers can be used for heating of schools, kindergartens and other public institutions. Since 2010, Moldagrotehnica JSC also started the production of boilers for pellets (chips, saw-dust and other waste) with a capacity of 17 to 75 kW.
- Biogas reactor for wine (cognac) bards recycling. The project plant "Vinaria-Bardar" has a bioreactor of 40 m³ which uses the distillery wastes. Also other 32 wineries have similar equipment which enables the recycling of harmful discharge up to the total volume of 5 mln. m³.

Returning to the bio-fuel theme, the project supervised by dr. Covaliov V. should be mentioned. It is entitled "BIOGAS TECHNOLOGY IMPROVEMENT", and aims to: finding a competitive and flexible technology to achieve high yield of biogas as an alternative energy source, reaching an efficient and energy saving biochemical treatment of wastewaters that ensure repeated use, obtaining the vitaminized sludge that can be used as a feeding additive for cattle or as fertilizers.

6. Ecological Chemistry and Healthy Lifestyle

It is well-known that the current ecological situation has a strongly negative impact on the normal vital activity of living organisms. This is the reason why the direction wich connects the ecology of environment and the chemistry and biochemistry of living matter, had appeared under the name of ecological chemistry and healthy lifestyle.

Among the gravest consequences of environmental pollution on human organism are the destructive processes taking place on the cellular level, because namely they regulate the state of entire organism. One of them is considered to be the oxidative stress, which is associated with the increased production of oxidizing species or the significant decrease in the capability of antioxidant defenses. In the Republic of Moldova this problem is also studied.

The interest in plant metabolites as sources of antioxidants appeared a long time ago. Dihydroxyfumaric acid is one of the leaders, due to its potential, in the series of natural reductons. "THE OBSERVATIONS ON THE ANTIOXI-DANT ACTIVITY OF NOVEL DIHYDROXYFUMARIC ACID DERIVATIVES" is the project, directed by the Institute of Chemistry of Academy of Sciences of Moldova, dr. hab. Fliur Macaev and Natalia Secara. The obtained results show that the presence in the molecules of amides or heterocyclic derivatives of additional nitrogen atoms has a negative effect on the antioxidant activity in comparison with the activity of anilide or bis-benzimidazole, benzimidazole with a free carboxyl- group which showed antioxidant activity twofold higher than the ether derivative of dihydroxyfumaric acid.

Another factor, which indicates the ecological chemistry to be very actual, modern and popular direction in the world is the number of scientific conferences and forums, dedicated to it. The organization of International symposia aimed to generalize and to exploit the experience in ecological chemistry, has become a tradition in Chisinau. The first Symposium of such kind was organized in Moldova in 1995 and brought together 150 participants. The second international symposium (2002) gathered 260 scientists from 26 countries. In the third international symposium on ecological chemistry (2005) 470 participants from 38 countries were registered. In autumn 2008 Chisinau again was hosting a major scientific forum (the fourth) on ecological chemistry. The workshop organized with the NATO support has a scope to analyze the level and quality of research in ecological chemistry in the CIS countries, those of Middle East and Western Europe, to present the new approaches and strategies referring to the technological development tolerant to the environment, to the sustainable development and capacity building, including education. Thus, researchers from 12 countries had analyzed the entire complexity of ecological chemistry problems - from pollution research to sustainable development for relevant regional and international collaboration, had discussed the increasing role of researchers in decision making on the environment in regional and international context, had elucidated the role of ecological chemistry to build the knowledge-based society in Western and Eastern Europe, USA and Arab countries. The pollution knows no national borders. It's an issue of international security. A continuation of these major events is the present one, the 5th International Conference "Ecological Chemistry – 2012", which will provide opportunities to exchange the latest ideas, to present and discuss recent results on the physical-chemical and biochemical aspects of processes occurring in the ecosystem - soil, air and water, and promising approaches for sustainable development, including engineering and modeling with social and educational implications.

A logic continuation of the achievements which have been reached in the field of ecological chemistry are the tasks that we set for the next five years:

- Development of research in the field of theory of the redox phenomena in aquatic environment.

- Study of the processes and mechanisms of oxidation-reduction, migration, accumulation, sorption of persistent organic pollutants, heavy metals, nitrogen and phosphorus compounds, surfactants, petroleum products etc. in the aquatic environment with the scope to reveal the formation principles of surface, phreatic and underground water quality.

- Elaboration of ecological sustainable technologies for natural water conditioning and industrial wastewater treatment by coagulation, flocculation, flotation, decantation, adsorption, UV radiation, oxidation-reduction using homogeneous and heterogeneous catalysts, ion exchange, etc.

- Elaboration of nanoporous catalysts on the basis of activated carbon, natural and synthetic adsorbents, transition metal oxides. Study of the structure, chemical and physicochemical properties of these catalytic supports, useful for water treatment.

- Target oriented synthesis, study of structure, magnetic and catalytic properties of coordination nanocatalysts for use in the processes of water photolysis to obtain hydrogen.

- Elaboration of chemical, physico-chemical, including electro-chemical, spectral methods of determination of toxic substances in environmental objects, food, etc.

- Elaboration of efficient methods for extraction of useful substances from agricultural secondary and waste

products and study of the structure, chemical, physicochemical and microbiological properties of new chemical preparations.

- Target oriented synthesis of new chemical compounds from natural sources. Study of their chemical structure, chemical and physicochemical characteristics, as well as antioxidant, antifungal, antibacterial, anticancer properties, etc. in order to obtain pharmaceutical and phytochemical products for human health and plant protection.

- Gradual implementation of new production technologies oriented to low energy consumption and low waste generation.

All these goals represent real challenges for our scientific community, but we are confident that they could be turned into reality. Modern society needs our contribution and there is no other way.

Finally, I would like to express my gratitude to all Symposium participants for their presence and active participation. We do really hope that our event will bring new ideas and collaborations, new solutions and perspectives.

BLACK SOILS DEGRADATION IN THE SOUTH-WESTERN BLACK SEA REGION AT IRRIGATION AND IN THE POST-IRRIGATION PERIOD

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Abstract: many years' studies of process of changes in composition, properties and fertility of the black soils of the North-Western Black Sea area in Ukraine with irrigation have revealed mobility of carbonates and humus in them, decrease of capacity of cationic consumption and content of consumed calcium, increase of share of consumed magnesium and sodium. Indicators of agro-physical state of soils under irrigation conditions are worsening significantly. In the last 15-20 years, due to sharp decrease in area of irrigated fields an extensification of agriculture the display of agro-physical solonetzicity of previously irrigated lands is strengthening, as well as processes of their dehumification; indicators of ecological&agrochemical state are degrading. Substantiated is the system or agro-amelioration measures aimed to prevent from degradation processes of irrigated black soil missives and increase their fertility.

Key words: black soils, irrigation, post-irrigation period, degradation processes.

Introduction

South-Western Black Sea region with high resources of sun warmth and potentially highly fertile black soils suffers insufficiency and non-stability of atmospheric precipitation and in the last decades – growth of weather aridness and increase of temperature in summer and autumn months. Only irrigation could be a reliable means of agriculture intensification in the region and decrease of its dependence upon unfavourable weather conditions. In 1960-1990 here were the periods of wide-scale irrigational development and regular irrigation of lands, both black soils southern and black soils common. In the last 15-20 years area of regular irrigation in the region decreased sharply (5-8 times). At that, significantly decreased input into the fields of irrigated massive of organic and mineral fertilizers, chemical ameliorants, pesticides and herbicides. As the black soils are very sensitive to the influence of irrigative water and differ with increased selectivity to Na-ions consumption, significant change is stated in changes of composition and property of these soils both during irrigation and during current post-irrigation period of their evolution on the background of decrease of agro-amelioration culture and extensivication of agriculture [1-3]. More often the changes of degradation direction taking place, and measures must be grounded and elaborated for protection and sustainable use of those highly fertile soils.

The objective of the paper is to reveal the essence and ecological & soil consequences of degradation processes of South-Western Black Sea Black soils during irrigation and in the current post-irrigation period of their evolution, development of measures to prevent degradation processes in irrigated massive and to increase their fertility.

Organization and Performance of Work and Researches

Studies of essence, spatial regularities and ecological & soil consequences of processes of North-Western Black Sea black soils' changes at irrigation and in the current post-irrigation period of their evolution, beginning from 1971, are carried out on the stationary plots for soil monitoring and irrigated massive monitoring in Odessa Region. The plots differ in their landscape & geochemical and soil & agro-amelioration conditions, duration and intensity of irrigation, quality of irrigation waters, duration of the period of post-irrigation landscape and black soils' evolution in the last 15-20 years. In parallel similar soil monitoring studies were carried out on the adjacent bogharic (non-irrigated) lands. Every year density of consistence is determined, and in the selected samples – pH, grain size, structural & aggregate and microaggregate composition, salinization and carbonate content, content and composition of absorbed bases, total and active fluorine, as well as NPK forms available for plants. In the samples of irrigation and drainage waters pH, ions composition, mineralization, content of different NPK forms and fluorine were determined. Stock of yields' sizes is taken. Since 2010, in experimental & production conditions, agro-ameliorative efficiency of measures developed by us to prevent black soils degradation in irrigated massive, liquidate unfavourable soil conditions-soil & ecological consequences of irrigation and increase of fertility are studied.

Main Results of Studies and their Discussion

With the beginning of irrigation in the region, mainly on plain watersheds and high river terraces, conditions of functioning of all components of nature-geographic environment changed, as well as the established in centuries scheme of landscape and geochemical junction. Our studies of many years (1971-2011) have established that under the influence of irrigation main characteristics and indicators of source state of natural- and soil-ameliorative environment change

significantly, direction and intensity of landscape & geochemical and soil-forming processes. As the result, landscape & ecological situation change in general – composition, properties of soils, often structure of soil cover of the territory of irrigated massive. At that landscape & geochemical and soil-forming processes could have reversible, partially reversible or irreversible character (usually of *degradation* direction) [1-3]. Among negative events and processes are; rising of undersoil waters to the surface, activation on local and regional levels of galo-geochemical processes. Practically everywhere the consequences of irrigation are increase in soils of mobility of carbonates and humus substances, decrease of capacity of cationic exchange and content of consumed calcium. The share of consumed magnesium and sodium grow which evidences of alkalization and salinization processes of black soils with irrigation. At that, significantly degrade indications of their agro-physical state as the result of processes of aggregates destroying – incrustation, increase of density and hardness, decrease of permeability, increase of hydrophility of irrigated soils. More intensive degradation in initially non-carbona5te black soils at irrigation with low-quality waters with high mineralization (1-3, sometimes 4-5 g/dm³) of sodium & magnesium chemical properties. Significantly less change at irrigation parameters of black soils in case if they are carbonate, with no-deficit balance of humus and calcium, at careful regimes of irrigation with good quality waters, as well as at observance of corresponding culture of irrigated agriculture.

In conditions of significant decrease in the last 15-20 years of area and intensity of irrigation in the region there is evident tendency to eventual re-naturalization of landscape & ecological and soil & amelioration situation on massive of irrigation, re-gradation soils changed by irrigation in the previous years, black soils first of all. Eventual change of agricultural & amelioration conditions on irrigation massive entail changes and certain difficulties with existing nature & economic and agro-amelioration and soil situation. On this background it is stated that new landscape & geochemical and soil-formation processes of previous period of large-scale irrigation develop, or their essence transform. Correspondingly, characteristics of soils state eventually change. First of all, those are salt characteristics of black soils of irrigation massive, composition of soil solution and soil adsorption complex (SAC), and largely - parameters of humus and agro-physical state. In particular, in soil massive on watersheds elution of salts from upper horizons of profile grow. At the same time the soil depth of geochemically subordinated landscapes differ with higher content of easily-soluble salts. In upper horizons of black soils after irrigation had stopped content of soluble and absorbed sodium decreased. At that the share of calcium in SAC grows. In lower hor5izons of black soils, especially those which were irrigated in past years with highly mineralized water of sodium chemical properties, ratio Ca2+: Na+ stays narrow (0,3-0,6) and content of exchangeable sodium is quite high (up to 3-6% of capacity of cationic exchange). At the background of desalination - dealkalinisation of black soil irrigated massive in post-irrigation period of their evolution the indicators of agro-physical state eventually improve. Tendency to decompactization is observed, quantity of blocky aggregates decreases, share of agronomic valuable aggregates and coefficient of soil pedality. Speed of agro-physical properties degradation is significantly higher in soils, which were previously irrigated with good quality (fresh) water.

In the last 20 years under both under bogharic conditions and in irrigated massive of the region humusness of soils eventually go down. I.e. process of dehumification of studied soils take place. The reason of this very unfavourable degradation process is domination of grain and sunflower in crop structure without application of necessary quantities of organic and mineral fertilizers. Only under perennial grasses, starting with 2nd year of cultivation, content and amount of humus grow, which enables us to recommend increase of grasses share up to 25-35% of area in the structure of crop rotation.

Thus, based on experimental & research materials, both our an other authors' it is revealed that irrigation of the South-Western Black Sea black soils cause significant changes (most often negative) in soil natural regimes and processes, and connected with them morphological indicators, soil composition and properties. Tendency of degradation of many soil parameters is preserved in current post-irrigation period of evolution of landscapes and soils of irrigated massive. Most often, as it was pointed out above, processes of decarbonization and irrigation salinization take place, and locally – salting of soils, degradation of their humus and agro-physical state, supply of nutrients for plants. Usually these degradation changes of the indicators of state and properties of soils reveal simultaneously [1-3]. Taking into account size of values of salinization, salting of soils and alkalinization of black soils of irrigated massive, their humus and agro-physical state, pollution with heavy metals and water-soluble fluorine, recently an integral classification of soils according to the level of their degradation had been proposed [2, 3]. At that, it had been established that at low level of soil degradation in irrigated massive crop yields decrease 15-20%, at medium level – 20-30%, at high level – 30-50% and over.

On the basis of results from researches and work of many years' system of agro-ameliorative measures had been substantiated and developed to prevent from the processes of degradation of black soil irrigated massive in the region and to increase their fertility. At present the system of measures proposed by us is being introduced in two farms of Odessa Region as a pilot-experimental activity.

Conclusions

1. Studies of the processes of composition, properties and fertility changes in the Ukrainian South-Western Black Sea black soils have been carried out both under irrigation conditions and during current 15-20 years long post-irrigation period of their evolution. Essence and ecological & soil consequences of processes of degradation of black soil massive of irrigation in the region have been studied. 2. In the black soils studied most often revealed are the processes of decarbonization, irrigational salinization and local salting of soils, degradation of humus state, ecological & agrochemical state and agro-physical state indicators.

3. System of agro-ameliorative measures has been substantiated and is being introduced as a pilot experiment to prevent processes of degradation of the black soils of irrigated massive and to increase their fertility.

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TRENDS OF CURRENT DYNAMICS OF CHEMICAL PROCESSES IN THE SOILS OF ODESSA REGION

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Abstract: Results of chemical processes' dynamic studies of many years (1971-2011) in the soils of Odessa Region have been described. The most significant in the last 15-20 years have been changes of humus content and ecological & agrochemical status of black soils. In spite of some increase in the norm of fertilizers input for the last 5 years the negative balance of plant nutrition elements in soils is preserved. Ways of optimization of humus and ecological & agrochemical status of soils in the region and increase of their fertility have been proposed.

Keywords: Odessa Region, black soils, humus content, ecological & agrochemical status.

Introduction

Exchange of matter and energy between soil and other natural bodies (soil, water, atmosphere, living matter), inflow of radiant energy, life activity of soil living phase are the most important factors and reasons of transformation and displacement of substances and energy in soil. It combination they form the reason for emergence of different in their nature, direction and essence interconnected and mutually caused soil processes.

Different phenomena of physical, chemical, physicochemical and biological nature are taking place in soil permanently. Their joint influence ensures development and functioning of soil as non-living material of nature and the main means of economic development and use. According to their character the processes in the soil could be divided into three groups [1]: processes of exchange of matter and energy between soil and other natural bodies (processes of inflow into the soil and removal from soil); processes of matter and energy transformation in soil; processes of moving of matter and energy in soil body.

In soil cover of Odessa Region (Ukraine) over 90% of area are black soils. In the north of the Oblast, in the foreststeppe part of its territory these black soils are typical and regraded, in the steppe central and southern part of the Oblast territory – black soils common and southern. In the Dnister Region the black soils are southern and common micellar and carbonate. The level of plowing up the area is quite high in the Oblast – around 80% of agricultural lands. The irrigated area in the Oblast (mainly black soils southern) is 226,9 thousand Ha. The waters used for irrigation are fresh water (0,4-0,6 g/dm³) of the Danube and Dnister Rivers, as well as water of lower quality and higher mineralization (1-2 g/dm³ and more) from the Lower Danube Lakes-reservoirs.

The main chemical, physical and biochemical processes in the black soils of the Oblast, which are forming their composition, regimes, properties and determining fertility level – dissolution-migration-accumulation, exchange and consumption of matters and elements; various reactions of decomposition of organic substances in the plant litter; similarly diverse phenomena of microbial decomposition and synthesis, formation and degradation of organo-mineral compounds, humus included; mineralization of humus substances; gas-atmospheric fixation and emission including the processes of ammonification, nitrification and denitrification; two-sided exchange of ashes, elements and nitrogen in the system soil-higher vegetation and many others.

Since 1990 the direction and intensity of chemical processes in the Region changed both in the zonal non-irrigated and in irrigated soils, which entail gradual changing of their composition and properties, most often soil degradation. The most vivid is degradation of humus content and ecological & agrochemical state of the black soils dominating in the Region, as the result of which in the last 15-20 years evident trend towards decrease in their fertility has been observed [2,3]. In connection with the above, this paper focuses on the results of studies of modern dynamics of the main parameters of humus and ecological & agricultural state of black soils in the Region, both under the background rain-fed (boharic) conditions and in the irrigated areas.

The objective of the paper is to reveal trends in modern dynamics of chemical processes in the soils of the Region and substantiate the ways of optimization of the main parameters of their composition and properties, first of all of their humus content and ecological & agrochemical state.

Organization and Performance of Work and Researches

Studies of chemical processes' dynamics in the soils of Odessa Region have been carried out for many years (1971-2011) in the key stationary plots of soils and lands state monitoring, both under the background non-irrigated land use and on the irrigated areas in the steppe part of the Region [2]. In accordance with programme and methodology of monitoring we determine periodically the parameters and characteristics of soil morphology and tendencies of their changes, general physical and water-physical properties, pH, grain size, soil structure and micro-structure, humus content and humus store, content of total NPK and accessible for plants NPK. In the framework of the NitroEurope International Project processes of nitrification and mineralization of organic nitrogen, as well as nitrification capacity of the black soils had been studied since 2007 in the area of "Petrodolinskoye" monitoring station with background southern black soils [4]. For all the key plots (stations) of monitoring source values (characteristics) of soil parameters controlled had been borrowed from the materials of large-scale survey of soils of the Region (1958-1961) and subsequent correction of those materials in 70-80th. Apart from our own results of study of chemical processes in soils of the Region we also used materials of soil and soil-amelioration surveys of lands carried out in the last 30-40 years by Odessa Branch of the "Ukrzemproekt" Institute, Odessa Hydro-Geological & Amelioration Expedition and Odessa Research & Production Centre "Oblderzhrodyuchist".

Main Results of Studies and their Discussion

The most important in the soil forming of the Region, and black soils forming in particular, are the processes of sod pedogenesis and calcium hydrocarbonate migration in profile. These two processes are forming two main profiles of black soils – humus and carbonate ones, as well as the main parameters and characteristics of their material & chemical composition and properties. As the result of sod process much humate- and calcium humus accumulate in profile being closely connected with mineral part, as well as much biophil elements – nitrogen, phosphorus, sulfur, calcium, iron, manganese and other macro- and micro-elements. At that structuring of soil mass is taking place, with forming of water-resistant crumb structure. The last is the result of influence of thin grassy roots, activities of invertebrates, waste products of microorganisms and adhesive characteristics of humus substances. Due to their structure the black soils, especially those typical for the north of the Region, have good physical and water-physical properties and the upper humus horizon is loose and have optimal porosity, density, permeability and water capacity.

Migration and accumulation of carbonates in the profile of black soils provide for high level of saturation of their colloids with calcium and absence on the most part of the Region's area of eluvial - illuvial differentiation of profile of silt (grain under 0,001mm), iron and aluminum. Humate – calcium composition of humus, as well as neutral and weakly alkaline reaction of soils are formed. This means that migration of calcium hydrocarbonate and presence of horizon of carbonate accumulations in the profile contribute to stability of soil mass of black soil, its properties, and in significant measure to potentially high fertility.

We would like to present some analytical characteristics of the processes of formation and values of the main parameters of material & chemical composition and properties of black soils in the Region as of the beginning of our studies. In the typical black soils and regarded black soils in the north of the Region humus content in the upper horizons makes 4,5 (4,7)-6,0 (6,5)%. With the depth of profile its quantity decreases gradually, and in the depth of 50-60 cm makes 3,0-3,5%. Capacity of cationic uptake is 40-50 mmol/100 g of soil, share of exchangeable calcium is in average 80% of capacity. Environmental reaction is neutral or close to neutral. Plant nutrition elements' content is: nitrogen - on the level of 70 mg/kg, phosphorus 60-90 and potassium 100-120 mg/kg. In the common black soils of steppe zone that are dominating in the territory of the Region humus content in the upper horizons is 4,4-4,6 to 5,5-6,0% to the east of the Dnister valley and 3,0-4,0 (4,5)% in the micellar - carbonate warm facie of the areas to the west of the Dnister. At the depth of 50-60cm humus content is 2,9-3,4 and 1,8-2,4% accordingly. Significantly lower quantity of humus in the black soils of warm facie is explained by their high bioactivity and strengthening of the processes of plant residues mineralization. Capacity of cationic uptake in the common black soils in most cases is 40-45 mmol / 100 g, share of calcium makes 80-85% of capacity. Environmental reaction is neutral and weakly alkaline. These soils are medium and well provided with plants nutrition elements: nitrogen - 70-90 (up to 100) mg/kg, P₂O₅ 80-110 and K₂O - 100-120 (up to 140-160) mg/kg. And, finally, in the most xeromorphic southern black soils in the south of Region humus content in the upper horizons makes usually 3,5-3,9% to the east of the Dnister and 2,7(2,9) - 3,1(3,3)% to the west of the Dnister. At the depth of 50-60 cm its quantity goes down quite sharply to 1,3-1,5%. Capacity of cationic uptake is 32-38 mmol / 100 g. Share of consumed magnesium and sodium increases here, especially in the coastal zone to the east of Odessa, which evidences residual and sometimes current alkalinity of southern black soils in the Region. Reaction of soils is weakly alkaline and alkaline (usually pH is 7,5-7,8). Content of nutrients goes here down if compared with black soils of middle and northern parts of the Region which are better watered [2,5].

Under conditions of agricultural development of black soils the sod process becomes significantly weaker due to decrease of mass of roots and invertebrate fauna. Under extensive system of land-use dominating in the Region the process of humus accumulation slows down compared to its mineralization and the processes of damaging of structure with agricultural machinery prevail over the structure-formation processes. Under these conditions black soils slowly become poor in humus (dehumification), their valuable for agronomy crumby structure eventually diffuses. These

processes especially intensified in the last 20 years because of extensification of land use and decrease of the norms of input of organic and mineral fertilizers, as well as ameliorants. Thus, the quantity of organic fertilizers applied in the Region in 2001-2005 was 17,7 times less if compared to 1986-1990.

Irrigation of black soils in the south of the Region practically always cause increase in them of mobility of carbonates and humus substances, decrease of capacity of cationic exchange and content of adsorbed calcium. Shares of adsorbed magnesium and sodium increase, which evidences the processes of alkalinization and salinization of black soils with irrigation. At that the parameters of their agro-physical state worsen as the result of aggregates destroying – slitization and crust forming, increase of density and hardness, decrease of permeability, increase of hydrophilic properties of irrigated fields. More intensive degradation in the source non-carbonate black soils irrigated with low quality waters having high mineralization (1-3, sometimes 4-5 g/dm³) containing sodium and magnesium. And significantly less changes take place with irrigated black soils in case of their carbonateness, no-deficit humus and calcium balance, careful regime of irrigation with water of respective quality, as well as keeping to respective culture of irrigated agriculture [2,3].

As it was shown by results of many years' studies of dynamics of modern processes in the soils of the Region, the most significant changes took place with humus and ecological & agrochemical state of black soils in the last 15-20 years. In particular, comparison of results of agrochemical survey of soils of the Region in 1986-1990 and in 2001-2005 shows the tendency towards dehumification of soils, especially black soils. While in 1986-1990 in the arable horizons of the soils surveyed humus content made 3,76%, in 2001-2005 it decreased to 3,20%. This means that humus content in the soils of the Region for the 15 years being analyzed had decreased in average 0.56% or 14.9% of the humus content in 1986-1990. In the Region in average loss of humus for those 15 years made 17,50 t/Ha or 1,17 t/Ha annually (for comparison – in the period of intensification of agriculture in 1960-1980 losses of humus were on the level of 0,5-0,7 t/Ha a year). The most significant dehumification losses took place in the initially better humused typical and regraded black soils in the north of the Region - decrease in humus content made here 0,79% of weight and 17,9% compared to source quantity, 24,69 t/Ha for 15 years and 1,65 t/Ha in average for a year. In less humused common black soils of steppe zone values of humus losses for 15 years somewhat decreased if compared to black soils of northern forest-steppe part of the Region - respectively 0,50 and 14,0%, 15,63 and 1,04 t/Ha. The lowest losses in humus for the period analyzed were suffered by southern micellar-carbonate black soils in the areas to the west from the Dnister. As it was pointed out above, those are relatively most xeromorphic and initially less humused $(3,0\pm0.3\%)$ black soils in Odessa Region. Loss of humus for the analyzed 15 years here made 0.28 and 9,0%, 8,75 and 0,58 t/Ha respectively.

Results of many years' monitoring of soils state in the Region also show the tendencies to eventual decrease in the last 15-20 of black soils' provision with plants nutrition elements. Despite somewhat increase of the norm of fertilizers application in the last 5 years and visible decrease of intensity of black soils dehumification processes, they still have negative balance of plants nutrition elements (NPK).

Based on the results of many years study of dynamics of chemical processes in the soils of Odessa Region a system of measures has been substantiated to improve black soils' state and rise their fertility. Those are measures to protect soils from water and wind erosion, introduction of scientifically grounded crop rotation with soil-amendment crops. Very important is to increase the norms of fertilizers application, both mineral (first of all nitrogen and phosphorus) and organic. Without organic fertilizers it would be impossible to overcome modern tendency towards decrease of black soils' humus content, worsening of parameters of their agro-physical and ecological & agrochemical state and biochemical regime. In the irrigated massive it is also recommended to apply calcium-containing ameliorants and deep tillage from time to time.

Conclusions

1. Results of chemical processes' dynamic studies of many years (1971-2011) in the soils of Odessa Region forming morphology of their profile, material & chemical composition, regimes and properties determining fertility level have been described and discussed. The main volume of studies has been done on the dominating in the Region (over 90% of area) black soils – regraded, typical, common and southern, both non-irrigated and irrigated.

2. It has been established that in the past 20 years on the background of tendency towards agriculture extensification the direction and intensity of chemical processes in the soils of Odessa Region have changed, which entails changes in their composition and properties, most often degradation. Degradation changes in humus content and ecological & agrochemical state of black soils are the most notable, as the result in the last 15-20 years evident tendency towards fertility decrease is observed.

3. System of measures has been substantiated to improve black soils' state and to rise their fertility. The system comprises in particular a complex of measures to protect soils from erosion, introduction of scientifically grounded crop rotations with soil-amendment crops, application of recommended norms of mineral and organic fertilizers. Especially effective is application of organic fertilizers which would enable us to stop development of current processes of black soils' dehumidification, worsening of their agro-physical and ecological & agrochemical state and characteristics of biochemical regime.

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WASTE DUMPS IN LATVIA: FORMER LANDFILLING, CONSEQUENCES AND POSSIBLE RE-CULTIVATION

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Abstract: Waste and the pollution of ecosystems are direct consequences of the use of resources for the modern society development. Waste dumps are evident witnesses of former economic and industrial management without sustainable waste management planning.

Particular attention in this article is paid to the research of two waste dumps in the capital city of Latvia – Riga, which are planned to be re-cultivated in the nearest future and one site, which is former toxic hazardous soil dump site, where the remediation of site is of priority need. Waste dumps, mentioned in the article below, were created without environmental planning and in many cases the location of waste dumps are very problematic for secondary use of dumped material, remediation and re-cultivation. The present study is giving a general overview of contamination level in two waste dumps in Riga, which were made in the period from 50-ties to 70-ties of the 20th century, also the case of hazardous soil dump site formed in a period of more than 100 years is described. Planned actions as well as direct remediational technologies to reduce the poisonous impact to the urban environment and the role of recultivation in the urban planning in general are proposed.

The fate of contaminants, including heavy metals, macrocomponents, oil products, various chemicals and other chemical oxygen demand (COD) and biological oxygen demand (BOD) agents must be estimated in the process of re-cultivation. Special technologies can be applied such as:

a) *in-situ* and *ex-situ* technologies – excavation with separation and concentration of valuable materials, stabilization/ solidification of some areas in site and out of site;

b) *in-situ* technologies – soil as the infiltrate source flushing for groundwater contamination level diminishing, barriers/ treatment walls around the waste dump areas, chemical treatment and phytoremediation;

c) ex-situ technologies - soil washing and bioremediation in some cases.

Legislative aspects as well as the possible land remediation technologies for reduction of the waste dump generated contamination in Latvia are also discussed.

Keywords: dump sites, remediation, re-cultivation

Introduction

Soil and groundwater are environmental compartments that are primarily influenced by industrial development with increasing amount of industrial wastes and inadequate their dumping. It causes a large number of contaminated sites that are disseminated in post industrialized countries [1; 2]. Environmental contamination as a result of anthropogenic activities is not a recent phenomenon. Contaminated sites can be found in functioning as well as abandoned industrial (brownfield) territories, landfills, residential areas with historical contamination, road sides and rarely in polluted sites by natural activities. Pollution data on its amount and concentrations is known from historical studies and monitoring nowadays, but it should be periodically updated for the use of territorial planning or in case of a change of the land use. A special attention should be paid to the contamination in former dump sites, because in many cases this contamination is most problematic for remediation and is disturbing the territorial planning of cities. 242 territories now are numbered as contaminated and fixed in the National Register of contaminated territories of Latvia, 14 of those can be counted as main in the sense of the amount of contaminants, concentration of those and the priority aspect of the importance to be remediated and re-cultivated.

Legislation

The Law "On Pollution" (came into force on 1 July, 2001) defines the procedures in the sphere of contamination [3]. The purpose of the Law (Section 2) is to prevent or reduce harm caused to human health, property or the environment due to pollution, to eliminate the consequences of harm caused. A local government in co-operation with the relevant Regional Environmental Board of State Environmental Service has an obligation to ascertain and initially assess polluted and potentially polluted sites in a relevant administrative territory (Section 33). The Ministry of Defense has to ascertain and initially assess polluted territories in its possession and notify the relevant local government and regional environmental board thereof (Section 34).

Methods and procedures for the ascertaining of polluted and potentially polluted sites, as well as the procedures

for financing, conditions for data collection and utilization are regulated by the Cabinet of Ministers Regulations No 483 adopted on November 20, 2001 "Inventory and registration of contaminated and potentially contaminated areas" [4].

Latvian Environmental, Geological and Meteorological Centre (supervised institution of Ministry of Environmental Protection and Regional Development, MEPRD) has an obligation to maintain all collected and processed information about contaminated sites.

The main legislative Act at the moment in Latvia is the State Waste Management Plan 2006-2012 [5], which is issued based on several Directives of the European Union, such as 75/442/EEK on Waste [6], 91/689/EEK on Hazardous Waste [7] and several other Directives on specific kind of wastes. Most important topics in this management plan is to prevent the formation of waste, guarantee that the waste is not hazardous to health and environment, promotion of the reuse and recycling of useful material, minimizing the amount of waste and processing as close as possible to places of generating of the waste. The organizing of waste management should contain such demands in a priority queue:

1. Causes of waste formation must be diverted, including the implementation of clean technologies;

2. Reduction of the amount of waste and hazardousness;

3. Waste must be reused and recycled as much as possible;

4. Waste must be landfilled in a relevant way, in order to guarantee the safety for environment and health of people;

5. Dump sites must be closed in an appropriate way, also the re-cultivation of closed dump sites and polygons must be guarantee according the Waste Management Plan.

Latvia is divided in 10 waste management regions, one of separate regions is the Great Riga region. The Great Riga region has individual Riga Waste Management plan 2006-2012 [8].

Law "On Waste Management" [9] is regulating the management of waste on legislative base, it is in force from 01.01.2011. The aim of the law is to adopt the requirements of new Directive 2008/98/EC [10] in the legislation of Latvia. Draft law states the measures to encourage the options that deliver the best overall environmental outcome, including specific waste streams departing from the hierarchy where this is justified by life-cycle thinking on the overall impacts of the generation and management of such waste. It also states that the first objective of any waste policy should be to minimize the negative effects of the generation and management of waste on human health and the environment. Waste policies and waste management plans should be renewed because of the administrative reform. According to this Law the waste is divided in two main groups: hazardous and municipal waste. Hazardous waste is defined as the waste which has one or more hazardous property, which is making those hazardous for environment or human health. All other waste is classified as the municipal waste. The law says that collecting, sorting, storing, transport, landfilling or recycling of waste must be done only at special places defined by legislation. The responsibility on waste management in regions is taken by the municipalities.

Waste Management Law tells that the management must be done according the State Waste Management plan, Regional waste management plans and norms issued by local municipalities.

Dump Sites in Latvia

Former dump sites of mixed waste can be composed of hazardous waste as well as all other types of waste. In the former USSR, various kind of municipal, residential and construction waste as well as hazardous substances and materials were often dumped in these dump sites. Table 1 shows the main contaminated dump sites, type of contamination as well as probable recommendations for remedial actions in these sites in Latvia, numbers in the table correspond to numbers in Figure 1, where the geographic location is given.

Especially hazardous sites must be counted separately, e.g. liquid toxic substances dump site in Jelgava (mostly groundwater contamination), biomedical and chemical industry dump site (Olaine), former treatment facilities of the city Riga and similar ones. Sites mentioned further are the most important which must be remediated and re-cultivated at the end. After all these actions the risk assessment for ecological threats must be done. Several sites mentioned in the table are described in a more detailed way, some of those has had assessment of threats to environment and given recommendations for the type of remediation to use. Case studies for more detailed research are described for two former "municipal waste" sites – Deglava and Kleisti Dumps, which were used for disposal of various type of waste in 50-ties to 70-ties of 20th century, also the example of "BLB Baltijas Terminal" territory is given - hazardous soil dump site formed in a period of more than 100 years.



Figure 1. List of main contaminated dump sites

Liquid toxic substances dump site in Jelgava (nr.1) was used for almost 40 years and the history of this dump site can be divided in 3 parts: 1) solid waste disposal (1951-1969); 2) liquid toxic waste disposal (1970-1989); 3) uncontrolled periodic disposal (1990-2005). Now this dump site is included in the priority list of remedial sites in Latvia and detailed research has been done in 2005 and 2008. Contamination of groundwater with heavy metals, nitrates, chlorides are unacceptable, exceeds norms and must be treated. Recommended technologies for treatment would be pump-and-treat groundwater remediation technology, also the possible way would be the treatment by passive wall or reactive wall technologies with soil stabilization/solidification in some most contaminated sectors. Most toxic for the environment are heavy metals, but the complex contamination of groundwater is one of the highest in Latvia – concentration of chlorides in groundwater exceed 7500 mg/l, sulfates >1200 mg/l. Contamination is distributed in area of 23 ha, the depth of the plume reaches 12 m. [12]

Two sites of toxic liquid hazardous substances are in **Olaine (nr.2;3)**, where the contamination of 2500 m³ liquid and 4000 m³ solid wastes are found up to 12 m of depth. Main substances are pyridine, butanol, isopropyl alcohol, chlorides and ammonia. Maximum concentrations of those in groundwater exceed 4500 mg/l, 3200 mg/l, 1400 mg/l, 1400 mg/l and 3000 mg/l sequentially. Contamination is widely distributed in area of 45 ha and is polluting groundwater and surface water further. Treatment technologies could be used similar as in the previous case in Jelgava – reactive walls, pump-and-treat and soil flushing in most contaminated parts. [13]

Inčukalns Southern and Northern goudron ponds (acid tar lagoons) (nr.11) are historical contaminated sites located 30-35km from Riga. During 1950-1980 goudron was waste generated as the result of medical and perfumery oil production. Disregarding environmental protection measures goudron and other chemical waste was dumped in a sandy pit in a foresed area. In 1986 the dump site was closed. Considering permeable and percolation sandy layers contamination reached groundwater and artesian waters in the depth of 70-90m by infiltration. In 2010 a remediation project of the Inčukalns goudron ponds began, the main task of which is to prevent contamination of the territories alongside with goudron ponds. Project implementation will include treatment with lime and replacement, excavation and disposal to landfill (with or without treatment) and un-engineered capping as well as pumping out the contaminated waters, then treating and injecting it back into the subterranean in order to stop mobility of the contaminated plume. Otherwise contaminated plume together with the groundwater flow will reach the river Gauja horizon. Strong precautions will be taken during excavation and neutralization works in respect to air emissions, behaviour of tar and its ingredients in the soil and groundwater. The remediation in Inčukalns will demand multidisciplinar approach in order to have the successful result. The gudron in ponds and in the soil has complicated chemical properties, therefore, similar case studies abroad should be used for application in the Republic of Latvia. The sandy layers, in which goudron is placed in, are permeable and have good filtration properties, what means, that there is a deal with potential hazard in closest 25 years, if the movement of contamination plume in groundwater will not be stopped to the river Gauja. The main idea is to stop the plume with pumping out the polluted waters, then treating and injecting back into the subterranean. Soil flushing may be applied to reduce the impact of long migrated contamination plume. Environmental impact and monitoring of it should be made using previous knowledge in other case studies. Air emissions as well as behavior of tar and its ingredients in soil and groundwater during excavation and neutralization works must be strongly taken in account. The legislation and funding should be taken in account while planning any time of remediational activities, also emissions and residuals during works must be utilized under the strong control.

Table 1

List of Main Contaminated Dump Sites, Type of Contamination and Recommendations for Probable Remedial Actions [11]

No	Location, name	Type of contamination	Recommendations for probable
			remediation actions
1.	Liquid toxic substances dump,	HM^1 , EAF ² , OP ³	Groundwater treatment technologies, soil
	Jelgava		flushing, reactive walls
2.	"BIOLAR" dump of toxic	Toxic hazardous substances,	Groundwater treatment technologies, soil
	substances, Olaine,	HM	flushing, reactive walls
3-	Olaine dump site	Heavy metals	Risk assessment, monitoring, re-cultivation,
			soil amendments
4.	Mārupe Landfill	COD ⁴ , N, P	Risk assessment, monitoring, re-cultivation
5.	Priedaine dump site	COD, ammonia, OP, HM, N	Risk assessment, monitoring, re-cultivation,
			reactive walls
6.	Ķilupe Landfill, Ogresgals	COD, EAF, N	Risk assessment, monitoring, re-cultivation,
			reactive walls
7.	Getliņi Landfill, Riga	EAF, N, COD	Risk assessment, monitoring, reactive
			walls, soil flushing
8.	Kleisti dump site, Riga	HM (Zn, Pb, Cr), COD, N,	Risk assessment, monitoring, re-cultivation,
		EAF	reactive walls, groundwater treatment
			technologies, soil amendments
9.	Deglava Street dump site	COD, N, V, EAF	Risk assessment, monitoring, re-cultivation,
			reactive walls, groundwater treatment
			technologies, soil amendments
10.	Bieķengrāvis, former	COD, OP, N, HM (Cu)	Environmental contamination assessment,
	hazardous waste dump		groundwater treatment technologies
11.	Inčukalns Goudron Ponds –	OP, EAF, COD	Groundwater treatment technologies, soil
	Southern and Eastern (2 sites)		flushing
12.	Gas Station near	OP	Groundwater treatment technologies, soil
	Sarkandaugava Ditch		washing
13.	BLB Baltijas Terminal	HM, OP	Solidification/stabilization, risk assessment
14.	Hazardous Waste Dump Site in	OP	Groundwater treatment technologies, soil
	Zvāre quarry (Tukums area)		washing

¹ – heavy metals and their compounds

²– elements in anionic form

 3 – oil products

⁴ - contamination with organic substances, creating a high level of chemical oxygen demand

In autumn 2010 the promotion of two great scale stabilization/solidification projects were started in the sites of Liepāja and Riga ports. These projects should be viewed as the pilot ones for further development of remediation works in Latvia. The use of the stabilization / solidification (S/S) technology is not the only solution for the remediation and immobilization of toxic compounds; the further research might be done to draw a sketch for the use of other heavy metal remediation technologies, but the case study, described below has improved that S/S technology still would be one of most effective for active and former industrial territories, because stabilized areas does not threat the environment around and also can be used for industrial construction use in future. The present study in the former hazardous soil dump site describes preliminary research before the use of this technology. This site is situated in Riga, Jaunmīlgrāvis - **BLB Baltijas Terminal (nr.13)** – it is economically active from the beginning of the 20th century. In earlier years (1894-1967) the territory was used for several industrial purposes including the manufacturing of superphosphates, but just nearby the dump site for tailings was made. Later in this area the oil product storage, reloading and transit terminal was founded. In 60-ties of the 20th century the factory-workshop was functioning, but later the oil product terminal facility overtook

the area. Soil pollution source mainly was superphosphate production waste (slag), where the highest concentration was received for lead, copper, zinc and arsenic. Total amount of toxic heavy metals throughout the whole research area was estimated 1264 t or 15 kg/1m² of slag or: 755 t of copper, lead 85 t, zinc 358 t, 66 t of arsenic. [14]

Stabilization / solidification technologies are based on the treatment of contaminated soils with materials such as cements and siliceous pozzolans and it can be employed *in situ* or to the excavated material. As a result, the mobility of the contaminant is reduced by physical-chemical processes. Solidification of the polluted substrate with cement restricts its contact with groundwater and air. Cement and siliceous pozzolans react with metals and cause the formation of hydroxides, carbonates and silicates of very low solubility. This treatment is not efficient for heavy metals that form soluble hydroxides or anions species. It should be emphasized that the mixing process and the heat generated by cement hydration reaction can increase the vaporization of organic pollutants. [15]

S/S technologies have been used for decades as the final treatment step prior to the disposal of both radioactive and chemically hazardous wastes. The stabilization refers to an alteration of waste contaminants to a more chemically stable form, thereby resulting in a more environmentally acceptable waste form. Typically, the stabilization processes also involve some form of physical solidification. [16] The preliminary study before the start of remediation was done in the Terminal territory and it gave results of leaching tests for experimentally solidified average soil samples, thus the perspective use of the S/S technology was approved.

Table 2

Parameter	Soil contamination in sample for S/S testing	Zero sample (pH level 3,2)	5% cement (pH level 10,5)	13% cement (pH level 10,5)	Acceptable leaching level after the use of S/S method (Finland)
As	350	0,02	0,02	0,08	0,5
Cd	2,3	0,27	<0,002	0,002	0,02
Cr	13	<0,01	0,03	0,01	0,5
Cu	2100	600	0,25	0,27	2
Hg	0,54	<0,002	<0,002	<0,002	0,01
Ni	8,1	0,65	<0,01	<0,01	0,4
Pb	400	0,02	<0,01	<0,01	0,5
Zn	1200	36	0,04	0,03	4

BS EN 12457-2 leaching test results compared to soil contamination (mg/kg). [17]

Leaching test [18] for solidified samples has shown that "zero sample" is leaching out unacceptable amounts of heavy metals – Cd, Cu, Ni and Zn, but in the stabilized solidified form leaching is diminished and are at the acceptable level. Besides, the emission of cadmium and nickel leaches more even the total amount is under the acceptable level. The results show that S/S remediation technology has high efficiency on heavy metals, which are most important contaminants in the former hazardous soil dump site.

Deglava and Kleisti waste dumps in Riga (nr.8;9). Riga Development Plan 2006-2018 [19] says that old former dump sites must be remediated and re-cultivated according environmental legislation, thus two cycles of research were done for each of these dump sites. Examinations of studies indicated that the most contaminated parts are the soft dry waste layer and the water saturation (infiltrate) under the waste layer. Dry waste layer in both dump sites is seen to have very strong heavy metal pollution, but leachate - a high content of heavy metals and organic substances, including petroleum products. Also concentration of total nitrogen, nitrogen compounds, total phosphorus, chlorides, sulfates were detected, as well as indicative parameters of COD and BOD. Additionally microbiological analysis was done (*Escherichia coli*, total coliforms, microbial colonies and enterococci).

The research was done in order to assess how much waste must be removed and what environmental impact remedial and re-cultivation actions will have. Deglava Dump site was researched in 2010, but Kleisti Dump site – started in summer 2011 and was finished in October 2011 [20;21].

Research Stage. Drilling sites in both studies were chosen after careful analysis of historical research study materials. Drilling works were done with *Fraste ,, Terra - in*" and "*Iveco*" drilling machines. The auger drilling method has been chosen, and boreholes 1-12 m of depth were drilled, including those done through the waste. Temporary monitoring wells were input in sites around and on the dump hill sites. Groundwater sampling and further analysis of possible contamination parameters were done.

Surface waters, sediments from ditches were sampled in closest area around these two dump sites on indicative parameters. The odor testing was done for the air sampled from the waste massif, in order to quantify the possible smell emissions while works of re-cultivation would be done. Emissions and gases were calculated based on the U.S. Environmental Protection Agency developed model LandGEM (Landfill Gas Emission Model - Version 3.02.). [22]

LandGEM calculations are used for household waste decomposition rate of the first order equation which takes into account the decomposition of waste disposed in municipal solid waste landfills. This program provides a relatively simple approach gases emissions assessment. Models developed in the U.S. dumps empirical observation basis. The model adopted in the data may instead be placed in the field observations.

Planning Stage. The waste from these two sites is planned firstly to be transferred to another part of the dump. One part of the waste, which is the so-called dry waste (the thickness varies from 2 - 11m) can freely be moved to other parts of the dump, after what all must be sorted, useful compounds recycled and the remained part remediated using technologies of separation and concentration of elements by particle size and magnetic properties. The remaining mass of the waste, if it does not contain hazardous components, which are exceeding legislative values, can be used for filling the ground where excavation works will be done and soil removed also from below the groundwater level. It is necessary to practice the lowering of groundwater by pumping system of wells. After a very rough estimate, lowering the water level should be an average of 1-2 m, biodegradable leachate together with the ground water will reach 100 000 to 300 000 m³ at each of sites. Averagely a day each pumping well can pump out around 200 m³ of groundwater in combination with leachate. If there are many, such a huge volume of water and leachate must be directed to the city sewerage system only after additional treatment, because research was carried out and groundwater and leachate analysis results have shown that certain characteristics of chemical concentration exceeds the Latvian legislation set out criteria for groundwater contamination.

Recommendations for Re-Cultivation.

After these "hills" will meet up environmental expectations after remediation procedures, those should be covered to prevent the infiltration of precipitation and stop the waste pollutant runoff from the waste. Preferably is to cover the waste with sandy loam, organic soil and then greening the area. Depending on the planned land use the bearing capacity for the soil must be adjusted in accordance with expectations. Before moving the waste redesigning of drainage system must be done from the dump outgoing leachate. After the re-cultivation a network of groundwater monitoring wells in both areas of former dump sites and surroundings must be developed, where regular monitoring of groundwater will be performed to control the leachate / groundwater quality and flow direction in future. It is recommended to carry out a risk assessment based on a planned regular (2-4 times per year) of groundwater monitoring data, and in addition according to the random selection method should be regularly monitored groundwater quality in farther situated (up to 0,5-1 km) surroundings and residential building areas. Some contaminated areas will remain and those will be detected and mapped after the finishing of the main part of the re-cultivation during the after remedial research. These separate areas can be remediated using several methods, a brilliant example is phytoremediation technology - with the use of plants to remove, destroy or sequester hazardous substances from the environment. In some cases different soil amendments can be used, e.g. where the pH level of the soil is low and, therefore, mobility of the polluting metals is high. This can be a strong approach for cationic metals, if those are concentrated in the upper part of the soil – these amendments must be chosen after deep evaluation and chemical experiments.

Remaining contamination of areas under the "reprocessed hills" can be diminished by chemical treatment. Chemical processes include reduction of the bioavailability/mobility of heavy metals as well as other contaminants upon chemical reactions with specific reagents. Technologies for heavy metal remediation are based on precipitation, oxidationreduction, and organic-metal complexation reactions that are well known and widely studied technique in chemistry, for example, chemical technology, environmental, analytical, and radioanalytical chemistry. The use of chemical processes has a drawback conjugated with the possible side effects on the environment that has to take into consideration in the choice of the chemical reagents in treatment process [23; 24]. Dissolved heavy metals in groundwater can be precipitated out of solution in various insoluble compounds. The most common heavy metal precipitates are hydroxides, sulphides, carbonates, phosphates, oxalates, and some others. Solubility product constant (K_{sp}) of the insoluble compound characterizes perfection of precipitation reaction. Using, for example, phosphate containing precipitating reagent (apatite II) U, Pu, and heavy metals (Cu, Cd, Pb, and Zn) are transformed into low-solubility phosphate mineral phase in which they are not bioavailable. Effectiveness is provided due to the extremely low K_{sp} value, for example, solubility product constant of U-phosphate (autunite) is $K_{sp}=10^{49}$, and of Pb-apatite (pyromorphite) is $K_{sp}=10^{-80}$ [25]. The lower the solubility product constant, the more insoluble the precipitated compound is. Precipitation of insoluble substance causes two another processes: coprecipitation and sorption that occur simultaneously with formation of precipitates. Thereby heavy metals that stand in aqueous media in minor and micro concentrations are also concentrated in solid phase. Precipitation, coprecipitation and sorption are spontaneous processes with tendency of disorder (entropy) decreasing, i.e., contaminants arrive into the solid phase.

Conclusions

The in situ and ex situ technologies are used for remediation of contaminated sites. The future of dump site remediation in Latvia mostly could be done by S/S, separation / concentration, chemical treatment, soil flushing, phytoremediation technologies or combined. In Latvia the assessment and evaluation of contaminated and potentially contaminated sites began in the 1980s. The National Register of Contaminated Territories (NRCT) covers the territories

that are contaminated with various materials, inorganic and organic including hazardous and non-hazardous substances. In the NRCT the sites are divided in 3 categories: the first includes 242 contaminated sites, 14 of which can be counted as the main contaminated dump sites in Latvia – former municipal or industrial. Heavy metal remediation in the case study of "BLB Baltijas Terminal" is mostly connected with the treatment of soil and immobilizing of toxic elements in situ - S/S technology is the most appropriate in this case. Leaching test shows that in the stabilized and solidified soil leaching is diminished and is at the acceptable level. It certifies effectiveness of the applied S/S technology. Leaching tests and economical evaluation has shown that this technology is among the best applicable. Separation/ concentration technologies, chemical treatment, soil washing and groundwater treatment technologies can be applied in remediation of two former municipal waste dumps in Riga: Kleisti and Deglava sites. Remediation of dump sites in the capital city Riga is urgent and important need in the way of the future development.

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SILVER LOADED CLAY AND INVESTIGATION OF THEIR ANTIMICROBIAL ACTIVITY

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Abstract: To guarantee the microbiological quality of the water, which has been affected by the pollution of the bacterium, it is necessary to implement a tertiary drinking system in the resident houses. A possible complementary system is the utilization of clay for treatment of drinking water.

Keywords: Escherichia Coli, Staphylococcus Aureus, Clay, Silver, Silver Nanoparticles

1. Introduction

Clay can be used for dispersion of Ag^+ and Ag^0 nanoparticles activity having permanent removal of coliforrm bacteria normally found in natural water. Clinoptilolite resulting with antibacterial effect of Ag^+ and Ag^0 nanoparticles/ clay was made from two bacteria: *Escherichia coli ATCC 25922* and *Staphylococcus aureus ATCC 25923*.

In this study, local natural clays from Răzoare areas have been investigated. Samples were used in the raw form (polycationic form) as well as in the Na-exchanged form.

2. Experimental

The Na-forms of the natural clays from Răzoare were prepared by treating the clay polycationic forms with NaCl 1M in a solid/liquid ratio of 1:10, at room temperature, under magnetic stirring for 12 hours, followed by centrifugation, water washing and air drying at 80°C for 5 hours.

The samples of clay in homoionic form of Na⁺ (R1-Na⁺) were suspended in $0.1M \text{ AgNO}_3$ solution at room temperature for 24 h in the dark (threefold with fresh solution), adjusting the solution to pH 5.0, with intermittent shaking to obtain the silver – loaded clay named R1-Ag⁺. The resulting solids were separated by filtration, washed with deionized water several times and dried overnight at room temperature. If the pH of exchange is greater than 7.5-8, the samples become dark because Ag⁺ in the zeolite turned to Ag⁰. Samples results were analyzed by FTIR and SEM. (Fig. 1).

After preparing inoculum and culture media, clay species were put in contact with the surface agarizat environment. After 24 hours of incubation was followed by inhibition of bacterial growth phenomenon by the appearance of lysis zones around clinoptilolite with Ag^+ and Ag^0 nanoparticles clay (Fig. 2 and 3).

3. Results and discussion

We incorporate Ag^+ in a clay carrier. The ion exchange of Ag^+ with other positive ions (often sodium) from the moisture in the environment, will give a release of silver "on demand". The structural location of Na⁺ cations, the channel dimensions and the hydrated ionic radii of Na⁺ (3.58 Å) are involved in the easily exchange with hydrated Ag^+ ion (3.43 Å) [1-4] The Na⁺ cations are preferable for Ag^+ instead of Ca²⁺, K⁺ and Mg²⁺ found in the natural clay.

FTIR spectra of the samples and silver exchanged, R1-Na⁺ and R1-Ag⁺, were investigated between 600 and 4000 region (fig.1). The vibration band assignments of the samples are summarized in table 1.

The SEM micrographs in fig.1 confirmed the phase purity of the crystal morphology. Also, the SEM micrographs showed that the particles were closely similar in size and appearance, which suggests that the loading of silver ions into framework seems to have little or no effect on the size of the zeolite.

After 24 hours of incubation was followed by inhibition of bacterial growth phenomenon by the appearance of lysis zones around particles of Ag^+ clinoptilolite.(Fig. 2 and 3)



SEM micrographs of R1-Na $^+$ (a) R1-Ag $^+$ (b)

						Ta	ble 1
Assignments	of	vibration	bands	of	the	initial	and
$\Lambda \sigma^{+} / \Lambda \sigma^{0}$ over	ana	od forms a	f the al	0 1 7 6	omn	los [4 7]	

Ag /Ag exchanged for ins of the etay samples [4-7]						
Vibration modes	Frequency (1/cm)					
	R1	$RR1-Ag^+$	R1-Ag ⁰			
External double ring	719.15	698.55	706.43			
External asymmetric stretch	792.94	795.91	802.53			
External symmetric stretch	996.95	1008.91	995.46			
Internal asymmetric stretch	1297.25	1248.10	1299.49			
OH bending	1633.27	1627.94	1632.05			
H-bonded OH stretching	3432.81	3432.17	3423.64			
Isolated OH stretching	3616.77	3618.70	3611.49			



Fig.2. Antibacterial test results using *Escherichia* coli ATCC 25922 P₁-P_b treated with AgNO₃, P₂-P_a treated with AgNO₃.



4. Conclusions

Ag⁺-clay was tested for their antimicrobial activity against *E. coli* and *S. aureus*. The bactericidal activity depended on the permeability and penetration rate against the bacteria cell wall.

Results showed that the silver highly dispersed on clay structure exhibited a good inhibition effect on the growth of *Escherichia coli ATCC 25922* and *Staphylococcus aureus ATCC 25923*. The inhibitory effects of silver were dependent on amount of Ag^+ -clay and Ag^0 -clay added.

The R1-Ag⁰ sample is more prolific than R1-Ag⁺ sample. Thus R1-Ag⁰ sample is a good candidate with potential antibacterial applications.

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MERCURY-CONTAMINATED FISH AND ESSENTIAL FATTY ACIDS: PROBLEMS AND SOLUTIONS

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Abstract: Fish consumption is an important part of human diet due to essential omega-3 fatty acids found naturally in this product. Many researchers from all over the world found that high mercury concentrations in the body reduced the heart-protective effects of the fatty acids in fish oils. People shouldn't be constrained by choosing between the health hazards related to toxins caused by industrial pollution and the nutritional benefits provided by consummation of essential fatty acids contained in oily fish. It is very important to find an alternative natural source of essential omega-3 fatty acids in the human diet. The Laboratory of Food Additives of the Practical Scientific Institute of Horticulture and Food Technology focuses on developing new essential vegetable oil blends with optimal ratio between omega-6 and omega-3 fatty acids.

Keywords: pollution, fish, mercury, fatty acids

Introduction

Health benefits of fish are linked to the essential nutrients such as omega-3 fatty acids, vitamins (D, B_2 , etc.) macro- and microelements such as calcium, phosphorus, iron, zinc, iodine, magnesium, and potassium. Two essential omega-3 fatty acids EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) aren't produced by the human body and are found in every kind of fish, especially in fatty fish. Fish do not actually produce omega-3 fatty acids, but instead accumulate them by consuming either microalgae or prey fish that have accumulated omega-3 fatty acids, together with high quantity of antioxidants as iodine and selenium, from microalgae, where these antioxidants are able to protect the fragile polyunsaturated lipids from peroxidation [1, 2, 3]. Practically all fish contain trace amounts of methyl mercury. As mercury moves up the food chain, it accumulates. As a result, larger predator fish, such as swordfish or tuna, tend to contain higher levels of mercury than salmon and other fish farther down the food chain. For this reason, the U.S. Food and Drug Administration recommends limiting consumption of certain predatory fish species due to high levels of toxic contaminants [4].

Organic mercury is most commonly found in the environment as methyl mercury: CH₃Hg⁺, or MeHg. Natural sources of elemental mercury include: degassing of the earth's crust, geologic deposits of mercury, forest fires and volatilization from the ocean. Levels of elemental mercury in the environment increase due to human industry: burning of fossil fuels, especially coal-burning plants, chemical industries, world-wide mining of gold and mercury. Once in water, mercury enters a complex cycle in which one form can be converted to another. Aquatic anaerobic bacteria identified as *Enterobacter aerogenes* convert elemental mercury to methyl mercury, which is taken-up by other microorganisms. Fish absorbs the toxic methyl mercury (from these microorganisms or other fish with concentrated MeHg) as they feed in these contaminated waters, and so the toxin builds up in them.

There is concern about mercury contamination through fish all around the world, but specific recommendations vary. For example, Health Canada advises consumers to limit their consumption of swordfish, shark or fresh and frozen tuna to one meal per week; for young children and women of child-bearing age, the recommended limit is one meal per month. Health Canada's guideline is 0.5 ppm total mercury content [5].

The FDA has released guidelines for children, women who are pregnant and women who are trying to become pregnant. These guidelines state that no more than 12 oz of low mercury fish should be consumed weekly. "Highest" mercury fish should be avoided and "high" mercury fish should be kept to only three 6-oz servings per month [6].

Eating fish high in mercury puts middle-aged men at a greater risk for coronary heart disease and may offset the protective effects of omega-3 fatty acids in some seafood, according to an important new Finnish study. The findings were based on an ongoing 14-year study by the Research Institute of Public Health at the University of Kuopio of 1,871 men ages 42 to 60 and free of previous heart disease or stroke [7].

The purpose of this paper is to determine balanced fatty acid profile using computer-assisted analysis of vegetable oil composition in accordance with WHO guidelines for further elaboration of the oil blends with optimal ratio of omega-3 and omega-6 fatty acids, through the replacement of "high mercury" fish consumption with intake of essential vegetable oil blends.

Results and Discussion

Computer-assisted analysis was used to identify nutritionally beneficial blends of commercially available edible oils, and to verify that their inclusion in alimentation is compatible with all current nutritional recommendations (WHO).

Computer-assisted analysis through Microsoft Excel by using Pearce's rule allows the translation of nutrient recommendations of polyunsaturated fatty acids into vegetable oil combinations. The preliminary forecasts have been developed through detailed calculations of experimental data from literary sources.

<i>Table</i> I	Та	ble	1
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	Fatty acti composition of some vegetable ons [8]								
Nr.	Vegetable oils	Fatty acid composit	Ratio (calculated)						
		linolenic acid (ω-3)	linoleic acid (ω-6)	oleic acid (ω-9)	between ω-3 and ω-6				
1	Sunflower oil	*ND – 0,3	48,3 - 74,0	14,0-39,4	< 0,004				
2	Rapeseed oil	5,0-13,0	11,0-23,0	8,0-60,0	0,45 - 0,56				
3	Maize oil	ND – 2,0	34,0-65,6	20,0-42,2	< 0,03				
4	Soybean oil	4,5 - 11,0	48,0 - 59,0	17,0-30,0	0,09 - 0,19				

Fatty acid composition of some vegetable oils [8]

*ND - non detectable, defined as 0,05%

The most adequate resultant blends of vegetable oils with optimal ratio of omega-3 and omega-6 fatty acids were next: a) 89% sunflower oil with 11% flaxseed oil; b) 80% sunflower oil with 20% flaxseed oil; c) 62,5% sunflower oil with 37,5% rapeseed oil; d) 69% maize oil with 31% rapeseed oil; e) 35% sunflower oil with 65% soybean oil.

The present results, obtained using a simple computer-assisted analysis, confirm our suggestion that not single oil provides the recommended amounts and balance of essential fatty acids, but specific blends of vegetable oils in the proper proportions.

Conclusion

To resolve the problem associated with nutritional advantages of fish consumption while reducing exposure to mercury and keeping optimal ratio of essential fatty acids, it's necessary to replace "high mercury" fish consumption to vegetable oil blends and to consume mainly types of fish low in mercury, non-contaminated.

The consumption of essential oil blends composed of several vegetable oil in adequate proportion which can provide optimal ratio between omega-6 and omega-3 fatty acids is an inexpensive and simple way to meet current dietary recommendations for polyunsaturated fatty acids, favoring overall dietary nutrient requirements. Combination of vegetable oils instead of single oil is more likely to achieve current nutritional recommendations. Such combinations are relatively easy to create via compositional modifications to improve the nutritional value of vegetable oils used for seasoning.

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THE POLLUTION SPECTRUM OF OLD PESTICIDES STORAGES IN MOLDOVA

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Keywords: soil pollution, POPs, pesticides.

Introduction

The inventory of old pesticide storages in Moldova performed by Ministry of Environment and World Bank showed that a large quantity of polluted sites (near 1500) remains after the repacking and evacuation project [1,2]. This work was made first of all for Persistent Organic Pollutants (POPs). More that 15 % sites were determined as extra high polluted territory with the POPs concentration in soil exceeding 50,0 mg/kg. They include some of the world's most harmful chemicals including highly toxic pesticides such as HCH, DDT; industrial chemicals such as PCBs. The management of domestic and hazardous wastes is considered as one of the most urgent environmental problems in Moldova. Old pesticide storages are in different conditions and can be classified as important pollution sources for environment. Other toxic substances were detected also in soils which are in list of monitoring substances of Water Framework Directive: PAHs, trifluralin, triazines and others. They are synthetic chemical substances with high toxic characteristics to wildlife and humans. However the information about the actual status and complete pollution spectrum is not sufficient at present. This investigation is important also for the assessment of remediation technologies that can be used for the future soil detoxication.

The aim of the study was an assessment of the pollution spectrum in soil at obsolete pesticide storages. This objective was realized by the determination of different type of pollutant in Gas Chromatographs equipped with μ ECD and mass selective detectors.

Analytical procedure

<u>Sampling</u>

Samples have been collected according to the Standard Guide for Composite Sampling and Field Sub-sampling for Environmental Waste Management Activities, EPA's Guidance for Choosing a Sampling Design for Environmental Data Collection, EPA QA/G-5S (USEPA 2000c), and other standardized procedures [3-8].

Extraction and clean up.

The extraction and analytical procedures were made by appropriate normative documents [9-13]. Prior to the extraction, the soil samples (1 g) were spiked with 1 ml of the solution of two internal standards (PCB29 and DCB) of appropriate concentration in the final solution. Extraction was carried out by Microwave Extraction System in the mixture of hexane-acetone (proportion 1:2, 20 ml) three times. After cooling down, the extracts were collected in the glass condenser and concentrated in n-hexane to a volume of 1 ml. The extracts were cleaned up on adsorption on chromatography columns filled up with 1 g of activated silica gel (activated at a temperature of 135° C for 16 hours. The column was conditioned with 5 ml of hexane. Interested substances (PAHs and POPs) were eluted from column with 5 ml of n-hexane/dichloromethane mixture (1:1). Final elutes were evaporated in argon flow to 1 ml. Sulfur interference was removed by copper powder activated in nitric acid.

Analytical determination.

All reagents (solvents, standard solutions, anhydrous sodium sulfate, and pure gases) were of the pesticide grade purchased from Supelco-Aldrich. Agilent 6890 gas chromatograph equipped with 63Ni µECD detector, split–splitless injector, and capillary column HP5 were used for the pesticide analysis. PAHs and trizines analysis were performed on an Agilent 6890 gas chromatograph equipped with Agilent 5973 Mass Spectrometer (CG/MS 6890/5973) based on the selected ion monitoring system (SIM) of molecular ion peaks and associated characteristic fragment ion peaks. Method conditions are presented in tables (1-4).

Instrument calibration parameters included calculation of the sensitivity (I-SE) as the slope of calibration curve at P-value < 0,05, linearity (I-LI) as a correlation coefficient for the calibration regression line and the instrument detection limit (IDL) corresponding to 3SD (standard deviation) of five replication of the lowest standard solution.

Table 1

System elements	Method parameters
Injection ports	Split/splitless inlet; injection – Split 5:1, 2 µl, inlet temperature of 300°C
Column	HP-5: 30 m Length, 320 μm I.D., 0,25 μm Film, max 325 ^o C
Carrier gas	He, 1,4 ml min ⁻¹ or Average Velocity 30 cm/sec, Constant Flow
Oven	First ramp: 100°C (hold 1 min) to 200°C at 20°C min ⁻¹ hold 2 min;
	Second ramp: 200°C to 280°C at 10°C/min, hold time 2 min.
Detector	63Ni μECD, 320 ^o C, N2 makeup gas, 60 ml min ⁻¹
Data collection	ChemStation

Experimental Conditions for pesticide determination in GC 6890

Table 2

Experimental Conditions for PAHs and triazines determination in CG/MS 6890/5973

System elements	Method parameters
Injection	Autosampler Agilent 7683 B
Injection ports	Split/splitless inlet; injection – Splitless (keeping the split closed for
	1.0 min), 1 µl, inlet temperature of 300°C
Column	HP-5MS: 30 m Length, 320 µm I.D., 0,25 µm Film, max 325 ^o C.
Carrier gas	He, 1,5 ml min ⁻¹ or Average Velocity 46 cm/sec, Constant Flow
Oven	First ramp: 120°C (hold 1 min) to 200°C at 20°C min ⁻¹ ; Second ramp: 220°C (hold 1
	min) to 290°C at 5°C/min, hold time 2 min.
Detector	Mass detector, EI 70 eV, quadropole 150° C, SIM
Data collection	ChemStation

Results

Five POPs compound groups namely \sum DDT, \sum HCH, Chlordane, Heptachlor and Toxaphene have been found in soil samples taken at investigated sites, in concentrations exceeding the national standard for organochlorinated substances in soil (0.1 mg/kg). Six DDTs isomers, three HCHs isomers, and Toxaphene as a mixture of approximately 200 organic compounds were analyzed in soil and waste samples. The pollution of POPs sites with DDT (88,2%) and - to lesser extent - with HCH (74,9%) can be defined as widespread. The share of sites contaminated with Chlordane (31%) and Heptachlor (22,7%) is also significant. The less number of sites are polluted by Toxaphene mixture (10,2%), but this pollution is characterized usually by high level. Aldrine, Dieldrine, Endrine, HCB and Mirex were not detected in the investigated samples. The acquired data showed a severe level of soil contamination with DDTs and HCHs at some sites, in the order of hundreds and even thousands of mg/kg.

Table 3

GC/MS calibration parameters for PAHs and triazines determination							
Nr	Name	Mass ion	ISE	ILI	IDL ng/ml		
	·	PAHs			-		
1	Naphtalene	128	2.71	0.999	4.0		
2	Acenaphtylene	152	3.01	0.999	1.2		
3	Acenaphtene	152	2.57	0.999	0.5		
4	Fluorene	166	2.15	0.999	0.5		
5	Phenanthrene	178	3.03	0.999	0.4		
6	Antracene	178	3.05	0.999	1.0		
7	Fluoranthene	202	3.41	0.999	2.5		
8	Pyrene	202	3.49	0.999	2.1		
9	Chrysene	228	3.28	0.999	2.0		
10	Benz[a]anthracene	228	3.22	0.999	2.1		
11	Benzo[b]fluoranthene	252	3.51	0.999	3.5		
12	Benzo[k]fluoranthene	252	3.86	0.999	2.2		
13	Benz[a]pyrene	252	3.33	0.999	2.0		
14	Benz[ghi]perylene	276	3.26	0.999	2.2		
15	Dibenz[ah]anthracene	276	4.14	0.999	2.5		
16	Indeno[1,2,3-cd]pyrene	276	3.7	0.999	2.5		
		Triazines					
1	Ametryn	227	3.15	0.996	3.4		

2	Atrazine	215	4.15	0.998	2.5
3	Prometon	225	3.25	0.995	4.1
4	Prometryn	241	3.42	0.995	3.2
5	Propazine	229	3.54	0.997	3.5
6	Simazine	201	4.25	0.998	2.5
7	Terbutryn	241	3.64	0.996	3.8

Table 4

GC calibration parameters for organochlorine pesticides determination

Nr	Name	Range of standards, µg/ml	ISE	ILI	IDL ng/ml
1	Trifluralin	0.20 - 2.00	0.40	0.999	2.0
2	a_BHC	0.05 - 0.50	2.16	0.998	1.6
3	b_BHC	0.20 - 2.00	0.38	0.998	1.5
4	g_HCH	0.05 - 0.50	1.76	0.998	1.2
5	Heptachlor	0.15 - 1.50	0.36	0.995	2.5
6	Aldrine	0.10 - 1.00	2.14	0.998	1.8
7	Chlordane	0.20 - 2.00	0.52	0.998	2.9
8	o-p-DDE	0.20 - 2.00	1.34	0.999	3.5
9	p,p-DDE	0.20 - 2.00	1.38	0.998	3.6
10	Dieldrine	0.23 - 2.30	1.68	0.998	3.2
11	o,p-DDD	0.38 - 3.80	0.74	0.996	4.5
12	Endrine	0.40 - 4.00	1.20	0.996	4.0
13	p,p-DDD	0.40 - 4.00	0.67	0.996	3.5
14	o,p-DDT	0.50 - 5.00	0.75	0.996	4.2
15	p,p-DDT	0.40 - 4.00	1.09	0.997	4.5
16	Mirex	0.20 - 2.00	0.91	0.999	3.5

Some part of site is polluted by several groups of compounds. The toxicology impact on these sites is higher in the case of synergism of different toxicants. The distribution of high polluted sites (exceeding 50,0 mg/kg) by POPs compounds are following: 144 sites are polluted by one substance; 48 sites – by two substances; 9 sites – by three substances, and 3 sites – by four POPs. Actually there are 252 POPs polluted sites with the concentration more that 50.0 mg/kg (15,9 % of total 1589 sites).

Sites with high POPs concentration (more 50,0 mg/kg) have a additional complex pollution by Trifluraline (85%) triazines (64%) and PAHs (33%). Some sites with relative low POPs pollution have high concentration of these substances. Near 10% of investigated site have high pollution (more 50,0 mg/kg) by other toxic substances which are not included in the inventory program. From other site determined pollutants also have high toxicity and should be monitored. The interpretation of this large set of analytical results and wide interval of concentration may become easier by showing their distribution (assuming distribution is lognormal) in a few clusters: 0.10 - 1.0 mg/kg; 1.0 - 10.0 mg/kg; 10.0 - 50.0 mg/kg; and > 50.0 mg/kg. The distribution of five investigated groups by pollution clusters gives a better understanding of the extent and severity of contamination (Fig. 1 and 2).



Figure 1. Distribution of POPs polluted sites by pollution clusters



Figure 2. Distribution of polluted sites with other toxic substances by clusters

The spatial distribution of old deposits ranges with the different pollution level in soil is presented on figure 3. This map is illustrated total POPs concentration in complex soil samples. The density of high polluted sites is higher for raions with more intensive fruit production. 18 districts have more that 15% of extra high pollution sites (figure 4). The polluted soil with concentration exceeding 50,0 mg/kg can be classified as hazardous waste. This fact means these sites should be protected first from the population access and recommended for the remediation to minimize direct contact with animals and people. The sampling of these sites confirmed indirectly a relative big volume of the toxic waste residuals on old pesticide storages



Figure 3. Spatial distribution of contaminated sites with different pollution level.

Conclusion

1. The inventory of POPs polluted sites showed a huge number of extra high polluted sites (exceeding 50,0 mg/kg) remained after repacking projects in Moldova which need first of all a remediation or other action to eliminate direct contact with people and animals.

2. The pollution spectrum of POPs is a complex and consists of five groups: DDTs isomers, HCHs isomers, Toxaphene mixture, Chlordane and Heptachlor. The principal groups among these substances are DDTs and HCHs isomers.

3. The pollution spectrum is characterized also by other toxic substances as triazines, trifluraline, PAHs and others, which were not analyzed in this study. Determined pollutants are included in normative documents for the monitoring of water quality (Water Framework Directive of EU, etc).

4. The database of POPs polluted sites was created by inventory project in Moldova but this information needs development for other pollutants and classification for risk level for environment;

5. The character of pollution spectrum demonstrates a complex character of future remediation and other action by the elimination of negative impact from polluted sites to environmental and public health.



Figure 4. Distribution of high POPs polluted sites (> 50,0 mg/kg) by districts in Moldova.

The investigations are made in the frameworks of the project "Creation of the Center for the control and water quality monitoring" of the State Program "Scientific and Management Researches of Water Quality" of the Academy of Sciences of Moldova.

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BIOCHEMICAL PROCESSES IN CHERNOZEM SOIL UNDER DIFFERENT FERTILIZATION SYSTEMS

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Abstract. The aim of this research was to assess how biochemical processes driven by microbial activity and extracellular enzymes have developed in the long-term under farming system with different fertilizer and crop types. It was concluded that the crop types had a more considerable impact on the soil biochemical processes compared to long-term Org or mixed Min+Org fertilizers amendments.

Keywords: soil, fertilization, enzymatic activity, organic N mineralization

1. Introduction

The intensification of agriculture in the 20th century has caused several environmental problems [1]. High N fertilizer rates have increased nitrate leaching and N₂O emissions from cropping systems [2]. The intensive soil cultivation of arable land has lead to a loss of soil C, thereby contributing to anthropogenic CO_2 emission [3]. These issues spurred research interest in less intensive agricultural management practices, and their potential to reverse some of modern agriculture's negative side effects [4]. We are to increase our awareness of how soil management affects soil fertility (e.g. nutrient cycling, soil structure and water holding capacity).

Since mineralization of soil organic substrates and the release of nutrients and elements are due to the heterotrophic activity of microbial decomposer compartment, this subsystem of terrestrial ecosystems gained importance [5]. The impact of management practices on the flow of C and N trough ecosystems is largely mediated trough the soil microbial community. The C derived from fresh or native soil organic matter that is used by microbes is either mineralized to CO_2 or it is put to anabolic use in production of biomass (new or maintenance) or egested as cellular metabolites (e.g. enzyme production) [6]. Soil microbes produce extracellular enzymes that mineralize organic matter and release carbon and nutrients in forms that can be assimilated. The current understanding is that mineralization of soil organic matter is governed by several concurrent processes: 1) destabilization via oxidation/hydrolysis, desorption and diffusion and 2) the size, community composition and metabolic activity of the microbial biomass [7-8]. It is thought that substrates must pass through the dissolved phase of organic C pool to reach and pass through microbial membranes [9].

Microbial biomass carbon (C_{mic}) and related parameters such as microbial quotient ($C_{mic}:C_{org}$), basal soil respiration (C-CO₂ rate), and metabolic quotient (qCO₂) are widely used with the objective of understanding of microbial responses to various soil management practices [5, 10-11]. These parameters were specified by Nannipieri et al. [12] as the general parameters of soil biochemical properties (directly related to microbial activity) and proposed as eco-physiological indicators of biological soil quality [5].

The aim of this research was to evaluate the intensity of certain soil biochemical processes (e.g. soil organic C mineralization) at Organic and mixed Mineral+Organic fertilization of typical chernozem in crop rotation dynamics (for 6 years) by use of eco-physiological indicators of biological soil quality: microbial biomass carbon, basal soil respiration, as well as, microbial and metabolic quotients.

2. Experimental

Soil sampling was performed from a long-term field crop experiment, which has been established in 1971 at the Balti steppe and are supported by Research Center "Selectia" (Balti, 140 km North of Chisinau) [13-14]. The soil is classified as a typical chernozem (black) soil (silt loam) with C_{org} reach horizon up to 92 cm. Soil organic matter (SOM) content initially was 4.65% (correspondingly, C_{org} constituted 2.70%) in 0-20 cm layer. The pH value – 6.6-7.1 (water) and 6.2 (salt solution). Total N constituted 0.24-0.26%; P – 0.12-0.13%; K – 1.2-1.4%. Two treatments representing organic (Org) and mixed mineral-organic (Min+Org) fertilization were selected for comparative research of soil biochemical properties, because of both are known to be able to maintain the SOM. But both provide different increases of crop productivity in frames of studied crop rotation [15]. Soil samples were taken June 14-16, 2010 (at active crop growth phase) by an auger from the top layer (0-20 cm) of arable field plots. Samples were taken from each of 4 replicates per treatment by combining 5 soil cores inside of each replicate, in total 48 samples. After removing vegetal rests and stones soil was passed through 2 mm sieve. Samples were stored at 4°C no longer than one month necessary for set of biochemical analysis. The aliquots of air-dried soil samples were used for chemical parameters determination.

Microbial biomass carbon (C_{mic}) assay was conducted by use of rehydratation method [16]. Soil samples (2 replicates till 5g for each of treatment) were oven dried at 65-70°C for 24 h, resulting in disruption of the microbial cell wall permeability. Repeated rehydratation of dry soil samples with 0.5 M K₂SO₄ at a ratio 1:2 (w/v) resulted in

microbial cell destruction and release of microbial carbon into solution. An additional 2 replicates of 5g fresh soil samples were placed in refrigerator to serve as controls which were treated in the same way. K₂SO₄-extractable organic C concentrations in the dried and fresh soil samples were simultaneously measured using dichromate oxidation. The aliquote 1.6 ml of filtered soil extract was carefully mixed in tube with 2.4 ml of dichromate solution: 1.28 g K₂Cr₂O₂ in 400 ml of deoinized water is dissolved in 2 L of H₂SO₄ (d=1.84 g/cm³). The mixture was incubated at 140°C for 20 min. The optical density after cooling is measured at 340 nm against of blank mixture of reagents without soil salt extract. The amount of carbon in the samples was calculated by the following formulae: $C_d = (OD_d - OD_b) \cdot V/k_1 \cdot a$, and $C_f = (OD_f - OD_b) \cdot V/k_1 \cdot a$, where OD_d and OD_f are the optical densities of dried and fresh samples, respectively; OD_b is the optical density of blank probe with salt solution instead of salt extract; V represents the volume of salt extract, ml; a - is the the weight of soil sample, g; and k, is the coefficient for transfer from optical dencity to carbon concentation according to calibration curve with glucose. Biomass C (µg C per g oven dry soil) was calculated from the expression $B_c = (C_d - C_f) / k_c$ where $(C_d - C_f)$ is the difference of C measured in dried and fresh sand samples, $\mu g C$; k_c (the portion of cell components released in solution after drying-rehydratation procedure) was 0.25 [16].

Basal soil respiration (C-CO, elimination) was determined by adopted method proposed by Isermeyer [17]. Briefly, soil (25-50 g of dry soil, adjusted to water content 40% WHC was weighted at the bottom of 1L glass jars containing two vessels with 10 ml of distilled water for air humidifying and 20 mL of 1 M NaOH for CO, trapping. The jars were sealed (air-tight) and incubated at 21°C in the dark for 7-14 d. CO, released during soil incubation was trapped in NaOH and determined by titrimetric analysis. Before analysis 0.5 M BaCl, was added to the NaOH solution to remove carbonates. Residual NaOH was titrated with 0.1 M HCl in the presence of phenolphtalein indicator. The soil respiration was recorded as μ g C-CO₂ g⁻¹ dry soil h⁻¹ at 21°C as the average rate during the whole 7-14 days incubation.

Metabolic quotient (qCO₂) or the quantity C-CO₂ produced per unit of microbial biomass C per unit of time was

calculated as a ratio $C-CO_2$: C_{mic} and was expressed in mg C-CO₂ g⁻¹ C_{mic} h⁻¹ [18]. *Total organic carbon* (C_{org}) was assayed using air-dried soil samples by wet oxidation with dichromate in an acid medium and evaluation of the excess of dichromate according to the method of Tiurin [19]. The value of total C is recorded as % of dry soil mass.

Microbial quotient was calculated as a ratio C_{mic} : C_{orr} and expressed in % of total organic C [20].

Statistical analysis. Two-way ANOVA (StatSoft STÅTISTICA 7.0) was conducted with fertilizer and crop type as fixed factors. Differences between means within a crop types for each investigated parameter were identified using paired t-tests.

3. Results and Discussion

Total organic carbon (Care) of typical chernozem soil from Balti steppe under long-term agricultural use with 6-field crop rotation and two fertilization systems was ranged, between 2,36%-2,59% and 2,40%-2,63%, respectively, for Org and Min+Org fertilization systems (table 1). According to two-way ANOVAs data, both independent factors the fertilization system and the crop types significantly (P < 0.05) influence the principal component of soil fertility (table 2). Mean values of total organic C were significant higher in soil amended by mixed Min+Org fertilizers and revealed the significant differences depending on cultivated crop type. The soil under winter wheat was characterized by the least values of C_{org} at both fertilization systems, though it follows after mixture of vetch + oats in crop chain.

Microbial biomass carbon (C_{mic}) serves the index of soil microbiological and biochemical potential. The size of microbial biomass carbon in typical chernozem soil cultivated with six different crops was influenced significantly (P < 0.05) by the crop type and the kind of investigated fertilizers, with the evident tendency of increase at mixed Min+Org fertilizers amendment (tables 1-2). It could be explained by bigger amount and accessibility of mineral nutrients for soil microorganisms at sum action of Min+Org fertilizers. Of six crops cultivated in studied crop rotation the soils under winter wheat and sugar beet characterized with significantly (P < 0.05) lower size of microbial biomass despite of adequate soil fertilization. It seems in contradiction with results of other researchers [21-23], which reported that the identity of the plant species did not influence the soil microbial biomass. However, it was shown that though soil bacterial and fungal biomass did not differ between soils of different plant species the microbial community structures did, due to the quality of rhizosphere carbon [24]. The idea that plant species may have specific effects on the carbon flow into soil microorganisms was confirmed by Ladygina and Hedlund [21] when regarding the active carbon allocation from a plant to the microorganisms. These data allow the assuming that winter wheat's and sugar beet's root exudation (organic rhizodeposition) could allocate less carbon into microbial biomass in comparison to the other four crops in studied crop rotation.

Microbial quotient (C_{mic} : C_{ar}) gives the insight into the capability of a soil to support the microbial growth [25], that is, it reflects the soil carbon available for growth [20]. Thus, it is expected that soils with better quality will have higher microbial quotient [10, 20]. According to results of this study (table 1) the two used fertilization systems (Org vs. Min+Org) insignificantly differed by the available C resource for microbial growth, but crop types did (table 2). Typical chernozem cultivated with winter wheat and followed sugar beet demonstrated the lowest microbial quotient

Eco-nhy	viological indicators	of soil quality re	flecting the intensity	of soil biochemical	Table 1		
Parameter	Microbial biomass carbon, C _{mic}	Microbial quotient, C _{mic} : C _{org}	Basal soil respiration, C-CO ₂ rate	Metabolic quotient, qCO ₂ ,	Total organic carbon, C _{org,}		
Units of measurement	µg C g ⁻¹ dry soil	%	μg C-CO ₂ g ⁻¹ soil h ⁻¹ at 21°C	$\mathop{\text{mg C-CO}}_{{h^{-1}}} g^{\text{-1}} C_{\text{mic}}$	%		
Crop type		Organic fertilization system					
Winter wheat	176 ± 8^{a}	0.75 ± 0.02	0.56 ± 0.14	3.2 ± 0.74	2.36 ± 0.04		
Sugar beet	177 ± 23	0.69 ± 0.09	0.61 ± 0.08	3.5 ± 0.80	2.59 ± 0.04		
Corn for grain	217 ± 24	0.88 ± 0.10	0.77 ± 0.08	3.6 ± 0.25	2.46 ± 0.03		
Spring barley	240 ± 11	1.00 ± 0.07	0.35 ± 0.16	1.5 ± 0.65	2.40 ± 0.20		
Sunflower	236 ± 5	0.96 ± 0.03	0.60 ± 0.16	2.5 ± 0.66	2.45 ± 0.05		
Vetch+oats	228 ± 31	0.89 ± 0.10	0.56 ± 0.17	2.6 ± 1.00	2.55 ± 0.10		
		Mineral	+Organic fertilizatio	on system			
Winter wheat	182 ± 18	0.76 ± 0.08	0.43 ± 0.13	2.4 ± 0.80	2.40 ± 0.04		
Sugar beet	197 ± 8	0.75 ± 0.03	0.25 ± 0.02	1.3 ± 0.13	2.63 ± 0.02		
Corn for grain	220 ± 10	0.84 ± 0.05	0.52 ± 0.13	2.4 ± 0.69	2.60 ± 0.04		
Spring barley	254 ± 5	0.99 ± 0.02	0.48 ± 0.11	1.9 ± 0.46	2.57 ± 0.03		
Sunflower	257 ± 14	0.99 ± 0.06	0.68 ± 0.05	2.6 ± 0.26	2.60 ± 0.03		
Vetch+oats	242 ± 34	0.96 ± 0.13	0.69 ± 0.11	2.9 ± 0.48	2.53 ± 0.03		

(0.7-0.8%), the sequence of crops spring barley-sunflower-vetch+oats characterized by higher values (0.9-1.0%), but the soil under corn for grain placed in the middle of crop chain was the bend point (0.8-0.9%).

^a Mean \pm S.D. (standard deviation, σ), n=4;

Table 2

Summarized results of two-way ANOVAs for soil general biochemical parameters				
Dependent variables	Independent variables ^a	d.f. ^b	Fc	P-value ^d
Microbial biomass carbon (C_{mic})	Fertilization system	1	6.24	0.017*
	Crop types	5	20.79	< 0.001***
	Interaction	5	0.32	0.89
Microbial quotient $(C_{mic}: C_{org})$	Fertilization system	1	0.90	0.35
	Crop types	5	20.21	< 0.001***
	Interaction	5	0.66	0.66
Basal soil respiration (C-CO, rate)	Fertilization system	1	0.88	0.35
_	Crop types	5	4.61	0.002**
	Interaction	5	2.98	0.023*
Metabolic quotient	Fertilization system	1	0.88	0.35
$(qCO2 = C - CO_2 : C_{mic})$	Crop types	5	4.61	0.002**
	Interaction	5	2.98	0.024*
Total organic carbon (C_{ave})	Fertilization system	1	15.91	<0.001***
(0-20 cm)	Crop types	5	7.95	<0.001***
	Interaction	5	2.21	0.07

^a Fertilization system (Min+Org vs Org) and crop types (six cereal and row crops in 6-years crop rotation) were the independent variables.

^b degree of freedom – the number of given elements (e.g. two fertilization systems or six crops) inside of independent variables minus 1;

^eF-test or Fisher's criteria;

^dconfidence level. P values: *** = P<0.001; ** = 0.001<P<0.01; * = 0.01<P<0.05

Basal soil respiration (C-CO₂ rate) serves as the indicator of metabolic activity of soil microorganisms or the content of organic carbon potentially mineralizable up to CO_2 . The CO_2 emission from the soil to the atmosphere is the main cause of soil C loss [26] and it provides an early indication of soil C level when changes in organic C

due to management practices are not detectable over a short period [27-28]. According to two-way ANOVAs analysis data (table 2) the fertilization systems revealed no significant influence upon C-CO, rate. But differences between means within a crop types identified using paired t-tests were significant for microbial communities from soils under winter wheat, sugar beet, corn for grain, and spring barley. The most evident differences were observed for microbial communities from soils of Org plots cultivated with corn for grain and followed spring barley, respectively, with the most enhanced and the most reduced levels of CO₂ elimination. At mixed Min+Org amendment the soil cropped with sugar beet characterized by the least quantity of CO₂ elimination, but the soil under sunflower and followed vetch + oats – by the biggest intensity of respiration. Thus, the soil respiration was significantly higher in soil under crop chain the winter wheat - sugar beet - corn for grain at Org fertilizers amendment in comparison to Min+Org system. It was found, the nitrogen fertilization inhibits soil microbial respiration regardless of the form of nitrogen applied [29]. The possible explanation of enhanced CO, evolution from soil, cropped with corn for grain, may be the existence of significant deficiency of mobile N. Conform to general N-regulation processes by the content of soil accessible N [30], the decomposition of soil organic carbon substances, containing simultaneously and organic N, can be initiated and the elimination of CO₂ is enhanced. It was hypothesized that organic cropping systems would reduce soil CO₂ emission and increase C storage compared to conventional cropping systems [31]. Last cited researchers have found that the soil CO, emission rate at peak times in the Org system was higher than the conventional (Conv) that is mineral fertilization system. However, even if the cropping systems had a temporary impact on the rate of soil CO, emissions, the soil C output calculated as the average of cumulative CO₂ emission over the 3-year period did not show significant differences between the Org and Conv systems. On the other hand, according to last researchers, the C input in the Org system was higher than in the Conv (9.46 Mg C ha⁻¹ vs. 5.57 Mg C ha⁻¹) as well as the C input/output ratio (1.10 vs. 0.72) [31]. The 3-year average of C_{org} content and C stock was higher in the Org than in the Conv system. Still, are needed to verify if C limitation for soil microbial growth and nitrogen limitation for crop growth in the organic system could hinder soil C accumulation over a longer period.

Metabolic quotient (qCO₂) was offered for the quantification of environmental effects on the microbial communities in soils [9]. The parameter qCO₂ indicates the efficiency by which soil microorganisms use C-resources in the soil, and it is expected that stressed soils will provide higher qCO₂ values than less-stressed soils [32]. The same, it reflects the microbial requirements of maintenance energy [20]. The results of our research show two certain crop chains (table 1). First one: spring barley-sunflower-vetch+oats were not affected by fertilizers, second – winter wheat-sugar beet-corn for grain had lower qCO₂ values at Min+Org than at Org fertilization. It could be explained by better supply of microorganisms and crops with mobile N at Min+Org, while at Org farming it may be as was noticed by Mancinelli et al [31] the temporary impact on the rate of soil CO₂ emissions due to use of organic substances which simultaneously contain C and N elements. In general, the metabolic quotient was not influenced significantly by fertilization system (P > 0.05) (table 2). The qCO₂ values of typical chernozem (soil pH 6.6-7.1) under 6 crops (Northern Moldova) ranged between 1.5-3.6 mg CO₂-C g⁻¹ C_{mic} h⁻¹ (mean=2.8) and 1.7-2.9 mg CO₂-C g⁻¹ C_{mic} h⁻¹ (mean=2.3), respectively, at Org and Min+Org fertilization, and were lower in comparison to the mean values 3.5 and 3.6 mg CO₂-C g⁻¹ C_{mic} h⁻¹, published by Trasar-Cepeda et al [33] for 40 climax soils and 45 cropped soils (respectively, soil pH 4.29 and 5.83) in Galicia (NW Spain). It is known under acidic conditions the qCO₂ is elevated since maintenance energy requirements of microbes are higher [20].

In summary, having in mind the interlinkage between the soil biotic component and biogeochemical cycling this research has followed the approach proposed by T.-H Anderson [5, 20] to use the eco-physiological indicators to estimate that one of soil management practices would be more or less detrimental than another, that is, Min+Org fertilization system versus Org one, only. It was published [20], that the $C_{mic} : C_{org}$ ratio of agricultural and forest soils at neutral pH is very similar and in the range between 2.0 and 4.4% C_{mic} of total C_{org} , depending on nutrient status and soil management. The metabolic quotient qCO₂ ranged between 0.5 and 2.0 mg C-CO₂ g⁻¹ C_{mic} h⁻¹ in neutral soils. Values below 2.0 for the $C_{mic} : C_{org}$ ratio or above 2.0 for the qCO₂ could be considered as critical for soils with a neutral soil pH. The evaluation of our results on typical chernozem soil from North Moldova steppe under long-term agricultural use with the most protective fertilization systems (Org and Min+Org) and crop rotation has shown the $C_{mic} : C_{org}$ ratio twice and more below 2.0 and the mean qCO₂ values above 2.0. It means that Org fertilization system still doesn't make it possible to avoid the loss of organic C in arable typical chernozem. These data coincide with another published results, that soil C_{org} accumulation declines in long-term experiments (>50 yr) with farm manure applications as a new equilibrium is approached [34]. The both Org and Min+Org fertilization systems equally provides the soil carbon available for growth according to $C_{mic} : C_{org}$ ratio, but last one revealed lower level of qCO₂ values. It could mean the mitigation of the negative consequences of long-term mineral fertilization by organic fertilizers.

4. Conclusion

The crop types had a more considerable impact on the soil microbial biomass and community biochemical activity compared to long-term Organic or mixed Mineral+Organic fertilizers amendments. The chain of crops: winter wheat – sugar beet – corn for grain revealed a strong influence on soil microbial communities resulted in higher metabolic
quotient (qCO₂) at Org system of fertilization. It could demonstrate a less efficiency of soil organic carbon use may be because of a more acute need for accessible N. Next chain of three crops: spring barley – sunflower – vetch+oats revealed higher microbial quotient (C_{mic} : C_{org} ratio) and least qCO₂ values. The organic fertilizer is able to mitigate the negative consequences of long-term mineral fertilization.

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PHYSICOCHEMICAL PROPERTIES OF THE WATER DEPLETED OF HEAVY ISOTOPES

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Abstract: Data on physicochemical properties of the light water (deuterium concentration is reduced to the ratio D/H = 4 ppm and that of the isotope of oxygen 18 – to the ratio ${}^{18}O/{}^{16}O = 750$ ppm) have been obtained. They include the melting and boiling points, kinematic viscosity, density, the spin-spin proton relaxation time, self-diffusion coefficients, and the small-angle laser light scattering. An explanation was proposed for the detected significant changes of the light water as compared with the high-resistivity water having geochemically ordinary isotope composition. This explanation was based on the mechanism of formation of supramolecular density inhomogeneities in water representing the deuterium-stabilized heterophase clusters.

Keywords: Physicochemical properties of water, protium water, light water, heavy water, water toxicity

Introduction

Water as a chemical matter has no color, no taste, and no smell. Drinking water this is the water, whose organoleptic, physicochemical, and biological properties meets human biological needs and having neither color nor smell should have taste determined by the presence in it of physiologically necessary salts of calcium, magnesium, sodium, and potassium in corresponding concentrations without which metabolism in human organism is impossible.

Life on earth was revived thanks to the presence of water. The greatest secret is unknown origin on the Earth of genome. However, the emergence of the biological life, most likely, is determined by the presence of bacteria having space origin, which created a fruitful layer of soil and which still penetrate our earth from space together with space objects. Therefore, the appearance and development of biological variety on our planet is determined, mainly, by the presence of water unique by its physicochemical properties rather than the presence in it of various microorganisms capable of surviving under extreme conditions of the space. The unique properties of the water, we are accustomed of, belongs to one of the simplest and well studied substances, is determined by its isotope composition and, first of all, by the ratio of protium and deuterium. The natural concentration of deuterium in the world ocean, in salt and fresh waters varies within the interval 90–180 ppm. The stable optima; concentration of deuterium in water constitutes 150 ppm. It is within this concentration interval that our ordinary water possesses maximum biological activity both in sea and in fresh water bodies. A decrease or an increase of the deuterium concentration in water results in a radical change of its physicochemical and biological properties, namely: density, viscosity, refractive index in water, speed of sound in it and many other properties right to boiling and freezing points. Light water (~ 5 ppm) freezes and boils at temperature by ~ 3°C lower than the ordinary water in other standard conditions. The most astounding is that fact that both protium and deuterium water is toxic for biological life on earth. Therefore, in order to detect life on other planets like our, one has to find not simply water, but the water with the same ratio in it of deuterium and protium.

Natural water represents a multicomponent mixture of molecules of different isotope composition (isotopologues). In natural water one million molecules on average includes 997284 molecules of ${}^{1}\text{H}_{2}{}^{16}\text{O}$, 311 molecules of ${}^{1}\text{HD}{}^{16}\text{O}$, 390 molecules of ${}^{1}\text{H}_{2}{}^{17}\text{O}$, and about 2005 molecules of ${}^{1}\text{H}_{2}{}^{18}\text{O}$ [1]. The concentration of water molecules containing heavy isotopes (D, ${}^{17}\text{O}$, and ${}^{18}\text{O}$) in natural waters fluctuates within the limits established in two basic international standards for the isotope composition of hydrosphere: VSMOW (Vienna Standard Mean Ocean Water) and SLAP (Standard Light Antarctic Precipitation) introduced by the International Atomic Energy Agency (IAEA) [1, 2]. The VSMOW standard determines the isotope composition of the deep water of the world ocean, while the SLAP standard determines the isotope composition of the natural water from the Antarctic (deuterium concentration varies depending on the depth of ice and its location on the mainland within the limits of ~ 10 ppm) at the current level on the surface equal to ~ 90 ppm (EDC station [3]). In accordance with the VSMOW standard the absolute contents of deuterium D/¹H and oxygen – 18 (${}^{18}\text{O}/{}^{16}\text{O}$) in the ocean water amount to 155.76 ± 0.05 and 2005.20 ± 0.45 ppm, respectively, [4]. The content of the lightest isotopologue (${}^{14}\text{H}_{2}{}^{16}\text{O}$) in the water corresponding in terms of the isotope composition to the VSMOW standard

and the SLAP standard amount to 99.73 and 99.76 mol. %, respectively. The SLAP standard characterizes the lightest natural water on Earth. Hence, the concentrations of deuterium D/H and oxygen – 18 ($^{18}O/^{16}O$) in this water amount to 89 and 1894 ppm, respectively, [5]. The light water can be defined as follows: as a result of special treatment the share of the lightest isotopologue 14 , ^{16}O in this water was increased as compared with standard SLAP.

The reason for designating the light water as deuterium-depleted was, probably, the fact that the most significant reduction of deuterium content was achieved by rectification in the process of removing heavy isotopologues and also due to technological difficulties of determining the content of oxygen isotopes. As shown in papers [6-8], "deuterium-depleted water" as a particular case of the light water possesses pronounced antitumor properties. The light water speeds up the exchange processes (both, physicochemical and biological) resulting in different physiological effects: immunostimulating, reproductive, and also in changes of the receptor sensibility to catecholamine and angiotensin [9, 10].

It can be assumed that such important biological responses registered according to the results of experimental investigations must have at the bottom common reasons, to which primarily we should refer the variation of the physicochemical properties of water. That is why the present paper is devoted to determination of the classical "tabular" physicochemical parameters of the light water and the comparative description of results (for preparations of the light, natural and heavy waters) obtained by new methods of investigating the associated liquids.

Materials and methods

The deionized (high-resistivity) water (18 M Ω ·cm at 25°C) was prepared by subjecting the pyrogenic distilled water to purification on the Milli-Q system produced by Millipore, Great Britain. The light water was produced at OJSC Almaz by the vacuum rectification method. The content of deuterium (D/H) and oxygen – 18 (¹⁸O/¹⁶O) in light water amounted to 4 and 750 ppm, respectively, as compared with D/H = 140 and ¹⁸O/¹⁶O = 1966 ppm for deionized water. The heavy water (99.9%) corresponded to Technical Specifications TU 95-1893-89. As a result of the performed investigation using the atomic absorption spectrometry with electrothermal atomization and Zeeman background correction [11], such elements as Al, V, Cr, Mn, Ni, Fe, Cu, Zn, As, Cd, and Pb were not detected in any preparations of water (concentration < 0.1 nM; the detection limit was 0.1–10 nM).

In addition, the presence of impurity particles of the dispersed phase was not registered in the specified preparations of water by using the methods described in paper [12].

Determination of the isotope composition. The methods used for measuring the deuterium concentration included the mass-spectrometry method [13] and the method of multiple-pass laser absorption spectroscopy using an LWIA-24d instrument produced by Los Gatos Research, Inc. [14]. The content of oxygen-18 was also controlled by using the multiple-pass laser absorption spectroscopy. Density of water was measured by a digital densimeter of model LiquiPhysics Excellence DM45DR produced by Mettler Toledo, and also by using a PZh_2 picnometer in accordance with GOST 18995.1-73. The relative error of the method was 0.016%; P = 0.95.

Viscosity of water was determined by using a VPZh-2 capillary glass viscosimeter (capillary internal diameter of 0.56 mm) with an additional thermostating device [15]. The relative error of the method was 0.89%.

Boiling point of water was measured on the Sventaslavskii instrument [15].

Melting point of water was determined by the method of differential scanning calorimetry (DSC). The scanning speed was 4 deg/min. The solution under investigation in the amount of 0.0010–0.0100 g was pressed into container and subjected to cooling with liquid nitrogen for one–two minutes to a temperature of 223 K. It was assumed that at such high speeds of cooling the changes of its phase state will be minimum. Next, the system was held during several minutes at temperature 223 K for the establishment of equilibrium. Thermograms were registered in the interval 223–293 K. In order to determine the reproducibility of results of DSC

and their dispersions, 10 measurements were performed for each water sample. The calculated sample variance amounted to 0.012.

Spin-spin relaxation time of water protons was determined by the NMR spin-echo method [16].

Molecular dynamics characteristics of water were determined by the method of monoenergetic neutron scattering (13.289 meV) [17]. In order to calculate the self-diffusion coefficients, the results were processed by using the Oskotskii-Ivanov model.

Variance analysis of water samples and nanoparticles was conducted by using the method of low-angle laser light scattering (LALLS) and the dynamic light scattering (DLS) (photon correlation spectroscopy) on instruments MasterSizer 2000 and ZetdSizer Nano ZS (Malvern Instruments) [11, 12].

Values of the pH were measured on a PP-20 pH-meter produced by Sartorius and equipped with a thermal sensor and by using the spectrophotometry with an acid–base indicator of phenol red ($pK_a = 7.4$) at the concentration 30 μ M [18].

Result and discussion

Physicochemical properties of light water. It occurred that the "classical" physicochemical properties of the light water differ from the deionized water having natural isotope composition (Table 1). The differences is surface tension (at the water-air interface) for the light water-deionized water pair are comparable with such for the deionized water of natural isotope composition than the deionized water to the heavy one. It should be stressed that the density of the light and deionized waters to a great extent is determined by the concentration of heavy isotopes of oxygen (see Table 1, line 3).

The temperatures of phase transitions in the light water are significantly reduced. The reduction of the freezing point of light water ($< -1^{\circ}C$) exceeds the similar value for a 100 mM sodium chloride or saccharose solution. This apparent violation of the Raoult law as also the probable variation of the ebullioscopic constant of the light water (the boiling point is reduced by 7°C) make it possible to suggest that the light water represents a new associated liquid that have not been earlier scientifically described in literature. It should be noted that the modern quantum mechanical models function by adopting during the simulation other physicochemical constants obtained for a real liquid heterogenetic in terms of isotope composition [19, 20].

Table 1

Item No.	Parameter	Light water D/H = 4	Deionized water having natural isotope composition D/H = 140	Heavy water 99% of D ₂ O
1	Kinematic viscosity, mm ² /s	0.987	1.012	1.274
2	Surface tension, mN/m	75.172	72.860	67.800
3	Density, g/cm ³	0.99692*	0.99820**	1.1042
4	Boiling point, °C	93.7	99.9	101.4
5	Melting point, °C	from -4 to -1***	0	3.8
6	Time of spin-spin proton relaxation (T_2) , s	0.347 ± 0.024	2.000 ± 0.140	—
7	Contribution to the total self-diffusion coefficient from collective movements $(D_1 \times 10^9)$, m ² s ⁻¹	0.63	0.46	0.52
8	Contribution to the total self-diffusion coefficient from single-particle movements $(D_i + D_j = D$ is the total self-diffusion coefficient), $(Df \times 10^9)$, m ² s ⁻¹	1.60	1.77	1.50
9	Life time of molecules in the oscillating state around the equilibrium center, ($\tau_0 \times 10^{12}$), s	2.08	2.80	2.79

Physical properties of preparations having different isotope composition ($T = 20 \pm 0.04^{\circ}$ C)

Notes: $*^{18}O/^{16}O = 757$ ppm; $**^{18}O/^{16}O = 1966$ ppm (at $T = \pm 25^{\circ}C$); *** depending on the weight of sample.

The variation of properties of liquid during the reduction of concentration of heavy isotopes testifies the presence of a "control" function of water molecules containing heavy isotopes: one molecule of ${}^{1}\text{H}_{2}{}^{16}\text{O}$, one molecule of ${}^{1}\text{H}_{2}{}^{16}\text{O}$ fall on ~3000 molecules of ${}^{1}\text{H}_{2}{}^{16}\text{O}$ [1]. Such realization of the isotope effect is possible via the cluster organization of associated liquid. The growing number of tie molecules in light water and its higher "homogeneity" in terms of the structure is corroborated by the variation of its molecular dynamics properties (see Table 1, lines 6 – 8). The diffusion coefficient increases in the light water reflecting the contribution from collective movements ($D_{l} = 0.63 \times 10^{-9} \text{ m s}^{-1}$) as compared with the deionized water ($D_{l} = 0.46 \times 10^{-9} \text{ m s}^{-1}$). The same tendency is also observed by the replacement of protium with deuterium in the preparation of heavy water.

A significant reduction of the spin-spin relaxation time $(0.35 \pm 0.02 \text{ s versus} 2.00 \pm 0.10 \text{ s for light}$ and deionized water, respectively, (see Table 1, line 6) means a sharp reduction of the degrees of freedom in light water. The removal of deuterium, probably, results in the elimination of geometrical effects in the network of hydrogen bonds. The obtained results well agree with the reduction of the freezing point of the light water.

The possible mechanism of implementation of the isotope effect during the formation of the water having the *natural isotope composition*. In our view the causes of differences in the properties of the light and deionized water can be explained as follows. In earlier papers [21 - 26] we put forward a hypothesis on the formation in the water of natural

isotope composition of density inhomogeneities of submillimeter range – deuterium-stabilized gigantic heterophase clusters (GHC) with the relaxation time of more than one second. It should be stressed that the long-living supramolecular density inhomogeneities (gigantic in comparison with the well known nanoclusters) were experimentally observed by other teams of researchers [27, 28]. These density inhomogeneities of water are stabilized by heavy isotopologues at their probable arrangement on the surface of density inhomogeneities that allows them to implement the "control" function.

As was noted in papers [21, 23], the rise of water homogeneity in terms of the isotope composition leads to disintegration of GHC. The opposite statement is also true: formation of GHC in the light water is hampered. This is demonstrated by the performed experiment on induction of the GHC formation by latex nanoparticles. As for any dispersed formation in colloidal solution, a "primer" is required for formation of GHC. In measuring the dimensional spectra of density inhomogeneities by using the LALLS method [12] it is possible to register GHC of the submillimeter range induced by nanoparticles (the same preparation of water, where the solution of nanoparticles is introduced to, is used as a background). Table 2 shows that such induction of GHC formation is only possible in the water having the natural isotope composition.

Table 2

in	in water preparations of different isotope composition				
True (certified) size of	Measured size of density inhomogeneities in different solvents,				
nanoparticles, nm	light water	ordinary water			
20	25 ± 5	10000 ± 500			
40	50 ± 7	10100 ± 200			
		26100 ± 1000			
80	85 ± 7	30100 ± 900			
35	315 ± 5	315 ± 5			
1200	1300 ± 50	1400 ± 100			

Initialization by latex nanoparticles of the formation of density inhomogeneities – gigantic heterophase clusters in water preparations of different isotope composition

Note. The concentration of sulfated nanoparticles (Invitrogen Corporation) amounts to 0.00005 vol %. All measurements were performed in the circulation mixing mode.

The analysis of suspensions of the standard latex microspheres of nanometer range by the method of low-angle laser light scattering revealed significant differencesz3332ds in dimensional spectra with respect to standard values. Hence, for hydrophobic nanospheres having the diameter < 100 nm their diameters appear to be exceeded by orders of magnitude that can be explained by the initiation of the formation of water GHC by nanoparticles. The investigation of larger microparticles does not reveal any variations of dimensional spectra: the diameter coincides with the declared value.

In the light water new GHC are not initialized by nanoparticles. Sulfated latex particles having the size >100 nm do not result in formation of GHC. In separate experiments it was revealed that hydrophobic nanoparticles are more effective in respect of GHC induction, i.e. they induce the formation of latter at lower concentrations than the sulfated nanoparticles. The collection of the specified data makes it possible to assume that in addition to our earlier descriptions of the Kolmogorov mechanisms of the white cascade of dissipation of kinetic energy into thermal one [23], a considerable contribution to the formation of GHC can be made by the dispersion (Deryagin) interactions.

The reduced concentration of GHC in light water [23, 24] results in the formation of a liquid that is closer to the model of "continual" water having the reduced values of the temperature of phase transitions, viscosity, and enhanced surface tension. It is not excluded that the investigation of the properties of samples of the light water with a higher concentration of isotopologue ${}^{1}\text{H}_{2}{}^{16}\text{O}$ shall reveal further reduction of the melting and boiling points in the direction "specified" by the Mendeleev periodic law for hybrids of elements of the II period and VI group of the main subgroup.

Conclusions

On the basis of obtained data the following conclusions can be made:

1. In terms of a series of physicochemical properties the light water is a new associated liquid that has not been scientifically defined earlier.

2. Possible mechanism of implementing the "control" function of heavy isotopologues of water molecules is the formation of density inhomogeneities, i.e., deuterium-stabilized gigantic heterophase clusters.

3. Biological effects of the light water are not related to the ligand-dependent toxic effect, but determined by the adaptive reasons.

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COMPUTER-BASED PREDICTION OF TOXICITY USING THE ELECTRON-CONFORMATIONAL METHOD. APPLICATION TO FRAGRANCE ALLERGENS AND OTHER ENVIRONMENTAL POLLUTANTS

Dedicated to the 60th birthday of Academician Gheorghe Duca Natalia N. Gorinchoy^a* and Isaac B. Bersuker^{a,b}

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Abstract: The electron-conformational (EC) method is employed for the toxicophore (Tph) identification and quantitative prediction of toxicity using the training set of 24 compounds that are considered as fragrance allergens. The values of $a=LD_{s0}$ in oral exposure of rats were chosen as a measure of toxicity. EC parameters are evaluated on the base of conformational analysis and *ab initio* electronic structure calculations (including solvent influence). The Tph consists of four sites which in this series of compounds are represented by three carbon and one oxygen atoms, but may be any other atoms that in the compound under consideration exhibit the same electronic and geometric features within the tolerance limits. The regression model taking into consideration the Tph flexibility, anti-Tph shielding, and influence of out-of-Tph functional groups predicts well the experimental values of toxicity ($R^2 = 0.93$) with a reasonable leave-one-out cross-validation. The methodology worked out in this paper can be applied to other environmental pollutants.

Keywords: Structure-activity relationships, Electron-conformational method, Toxicity screening and prediction, fragrance allergens.

Abbreviations: Tph-toxicophore

EC – electron-conformational ECMC – electron-conformational matrix of congruity ECSA – electron-conformational sub-matrix of activity QSAR – quantitative structure-activity relationships

1. Introduction

Fragrance chemicals are of increased interest to both the fragrance industry and environmental protection agencies, as well as to dermatologists, due to a number of allergies caused by these chemicals in cosmetics and toiletries. For this reason, use of fragrance chemicals is subjected to strict restrictions. Considerable attention is paid to safety, stability and innocuity of the substances (natural or artificial) used as fragrance ingredients. Checking the latter on toxicity using animal testing is extremely expensive and of long duration. Therefore alternative, computer-based methods of screening and prediction of toxicity for large number of the fragrance chemicals based on limited numbers of experimentally studied ones, becomes very attractive.

Computer-based toxicology is presently a well recognized and rapidly developing trend in environmental chemistry. The QSAR (Quantitative Structure-Activity Relationship) methodology is usually used to reveal relationships between the chemical structure of the compound and its specific toxicity in order to predict the latter in new chemicals. There are a number of QSAR systems and commercial programs, which allow prediction of a wide range of biological endpoints and toxicity (see, e.g., [1-5] and references therein). The main problem in this approach is to choose the molecular features (descriptors) that properly represent the possible interaction of the toxicant with the bioreceptor to produce the toxicity, and to correlate the descriptors with the toxicity by means of some regression relationships. There is a common shortcoming in all the approaches to QSAR problems: the choice of molecular descriptors deemed to be responsible for the toxicity is not directly based on first principles, meaning it is arbitrary, some (or all) descriptors being thus artifacts with no physical meaning implied in their initial choice (see also [2]).

Distinguished from the traditional QSAR approaches, the electron-conformational (EC) method [1, 2] does not employ arbitrary descriptors and statistics in evaluation of their weight. Instead, the electronic structure and topology of the molecule evaluated by quantum-chemical calculations is used as one (a unique) descriptor, presented in a computer friendly digital-matrix form. The comparison of these matrices with a certain type of toxicities allows one to reveal a group of matrix elements that are common to the active compounds under consideration, and represent the numerical picture of toxicophore (Tph). This approach has been applied successfully to study several types of biological activities (see, e.g., [6-10]) and to predict aquatic toxicity to fish [11].

In this paper the predictive power of the EC method and its efficiency is demonstrated by revealing the origin of toxicity in a series of 24 compounds which are identified as fragrance allergens. Using the EC method we performed conformational analysis (optimization of geometries of the low-energy conformers) and electronic structure calculations (by *ab initio* method including solvent influence) for these allergens and constructed the Electron-Conformational Matrix of Congruity (ECMC) for each of their (room-temperature) conformations. Then the toxicophore (Tph), the group of atoms (atomic characteristics) responsible for the toxicity, is revealed by evaluation of the EC sub-matrix of activity (ECSA), a sub-matrix with matrix elements common to all the active compounds under consideration within minimal tolerances. Starting with only five most toxic compounds, their ECSA (toxicophore) was found to consist of a 4x4 matrix (four sites with certain electronic and topologic characteristics) which was shown to be present in all the most active compounds. In addition to the Tph identification, a regression structure-toxicity model was employed to take into account the influence of Tph flexibility, anti-Tph shielding (ATS), and other out-of-Tph functional groups (auxiliary groups, AG) on the quantitative value of toxicity. The results predict well ($R^2 = 0.93$) the experimental values of toxicity with a reasonable leave-one-out cross-validation. The methodology worked out in this paper can be applied to screening and prediction of toxicity of other environmental pollutants.

2. Data set and method

The chosen data set contains 24 compounds that are included in the list of fragrance contact allergens by the European Union scientific committee SCCNFP's [12]. The values of $a=LD_{50}$ in oral exposure of rats were chosen as a measure of toxicity. These values were obtained from the site ChemIDplus of the United States National Library of Medicine [13]. Structural formulas for chemicals studied, along with the experimental LD_{50} values, are given in the Table 1.

wioieculai su u	Molecular structures and experimental toxicities (11250) of 24 in agrance contact and gens				
Structure	LD ₅₀ (mg·kg ⁻¹)	Structure	LD ₅₀ (mg·kg ⁻¹)	Structure	LD ₅₀ (mg·kg ⁻¹)
1 Coumarin	196	9 Cinnamyl alcohol	2000	он 17 Amylcinnamyl alcohol	4000
2 Anisyl alcohol	1200	10 Cinnamal	2200	HO HO 18 Linalool	4180
3 Benzyl alcohol	1230	11 Benzyl salicylate	2227	19 d-Limonene	4400

Iolecular structures and experimental toxicities (LD₅₀) of 24 fragrance contact allergens

Table 1



The electron-conformational method used in this study of structure-toxicity relations is outlined in detail elsewhere [1, 2]. It includes as a first part the conformational analysis and quantum chemical calculations of the equilibrium geometries and electronic structure of compounds under consideration. Next, using results of calculations (molecular orbital population analysis, Mulliken atomic charges, and bond orders), the so-called Electron-Conformational Matrices of Congruity (ECMCs) are constructed for the low-lying conformations of molecules of the training set. These ECMCs contain a rather full description of both the geometry and electronic properties of molecules presented by the corresponding matrix elements.

Figure 1 illustrates an example of the ECMC calculated for the lowest conformation of compound **3**. The hydrogen atoms are excluded from consideration here for simplicity. Diagonal matrix elements reproduce atomic interaction indices (II) [1], which are measures of electron-donor properties of the corresponding atoms in the molecule:

$$II^{A} = g^{A} \exp\left(-R_{0} \sqrt{2 \operatorname{VOIP}^{A}}\right)$$
(1)

Here g^A is the Mulliken electron population of the outermost orbital of the atom A (g^A for *n*p-elements is equal to one-third of the total occupancy of valence p-orbitals, p, p, and p, of the atom), and VOIP in atomic units refers to the Valence Orbital Ionization Potential of this atom-in^{*}-molecule ⁷orbital calculated as a function of the Mulliken charge and the electronic configuration of the atom using the reference data [14]. A value of $R_0 = 1.51$ Bohr radii (0.8 Å) is conventional [9]. Off-diagonal matrix elements represent Mulliken bond orders for chemically bonded atoms and interatomic distances for non-bonded pairs (see Figure 1).

	C1	C4	C2	C6	C5	C3	C7	01	- C-7
C1	0.13								<u></u>
C4	2.78	0.16							C ₆
C2	1.39	2.41	0.16						
C6	1.39	1.40	2.41	0.17					
C5	2.41	1.45	2.78	1.39	0.16				c-S (
C3	2.41	1.39	1.44	2.78	1.43	0.16			C ₂
C7	1.50	4.29	0.93	2.51	3.78	3.79	0.11		
01	2.36	4.92	2.94	3.44	4.60	4.24	1.41	0.38	C_4 C_3

Figure 1. The electron-conformational matrix of congruity for molecule 3. Hydrogen atoms are omitted for simplicity. The diagonal elements refer to the atomic (e.g., O_1 in the picture) interaction indices calculated by Eq. 1, while the off-diagonal elements reproduce Mulliken's bond orders for chemically bonded pairs of atoms (e.g., C_5 - C_6) and interatomic distances for non-bonded pairs (e.g., C_7 - O_1).

The next step in the EC method is the one-by-one comparison of the matrix elements of different ECMCs in order to reveal the so-called electron-conformational submatrix of activity (ECSA), namely the set of matrix elements (geometry and electronic parameters) that, within some tolerances, are common for all the toxic compounds. The ECSA describes a group of atoms in the molecule, which are characterized by means of interatomic distances, bond orders, and interaction indices, the toxicophore (Tph).

Numerical values of toxicities can be evaluated using a general formula for biological activity [1]:

$$a_{i} = a_{ref} \exp\left(-\frac{E_{i}^{Tph} - E_{ref}^{Tph}}{k_{B}T}\right) \exp\left(-S_{i}[R]\right),$$
⁽²⁾

$$S_{i}[R] = \sum_{j=1}^{P} k_{j} \left(R_{j}^{(i)} - R_{j}^{ref} \right),$$
(3)

where a_i and a_{ref} stand for numerical values of activity of the *i*-th compound and the reference compound, respectively, E^{Tph} is the relative energy of the lowest energy conformer that contains the Tph, and $S_i(R)$ is a function of the electronic and geometric parameters of the substrate molecule, parameters *R* stand for the toxicophore flexibility and ATS/AG influence in the substrate molecule (see below).

Regression coefficients k in Eq. 3 are calculated by means of a least-squares fit of the values of toxicity a_i (calculated by Eq. 2 for all the compounds in the training set, which contain the Tph) to corresponding experimental

values a_i^{exp} . The quality of the linear regression can be evaluated by the analysis of variance [15]:

$$SE = \sqrt{\frac{\sum_{i=1}^{N} (x_i - \bar{x})^2}{N - 1}}, \qquad \bar{x} = \frac{1}{N} \sum_{i=1}^{N} x_i$$
(4)

$$F\left(\frac{\nu_1}{\nu_2}, \alpha\right) = \frac{R^2}{1 - R^2} \frac{\nu_2}{\nu_1},$$
(5)

where SE is a standard error, $x_i = a_i^{exp} - a_i^{theory}$, and *R* is the correlation coefficient. The F-statistics value can be compared to tabulated values [15] for *F* with the confidence α and the degrees of freedom v = P, v = N - P - 1 (*P* is the number of descriptors *R* in Eq. 3, *N* is the number of molecules that have the toxicophore). The E-statistics values are calculated as a ratio:

$$E = \frac{PRESS_P}{PRESS_{P-1}}, \qquad PRESS = \sum_{i=1}^{N} \left(a_i^{\exp} - a_i^{theory} \right), \tag{6}$$

The E-values characterize the weight of each eliminated parameter R in the numerical value of toxicity. If E > 0.4 for any (eliminated) parameter R in Eq. 6, the latter is considered to be statistically irrelevant [16], meaning this parameter does not influence significantly the activity.

3. Results and discussion

Construction of electron-conformational matrices of congruity. Conformational analysis for all compounds from Table 1 was performed using the methods of molecular mechanics with Merck force field [17] and the Monte-Carlo randomized search method with the help of the SPARTAN package [18, 19]. The total energies of conformers in their equilibrium geometry were calculated by *ab initio* RHF method in the 6-31G* basis sets and corrected for the aqueous solvation effect by the polarizable continuum model. This part of calculations was carried out using the PC GAMESS version [20] of the GAMESS (US) QC package [21]. The results of single-point *ab initio* calculations of the electronic structure (molecular orbital population analysis, Mulliken atomic charges, and bond orders) were used then for construction of the ECMCs for all conformers that are significantly populated at room temperature.

Pharmacophore Identification. The electron-conformational submatrix of toxicity was revealed first using 5 compounds **2-6** with statistically significant values of toxicity (see Table 1). The Tph consists of four molecular sites with specific interaction indices, which in our set are occupied by one oxygen and three carbon atoms, but they can equally well be occupied by any other atoms, that have the same electronic structure parameters and geometric positions in the compound within the limits of tolerances (Figure 2).

	A ₁	\mathbf{A}_{2}	\mathbf{A}_{3}	$\mathbf{A_4}$
\mathbf{A}_{1}	0.151 ± 0.034			
A_2	2.791 ± 0.754	0.166 ± 0.030		
\mathbf{A}_{3}	2.209 ± 0.689	1.423 ± 0.102	0.154 ± 0.033	
A_4	2.620 ± 0.833	4.903 ± 1.195	3.840 ± 1.130	0.332±0.091

Figure 2. The electron-conformational submatrix of toxicity obtained via identification of the toxicophore in the series of compounds under consideration. The tolerances reflect the toxicophore flexibility.

Next, we examined the rest of the molecules from the series to reveal the ECSA for their low-lying conformers with energies below 1 kcal/mol (read off the lowest energy one). We found that in addition to 5 compounds with the Tph, all other compounds except **19** also contain the Tph. Examples of molecular structures of some of the toxic compounds and their ECSAs are shown in Figure 3.



Figure 3. Electron-conformational submatrices of toxicity and molecular structures of compounds 2 (a), 4 (b), and 7 (c). Hydrogen atoms are omitted for simplicity. The four toxicophore atoms are marked by circles. The interatomic distances D₂, L₂ and L₂ are given as an example of descriptors used below for quantitative toxicity prediction.

Quantitative prediction of toxicity. For quantitative prediction of the values of toxicity of compounds containing the Tph the general formula for biological activity (2) and (3) can be rewritten as follows

$$\log(LD_{50})_{i} = \log(LD_{50})_{ref} - 2.30259 \left\{ \frac{E_{i} - E_{ref}}{kT} + S_{i}[R] \right\}$$
(7)

We performed parameterization of the Tph flexibility and ATS/AG influence with a training set of 19 compounds containing the Tph (see Table 1 for 1–18 and 20). Compounds 21-24 were considered inactive due to their statistically low experimental toxicities (LD_{50} >5000 mg/kg) and were not included in this training set. Compound 1 with the most significant parameter of toxicity was used as the reference one.

A regression model for quantitative prediction of toxicity was constructed. It is composed of as few as six descriptors, selected to parameterize the toxicophore flexibility and ATS/AG influence using Eq. (3) with the coefficients determined from the best possible correlation of the theoretical values of LD_{50} with the experimental data. Three parameters describing the toxicophore flexibility, $R(A_1-A_2)$, $R(A_1-A_3)$ and $R(A_2-A_3)$, are just the distance between A_1

and A_1 sites of the toxicophore (Figure 2). For instance, the sites A_i (*i*=1–4) appear in the case of **2**, **4** and **7** as atoms C(1), C(2), C(3), and O(1), respectively (Figure 3). The interaction index $II(A_1)$ is a function of the Mulliken charge and the electronic configuration of the atom C(1) in the molecule and stand for the donor-acceptor activity of this atom..To describe the anti-toxicophore shielding, ATS groups we introduce the parameter D_2 which is the distance (in Å) from the toxicophore site A_2 to some non-hydrogen entity defined as the out-of-Tph atom *most distant* from the geometric center of the Tph (Figure 3, c) [22]. Large values of D_2 correspond to relatively large (mainly hydrophobic) groups (for instance, phenylalkyl groups in **7**, **9**, **11**, **21** or long alkyl chains in **5**, **13**, **14**, **15** and **17**) that obstruct the toxicity.

To describe the auxiliary out-of-toxicophore AG groups we introduce parameters l_i (in Å⁻¹):

$$l_i = \frac{II^B}{L_i},\tag{8}$$

where L_i is the distance from the Tph site A_i to the *nearest* non-hydrogen atom *B* with the interaction index II^B belonging to the auxiliary (mostly electron donor) group (Fig. 3, a, c). The influence of such groups increases with the growth of the electronic density near the atom *B* (larger value of II^B) and with the decrease of L_i . The function l_i reflects the electrostatic potential induced by a point charge *B* at the distance L_i from the charge.

Table 2 shows the optimal values of the linear regression coefficients k_i (Eq. 3) corresponding to the six descriptors. Cross-validation and E-statistics (Eq. 6) of regression parameters indicate that the most statistically significant descriptors are two values of $R(A_i-A_j)$, followed by less important $II(A_1)$ and D_2 , and statistically irrelevant l_2 ; removal of the latter results in only a slight loss of accuracy with the value of R^2 dropping to 0.85 (Table 2).

			Table 2
Regress	sion Coefficients k _i and	d Cross-Validation St	atistics ^a
Descriptor	k _i	R ²	E-statistics
$II(\mathbf{A}_1)$	-2,71	78,37	0,31
$R(A_1 - A_2)$	0,17	83,39	0,41
$R(A_1 - A_3)$	-0,19	76,63	0,29
$R(A_2-A_3)$	-1,26	40,72	0,11
l_2	0,68	85,20	0,46
D_{2}	-0,01	80,05	0,34

^{*a*} Dimensions of the regression coefficients k_i (i = 1 - 6) are reciprocal to the dimensions of corresponding descriptors. Values of the correlation coefficient *R* and the E-statistics are calculated by means of consequent removal of one of the six descriptors out of the regression analysis.

Theoretical values of toxicity (LD_{50}) calculated by the Eq. (7) with the regression coefficients from Table 2 are presented in Table 3. The plot of predicted versus experimental data is shown in Figure 4. The quality of the model is characterized by following values of statistical parameters: square correlation coefficient $R^2 = 0.93$, SE = 0.029 (standard

error, Eq. 4), and a value of $F\left(\frac{6}{12},\alpha\right) = 26.6$ for F-statistics (Eq. 5) with 95% of confidence α . Cross-validated

"leave-one-out" values for toxicities (LOO in Table 3) are calculated by means of consequent removal of one of the compounds from the regression analysis and then calculating the predicted activity for the removed compound. These LOO values still predict the experimental data rather well (Table 3), the value of $R^2 = 0.93$ being lowered to $R^2 = 0.79$.



Figure 4. Theoretical versus experimental values of LD_{50} (mg/kg). Linear regression (points marked by squares) results in the value of $R^2 = 0.93$ for the correlation coefficient *R*. Leave-one-out cross-validation (points marked by crosses) predicts $R^2 = 0.79$.

Table 3

Correlation Between Experimental (Exp) and Calculated (Theory) Values of LD₅₀ (mg/kg) and Prediction of Toxicity in the "Leave-One-Out" Cross-Validation Scheme for the Training Set of 19 Compounds^{*a*}

Cpd	Exp	Theory	LOO	Cpd	Exp	Theory	L00
1 (Ref)	196	196	196	11	2227	1893	1663
2	1200	1242	1305	12	3100	3122	3167
3	1230	1126	960	13	3250	3189	3164
4	1390	1489	1713	14	3450	3840	4035
5	1530	1850	2246	15	3450	3298	3338
6	1560	1644	1609	16	3730	3109	2786
7	1700	1718	1767	17	4000	4404	4527
8	1930	1996	2035	18	4180	4331	4521
9	2000	1734	1674	20	4960	4058	3796
10	2200	2697	3142				

^{*a*} Experimental data (exp) on acute toxicity to rats are quoted from ref [13]. Only 19 compounds with the Tph (Tables 1) are included in the linear regression. Theoretical values of LD_{50} (Theory) calculated with use of regression coefficients *k* and corresponding descriptors *R*, *II*, *D*, and *l* (Table 2) are compared with experimental data.

Screening of new compounds. Next, we applied the obtained above results of the toxicophore identification and toxicity predictions to a new set of compounds that are included in the third European Union scientific committee SCCNFP's list of 78 fragrance chemicals that may be used subject to certain restrictions. Only for 20 compounds from them listed in Table 4 the chemical structures and the toxicity data were found in the literature, but the reliability of these data is uncertain. The electron-conformational matrices for their low-lying conformers (below 1 kcal/mol) were calculated and checked whether or not they contain the electron-conformational submatrix of activity, the Tph. Then for the compounds that have the Tph theoretical values of toxicity (LD_{50}) were calculated by Eq. (7) with the regression

coefficients from Table 2. The results are shown in Table 4. From the 20 compounds 17 have the Tph and three (6, 15 and 18 inTable 3) have not.

Structure	$\frac{\text{LD}_{50}^{a)}}{(\text{mg}\cdot\text{kg}^{-1})}$	Structure	$\frac{\text{LD}_{50}^{a)}}{(\text{mg}\cdot\text{kg}^{-1})}$
	218	H ₂ C H ₃ C H ₃ C CH ₃	1400
1 Allyl hexanoate	2500	сн ₃ 11 Allyl 3,5,5-trimethylhexanoate	424
H ₃ C CH ₂	230		1520
CH ₃ Ö	322		1240
		12 allyl cinnamate	
	250	Å	1650
	2490		1760
3 Allyl butyrate		13 3-Propylidenephthalide	
	475		1720
	409		3200
4 Allyl phenoxyacetate		14 4-(prop-1-en-2-yl) cyclohex-1- enecarbaldehyde	
H ₃ C	500	H _s c	1720
ö	2630	CH3	no Tph
5 Allyl phenoxyacetate		15 3-Methyl-2(3)-nonenenitrile	
ů II	570	CH3	2200
H ₂ C	no Tph		2160
6 Allyl octanoate			
		16 Amylcyclopentenone	
	585	СНа	2220
	407	H _a C ⁰	1780
7 Allyl cyclohexanepropionate		17 Methyl octine carbonate	

Molecular structures and toxicities (LD_{50}) of the set of new 20 compounds

Table 4



^{a)}The first row in the second and fourth columns presents the experimental values of LD_{50} (mg·kg⁻¹), while the calculated values are in the second row.

It is seen from Table 4 that for 9 compounds of this series (2, 4, 7, 9. 12, 13, 16, 17, 19) calculated values of toxicities agree rather well with the experimental data (triangles in Fig.5); note that they are all of higher toxicity where the experimental measurements are more reliable. For these compounds, taking into account the uncertainties in the toxicity measurements, the theoretical predictions are excellent. For the rest 8 compounds (1, 3, 5, 8, 10, 11, 14, 20) the theory predicts that they are toxic, but there is a significant discrepancy between the quantitative values of the toxicity predicted by the theory and the experimental data. Three compounds (6, 15, 18) have no Tph. The remaining discrepancies between the EC theory and experimental measurements of toxicities and chemical structures; it may stimulate additional investigation of the experimentally measured toxicity of these compounds, as well as their chemical composition.



Figure 5. Calculated versus experimental toxicities in two series of chemical allergens. Green triangles mark the calculated values of LD_{50} (mg/kg) for the testing set.

4. Conclusions

The electron-conformational method applied to the acute toxicity of 24 fragrance allergens reveals that this toxicity is controlled by a toxicophore that consists of four sites with certain electronic and topologic characteristics. In the series of compounds under consideration they are occupied by three carbon and one oxygen atoms, but may be substituted with any other atoms that have the same electronic structure parameters and geometric positions within the derived tolerances. A model composed of six parameters describing the toxicophore flexibility and the influence of anti-toxicophore shielding and other out-of-Tph groups reproduces rather well the experimental data [13].

The method worked out in this paper can be used in a similar way for screening and prediction of toxicity of other environmental pollutants.

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STUDY OF DESTRUCTION PROCESSES OF SYNTHETIC SURFACE-ACTIVE SUBSTANCES (SURFFACTANTS) IN BARRIER DISCHARGE

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Abstract: Kinetic data on degradation of aqueous solutions of surfactants in the plasma of barrier discharge are presented. The possible mechanism of proceeding processes is offered and considered. It is shown that the treatment in a barrier discharge results in decomposition efficiency of 95%. It is established that the main product of degradation are carboxylic acids.

Keywords: water, barrier plasma discharge, pollutants, surfactants

1. Introduction

A pollution of natural waters is one of the global environmental problems. Also, the problem becomes aggravated that the world enters an era of deficiency of pure water. This is the problem for 40% of world population. And it will be the problem for the 60% of population on keeping this rate. Situation aggravates by a constant increase in technogenic loading at natural sources of water. Therefore, the problem of providing of the population with qualitative drinking water has been recognized as one of priority at last session of National Security Council.

Considerable part of the anthropogenic loading on superficial water objects falls on the sewage containing synthetic surface-active substances which are a part of all domestic and the majority of industrial wastewater. Thereby 95-98 % of total applied in our country detergents is the synthetic washing-up liquids manufactured by the industry, both anionic and nonionic detergent agents on their basis which, as a rule, are characterized by low biological degradability and owing to the chemical nature have essential negative influence on water objects. A stability of surfactants to the biochemical oxidation is the cause of their accumulation in water objects especially in the bottom sediments, which in turn leads to a decrease in self-purification capacity of natural waters and creates the risk of secondary contamination of water bodies and watercourses. Therefore the aim of this study was to investigate the processes of destruction of aqueous solutions of surfactants in a dielectric barrier discharge.

2. Experimental part

The object of investigation was a sulfonol aqueous solution (sodium salt alkylbenzolsulfoacid) ($C_{12}N_{25}S_6N_4SO_3Na$). Experiment was carry out on the set-up as the basic element of which was a plasma-chemical reactor (Fig. 1). The outer tube of the reactor was made from molybdenum glass. For feeding plasma forming gas and the removing gaseous products in a glass reactor tube (insulator) were welded two sockets. A charge device was tightly closed by covers made from PTFE, the center of which was fixed with non-isolated electrode made from aluminum alloy. It was contained a hole with the depth of 50 mm, for passing treated solution (the object of research) coming from the tank directly on a layer of porous hydrophilic material (fiberglass) by 1 mm of thickness covering the central electrode.

The extraction of purified solution was carried out through the hole located at the bottom of the electrode. Flow rate of solution ranged from 0.02 to 2 mL/s. In case of increase in consumption of water containing a pollutant, there was malfunction of a film mode of fluid flow partial filling by solution of reactor volume and consequently the burnout barrier discharge.

The barrier discharge was excited by high-voltage transformer, the value of the variable (current at 50 Hz) voltage between the electrodes (U) was varied within the limits of $5 \div 20$ kV. The control of voltages was carried out with a voltmeter. The length of the discharge zone was 120 mm. As the carrier gas the technical oxygen was used the flow rate of which was varied from 1.1 to 5.3 cm³/s. A residence time (τ_1) of the treated solution with the discharge zone was determined as follows

$$\tau_l = \frac{\pi \cdot D \cdot h \cdot l}{Q}$$

where 1 - length of the discharge zone, m; Q - flow rate, m³/s; D - diameter of the inner electrode, m; h - thickness of the liquid layer, m.



Fig. 1. An element of the reactor with a coaxial arrangement of electrodes.

The determination of the sulfonol concentration and formaldehyde in aqueous solution was based on the fluorescence method using fluorometer "Fluorat-02-3M" [1].

Monobasic carboxylic acids were determined spectrophotometrically (spectrophotometer KFK-2MP, Russia). The method is based on the color reaction of the compounds with m-vanadate of ammonium [2].

The determination of carbon dioxide (converted to bicarbonate ion) in water was carried out potentiometrically [3]. The value of the pH of the solution before and after treatment was determined by ionometer IPL-311.

The method for determination of free CO_2 in the gas phase is based on the conversion of barium carbonate into a soluble chloride, after the absorption of carbon dioxide with cold ammonia solution of barium chloride (5 g BaCl₂·2 H₂O and 27.5 ml of 25% NH₄OH in 100 ml of solution) [4].

Measurements of ozone in gaseous and liquid phases was accomplished by the method of absorption spectroscopy - on the absorption of light at λ =253,7 nm attributable to the maximum of the photoabsorption cross section O₃ (σ =7,8·10⁻¹⁸ cm² [5]). Ozone concentration was calculated from the expression:

$$N_{O_3} = \frac{\ln(I_0 / I)}{\sigma \cdot l}, \text{ cm}^{-3},$$

where I₀ - initial intensity of the line Hg resonance without ozone, rel. units.;

I - then the same, but at ozone presence rel. units.;

 σ - Photoabsorption cross section, cm²;

l - length of a cell from 5.9 cm - in the case of measuring the concentration of O_3 in the gas phase or 0.999 cm - in the case of measuring the concentration of O_3 dissolved in an aqueous solution

$$C_{O_3} = \frac{M_{O_3} \cdot N_{O_3} \cdot 10^9}{N_A}, mg/m^3$$

where M_{03} - molecular weight of ozone (48 g/mol), and N_A - Avagadro number (6,02 • 10²³ mol⁻¹). The efficiency of decomposition surfactants were determined by the formula:

$$\alpha = \frac{C_i - C_f}{C_i}, \%,$$

where C_i , C_f - the concentration of phenol before and after treatment, respectively, mg·l⁻¹.

Figure 2 shows the decomposition kinetics of surfactants dissolved in the water under the impact of a barrier discharge. From the presented data it follows that degradation of surfactants is described by the reaction of pseudo-first order. The experimental data show that the decrease in flow of model solution, and, consequently, an increase in residence time of the treated liquid with the discharge zone influences on the degree of degradation of surfactants. So

the increase in residence time of a liquid with a discharge zone in the 3-fold (from 1.7 to 5.4 seconds) resulted in the decrease in concentration of the pollutant in solution in an average of 5 times.



Fig 2. The degree of decomposition of the surfactants in the solution vs the residence time of the liquid with the zone of discharge.

Figure 3 shows the kinetic dependences of the concentration of the pollutant – surfactant and primary oxidizing agent - ozone in a model solution at processing. It is known [6], that with increasing the power, whose magnitude is directly proportional to the applied, the output of active particles involved in chemical processes is increased, therefore, the efficiency and expansion of surfactants in aqueous solutions, was to be increased as observed during the experiments.



Fig 3. The change in concentration of ozone (1) and the synthetic surfactants (2) in a solution vs the residence time of the solution with the zone of discharge.

The dependence (Fig. 3) suggests that at short residence times there is a high consumption of dissolved ozone on the oxidation of synthetic surfactants (the initial part of the curve from 0 to 2.5 s). Later, with increasing time of residence rate of degradation falls, and runs a stationary process of oxidation (the smooth decrease in concentration of synthetic surfactants and the steady-state concentration of ozone at exposure time range of 2.5 - 5.6 s). At achievement of the maximum degrees of decomposition of synthetic surfactants, a concentration of ozone in water is increased (5.6 - 7.6 s). Hence, it is possible to assume that at times of contact more than about 6.2 ozone starts to participate in oxidation processes, both with an pollutant itself, and products of its decomposition (such as carboxylic acids and aldehydes), therefore the oxidation process does not proceed completely to CO_2 and H_2O . It agrees with experimental data on determination of the final and intermediate products of oxidation.

According to the stoichiometric equation for the complete oxidation of one molecule of sulfonol it needs 16 molecules of O_3 . Therefore, the complete oxidation of 14 mmol of sulfonol requires 224 mmol of O_3 . Maximum ozone concentration in the liquid phase, which is observed during the experiments was 52 mmol/L. Consequently the existing O_3 in these experiments is not sufficient for complete oxidation of synthetic surfactants (4.3 times the stoichiometric ratio). From experimental data follows that the maximum flow rate of ozone is observed in the initial period of treatment

and approximately 70% of the incoming ozone in the reactor. Thus, practically all ozone is consumed in the oxidation of synthetic surfactants and its degradation products. Straight in the range of residence time of 2.5 s to 5.6 s a maximum rate of oxidation of synthetic surfactants is observed (which is confirmed by the kinetic curves of oxidation of synthetic surfactants (Fig. 3).)

A model scheme of the process of oxidation of synthetic surfactants in the barrier discharge reactor can be represented in the following:



No alcohols in the processing of these compounds within the sensitivity of the measurement methods have been found. In fig. 4 the kinetics of formation of monobasic carboxylic acids (acetic acid), carbon dioxide and formaldehyde is presented in terms of carbon. The experimental data indicate that the kinetics of accumulation of carboxylic acids, as well as the kinetics of accumulation of carbon dioxide and formaldehyde was described by a curve with saturation. This suggests that carboxylic acid and formaldehyde, along with carbon dioxide, are the final products of oxidation of surfactants.



Fig. 4. Dependence of synthetic surfactants concentrations (1), carboxylic acids (2), the dioxide of carbon (3) and formaldehyde (4) in terms on carbon from the time of residence.

Complete chemical oxidation of organic compounds should proceed with the formation of carbon dioxide and water. Formation of CO_2 in the system depends on the pH. Under the conditions of the experiment (pH value of the solution after treatment in barrier discharge was 4.5) the carbonate system in solution was presented in the form of gaseous CO_2 [7].

Experiments have shown that the increase in processing time of model solution the value of the CO_2 concentration is increased, indicating the increase in the completeness of the destruction of organic compounds presented in the treated water. With increasing the processing time in 5 times the concentration of CO_2 has increased 1.7 times. While reducing the concentration of surfactants in 2 times. Maximum yield of carbon dioxide in the gas phase was 2.7 mg/L.

The maximum concentration of carboxylic acid was 6.2 mg/l (207 mol 'C'/L), the maximum CO_2 concentration was 2.3 mg/L (52.1 mol 'C'/L), and a maximal concentration of formaldehyde under study was 0.651 mg/L (21.7 mol 'C'/L). We can conclude that the main product of decomposition of sulfonol in the barrier discharge reactor under study, are monobasic carboxylic acids. The reliability of our data is based on the fact that the average convergence of the material balance on carbon in this case is 85%.

As it can be seen from the experimental data, we did not reach 100% destruction of organic compounds (maximum degree of decomposition was about 95%). This result can be explained by the fact that the solution contains an insufficient quantity of active particles, which are responsible for the oxidation of organic compounds. Results of experiments showed that the barrier discharge is effective for destruction of organic pollutants (for example, surfactants) in model solutions. However, at the transition from model experiments to the conditions of real systems it can be expected the

change in the efficiency of oxidative processes because of the increase in the number of reversible reactions due to a more complex component composition.

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INFLUENCE OF CLIMATE CHANGES ON WATER RESOURCES IN MOLDOVA

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Abstract: The paper aims to analyze the current state of affairs with water resources in Moldova, the challenges it faces for its national human and economic development, having in mind that the water resources are quite limited in Moldova, which encounters pollution, degradation influenced by climate change and unwise human activity to their biodiversity and ecosystems, availability and accessibility. It also attempts to highlight the relationship between climate change and water resources in Moldova, which has adverse effects on both environment and people's health, and raise significant hurdles to the international, regional and sectoral development.

Keywords: climate change, water resources, freshwater, pollution, degradation.

Water pollution is among the most critical environmental challenges worldwide having extremely serious consequences for both environment and people's health. One of such consequences is the degradation of water resources influenced by climate changes. The relationship between climate change and water resources are interconnected in complex ways.

One may say that climate change is only one of many factors influencing the future supply and demand for water. Indeed, population, technology, economic conditions, social and political factors, and the values society places on alternative water uses are likely to have more of an impact on the future availability and use of water than changes in the climate. Nevertheless, scientists around the world now agree that the climatic changes occurring internationally are the result of human activity, and the climate change, be it through heightened water insecurity, increased exposure to extreme weather events, environmental degradation or loss of natural resources, biodiversity and ecosystems, may raise significant hurdles to the international, regional and sectoral development.

Rising global temperatures as a consequence of environment pollution will lead to an intensification of the hydrological cycle, resulting in dryer dry seasons and wetter rainy seasons, and subsequently heightened risks of more extreme and frequent floods and droughts. Changing climate will also have significant impacts on the availability of water, as well as the quality and quantity of water that is available and accessible. The predicted changes in quantity, quality and accessibility to water resources will have important consequences for human populations, through impacts to agriculture and food security, health, economic activity, and conflict over water resources.

As concerns Europe, according to recent researches, climate change is posing two major water management challenges: increasing water stress mainly in southeastern Europe, and increasing risk of floods throughout most of the continent [1, p.95].

The climate change effects are already visible in Moldova. On the whole, Moldova is located in an area with quite limited water resources, which results in a high frequency of droughts. For example, from 1990 to 2010 the country has undergone nine droughts, and the most serious one in 2007, and two major floods in 2008 and 2010. The increase in natural disasters, primarily floods and droughts, will further exacerbate issues over water availability and water quality having major impacts on water resources.

And it is proved by the unequal distribution of available water resources and natural moistening. The northern part of Moldova (and the central part to some extent) is currently more or less secured from this point of view, while the southern part suffers from a natural water deficit. At the same time, medium and long distance water transfer systems are almost non-existent in the south [3. p.57]. This region is among the most exposed to water shortages. Moreover, local surface water resources in the south (and, less frequently, in the central part of the country) are exposed already today to depletion in drought years (like in 2007). However the water scarcity area, has, as it extends northwards, already reached the most populated areas, which place the biggest load on water resources and are most intensive in water use.

Under the 2010 OECD Report on Moldova the country's economy is very vulnerable to environmental factors, mainly because of its reliance on agricultural production [4]. Demand for agricultural and domestic water in particular increases significantly at hotter and drier times of the year. Agriculture has always been the dominant end-use of diverted water; this only intensifies with increasing needs for irrigation brought on by higher temperatures and reduced precipitation.

Besides the growing needs for irrigation there is another threat to freshwater resources and in particular ecological degradation. Ecosystems can be severely changed or destroyed by water pollution. Many areas are now being affected by careless human pollution, and this pollution is coming back to hurt humans.

The main source of freshwater pollution can be attributed to discharge of untreated waste, dumping of industrial effluent, and run-off from agricultural fields. Urbanization and the increasing use of synthetic organic substances have serious and adverse impacts on freshwater bodies. The protection of water resources and their quality is closely interrelated with health and reducing water-related diseases. It is a well-known fact that clean water is absolutely essential for healthy living. Adequate supply of fresh and clean drinking water is a basic need for all human beings.

As far as we know, in fact all types of water pollution are harmful to the health of humans. Water pollution may not damage our health immediately but can be harmful after long term exposure. The effects of water pollution are varied and depend on what chemicals are dumped and in what locations.

And the situation in Bubuieci village may serve as a strong example in Moldova of how badly pollution can damage water and people's health. Throughout recent years the waste mud from the Chisinau Water Treatment Plant and other domestic untreated waste is stocked on Bubuieci village outskirts. This has led to ground water pollution through runoff and hazardous substances trickling onto the soil, air, and wells. The negative human health effects are already evident. And in particular, during the last 4 years the number of serious diseases, such as cancer, increased by 30%. The physicians claim the growing number of respiratory diseases in children, tuberculosis, thyroid function abnormality, etc. because of use of ground waters, which proved to be seriously polluted with nitrates and nitrites, exceeding the maximum acceptable concentration (MAC) by hundreds of times, as well as the maximum permissible concentration of heavy metals such as lead, mercury, zinc, etc.

Furthermore, Moldova's water resources are made up of surface (70 percent) and underground waters (30 percent), including ground waters. Throughout the past twenty years, economic circumstances have had a much stronger influence on water supply dynamics than natural factors. However, both types of water resources in the Republic of Moldova, in particular surface waters, are vulnerable and sensitive to climate change with regard to their quantity and quality. Water quality is also threatened by degradation due to natural, non-pollution, factors. Thus, an increase in air temperature and excessive water pollution will lead to an increase of the temperature of the surface waters and diminishing dissolved oxygen (DO) level, which may result in degradation of surface waters in Moldova.

The surface water resources are the main source of water supply in Moldova, and the largest artery is the Dniester River (which is proved by the fact that 93 percent of water supply for the capital city of Moldova, Chisinau municipality, comes from the Dniester River), and its waters are degrading under influence of climate changes and technogenic disasters. The significant anthropogenic impact on the ecosystem of the Dniester River has the hydropower installations, built in Ukraine, which already lead to irreversible processes and degradation of the River as a whole, and extinction of a number of fish species.

Nowadays a number of power plants are functioning on the Dniester, in particular, the Dnestrovsk Power Plant (GES-1), the Hydropower complex (GES-2) in Naslavcea village, the Dubossar Hydroelectric Power Plant, the water reservoir of which, according to the recent researches by both Moldovan and Ukrainian scholars, is heavily silted and virtually all its bottom is covered with a layer of aquatic vegetation; and the Novodnestrovsk Power Plant.

Under the influence of exploitation of the Dnestrovsk Power Plants aimed only to satisfy the energy needs without appropriate environmentally sound downstream water releases the Dniester River turns into a lake-type reservoir with all the consequences. The physical and chemical properties of river water have been changing as well and in particular thermal, gas, water transparency regimes, composition of suspended substances. Unnatural water-level and temperature fluctuations in the river have had a negative impact on the fish reproduction, valuable fish species have virtually disappeared.

The Novodnestrovsk Power Plant functioning would possibly lead to catastrophic changes in the Dniester River ecosystem. Suffice it to note that besides the changes in the hydrological regime of the River, in the process of water injection and release through pipelines more than 80% of plankton organisms die. The negative effects for the river basin would also have the channel "align", which will inevitably lead to an imbalance between ground and surface waters. The Dniester River water level has already decreased by 1 meter. And the lack of water in the Dniester delta may lead to the destruction of the biodiversity of the region, because rare for Ukraine and the world otter, European mink, ermine, yellow heron, white-tailed eagle, black stork are breeding there. Therefore, the natural balance of the Dniester River is important for both Ukraine and Moldova, and its delta is protected under the Ramsar Convention (Convention on Wetlands of International Importance).

All the above have led to serious degradation of the Dniester River water quality, extinction or considerable decline of certain species of fish, animals and birds, and the river plants are also changing into species more characteristic to stagnant and boggy waters.

Unfortunately, it should be mentioned that Ukraine have been failing to submit so far the Environment Impact Assessment of the Novodnestrovsk Power Plant functioning, which it was bound to submit long before under Article 6 of the Espoo Convention (Convention on Environmental Impact Assessment in a Transboundary Context).

As concerns the underground waters quality in Moldova, their pollution is often caused by ammonium, sulphuretted hydrogen, and other chemical compounds. According to the data provided by the sanitary-epidemiological service, more than 60% of Moldova's population consumes water with inadequate concentrations of pollutants, because the quality of

groundwater in most cases fails to meet the requirements for domestic drinking water. Inspections of wells and springs proved that more than 71% of wells and 50% of springs are not safe for use in the household drinking water. And, the nitrate contamination found in 48% of wells and 35% of springs pose the greatest danger. Fluorine in unacceptable concentrations is found in 8.5% of springs and 90% of wells.

In recent times the pollution of groundwater with pesticides and other nutrients is increasingly serious challenge for Moldova. Pesticides are carried in rainwater runoff from farm fields, suburban lawns, or roadside embankments into the nearest creeks and streams.

Another challenge for water resources is posing deforestation, which in turn has its contribution to climate change, resulting in a drier climate. It is generally known that the lesser is the forest surface, the lesser water the region has. On the other hand, the lack of trees may also contribute to flooding should the erosion occurs due to increased runoff (unabsorbed rainfall). The Republic of Moldova is sparsely wooded and deficient in forest resources, and the forests are unequally distributed as well, the lesser degree of forestation is observed in the southern part. According to FAO [2], the area of forest (386 000 ha) accounts for about 11 percent of the land area, which is considerably less than in other European countries. Given the raggedness of the terrain, the low proportion of forest area, the high level of soil erosion, the aridity of the climate and the high population density, forests in Moldova are very important, both ecologically and socially, having in mind that land degradation is a major problem throughout the country. According to specialists, the negative influence of climate factors and the low biological resistance of the forests encourage periodic outbreaks of infestation and disease.

In conclusion, it may be said that the situation with the water resources in Moldova is alarming. Notwithstanding that the water resources and freshwater supply is of primary concern for Moldova and for its national human and economic development, freshwater-related issues need higher attention from both politicians and scholars, with active involvement of civil society, to help mitigate adverse effects of climate changes on water resources, improve the current water management practices, minimize and stop water pollution and deforestation, to jointly seek ways to address the above challenges. There is also a strong need in improved legislation, which would help water protection, conservation, its effective management, green economy implementation and environmental education.

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RESEARCH ON DETOXICATION OF SOILS POLLUTED WITH ARSENIUM AND DEVELOPMENT OF NEW SORBING MATERIALS

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Arsenium being a hazardous substance is harmful for plants, animals and humans. Having been applied to the soil arsenium is partly transformed as the result of interaction with the soil minerals. It may enter plants, animals as well as human body through the feed circuit. Nowadays issue of the anthropogenic impact diminishing as well as environmentally friendly farm production obtaining in the polluted soils becomes more and more urgent. Therefore development of soil detoxication techniques using natural sorbing materials is challenging and timely.

On the base of the previous research analysis and references natural sorbing materials (such as sapropel, vermiculite, diatomite, zeolite, kaolinite clays, peat) were selected to adsorb mobile arsenic [1]. To estimate sorbing materials efficiency simulated experiment was carried out. Sorbing materials were applied into the preliminary polluted (using Na_2AsO_3) soil at the application rate of 1 kg/m^2 , after solution stabilization soil arsenic content was estimated using Spectroscan. The sorption capacity range was determined for the different sorbing materials as the result of the experiment (Figure 1)





At the next stage of the research mixtures of different sorbing materials were made and tested during experiment. To improve sorption capacity some natural sorbing materials were treated with ferric iron. Vermiculite and diatomite mainly being comprised of silicon oxide (30% and 90% correspondently) and due to their porous structure were treated with ferric iron. This porous structure is a basic for ferric iron settling without its chemical linkage which provides high sorption capacity of pretreated sorbents in soils.

Sapropel and peat are treated with ferric iron too. The range of sorption capacity of sorbing materials mixtures and their pretreated modifications are shown on the Figure 2.



Figure 2 - The range of sorption capacity of sorbing materials mixtures and their pretreated modifications.

Mixture of diatomite pretreated with Fe^{3+} and blue clay having been applied into soil showed the best efficiency at mobile arsenic sorption (87%). As the result of the experiment the following mixtures were selected for the further research: mixture N 1 (diatomite pretreated with Fe^{3+} and blue clay) and mixture N 2 (sapropel pretreated with $CaCO_3$) [2].

To investigate natural sorbing materials influence on the arsenic accumulation in the crops plant experiment was carried out. Crops (garden radishes, oat and wheat) were planted in soil polluted with arsenic at the application rates – 20, 40 and 80 mg/kg, as well as in soil without arcenic application [3].

Mixture N1 provided high efficiency at detoxication of soil polluted with arsenic. Standard crops (oat, spring wheat, garden radishes, lettuce) were obtained in polluted soils in the case of corresponding application rates of sorbing materials and their mixtures (Figure 3).



Figure 3 – Arsenic content in lettuce.

Lysimeter experiment was carried out to confirm high efficiency of mixtures N1 and N2. There were the following variants of the lysimeter experiment:

- checking (soil without arsenic pollution),

- arsenic content in soil 40 mg/kg without sorbing materials application;
- arsenic content in soil 40 mg/kg + mixture №1 (1kg/m²),
- arsenic content in soil 40 mg/kg + mixture №2 (1kg/m²).

As the result of arsenic toxicity wheat productivity is reduced by 24% as compared to the checking variant in the first year of the lysimeter experiment. For the second year of the lysimeter experiment wheat productivity was higher by 24% in the variant with polluted soil as compared to the checking variant (Figure 3). As the result of self-purification capacity of the polluted soil arsenic content was reduced and it transferred into the useful microelement- phosphorus analog. However arsenic content in grain crop exceeded maximum permissible concentration. In the variant of diatomite pretreated with Fe³⁺⁺ blue clay application excess of Fe in soil causes wheat productivity reduction by 28% as compared to the checking variant for the first year of the lysimeter experiment, wheat productivity being increased by 43% during the second year of the experiment. For the variant of sapropel+ CaCO₃ application wheat productivity was increased by 51% as compared to the checking variant due to the fertilizing value of sapropel.

For the variant of diatomite pretreated with Fe^{3+} blue clay application arsenic content in grain crop was decreased up to 0-0,01 mg/kg (table 1). Arsenic content was reduced in roots-by 61%; in stalks – by 50%; for the plant in average –in 7 times (as compared to the variant of arsenic content in soil -40 mg/kg –without sorbing materials application) (2011 data). For the variant sapropel+ CaCO₃ arsenic content was decreased in grain crop - by 67-75; in stalks – by 73%; in roots-by 46%. Arsenic content in grain crop did not exceed maximum permissible concentration for above mentioned two sorbing materials application.

Nitrogen and protein content in wheat grain was higher for the variant of diatomite pretreated with Fe^{3+} +blue clay application as compared to the checking variant due to the fertilizing value of sorbing materials. It's important that quality of crops met the requirements of a standard good for the variant of diatomite pretreated with Fe^{3+} +blue clay application and it was not deteriorated for other sorbing materials application.

To estimate efficiency of mixture N1 (diatomite pretreated with Fe^{3+} +blue clay) at its application in locally polluted soils field experiment was carried out near the town Skopino in the Riazan region. Arsenic content in soil within the experimental plot was 54,45 mg/kg. Spring oat was planted. Sorbing material was applied at the rate of 10 t/hectare. Application plots' area was $2m^2$, shelter belt width was 1m, replication – 3 times.

A) in 2010.			
Variant of the experiment	Arsenic content in roots, mg/kg	Arsenic content in stalks, mg/kg	Arsenic content in grain, mg/kg
40 mg/kg	19,6	2,6	0,4
40 mg/kg + diatomite pretreated with Fe ³⁺ +blue clay	7,7	1,3	_*
$40 \text{ mg/kg} + \text{sapropel} + \text{CaCO}_3$	10,5	0,7	0,1
HCP ₀₅	2,43	0,35	0,05

Arsenic content in wheat

Note - * arsenic was not determined by atomic absorptive analyzer

Б) іп 2011.

Variant of the experiment	40 mg/kg	40 mg/kg + diatomite pretreated with Fe ³⁺ +blue clay	40 mg/kg + sapropel+CaCO ₃	HCP ₀₅
Arsenic content in plant (mean value), mg/kg	12,0	1,7	7,4	4,22
Arsenic content in grain, mg/kg	0,3	0,01	0,1	0,06

Variants of the field experiment were the following: sorbing material application; checking (without sorbing material application). The developed sorbing material application provided arsenic content decrease in plant (mean value) - in 5.3 times; in oat grain - in 6.5 times. Grain crop met the requirements of the Sanitary standard 42-123-4089-86 (Table. 2). After sorbing material application mobile arsenic content in soil was lower in 2,3 times as compared to the checking plots.

Thus application of diatomite pretreated with Fe³⁺+blue clay provides to obtain high quality grain crop in the polluted soils.

Additional soil analysis showed that the developed sorbing material didn't deteriorate agrochemical properties of soil (content of phosphates, Fe, pH) which is very important at detoxication of soil so long as qualitative characteristics being at the previous level.

Tentative mechanism of mobile arsenic sorbing on the developed sorbing material can be described as the following:

At sorbing material application insoluble iron arsenate is formed.

 $Fe^{3+} + AsO_4^{3-} = FeAsO_4$ $Fe^{3+} + AsO_3^{-} = Fe(AsO_3)_3$

Table 2

A reanic content in out coefficient of biological magnification, mobile arganic content in soil

AI senic content in	Ai sente content in oat, coefficient of biological magnification, mobile ai sente content in son					
Variant of the field experiment	Checking (initial As content 54, 5 mg/kg)	initial As content 54,5 mg/kg + diatomite pretreated with Fe ³⁺ +blue	HCP ₀₅			
		clay				
Arsenic content in oat, mg/kg						
in plant (mean value)	6,9	1,3	3,35			
in grain	3,1	0,18	0,27			
Coefficient of biological magr	nification					
in plant (mean value)	0,6	0,22				
in grain	0,47	0,2				
Content of mobile arsenic in s	oil, for the end of the vegetation, m	ng/kg				
Content of mobile arsenic	0,3	0,1	0,08			

As the result of hydrolysis in the water solution arsenic turns into soluble compound again.

 $\operatorname{FeAsO}_{4} + \operatorname{H}_{2}\operatorname{O} = \operatorname{FeOH}^{2+} + \operatorname{H}^{+} + \operatorname{AsO}_{4}^{3-}$

 $Fe(AsO_3)_3 + H_2O = 3FeOH^{2+} + H^+ + AsO_3^{-1}$

Table 1

Al ³⁺ being contained in blue clay provides the same effect. Al³⁺ + AsO₄³⁻ = AlAsO₄ Al³⁺ + AsO₃⁻ = Al(AsO₃)₃ Anionic form of arsenic is easily reduced to cationic form. AsO₃⁻ + H⁺ + e (electrons of the organic compounds) = H₂O + As³⁺ Ferric iron is reduced to the ferrous iron preventing arsenic reduction. Fe³⁺ + e (electrons of the organic compounds) = Fe²⁺ 2Fe³⁺ + As³⁺ + 3H₂O = 2Fe²⁺ + AsO₃⁻ + 6H⁺

Composition on the base of diatomite and blue clay contains both sorbing materials for sorption of anionic form of arsenic and oxidizing agents hindering arsenic reduction.

Thus the developed composition (diatomite pretreated with Fe^{3+} -blue clay) for detoxication of soils polluted with arsenic provides to obtain ecologically standard crop in the highly polluted soils, quality of crops and agrochemical properties of soil are not deteriorated.

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ENVIRONMENTAL REGULATION OF TRACE ELEMENTS IN SOILS OF MOLDOVA

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Abstract. The scale of environmental regulation (content and pollution levels) of trace element in soils of Moldova was proposed. Contamination levels are justified, depending on the adverse effects that have trace elements in soils, plants, groundwater and more - per person. Proposed scale has 6 content and 4 pollution levels. Along with equalized MPC it may help to consider a number of deficiencies that are met in this area and improve the practical application of the assessment of soil contamination. Scale Indicators are flexible values, taking the large variability content of trace elements.

Key words: environmental regulation, trace elements, soils, pollution level, indicators

Introduction

Saving the biosphere in the native status under current conditions is practically impossible, since the entire surface of the globe is more or less exposed to anthropogenic products. Consequently, the question is not whether to have a clean environment, but the levels of the chemical elements of biospheres are in amounts that not lead to negative consequences. The need to determine the maximum permissible concentration (MPC) of a number of elements emerged with increased reports of adverse effects of contamination of soil and water, which are manifested in the reduction of yield, worsening the hygienic quality of production, diseases of livestock, infringement of normal human life.

Heavy metals are polyvalent, well-absorbed by organically substances and soil colloidal complex, forming insoluble compounds, which contributes to their gradual accumulation in soils. The accumulation of chemical elements increases the toxic potential of soils, so its necessary to determinate what levels of concentration can bring the contents of each elements – pollutants, without fearing the consequences. Development of theoretical bases and practical methods of determination of MPC elements in soils is a difficult task and the work started later than in other environmental components.. Not by chance, currently established the MPC only for hundreds of substances that pollute the soil, while the number of MPC – polluting water sources, more than 800, polluting the atmosphere – about 300 [1].

Principles of chemical substances regulation in soils differ from those bodies of water, air, food. This is due mainly to the fact that direct intake from soil to human body is limited and most often goes on the biological (food) chains: from the soil to plants and animals, water and air, to man. The problem is complicated by the fact that the state of chemical substances in soils is determined by a large number of influencing factors of soil are geological character. So considering all factors affecting a specific geochemical conditions in the state of elements of contaminations is difficult, sanitation-hygienic services for regulations of elements in soils is based on those soil, climatic, geochemical, biological experimental condition that provide to the maximum possible extent the ability of chemical elements to pass into the adjacent environment. This work was carried out experimentally and the present sanitary-hygienic system MPC has played a great role in the environment protection of soils, plants and water [1, 2].

Moldova is a young state, and in spite of the great scientific potential, normalization of all chemical contaminants to re-complete the scheme is not possible. In the former Soviet Union the costs of establishing a standard in one environment habitat were 50 thousand rubles, in 2011 - 50 thousands of Euros [3]. Easy to re-approve the world (European) MPC or neighboring states MPC without analytical consideration leads to large errors and problems [4, 5]. Application of the calculation method for determining the approximate temporary MPC based on physic-chemical and toxic properties of individual contaminants classes of compounds requires further verification to complete the scheme.

The method proved to be reliable, but again needs a long time. Today's requirement is to have a real MPC or MAC (other indicators), to properly assess the degree of soil pollution by chemical elements – pollutants. Quantity of element-pollutant in the soil below MPC is considered hygienic standard MPC. For assessment and management of hygienic standards MPC are taken into account several factors, in particular:

- 1. Concentration of the element-pollutant in soil;
- 2. Adsorption capacity of the soil;
- 3. Migration capacity of the element-pollutant and possibility migration ways and in connection with the ability of soil to self-purification, or conversely, to an accumulation of element-pollutant;
- 4. Toxic level of element-pollutants on biota (microorganisms, plant roots).

Γ	
Table	

5)	Zr	ı	100- 870	450		<250	251- 400	401- 550	551- 700	701- 900	900- 1200	300	1200		1201- 2000	2001- 3000	3001- 5000	5000	1
(6,0-8,	Zn	1	10-166	71		<20	21-50	51-100	101- 150	151- 200	201- 250	300	250		251- 500	501- 1000	1001- 2000	>2000	>3000
action	Λ	3	15- 165	91		<40	41-70	71- 100	101- 150	151- 200	201- 250	150	250		251- 350	351- 500	501- 750	>750	>350
eutral re	Ti	ı	1600- 6800	4900		<200	2001- 4000	4001- 6000	6001- 10000	10001- 15000	15001- 20000	5000	20000		20001- 30000	30001- 45000	45001- 60000	>60000	
ne and n	Sr	3	50-400	240		<100	101- 150	151- 200	201- 300	301- 450	451- 600	600	600		601- 800	801- 1100	1101- 1500	>1500	
alkalir	Sn	ı	1-10	5,0		\Im	3,1- 5,0	5,1- 7,5	7,6- 10	11-15	16-20	4,5	20		21-30	31-50	51-75	>75	ı
ghtly	Sb	2	1-5	2,0		\sim	1,1- 1,5	1, 6- 2,0	2,1- 3,0	3,1- 4,0	4,1- 5,0	4,5	5		5,1- 10	11-25	26-50	>50	>300
vith sli	Pb	1	5-30	20		<10	11-20	21-30	31-40	41-50	51-60	32	60		61- 100	101- 200	201- 300	>300	>600
ation v	Ni	2	5-75	39		<15	16-30	31-50	51-70	71- 100	101- 150	50	150		151- 250	251- 350	351- 500	>500	>500
associa	Мо	5	0,9- 4,8	3,0		$\overline{\vee}$	1,1- 3,0	3,1- 5,0	5,1- 10	11-15	16-20	5	20		21-35	36-50	51- 100	>100	>200
emical a	Mn	3	150- 2250	062		<300	301- 700	701- 1100	1101- 1500	1501- 2000	2001- 2500	1500	2500		2500- 3500	3501- 5000	5001- 7000	>7000	
geoch	Ι	ı	0,5- 15	5,3		<2,5	2,6- 5,0	5,1- 7,5	7,6- 10	10,1- 25	25,1- 40	40	40		41- 100	101- 200	201- 300	>300	
or soil	Hg	1	0,01- 0,3	0,19		<0,1	0,1- 0,25	0,26- 0,50	0,51- 1,0	1,1- 1,5	1,6-2,0	2,1	2		2,1- 3,0	3,1- 5,0	5,1- 10	>10	>10
g/kg) f(F	1	64- 1047	485		<200	201- 300	301- 400	401- 600	601- 800	801- 1000	200	1000		1001- 1500	1501- 2000	2001- 2500	>2500	
ents (m	Cu	2	2-400	32		<10	11-25	26-50	51-75	76-100	101- 150	60	150		151- 250	251- 350	351- 500	>500	>500
ıl elem	Cr	2	25- 145	91		<40	41-70	71- 100	101- 150	151- 200	201- 250	06	250		251- 350	351- 500	501- 750	>750	>800
nemica	Co	2	4-18	13		<5,0	5,1- 10	11-20	21-30	31-40	41-50	30	50		51- 100	101- 150	151- 250	>250	>300
n of cł	Cd	1	0,2-0,8	0,41		<0,1	0,11- 0,25	0,26- 0,50	0,51- 1,0	1,1- 2,0	2,1- 3,0	2	3		3,1- 5,0	5,1- 10	11-20	>20	>20
ulatio	Be	2	0,9- 3,0	2,2		$\overline{\vee}$	1,1- 2,0	2,1- 3,0	3,1- 5,0	5,1- 7,5	7,6- 10	10	10		11-20	21-30	31-50	>50	
ntal reg	Ba	3	140- 640	460		<300	301- 400	401- 500	501- 700	701- 900	901- 1200	100	1200		1201- 1500	1501- 2000	2001- 3000	>3000	>2000
omno:	В	2	13- 220	70		<40	41-60	61-80	81- 100	101- 150	151- 250	100	250		251- 350	351- 500	501- 700	>700	
envir	A_{S}	1	1-10	5,0		<3,0	3,1- 5,0	5,1- 7,5	7,6- 10	11-15	16-20	2,0	20	on	21-30	31-50	51-75	>75	>50
cale of	Ag	ı	0,1-1,8	0,5	t	<0,3	0,31- 0,50	0,51- 0,70	0,71- 1,00	1,01- 2,00	2,01- 3,00	1	ŝ	aminati	3,1-5	5,1-10	10,1- 20	>20	ı
The s	Gradation	Hazard class [6]	Content range in the soils of MD	Average in soils of MD	Level conten	Very low	Low	Moderate	Improve	High	Very high	MPC existing and adopted [5, 1]	MPC proposed	Level of cont	Low	Moderate	High	Very high	M.O. [5]

Results and discussion

Generalizing the long-term data of numerous studies of trace elements in Moldova we propose a scale of environmental regulation of trace elements (table), for soils with neutral or slightly alkaline reaction (pH 6,0-8,5). Indicators of the scale are quite flexible values, taking into account the average content of chemical elements in soils of Moldova, their variability depending on the concentration.

Levels of pollutants justified in dependence on the adverse effects that have trace elements, especially on plants and ground waters, and then – on the human body. There is no doubt that for a correct assessment of the soil pollution degree by trace elements its needed to have a point of reference (background, native content) and knowledge of MPC (MAC). Proposed scale of the content levels and contamination may help to consider a number of shortcomings that are available in this area in Moldova.

First, eliminate the existing gaps and inconsistencies between background and soil content, and MPC of elements (B, Ba, Cr, As, Sn), reproved 22.10.2004 (M.O. № 189-192), which are, mg/kg:

1) Below average in the soils of Moldova (As -2,0 with an average 5,0; Sn -4,5 with an average 5,0);

2) Equal to the average content (Cr - 90 with an average 91);

3) Equal or below to the maximum content (Pb – 32 with a maximum of 30; V – 150 with a maximum of 165; Mn - 1500 with maximum of 2250).

A similar situation exists for Ba, F, Zn (first variant), Ti [2] and Ag, Ni, B, Cu and Mo [3]. Therefore, at this moment we can not correctly determinate the degree of soil contamination with 15 elements (23 analyzed), to identify preventive measures and methods of soil remediation. Secondly, to remedy the lack of data on the background content of some elements, as Ag, As, Be, Sn, Sb etc.

When comparing the pollution formal [5] and proposed levels (table), the concentration convergence is very strong on the pollution level named "very high". Values of Cd, Co, Cr, Cu, Mo, Ni, Hg are identical or very similar; for As, Bd, Zn – differ by 1,5 times; Pb and V – by 2 times. When compared with the developed MPC and MAC of different countries (WHO) shows that on the 11 items they are the same, on the 9 (B, Ba, Cr, F, Mo, Ni, Ti, Zr, V) they exceed them, because of the high content of these elements in the chernozems of in Moldova, and 3 (Pb, Sn, Zn) – lower due its weaker anthropogenic pollution of country soils.

Conclusion

The proposed scale of content levels and soil contamination (concentrations) are justified, depending on the size of the adverse effects that have trace elements in soils, plants, soil biota, ground waters and more - per person. Proposed scale of content levels (6 grades) and pollution level (4 grades) along with equalized MPC may help to consider a number of shortcomings that occur in this area and improve the practical application and validity of the assessment of soil contamination.

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i iou experiment									
Variant	pH	CaCO ₃	Humus	N-NO ₃	P ₂ O ₃	K ₂ O			
		0/	0	mg/100g soil					
	The contents in the 0-20 layer								
Control (without residues)	7.9	1.5	3.72	0.8	3.8	65.1			
BM, 100 +gypsum,10	7.6	1.7	4.15	1.3	4.5	78.0			
HL,50 +HS,50+gypsum,10	7.3	1.6	4.26	0.8	3.9	71.1			
HS,50 +DM,50	7.5	1.7	4.33	1.1	4.5	70.0			
HL,25+HS,25 +US,50	7.3	1.3	4.33	1.1	4.3	60.8			
HL,25 +HS,25 +DM,50	7.6	2.1	4.23	1.2	4.3	61.3			
HL,10 +HS,10+ US,30+DM,50	7.5	2.5	4.18	1.1	5.4	68.9			
LD, 5%	0.2	1.3	0.35	0.1	0.4	6.8			

Impact of the compositions of the hydrolytic residue on the indicators of chernozem solonets fertility. Field experiment

Table 6

The experimental compositions contributed to the increase of humus in the chernozem solonets. In the ploughed layer, the contents of humus increased by 0.43-9.61 % of the soil mass compared to 3.72 % in the control variant. At the same time, the contents of the mobile forms of the nutritive elements also increased. The average value in the three years of the nitric hydrogen in the 0-20 cm layer, for example, in the variant treated with hydrolytic lignin mixture, 25t + hydrolytic slime, 25 t + defecation mud, 50 t/ha constituted 12.2 me/100 g, while in the control variant this indicator was of 0.8 me/100 g of soil. An exception was observed in the variant treated with hydrolytic residues and gypsum, where the concentration of nitrates in soil was maintained at the level of the control variant. This effect is caused by the fact that the hydrolytic lignin and gypsum does not favor the nitrification process [4, 5].

We emphasize that the solonets of the experimental field contained increased quantities of mobile phosphorus and changeable potassium. The high contents of mobile phosphorus is explained by the previous fertilization with phosphorus fertilizers of these soils that were in the agricultural circuit. Though the contents of mobile phosphorus was high (3.8 me/100 gr) in the experimental compositions, an essential increase of mobile phosphorus by 0.5-1.6 mg/100 g of soil was stated.

Conclusion

The compositions of hydrolytic residues with urban sludge and defecation mud proved to be more efficient on the chernozem solonets than their separate application. When used in mixtures, the residues become more complex concerning the fertile elements that they contain, thus obtaining a multilateral impact on the soil fertility. Simultaneously the unfavorable parameters, which each separate residue has, are eliminated. In the variants with mixtures, the ameliorative action of organic residues increased by the optimization of the solonets reaction, the calcium and other elements' mobility. The experimental compositions contributed to the increase of humus contents and of the mobile forms of the nutritive elements in the chernozem solonets.

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ASSESSMENT OF MICROELEMENTS SOIL POLLUTION WITH ECOLOGICAL INDICATORS

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Abstract: Trace elements soil pollution leads to degradation of structure and aggregates stability which promotes the soil erosion and compaction. For more efficient management of the results on the content of trace elements in eroded soils the ecological indicators for characterization the accumulation in genetic horizons are used. The data confirms the soil pollution by erosion, the losses of humus, carbonates, and trace elements in arable carbonatic chernozems of catena. The humus losses in eroded soils consists 52%, the trace elements - 33-35%.

Keywords: trace elements, ecological indicators, chemical forms, soil erosion, chernozem.

Introduction

The eroded soils of Moldova constitutes about 80% from total soil surfaces [1]. On the catena with eroded soils exists the soil pollution by erosion, by excess or deficiency of plant nutrients, by soil compaction [2-7]. These soils are most vulnerable at degradation processes. The losses of humus, macro- and microelements in agricultural soils on the slopes are very considerable and become an ecological problem for agricultural production. The investigation on this research field was development in direction to verify the content of trace microelements accessible for agricultural plants and to determinate the total forms in biogeochemical aims. On the bases of these researches were developed the ecological indicators for assessment of microelement soil pollution in results of erosion degradation.

Methodical part

The investigation of catena carbonatic chernozems are presented by all degree of erosion: none eroded, weakly eroded, moderate eroded, strongly eroded and deluvial (accumulative) soil. The catenary's soils is presented by the sequence of soils with approximate age, formed on the same parental material in similar climatic conditions, but having a different characteristics in dependence of erosion degree. The soil samples were collected from all genetically horizons. The trace elements in soil samples were determinate by atomic absorption spectrophotometer, AAS-1. The total forms of microelements were determinate through classic methods of desagregations with hydrofluoric acid in combination with sulfuric acid. The chemical forms of microelements were determined in different regents.

Results and discussion

The microelements accumulation in humus horizons of soils is the results of different factors influence. However, in the first, the concentration of them in these horizons is result of bioaccumulation and actual anthropogenic pressing. The content of humus in eroded soils was in direct dependence of soil erosion degree. In the none eroded soil the humus content in the layer 0-10 cm consist 3,84 %, in the strongly eroded soil this content was 1,85 %. The average losses of organic matter by erosion consist 52%.

The deluvial soils from this zone incorporate the soils which formed in result of accumulation of pedolit deposits deluvial provenience in a very rapid temp as a result of erosion intensification on the slopes with carbonatic chernozems. In deluvial soils the content of humus has a stratification character of accumulation in superficial stratum, which depended of erosion manifestation. The some quantity of organic matter was washing from slope and deposited in the valley down. In accumulative soil the content of organic mater was same as in non eroded soils – about 3%. In the eroded chernozems the content of carbonates was changed considerable. In none eroded its content - about 1% in arable stratum (0-30 cm), in the strongly eroded – 7%, with a large values in depth, horizon C – 11%.

Copper. The total forms of trace elements include the all chemical forms from soils, inclusive: accessible for plants and actual inaccessible for plants. The distribution of Cu in soils is conditioned by following factors: parental rocks, soil genesis and erosion process. The accumulation of Cu in humic horizons depends of erosion intensity [2,4]. The total forms of Cu in humus layer of carbonatic chernozem none eroded is less under average level for chernozems of Moldova (34, 6 mg/kg) - 22, 7 mg/kg, in strongly eroded chernozem - 14, 7 mg/kg. The losses of Cu total forms in eroded soil consists 35%.

The content of Cu depends of value of humus that accumulated in organic matter. In the strongly eroded soil the content of Cu in surface horizon (0-20 cm) is 14, 7mg/kg. In the deluvial soil the accumulation of total Cu was less pronounced. In superficial stratums of the deluvial soil the content of Cu is 13,5-19,1 mg/kg. In the covering soil the

content of total Cu is keeping on the no eroded level -20 mg/kg. Cu total do not have accumulation varieties in depth of horizons B, BC, C of eroded soil (table 1). *Table 1*

			Chemical forms of trace elements associated with										
Horizon	Depth,	Total	mobile,		organic	Fe-Mn	minerals						
	cm	content	accessible	carbonates	matter	oxides	clay	primarv					
1	2	3	4	5	6	7	8	9					
Cu - none eroded chernozem													
Ар	0-20	22,7	<u>1,65</u>	<u>3,8</u>	<u>0,42</u>	<u>10,3</u>	<u>2,5</u>	<u>5,7</u>					
			2,9	16,7	1,9	45,4	11,0	25,1					
BC	110-140	16,2	<u>1,50</u>	<u>2,9</u>	<u>0,25</u>	<u>6,8</u>	<u>1,2</u>	<u>5,1</u>					
			9,3	17,9	1,5	42,0	7,4	31,5					
С	140-200	17,0	<u>1,10</u>	<u>2,7</u>	<u>0</u>	<u>6,8</u>	<u>1,6</u>	<u>5,9</u>					
			1,1	15,9	0	40,0	9,4	34,7					
		,	Cu	- weakly eroded	chernozem								
Ар	0-20	20,8	<u>1,5</u>	<u>3,7</u>	<u>0,25</u>	<u>10,6</u>	<u>2,5</u>	<u>4,8</u>					
			4,8	17,8	1,2	51,0	12,0	23,1					
BC	90-110	18,3	<u>1,0</u>	<u>3,4</u>	<u>0,21</u>	<u>6,8</u>	<u>7,9</u>	<u>8,5</u>					
			5,0	18,6	1,1	37,2	43,2	46,4					
C	110-180	17,6	<u>1,0</u>	<u>2,4</u>	<u>0</u>	<u>6,5</u>	<u>1,1</u>	<u>7,6</u>					
			5,7	13,6	0	36,9	6,3	43,2					
Cu - moderate eroded chernozem													
ABp	0-20	18,1	<u>1,3</u>	<u>3,3</u>	<u>0,25</u>	<u>9,2</u>	<u>2,4</u>	<u>4,0</u>					
	<u> </u>	1.5.0	5,5	18,2	1,4	50,8	13,3	22,1					
BC	60-90	15,0	<u>1,0</u>	<u>3,0</u>	<u>0,20</u>	<u>7,3</u>	<u>4,5</u>	<u>6,7</u>					
	00.150	10.0	6,7	20,0	1,3	48,7	30,0	44,7					
C	90-170	12,0	$\frac{1,0}{2,2}$	<u>3,0</u>	$\frac{0}{2}$	<u>6,1</u>	<u>2,9</u>	<u>2,0</u>					
8,3 25,0 0 50,8 14,2 16,7													
Cu - strongly eroded chernozem													
Вр	0-20	14,7	<u>1,0</u>	<u>3,0</u>	<u>0,21</u>	8,3	<u>3,2</u>	<u>5,5</u>					
DC	40.00	10.7	6,8	20,4	1,4	56,5	21,8	37,4					
BC	40-60	13,7	<u>1,0</u>	$\frac{3,0}{21,0}$	<u>0,20</u>	<u>3,3</u>	$\frac{5,0}{26.5}$	<u>2,6</u>					
	110 1 (0	11.6	/,3	21,9	1,5	40,1	36,5	19,0					
C2	110-160	11,6	<u>1,0</u>	<u>2,2</u>	$\frac{0}{0}$	$\frac{1,3}{20}$	<u>2,1</u>	<u>1,5</u>					
			8,0	19,2 none moded	0	<u>02,9</u>	18,1	12,9					
An	0.20	76.4	2.4	$\frac{1}{2}$	0.4	45.0	21.0	12.4					
Ар	0-20	/0.4	$\frac{2,4}{2,1}$	<u>3,2</u>	0,4	<u>43,0</u> 58,0	$\frac{21,0}{27.5}$	$\frac{12,4}{16,2}$					
DC	110 140	20.2	3,1	4,2	0,32	38,9	1 2	10,2					
DC	110-140	39.3	<u>2,2</u> 5.6	<u>0,5</u> 16.5	<u>0,3</u> 0.76	<u>36,0</u> 06,7	$\frac{1,3}{2,2}$	$\frac{14,5}{36,4}$					
C	140.200	48.1	2.6	10,5	0,70	35.0	12.1	23.1					
C	140-200	40.1	<u>2,0</u> 5.4	$\frac{1,7}{3.5}$	$\frac{\underline{0}}{0}$	<u>33,0</u> 72,8	$\frac{13,1}{27,2}$	$\frac{23,1}{48.0}$					
			<u> </u>		chernozem	72,0	21,2	40,0					
Δn	0-20	63.2	1.3	3 2	0.6	56.0	23.4	51.2					
rip	0 20	05,2	$\frac{1,5}{2}$	<u>5,2</u> 5,1	0.95	<u>88.6</u>	$\frac{25,4}{37.0}$	81.0					
BC	90-110	36.6	1.7	17	0.3	35.0	1.6	11.6					
DC	<i>y</i> 0 110	50,0	4.6	$\frac{1,7}{46}$	$\frac{0.5}{0.82}$	<u>95,6</u>	$\frac{1,0}{44}$	$\frac{11,0}{31.7}$					
С	110-180	42.7	1.6	1.7	0	33.0	97	97					
C	110 100	12,7	$\frac{1,0}{3,7}$	$\frac{1,7}{40}$	$\frac{\underline{o}}{0}$	773	$\frac{2.7}{22.7}$	$\frac{2.7}{22.7}$					
7n - moderate eroded chernozem													
ABn	0-20	60.6	13	2.4	0.45	50.0	10.6	23.6					
	0 20		2.1	4.0	0.74	82.5	17.5	38.9					
BC	60-90	68.0	1.3	1.7	0.30	50.0	16.0	45.0					
			1,9	2.5	0,44	73.5	23.5	66.2					
С	90-170	43.8	1,3	1.7	0	35.0	7.1	23.8					
		,	3,0	3,9	$\overline{0}$	79,9	16,2	54,3					

The content of trace elements in chernozems of catena, mg/kg/% from total forms
Continuation of the table 1

1	2	3	4	5	6	7	8	9
			Zn - s	strongly eroded c	chernozem			
Bp	0-20	52,7	1,3	1,7	0,55	40,0	10,5	29,7
_			2,5	3,2	1,04	75,9	19,9	56,4
BC	40-60	47,9	1,3	1,7	0,30	35,0	10,9	19,9
			2,7	3,5	0,63	73,0	22,8	41,5
C2	110-160	42,2	1,3	1,7	0	35,0	5,5	22,2
		,	3,1	4,0	$\frac{-}{0}$	82,9	13,0	52,6
				Ni - none eroded	l chernozem	, , , , , , , , , , , , , , , , , , , ,	,	/
Ap	0-20	48,9	3,0	3,3	0,5	43,0	1.7	10,9
1		,	6,1	6,7	1,02	88,0	3.5	22,3
BC	110-140	56,0	6,0	2,5	0,4	36,0	13,5	23,0
		,	10,7	4,5	0,7	64,0	24,1	41,0
С	140-200	56,7	5,3	2,5	0	29,0	25,2	26,7
		,	9,3	4,4	$\frac{-}{0}$	51,0	44,4	47,0
			,	Ni - weakly erod	ed chernozem	,	,	,
Ар	0-20	51,9	3,0	2,5	0,5	36,0	8,4	18,9
1		,	5,8	4,8	0,96	69,0	16,2	36,0
BC	90-110	52,2	4,0	2,5	0,4	34,0	11,7	20,2
		,	7,7	4,8	0,77	65,0	22,4	39,0
С	110-180	44,8	4,0	2,7	0	36,0	6,1	9,8
		,	8,9	6,0	$\frac{-}{0}$	80,0	13,6	22,0
			Λ	i - moderate ero	ded chernozen	n		· · · ·
ABp	0-20	77,6	3,0	3,0	0,4	<u>35,5</u>	<u>35,1</u>	48,6
			3,9	3,9	0,52	46,0	45,2	63,0
BC	60-90	64,6	4,3	2,5	0,3	36,0	23,1	<u>34,6</u>
			6,7	3,9	0,46	56,0	35,8	54,0
С	90-170	62,3	3,5	<u>2,9</u>	<u>0</u>	<u>33,</u>	26,4	34,3
			5,6	4,7	0	53,0	42,4	55,0
				Ni - strongly ero	ded chernozer	n		
Bp	0-20	83,5	<u>3,0</u>	<u>2,5</u>	0,35	<u>29,0</u>	<u>48,5</u>	<u>55,0</u>
			3,6	3,0	0,42	35,0	58,1	66,0
BC	40-60	75,0	<u>3,5</u>	<u>2,5</u>	<u>0,30</u>	<u>29,0</u>	<u>40,5</u>	<u>42,0</u>
			4,7	3,3	0,40	39,0	54,0	56,0
C2	110-160	90,6	<u>3,0</u>	<u>2,5</u>	<u>0</u>	<u>36,0</u>	<u>52,1</u>	<u>62,6</u>
			3,3	2,8	0	40,0	57,5	69,0
			(Co - none eroded	d chernozem			
Ap	0-20	21,0	<u>0,18</u>	<u>2,4</u>	<u>0,10</u>	<u>13,0</u>	<u>3,0</u>	<u>2,5</u>
			0,86	11,4	0,48	61,9	14,3	11,9
BC	110-140	15,0	<u>0,40</u>	<u>2,3</u>	<u>0,10</u>	<u>10,4</u>	<u>1,2</u>	<u>1,0</u>
			2,67	15,3	0,67	67,3	8,0	6,7
C	140-200	19,5	<u>0,44</u>	<u>2,0</u>	<u>0</u>	<u>14,0</u>	<u>1,0</u>	<u>2,5</u>
			2,26	10,3	0	71,8	5,1	12,8
			1	Co - weakly erod	ded chernozen	1		
Ap	0-20	19,5	<u>0,25</u>	<u>2,1</u>	<u>0,10</u>	<u>14,0</u>	<u>1,0</u>	<u>2,3</u>
			1,28	10,7	0,51	71,8	5,1	11,8
BC	90-110	15,8	<u>0,43</u>	<u>2,2</u>	<u>0,10</u>	<u>12,0</u>	<u>1,0</u>	<u>0,5</u>
			2,72	13,9	0,63	75,9	6,3	3,2
C	110-180	13,3	<u>0,50</u>	<u>2,1</u>	<u>0</u>	<u>9,7</u>	<u>1,0</u>	<u>0,5</u>
			3,75	15,8	0	72,9	7,5	3,8
	· · · · · · · · · · · · · · · · · · ·		-	Co – moderate e	roded chernoz	zem		
ABp	0-20	18,0	<u>0,25</u>	<u>2,1</u>	0,10	<u>13,0</u>	<u>1,0</u>	<u>1,8</u>
			1,39	11,7	0,56	72,2	5,6	10,0
BC	60-90	15,7	<u>0,47</u>	<u>2,2</u>	0,10	<u>11,1</u>	<u>1,0</u>	<u>1,0</u>
			3,00	14,0	0,64	72,6	6,4	6,4
C	90-170	14,1	<u>0,44</u>	<u>2,1</u>	<u>0</u>	<u>10,0</u>	<u>1,0</u>	<u>1,0</u>
			3,12	14,9	0	70,9	7,1	7,1

1	2	3	4	5	6	7	8	9
				Co - strongly ero	oded chernozei	т		
Bp	0-20	17,3	<u>0,35</u>	<u>2,0</u>	<u>0,10</u>	<u>13,0</u>	<u>1,0</u>	<u>1,2</u>
			2,02	11,6	0,58	75,1	5,8	6,9
BC	40-60	14,6	<u>0,50</u>	<u>2,1</u>	<u>0,10</u>	<u>10,4</u>	<u>1,0</u>	<u>1,0</u>
			3,40	14,4	0,68	71,2	6,8	6,8
C2	110-160	11,7	<u>0,51</u>	<u>2,1</u>	<u>0</u>	<u>8,0</u>	<u>1,0</u>	<u>0,6</u>
			4,36	17,9	0	68,4	8,5	5,1

Continuation of the table 1

The concentration of mobile and accessible forms of Cu was in limits 3-9% from total forms. In none eroded soil these forms have less content in humus horizon -0.65 mg/kg (2.9% from total forms). In depth the concentration increase to 9.3% in horizon BC. In strongly eroded soil this dependence does not keep, the mobile forms are 7-8% from total Cu. In deluvial soil the distribution of mobile forms of Cu was in I-VI stratums of soil 7.4-5.2%; in covering soil 4.9-7.3% from total Cu.

Zinc. The distribution of total Zn has been showed in table 1. In comparison with copper Zn have another distribution in soils profile. In none eroded soil the total Zn decrease from surface (0-20 cm) - 76,4 mg/kg to 27,8 mg/kg. The content of total Zn is more in strongly eroded soil, than in none eroded. The high concentration of Zn was accumulation on the geochemical barrier, when the concentration of carbonates is bigger. The losses of total Zn in 0-20 cm stratum of soil consist 33%.

The mobile forms of Zn in nonoe eroded soil consist in 0-40 cm layer of soil 3,1-4,5% from total forms. The high accumulation of mobile Zn takes place in horizon Bh, which are the barrier of transit of parental rock – 14,4%. In other horizons (BC, Ck) the mobile forms are about 5%. In the soil with strongly erosion the concentration of mobile forms have values about 3% in whole horizon. In the deluvial soil the accessible forms of Zn have bigger limits (5-13%) then in eroded soils. The higher concentration were accumulated in humic (9,2%) and carbonatic horizons (12,9%).

Cobalt. The reserves of total Co in investigation soils are under average level (20 mg/kg) for chernozems. The arable horizons to posses the higher degree of Co content, than inferior layers. The losses of total Co consist 5% in stratum of eroded soil 0-20. The carbonatic horizon of none eroded soil has about 20 mg/kg Co. This quantity of Co does not retain in eroded soil, it consists 12 mg/kg and another quantity was migrated after 200 cm of soil.

The content of total Co in accumulative soil from valley has stratification character in distribution in dependence of different texture and humus degree. Deluvial soil content in 0-7 cm - 19,7 mg/kg Co, with a little degree in depth - to 10 mg/kg. This distribution show that Co has leached in inferior stratums (B, Ck - 11-12 mg/kg) and accumulated in covering humic horizon (20 mg/kg Co).

The mobile and accessible forms of Co consists from 0,86% to 4,36% from total forms. These forms have the proportional correlation with global forms. The Co ions can be easy sediment by sulfides, carbonates and hydroxides. As a result the Co became an easy mobile element in soils. The concentration of plant accessible forms of Co maintain a little increase in 0-20 cm of soil stratum: 0,18 mg/kg in none eroded soil, 0,35 mg/kg – in eroded soil. There are emphasizing the covering horizons of deluvial soil – Ah, Bh, keeping a well correlation with total forms. The mobile forms of Co in deluvial soil consists 4,3% from global Co. In function of investigation methods the sufficient limits of Co supply for agricultural soil is 0,5 mg/kg [7]. The exanimate eroded soils is situated under supply limits with Co for plants.

Nickel. The carbonatic none eroded chernozem has in 0-20 cm stratum about 50 mg/kg Ni. In result of erosion processes the content of Ni increased to 84 mg/kg or 68% from total content. In depth of profile the total Ni has increase in both soils, but in eroded increase was significant, up to 90 mg/kg in 140-200 cm. The mobile and accessible forms of Ni consist 3-8% from global forms. The researches of chemical forms of Ni in eroded and deluvial soil it's necessary for determinate the factors which influence their comportment and are also as an ecological determinates in this soils [3,6].

The inaccessible forms of trace elements are presented by insoluble or heavy soluble salts, organically and organic-mineral compounds, primary and secondary minerals. It consists in this soil about 70-80%. The part of them can be successive in time accessible for plants through physic-chemical and biochemical processes of mobilization from insoluble to easy soluble and ionic status. These forms constitute the mobilized potential reserves of trace elements in soils. But in practically insoluble salts and in minerals are remain another part of elements which are immobilized for plants.

Ecological indicators The *degree of fixation* of trace elements by soil components showed that in the eroded soils the main role in their absorption is organic matter, oxides of Fe and Mn, clay minerals. The *transformation of chemical forms* of trace elements in soils is influenced by the action of various factors. The soil erosion increases the content of soluble forms and associated with clay minerals, decrease the forms of organic matter, oxides, and primary

minerals. In non eroded, fallow, cumulative soils reduction occurs content of mobile forms, chemical forms of clay minerals compounds is reduced. The arable soils of slope show, that in according to erosion increasing, the content of chemical forms associated with carbonates and oxides is higher. *Interdependence* of trace elements, their *deficiency* and *toxicity* in the soils is important for optimal development and grow of plants. Plant has valuable information related to soil ratio of elements: Fe/Mn, Ni/Co, Zn/Cu, etc. These correlations are well highlighted, especially when there is a surplus of chemical elements in soils. Deficiency affects physiological processes and consequently on plant productivity. Diagnosis deficiency and correction methods require further research. For different plant species, the concentration, which determines the deficiency and toxicity, is very different.

Conclusion

The regularity distribution of chemical forms content of trace elements in carbonatic chernozems eroded and none eroded are coordinated by eroded degree, content of soil carbonates, oxides and clay minerals and have a good correlation with them. The mobile chemical forms of trace elements in carbonatic chernozems are partial or total submissive transformations, in this case under erosion processes. Due time this forms can be passing from one forms to another, to maintain the dynamic equilibrium, but sometimes the accessible forms are immobile. The study of chemical forms transformation of different trace elements in soils complete the information about their provenience.

Between chemical forms of microelements in soils does not exist precise separation elements, but there are transitions, gradual passing. At the separation of chemical forms its necessary to select the adequate methods of determination and stabilization the equilibrium between them for each type of soil. The other ecological problem is study the factors which influence the mobility of elements in soil and anthropogenesis. Using the humus and chemical forms of trace element its can be diagnostically the level of erosion degree and sometimes the level of pollution by erosion.

The non eroded and eroded carbonatic chernozems contain the trace elements under average level, which are tolerated by plants. The losses of trace elements through erosion consist: Cu - 35 %, Zn - 33%; Ni - 15%, Co-5%. The distribution of elements in profiles depends of organic bioaccumulation and quantity of carbonates. The agriculture soil from slope needs in fertilization with organic fertilizers. These measures will conduct to increase the content of humus and accumulation of trace elements in mobile and accessible forms for plants.

Using ecological indicators in assessing chemical pollution of soils allows gaining more knowledge on the current status and trends that occur in soils and the environment.

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DEVELOPMENT OF HUMICS-BASED DETOXICANTS OF COMPLEX EFFECT

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Abstract: This research demonstrated development and properties of detoxicants of integrated effect based on humic derivatives. Set of samples of humic-based derivatives including carbonylated, hydrophobizated, oxygenated, cryodestructed and biosolubilized have been synthesized. It has been demonstrated that all the produced detoxicants possessed plant growth promoting activity and detoxifying potential in relation to heavy metals.

Keywords: humic substances, heavy metals, de-toxicants, detoxifying ability

Introduction

Chemical contamination makes significant harm to natural biocenosis, and sometimes causes their whole destruction. So, toxic metals (mercury, cadmium, lead) can be bound with different cell enzymes, disturbing normal functioning of the whole organism. Chlorine-organic hydrocarbons exhibit high mutagenic activity. Poly-nucleic aromatic hydrocarbons possess the expressed cancerous properties. Pesticides are of special danger: along with toxic effect they cause genome and structural mutations. To rehabilitate normal vital activity of biogeocenosis it is necessary to provide detoxification of the contaminated objects, the success of that is mainly dependent on the efficacy of detoxification agents applied. Consequently, an urgent problem is to create ecologically safe detoxification agents of complex effect with high rehabilitation potential in respect to the damaged biogeocenosis.

For the development of de-toxicants with such properties it is rather prospective to use humic substances (HS) and their derivatives. It is so, because humic substances comprise the properties of (1) reclaiming agents – favourably influence on physical and chemical properties of soil by their structuring, increasing moisture capacity, improving gas exchange, etc., (2) sorbents - bind hydrophobic organic compounds by a physical sorption mechanism, and ions of metals - by a ion exchange mechanism, (3) antidotes - enter into chemical reactions with a wide range as well as organic and inorganic compounds. Humic substances are natural organic compounds comprising 50 to 90 % of the organic matter of peat, lignites, sapropels, as well as of the non-living organic matter of soil and water ecosystems [1, 3, 20, 23]. Soil enriched with humic substances can endure significantly higher technogenic loads. Toxic effect of heavy metals and organic compounds decrease in them for biota, the penetration of eco-toxicants into the plants is reducing, the level of ground waters contamination is lower [9, 12, 21]. As a rule, protective effect of humic substances is explained by the formation of non-toxic and inaccessible for live organisms complexes with eco-toxicants [5, 16, 17, 19]. It is approved by data obtained on the reduction of SAH (surface active hydrocarbons) and heavy metal accumulation in water organisms in the presence of humic substances [14, 17, 22, 24]. At the same time it is known, that humic substances can speed up the processes of abiotic and biotic decomposition of eco-toxicants. So, humic substances increase the solubility of high-hydrophobic chlorine-organic pesticides in water [2], speed up photolysis of SAH and catalyze hydrolysis of sym-tryasines [25]. Thanks to redox - mediatory properties, humic substances are able to play a role of terminal acceptor of electrons, speeding up the processes of anaerobic decomposition of organic contamination substances [4, 15]. Adaptogenic activity of humic substances is of special interest, because it is exhibited in the increase of live organism resistance to stress loads, in particular to chemical stress [21].

The properties mentioned above allow considering humic substances as natural ecologically safe detoxicants of complex effect. In order to increase the specificity of humic de-toxicants it is prospective to produce the derivatives on their basis. The goal of this work is to demonstrate a new strategy whereby the directed design of humic detoxicants is used to bring about a desired remedial action. To reach this goal, the following objectives were formulated: (1) to produce the humic derivatives using the methods of fractionation, chemical and microbiological modification and complex-formation; (2) to characterize the structure and physical-chemical properties of the produced humic derivatives; (3) to evaluate biological activity of the obtained humic derivatives; (4) to evaluate detoxifying properties of humic derivatives with respect to primary ecotoxicants using laboratory express-tests and vegetation experiments.

The scope of work includes: isolation of the native humic materials from main types of raw materials and synthesis of their derivatives (more than 20 samples as a total) under laboratory conditions; characterization of the structure of the natural and modified humic materials using elemental analysis, functional group analysis, 1H-, 13C NMR-, FTIR-, Mössbauer spectroscopy; X-ray diffraction SEM and TEM analysis, evaluation of the biological activity of the produced natural and modified humic materials with respect to higher plants; and performance assessment of the detoxifying ability of the produced humic derivatives with respect to heavy metals under laboratory conditions.

Materials used and methods

Protocols of native humic substances isolation

Coal humic acids (HA) were isolated from two Kyrgyz oxidized brown coal deposits (Kara-Keche and Kyzyl-Kiya). To obtain HA samples, the potassium humates were dissolved in distilled water, centrifuged from the mineral components, and acidified until pH 1. The dark precipitate was washed out with distilled water, dialyzed against distilled water using dialysis and dried in vacuo at 60°.

Protocol for carbonylation of humic acids (CarbHP)

Carbonylated humic derivatives (CarbHP) were obtained by Hatterman-Koch reaction in the medium of the sulphuric acid. A weight of the HA (10 g) was placed in the three-neck flask equipped with mixer and reverse fridge. 50 mL of the mixture of $AlCl_3$ and CuCl as catalysts were added in the reaction system at vigorous stirring. 50 mL of concentrated H_2SO_4 was dropped in the reaction system. Gaseous CO and HCl were passed through reaction mixture. The retort was deeply lowered in the bath, filled by ice in the first hours of the process at 5°C, and then after 5 hours at 20°C. Total duration of the reaction is 12 hours. After the completion of the reaction the products mixture was diluted by water in the ratio 1:2, sediment was separated by the centrifugation, washed out from ions SO_4^{-2} and Cl^- and dried out.

Protocol for oxygenating of humic acids (Oxy-HP)

10 g of HA was suspended in 500 mL of 3.5% KMnO₄ in 1% KOH. The reaction mixture was placed in the threeneck flask equipped with the mixer and reflux condenser. Oxidation was carried out at 50°C in the water bath within 15 min and then was kept at 22°C up to a brown colour. Liquid product was separated from MnO_2 by filtration. Oxy-HP was extracted with methylethylketone (MEK). Filtrate was saturated by Na_2SO_4 . Extract was washed out from MEK and dried.

Protocol for hydrophobization of humic acids (HymA- hymatomelanic acids)

Hydrophobic fraction of HA was isolated from the parent HA by extraction with alcohol. 5 g of HA was put in the Erlenmeyer flask with 100 mL of 96% ethanol and kept for 24 hours at the continuous shaking. Then 50 mL of ethanol was added and shaked within 30 min and extract was centrifuged at 6000 rtm. Sediment was transferred into flask and processed with 50-100 mL of ethanol in dependence on content of HymA and kept at the continious shaking. The extract was separated on the centrifuge. This operation was reiterated 5-7 times till orange or brown colour of solution. Combined extracts were purified from impurities with ethyl alcohol using rotary evaporator at 40-45°C. The concentrated extract was air-dried at 30°C.

Protocol for biotransformation of humic acids (BioHP)

Natural microbial populations from a cultivated soil (Bishkek, Kyrgyzstan), a biohumus from *Eisenia foetida*, a wood rot from *Ulmus Pamila* were used as inocula. Colony forming units (CFU) were counted after 7 days at 25°C on MPA for bacteria and on Czapek agar for microscopic fungi (standard deviation of CFU counts: 10%). Numbers of bacteria were determined microscopially after fluorescent staining of cells according to Bloem.

The basal solution (per litre of distilled water) $KH_2PO_4 - 0.5$ g, $K_2HPO_4 - 0.5$ g, $MgSO_4 - 0.4$ g, NaCI - 0.1 g, $CaCl_2 - 0.01$ g, $(NH_4)_2SO_4 - 0.5$ g, glucose -15 g, was used in full strength ($(NH_4)_2SO_4$) or without (NH_4)_2SO_4 to receive a nitrogen-deficient nutrient solution. The salt solution, sterilized by autoclaving ($160^{\circ}C$, 30 min), 5 mL of inoculum, and 1 g of HS were added into flasks to final concentration of 1.0 mg/mL. pH was 6.8. The flasks were incubated in the dark at 28°C for 12 months. All experiments were performed in duplicate. After incubation biosolubilized HA were separated by centrifugation, washed out with H_2O , desalted using dialysis and dried in vacuo at 60°. Noninoculated sterile HS served as a control.

Protocol for cryodestruction of humic acids (CryoHD)

Sample of parent HA was freezed at -16°C with following defrosting at 30°C. This procedure was repeated fivefold.

Elemental analysis

Elemental analyses (C, H, N) were performed on a Carlo Erba Strumentazione elemental analyzer. Ash contents were determined manually. Oxygen contents were calculated as a difference. The H/C and O/C atomic ratios were derived from the contents of the elements calculated on ash- and water-free basis.

Determination of total acidity

5-10 mL aliquot of HA solution containing 5-20 mg HA was transferred into a vial (~ 22 mL) and 10 mL of 0.03 M Ba(OH)_2 were added. The vial was tightly sealed, shaken well and left for equilibration for 24 hours at room temperature. Aliquots of transparent solution above the precipitate of Ba humates were transferred to titration cell and titrated with HCl standard solution (~0.1 M) using phenolphtaleine as an indicator. Total acidity (TA, mmol/g) was calculated according to the formula

$$TA = \frac{(V_0 - V_{HA}) \cdot c_{HCl}}{m}$$

where V_0 and V_{HA} are the volumes of HCl (mL) consumed for blank and sample titrations respectively, C_{HCl} is the titrant concentration (mmol/mL) and m is the mass (g) of HA in the aliquot.

Saturated $Ba(OH)_2$ solution was prepared from BaO by dissolving it in CO_2 -free deionized water (boiled during 1 hour) in a sealed volumetric flask under intensive shaking. The solution was left for 3-4 days until complete precipitation of $BaCO_3$ occurred. Work solutions were prepared immediately prior to the analysis by diluting an aliquot of transparent supernatant and standardized against HCl.

Determination of carboxylic acidity and contents of strong acidic groups (COOH and SO₂H)

Calcium acetate method was used to determine carboxylic acidity or the content of strong acid groups (in case of sulphonated derivatives) in the humic samples. 5-10 mL aliquot of HA solution containing 5-20 mg HA was transferred into a vial (~ 22 mL) and 10 mL of 0.6 M Ca(CH₃COO)₂ were added. The vial was tightly sealed, shaken well and left for equilibration for 24 hours at room temperature. Aliquots of transparent solution above the precipitate of Ca humates were transferred into titration cell and titrated with NaOH standard solution (~ 0.05 M) using autotitrator. Carboxyl acidity (CA, mmol/g) was calculated according to the formula

$$D = \left(1 - \frac{R_d - R_{d+t}}{R_d} \middle/ \frac{R_o - R_t}{R_o}\right)$$

where V_0 and V_{HA} are the volumes of NaOH (mL) consumed for blank and sample titrations respectively, C_{NaOH} is the titrant concentration (mmol/mL) and m is the mass (g) of HA in the aliquot.

Fourier Transform Infra Red Spectroscopy

Fourier transform infrared (FTIR) spectra were obtained by pressing the HA sample into KBr pellet and analyzing with a FTIR spectrometer IR-200 (ThermoNicolet, USA) on spectral range of 400-4000 cm-1 (4 cm-1 resolution, 64 scans per spectrum).

Quantitative 13C NMR Spectroscopy

13C solution-state NMR spectra of HA samples were measured on solutions of humic materials in 0.3 M NaOD/ D₂O at concentration of 100 g/L. Measurements were made on a Bruker Aspect 3000 spectrometer operating at 100MHz 13C observation frequency using inverse gate decoupling. MeOH/D2O (d = 49.0 ppm) was used as an external standard. All the spectra were recorded at 8-s delay time. These conditions were shown to provide quantitative determination of carbon distribution among the main structural fragments of HA. To quantify the observed spectra, the assignments were made after Kovalevskii (Kovalevskii, 1998) and were as follows (in ppm): 5-50, aliphatic H and C-substituted C (CAlk); 50-108, aliphatic O-substituted C (CAlk-O); 108-145, aromatic H and C-substituted C (CAr-H,C); 145-165, aromatic O-substituted C-atoms (CAr-O); 165-187, - C of COOH/R groups (CCOO); 187-220 – ketonic/quinoic groups (CC=O).

Quantitative 1H NMR Spectroscopy

1H NMR spectra were acquired with Bruker DMX 500 NMR spectroscopy operating at 500 MHz proton frequency. The spectra were recorded at 303K using the 1-st increment presat-NOESY (90-deg), acquisition time = 4.7 s, relaxation delay = 15 sec.

All the spectra were acquired with 5-mm broadband probe, the samples were dissolved in 700 ml 0.1N NaOD/ D_2O .

Size Exclusion Chromatography (SEC)

SEC-Analysis was performed as described in Perminova et al. (1998). SEC system Abimed (Gilson, France) included HPLC pump, autosampler, and UV detector. The column 25 mm \times 20 cm packed with Toyopearl HW-55S gel (Toso Haas, Japan). The 0.028 M phosphate buffer (pH 6.8) was used as a mobile phase. The flow rate was 1 ml min-1. The absorbance of eluate was detected at 254 nm. The void volume and total permeation volume of the column were determined using blue dextran (2000 kDa) and acetone (48 Da), respectively. Sodium salts of polystyrenesulfonic acids of peak molecular weight of 14.00, 20.70, 45.10, and 80.84 kDa (Polymer Standard Service, Germany) were used as markers for molecular weight calculations. On the basis of the obtained data, the number-, weight, and z-average molecular weight and polydispersity (Mn, Mw, Mz, and Mw/Mn, respectively) were calculated as described in our previous publications (Kudryavtsev et al., 2000).

Protocol of bioassay with seedlings

Biological activity of the preparations was estimated by bioassay technique with seedlings. Seedlings of wheat *Triticum aestivum L*. were used as a target object, and a length of the longest root was used as a response. Ten wheat seeds were placed in Petri dishes with solutions containing 10 mL of 5, 15, 30, 50, 100 mg/L of humic derivative obtained during hydroxylation of humics.

The stock solutions of humic materials were prepared by dissolution of a certain amount of the sample in a small volume of 1M NaOH. Values of pH of all the solutions were adjusted to 5.5-5.9 using 0.1M HCl. Seeds were grown for 72 hours at 25°C in the dark.

Protocol of the quantitative assessment of detoxifying ability of the humic copolymers

For quantitative assessment of detoxification ability of HA preparations the detoxification coefficients (D) were calculated using approach described in (Perminova et al., 2001):

$$D = \left(1 - \frac{R_d - R_{d+t}}{R_d} \middle/ \frac{R_o - R_t}{R_o}\right)$$

where: R_0 – response of control; R_d - response in presence of HA; R_t - response in presence of toxicant (copper or mercury); R_{d+t} - responce in presence of toxicant (copper or mercury) and HA. On the basis of the D values, the toxicological constants of copper binding to HA normalized to the organic carbon content in HA preparation (K_{oc}^{tox}) were calculated as described previously (ibid.). The values of K_{oc}^{tox} were obtained by approximating the following expression:

$$D = \frac{K_{OC}^{tox} \times C_{HA}}{1 + K_{OC}^{tox} \times C_{HA}}$$

where CHA is a concentration of HA. The obtained values of K_{oc}^{tox} values were further used for comparison of detoxifying ability of different HA derivatives in relation to copper.

All preparations obtained using various modification techniques are listed in Table 1 below.

Table 1

Table 2

Sample designation	Description of the source humic material
HA1	Humic acids from oxidized brown coal of Kyrgyz Kyzyl-Kiya deposit
HymA1	Hymatomelanic acids, alcohol extraction from HA1
CarbHD1	Carbonylated humic derivatives from HA1
CryoHD1	Cryodestructed humic derivatives from HA1
BHD1-S3 (BHD1-S6,	HA biosolubilized by soil natural microbial populations in 3, 6, 9 months respectively
BHD1-S9)	
BHD1-W3	HA1 biosolubilized by wood-degrading natural microbial populations in 3 months
HA2	HA isolated from oxidized brown coal, Kyrgyz Kara-Keche deposit
HymA2	Hymatomelanic acids, alcohol extraction from HA2
CarbHD2	Carbonylated humic derivatives from HA2
CryoHD2	Cryodestructed humic derivatives from HA2
BHD2-S3	HA2 biosolubilized by soil natural microbial populations in 3, 6, 9, 12 months respectively
(BS6-HD2, BHD2-S9,	
BHD2-S12)	
HA2 (Fr3)	HA2, SEC-fraction 3
BHD2-S3 (Fr3)	BHD2-S3, SEC-fraction 3
BHD2-W3,	HA1 biosolubilized by wood natural microbial populations in 3, 6, 9 months respectively
BHD2-W6,	
BHD2-W9	
CryoBHD2-S3	Cryodestructed BHD2-S3
BHD1-B12	HA1 biosolubilized by biohumus natural microbial populations in 12 months

The sources and names of the humic derivatives

Results and discussions

Elemental and functional group composition of parent and modified HA are summarized in Tables 2 and 3.

Mass % **Atomic correlation** Index С Η Ν S 0 H/C O/C ώ HA1 65.99 3.86 1.08 0.43 28.71 0.70 0.36 -0.05 HymA1 63.15 4.41 0.67 0.25 31.61 0.84 0.38 -0.06 CarbHD1 64.59 3.60 0.90 0.24 30.73 0.50 0.26 -0.02 0.77 CryoHD1 64.30 4.05 0.80 0.23 30.58 0.36 -0.05

Elemental composition of humic derivatives

BHD1-S3	63.30	5.55	0.81	0.11	30.35	1.04	0.35	-0.35
BHD1-S6	64.60	5.83	1.24	n.d.	28.42	1.07	0.32	-
BHD1-S9	59.54	5.64	1.99	n.d.	32.83	1.12	0.41	-
BHD1-W3	65.20	4.98	0.82	n.d.	29.02	0.92	0.33	-
HA2	65.51	3.91	1.08	0.41	29.22	0.71	0.33	-0.05
HymA2	62.74	4.06	0.73	0.27	31.82	0.88	0.38	-0.08
CarbHD2	63.38	4.11	0.90	0.35	31.44	0.68	0.51	-0.04
CryoHD2	63.20	4.18	0.98	0.34	31.30	0.80	0.37	-0.05
CryoBHD2-S3	62.26	4.33	1.40	0.40	31.61	0.83	0.38	-0.07
BSHD2	58.40	4.61	0.86	n.d.	36.22	0.91	0.56	-0.01
HA2 (Fr3)	62.30	3.82	1.06	n.d.	32.64	0.73	0.39	0.05
BHD2-S3 (Fr3)	58.50	4.42	1.22	n.d.	35.91	0.90	0.50	0.01
BHD2-S3	62.02	4.55	1.06	n.d.	32.50	0.87	0.36	-
BHD2-S6	64.24	4.61	1.24	n.d.	30.05	0.85	0.34	-
BHD2-S12	59.54	5.28	1.95	0.40	32.83	1.06	0.41	-0.23
BHD2-W3	62.05	5.02	0.95	n.d.	32.17	0.98	0.40	-0.01
BHD2-W6	60.22	5.00	1.88	0.40	32.50	0.99	0.40	-0.19

As can be seen from the obtained data (Table 2), HA isolated from coal Kara-Keche deposit as well as Kyzyl-Kya had the lowest H/C ratio. Such values indicate of high content of non-saturated structural moieties in the corresponding molecular structure. Analysis formylated and biotransformed HS are characterized with more highest H/C ratio. Such values indicate of high content of saturated structural moieties of humic substances macromolecules.

Table 3

Sample	Functional groups					
	СООН		C	H	C	0
	mM/g	%	mM/g	%	mM/g	%
HA1	4.03	18.14	2.67	4.54	1.39	3.90
CryoHP1	4.08	18.36	2.72	4.62	1.61	4.51
BCryoHP1-S3	4.16	18.17	2.99	5.08	1.54	4.31
BHP1-S3	4.09	18.41	3.01	5.12	1.57	4.40
BHP1-W3	4.03	18.14	2.73	4.64	1.48	4.14
BHP1-B3	4.04	18.18	2.81	4.78	1.57	4.40
BHP1-S6	3.97	17.87	3.13	5.32	1.52	4.26
BHP1-S9	3.79	17.06	3.55	6.04	1.36	3.81
BCryoHP1-W9	3.86	17.37	3.53	6.00	1.34	3.76
BCryoHP1-B9	3.81	17.15	3.62	6.15	1.35	3.78
BHP1-W12	4.35	19.58	3.62	6.15	1.40	3.92
BHP1-B12	4.29	19.31	3.71	6.31	1.43	4.60
HA2	5.00	22.5	2.40	4.08	1.44	4.03
CryoHP2	5.34	24.03	2.76	4.69	1.60	4.48
BHP2-S3	5.34	24.03	2.65	4.50	1.48	4.14
BHP2-W3	5.47	24.62	2.47	4.12	1.47	4.12
BCryoHP2-S6	4.83	21.74	3.11	5.29	1.25	3.50
BHP2-S6	5.05	22.72	2.94	5.00	1.29	3.62
BCryoHP2-W6	5.00	22.50	2.59	4.41	1.30	3.64
BCryoHP2-B6	4.99	22.45	2.80	4.76	1.31	3.66
BCryoHP2-S9	4.57	20.57	3.61	6.14	1.42	4.17
BHP2-S9	4.59	20.66	3.45	5.87	1.43	4.00
BCryoHP2-W9	4.64	20.88	3.50	5.95	1.38	3.86
BCryoHP2-B9	4.75	21.37	3.32	5.64	1.35	3.78

Functional group composition of humic derivatives

Functional group analysis showed (Table 3) that a remarkable increase in the content of ArOH is observed for biosolubilized humic derivatives; it should be noted for all that derivatives studied, the content of ArOH increases along

with an increase in the inoculation time. For the cryodestructed derivatives an increase in the content of phenolic groups was observed as compared to the corresponding humic acids. For the synthesized humic derivatives enriched in carbonyl fragments a slight increase in the content of carboxylic groups was revealed as compared to the corresponding parent humic material.



Figure 1. FTIR spectra of humic derivatives

Figure 2. SEC-profiles of the parent humic material and carbonylated derivatives

FTIR-spectra of the parent and humic derivatives (Figure 1) turned out to be very similar and their shapes were typical for spectra of coal HA. The FTIR spectra of enriched humic derivatives exhibit the bands typical for parent humic material with negligible differences associated with the modification of parent material.

SEC analysis (Figure 2) showed that enrichment with carbonyl groups caused an increase in molecular weights of the derivatives, whereas oxy-, as well cryo-derivatives did not change molecular weight of the parent humic material. Humic derivatives enriched in carbonyl fragments exhibited the peak typical for the humics and also indicated the increase in peak molecular weight comparing to the parent humic material. The same tendency is also observed for biosolubilized derivatives in 9-12 months of inoculation.

¹³H-NMR and ¹³C-NMR spectra of the investigated humic substances are presented in Figures 3-4. As it can be seen from the figures, the spectra are typical for those of coal humic acids, characterized by large peaks corresponding to aromatic (100-165 ppm) and carboxylic/ester (165-187 ppm) signals and reducted hydrocarbon (48-108 ppm) signals. Spectral partial integrals, corresponding with 9 structural fragments of HS, are presented in the Table 4.

Table 4

¹³ C-NMR spectral integrals of studied samples (%)									
Structural fragments	C _{ALK}	CH ₃ O	CH ₂ - O,N	CH- O,N	OC-0,N	C _{AR}	C _{AR} -O,N	CO-0,N	С=0
Intervals, ppm	0-48	48-58	58-64	64-90	90-108	108-145	145-165	165-187	187-220
HA1	7.7	1.1	1	5.2	3.9	48.5	8.7	18.3	5.6
HymA1	11.9	1.7	0.8	1	1.5	51.7	7.8	18.8	4.8
HA2	9.5	1.5	1.5	4.2	5.5	44.1	12.1	14.7	6.9
HymA2	17	1.8	0.2	1.5	2	48.6	6.48	18.2	4.2
BHD1-W12	8.2	1.6	0.8	2.4	2.7	48.5	10.8	18.8	6.2
BHD2-W12	7.1	0.7	0.1	1.9	0.9	51.1	11.6	18.6	7.9
BHD1-S12	7.5	1.3	0.4	0.5	1.8	55.2	9.2	19.5	4.6
BHD2-S12	10.7	1.2	0.7	3.4	4.9	54.9	5.7	16.4	2.2
BHD1-B12	8.9	1.2	0.4	1.6	3.7	54.9	6.7	18.8	3.8
BHD2-B12	10.2	1.2	0.4	2	2.8	51.3	8.2	18.1	5.8



Figure 3. ¹³C-NMR spectra of humic derivatives: HA1; HA2; Hym1; HymA2; BHD1-W12-1; BHD1-W12-2; BHD1-S12-1; BHD1-S12-2; BHD1-S12-1; BHD1-S12-2

As it was expected, the ¹³C NMR-spectra of HymAs in comparison with the HAs demonstrated increasing in the intensities of the hydrophobic (alkyl and non-substituted by heteroatoms aromatic) groups signals. The Table 3 also shows increasing of aromatic spectral signals intensity (108-145 ppm) and decreasing of spectral integrals in 58-90 ppm area, corresponding to hydrocarbon and peptide functional groups of HS, as a result of the HS long time biotreatment.

Comparison of the ¹H and ¹³C NMR spectroscopy results shows that NMR-signals of alkyl-H and alkyl-C structural fragments increase from the humic acids to the hymatomelanic acids (Tables 4-5).

Aromatic carbon signals increase as a result of microbiological long treatment of the HAs. At the same time, aromatic hydrogen signals decrease from the HAs to the HymAs and to the microbiological treated HAs. It can be interpreted as a result of carbon substituted aromatic rings contribution relatively increased. The significant rise of alphagroups proton signals shown in the Table 3 confirms this supposition.



Figure 4. ¹H-NMR spectra of humic derivatives: HA1; HA2; HymA1; HymA2; BHD1-W12-1; BHD1-W12-2; BHD1-S12-1; BHD2-S12-2; BHD1-S12; BHD1-S12-2

Table 5

¹ H-NMR spectral integrals of studied samples (%)								
Structural fragments	Aromatic H	O-CH-O,N	CH-O,N	alpha-CH*	Alkyl-H			
Intervals, ppm	10.0-6.0	6.0-4.8	4.6-3.2	3.2-2.05	2.05-0.0			
HA1	52.7	1.8	2.7	8.2	33.6			
HymA1	48.2	0.0	6.3	10.7	45.5			
HA2	51.4	3.1	1.8	8.2	33.6			
HymA2	33.9	0.0	6.3	18.8	38.4			
BHD1-W12	49.6	0.9	4.5	9.9	33.3			
BHD2-W12	55.9	0.0	0.9	9.9	32.4			
BHD1-S12	53.6	1.8	3.6	9.8	29.5			
BHD2-S12	48.2	1.8	2.2	8.9	37.5			
BHD1-B12	49.6	0.9	3.6	9.9	33.3			
BHD2-B12	45.9	0.0	1.8	9.0	42.3			

* alpha-CH – protons of aliphatic groups in alpha-position to electronegative group or to aromatic ring

Assessment of stimulating activity and potential self-toxicity of the produced humic materials and synthesized derivatives has revealed that there was no toxicity observed for all humic derivatives studied: the root length was in the range of $(100\pm15)\%$ or $(100\pm17)\%$ of control, respectively. Stimulating activity towards wheat seedlings was the highest for the parent humic material reaching 120% of control. For the both hydrophobic and carbonylated derivatives, in most cases, it was lower than for other humic preparations and laid in the range of experimental error. The results on detoxifying ability activity of the humic derivatives are given in Figure 5.



Figure 5. Dose-response relationships in the presence of 1 mg/L of copper(II) for the parent humic materials and carbonylated derivatives

Estimation of phyto-hormonal activity of the produced humic substances and their derivatives in respect to higher plants (*Rosmarinus Officinalis L.*, watercress *Coronopus*, lettuce (*Lactuca Sativa*) with auxine and gibberellic tests has been shown that parent humic substances had no hormon-like activity. But, some produced derivatives (cryodestructed, hymatomelanic, carbonylated from Kara-Keche) displayed hormone like effect. Low molecular weight fractions of cryodectructed and carbonylated HP displayed two kind of biological activity.

Detoxifying properties of the produced humic preparations

Estimation of detoxifying ability of the parent and modified humic materials towards heavy metals revealed that all the studied humic derivatives significantly decreased toxicity of copper in bioassays with seedlings, vegetative experiments and field trials. The obtained results on copper toxicity are presented in Figure 6. At the low concentrations of HA (5-30 mg/L) the tendency that in most cases the detoxification activity toward copper of the carbonylated HA was higher than that of the parent HA was observed.



Figure 6. Dose-response relationships for copper(II) in the form of $CuSO_4 \times 5H_2O$ under conditions of lab vegetation experiments with a use of plant length (a) or weight (b) as a response. Bars represent standard deviation.

The calculated values of the toxicological binding constants of the humic materials studied with copper(II) are given in Table 6. Data presented in Table 6 confirm the above conclusion on detoxifying ability of the obtained humic derivatives. K_{OC}^{tox} values of modified humics were higher than K_{OC}^{tox} value of the parent HA. Maximum value of K_{OC}^{tox} was observed for BHP-B12 that was biosolubilized humic derivatives. Among derivatives the greatest detoxification ability toward copper was observed for biosolubilized derivatives. For the carbonylated derivatives a slight increase in detoxifying ability was registered comparing the initial humic materials.

Table 6

The	tovicological	constants	٨f	aannar	hinding	to	the	humia	mataria	le
Inc	toxicological	constants	UI	copper	Dinung	ω	une	nunne	materia	12

Derivative Cipher	K _{oc} ^{tox} , L/kg
Humate Kara-Keche	1.3×10 ⁵
Humate Kyzyl-Kiya	1.0×10 ⁵
CarbHD1	1.8×10 ⁵
CarbHD2	1.6×10 ⁵
BHP1-B12	3.6×10 ⁵
BHP2-B12	3.2×10 ⁵

Conclusion

Set of samples of humic-based de-toxicants have been synthesized. The experimental approaches undertaken to produce humic-based de-toxicants included enrichment with carbonyl groups; hydrophobic groups; oxygen groups; cryodestruction of humic substances and biosolubilization.

It has been demonstrated from both chemical characteristics and detoxifying ability point of view that all the produced de-toxicants possessed higher detoxifying potential in relation to heavy metals. All the derivatives of humics have been studied at express bioassay, laboratory vegetation experiments. It has been confirmed not only diverse detoxifying potential of those de-toxicants in relation to heavy metals but also their prolonged activity in the environment.

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UNUSUAL PROPERTIES OF WATER

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Water has been known for its unusual properties from antiquity when, e.g. was found that hot water freezes faster than cold water. Presently, on the web page 'water' Martin Chaplin [1] lists sixty seven properties of water which may be considered 'anomalous' when comparing to 'normal' chemical substances. Much of this can be attributed to the spatial structure of hydrogen bonding in condensed phases of water. Hydrogen bonding constitutes about 2/3 of cohesion energy of water. However, the remaining 1/3 is definitely not negligible. Combination of the two leads to properties of water in the systems where it plays a role. The very comprehensive range of such systems and common presence of water make the enormous variety of structures and properties of water-containing compounds. In the present paper the non-hydrophilic component of properties of water will be emphasized in combination with the structural aspects of supramolecular bonding of water molecules.

Basic physicochemical characteristics

<u>Structural features</u>. Inclusion of hydrophobic species in porous structure of water is closely associated with the idea of clathrate inclusion compounds. Although clathrate formation was first observed early in the last century [2], the physicochemical nature of clathrate compounds was not understood until the middle of the last century [3]. The concept of guest molecules 'closed or protected by cross bars of trellis' was originally proposed by Powell [4]. The presence of guest species in clathrate cavities is not simply of a fortuitous nature. Thermodynamic theory has made clear that guest-host intermolecular interactions are of primary importance, and the guest species may (as often happens) influence the host in such a way that the 'imprisonment' becomes more effective or is even made possible.

During the past five decades the chemical definition of *clathrate* has been extended to cover *inclusion-type interactions* in the solid state, and in liquid (or solution) phases. The latter issue seems to be one of the most important recent developments in the field because of its relevance to biological and geological systems.

In recent years the term '*lattice inclusion*' has been widely used [5] to describe, in a rather general manner, the situation whereby a foreign (guest) species is accommodated in a crystalline lattice of the host component. In such a way the distinction between pure '*clathration*' or '*complexation*' becomes less important, while special emphasis is put on the steric fit between the two components, i.e. the crystalline host and the guest species. The latter may be monomolecular or oligomeric, electrically neutral or ionic. If the crystal is a molecular solid, the term '*lattice inclusion*' is equivalent to description of the compound as interstitial. And, in turn, it follows that we consider *interstitial solid solutions* of the guest in the host lattice (provided there is no significant chemical bonding forces between the components, otherwise we should speak of *interstitial complexes*).

It is convenient to distinguish between two basic structural types of clathrate inclusion compounds. These are:

(i) *framework host structures*, in which building blocks (molecules) of the host are interconnected by directional bonds in such a way that some space is left empty, and thus available to incorporate foreign (guest) species, and

(ii) *packing structures*, in which close packing cannot be attained in a chemically homogeneous composition or, put in other words, the host molecules are of size and shape that preclude efficient packing. Thus, the unfilled intermolecular space which remains between the host molecules can be used to include a suitable guest.

A common characteristic of the two above mentioned structural types is that the host itself cannot form close packed crystal structures, and packing efficiency is attained by using a second (i.e. guest) component. As a rule, an '*empty*' clathrate (i.e. a '*clathrate without any guest'*) is thermodynamically unstable and may only be prepared as a metastable, and in rather rare cases only. Accordingly, one may consider desorption of a guest component from a clathrate structure as a possible strategy towards preparation of new polymorphic (metastable) structures of the host compound.

It is commonly assumed that no specific chemical bonds exist between the host and guest in clathrate compounds. There is even a system of nomenclature of inclusion-type compounds [6] in which the term *clathrate* is meant as the opposite of *complex*. In other words, *clathration* refers to *physical incorporation* rather than *chemical bonding*, for which the term complex is more appropriate.

<u>Clathrate Hydrates - Supramolecular Architecture of Water as Host</u>. Water molecule is commonly known to form, in condensed phases, four hydrogen bonds, donating two and acting as the acceptor of the other two. The resulting tetrahedral geometry of the intermolecular bonding may be realized in the dodecahedral arrangement of water molecules in which oxygen atoms occupy vertices while hydrogen bonds lie along edges of this regular figure. Indeed, almost a

perfectly regular dodecahedron may be found in clathrate hydrates [7], and has also been found as a *building block* of composite cavities [8]. It is interesting to note that such a geometric figure formed by water molecules has been identified in molecular beam experiments [9]. Accordingly, it may be taken as a proof that water molecules have inherent tendency to form dodecahedral cavity structure. An even more striking observation coming from the cited above experimental work is that the cavity contains an extra water molecule (or H_3O^+ ion) enclathrated inside the $(H_2O)_{20}$ cavity. Such a model has been suggested by L. Pauling in his theory of the structure of water. (An *extra* molecule inside the cavity might, presumably, be the reason for water's increasing density upon melting of ice and, bonded in a hydrophobic manner, contribute to the dynamic properties of liquid water, although this has never been confirmed by any solid state structure of water or its clathrates. The structures of $(H_2O)_{20}$, $H^+(H_2O)_{20}$ and $H^+(H_2O)_{21}$ were also analyzed theoretically [10] and their stabilization energies calculated. Water molecules can be replaced by methanol up to $(H_2O)_{13}(CH_3OH)_8$ with the formation of clathrate-like structures of mixed water-methanol $(H_2O)_{(CH_3OH)_m}H^+$ clusters [11].

Packing of dodecahedra leads to the formation of additional cages, since five-fold symmetry of the dodecahedra does not allow efficient space-filling in a crystalline form. Put in other words, packing of dodecahedra produces crystal structures which contain some additional cavities, usually larger than the dodecahedral ones. The simplest solution, known in the literature as hydrate type I, contains a combination of dodecahedra and tetrakaidecahedra in the proportion 3:1. Internal cavity radii of the cavities are approx. 3.9 Å (small cavity = dodecahedron) and 4.3 Å (large cavity), respectively. Common description of cavity structure of hydrates is 5^{12} for dodecahedra and $5^{12}6^2$ for tetrakaidecahedra what means the former is built of 12 pentagons and the latter of 12 penta- and 2 hexagons. This kind of notation is commonly used, thus, e.g. $5^{12}6^8$ represents icosahedron observed in hydrates type I, thus far the largest (radius = 4.06 Å) single cavity found in clathrate hydrates [12]. Clathrate hydrates type II are built of the 5^{12} and $5^{12}6^4$ cavities in proportion 2:1, the internal radius of the hexaidecahedron is equal 4.7 Å.

When considering the ability of hydrophobic molecules to form hydrates of the structures mentioned above, it is common to use, as the first approximation, geometric fit of molecular sizes to the cavities. Small molecules, like nitrogen N_2 , may enter both small and large cavities but their contribution to stabilize the cavities is not high, however. On the other hand, methane or hydrogen disulphide fit very well to the dodecahedral cavities, thus being efficient hydrate 'formers'.

Although hydrate types I, II and H are commonly listed as the basic ones for hydrophobic hydration, the complete structural information may still be far from completeness. Although this problem will not be discussed here, it seems important to mention that basic structural chemistry of gas hydrates is still an open field for interesting discoveries.

Very important and interesting is the formation of large polyhedra, typically combined of, four basic units, which take on open structures in which the tetrahedral, intermolecular bonds of water molecules, are preserved only partially. This leads to a variety of possible three-dimensional structures.

<u>Guest-host interactions</u>. In recent years much new information concerning clathrate hydrates has been provided both from experimental and theoretical studies. In the latter a major breaktrough may be consideration of the host-guest coupling in the normal mode analysis for the accommodation of large (relative to the cavity size) guests [13] when cage distortion occurs (like CF_4 guest molecules in the small cavities of clathrate hydrates). The classic theory of van der Waals and Platteeuw is based on an ideal solid-solution model and thus ignores the guest-host vibrational coupling and host lattice distortions. These contributions cannot, in real structures, be neglected. By taking them into account [14] the stability of Xe and CF_4 clathrate hydrates could be calculated. Encaging non-spherical propane molecules required taking into account the anharmonicity of vibrational free energy associated with the non-spherical nature of the guest. The calculated thermodynamic properties are, reportedly, better approximation to the experimental data [15] than those previously available. By examining the temperature dependence of the dissociation pressure of Xe and Ar clathrate hydrates it has been concluded that the predicted pressures agree well with experiments at higher temperature range [16].

It has also been demonstrated, in an elegant series of advanced NMR studies, that the distribution of guest species (Xe) between small and large cages is more complex than predicted from simple theory of clathrate solutions [17], or by simple rules of classical size-structure dependence. As shown by spectroscopic methods, carbon dioxide [18], oxygen [19], nitrogen and carbon monoxide [20] can form hydrates of type II from vapour-deposited amorphous solid water. X-ray studies give similar results with respect to the natural air-hydrate in Greenland Dye-3 deep ice core [21]. It seems likely, however, that some of the structure II hydrates undergo the II - I phase transitions at high-pressure conditions [22]. There are many subtle structural features of clathrate hydrates, and related structures like e.g. semiclathrates, which are a subject of interest in advanced studies which employ solid state NMR ²⁷. In addition to guest order-disorder phenomena, rather common in clathrate compounds, hydrates show proton disordering around host water oxygen atoms. These systems are dynamic at room temperature, freeze down to rather complex static patterns at lower temperatures [23]. It has been found by neutron diffraction study of the structure II clathrate hydrate of xenon/carbon tetrachloride (3.5 : 8 : 136) that the D₂O molecules are disordered at 13 K and 100 K in 6 H-bonded orientations [24]. It has recently been demonstrated that polar guest molecules may alter the rearrangement process in a defect-bearing solid clathrate hydrate network [25]. It was found that the rearrangement dynamics in a clathrate hydrate encaging polar guests follows a different mechanism from that proposed for Bjerrum for crystalline ices.

Hydrophobic hydration

Methane hydrate. The apparently most important clathrate hydrate at present is methane hydrate since its potential as an energy source is becoming widely recognized, as well as hazards associated with conceivable gas eruption on clathrate decomposition [26]. However, it is not unlikely that clathrate hydration plays a more substantial role, at least for the biosphere, in formation and stabilization of biological supramolecular systems. For example it has been shown that the behavior of protein-containing reversed micelles may be significantly modified when the species are subjected to clathrate hydrate formation conditions [27]. It was shown that clathrate hydrates of methane can form in protein-containing reversed micellar systems; their effect is to reduce the water content in the micelles. This observation has possible important implications since it has been known that, e.g., enzyme activity is dependent on water content.

Semiclathrate structures. A series of novel semi-clathrate structures of water have recently been found. In semiclathrates the water intermolecular structure contains some deviations from a four-connected, threedimensional polyhedral network. Typically, as found e.g. in the structure of the 7.25-hydrate of tert-butylamine [28], the guest species are not only enclathrated but also participate in the hydrogen bonding. Another possibility is to replace some water molecules of the host framework with hydroxyl [29] or fluoride ion. In such a way the host water connectivity is subjected to significant modification and also adopts an ionic character. Tetramethylammonium hydroxide has been reported to form solid hydrates ³⁹ Me₄NOH•nH₂O with, at least, eight different structures (n = 2, 4, 5, 7.5 and 10, at some compositions polymorphism has been observed); tert-butylamine has been reported to form as many as seven different solid hydrates [28].

It would be conceivable to include a metal cation into small cavities present in such semiclathrate lattices having an anionic nature. As clearly demonstrated [30] cesium ions can be enclathrated in small decahedral cavities; the Cs...O distances of 3.43 and 3.53 Å to two non-related by symmetry oxygen atoms (two sets of eight atoms each form the cavity) being evidence for a good fit.

Another series of recently reported structures of this type is based upon cavity architectures in which the network formed by host water molecules is more or less distorted from the regular shape. This is the case of 1,10-diaza-18crown-6 hydrate. Rather characteristic for these systems is that more than one hydrate is formed under slightly different experimental conditions. The next example shown represents a somewhat more open structure in which guest cations (methylammonium) are arranged, together with 18-crown-6 receptor molecules, parallel channels to the water framework which assumes an open channel structure presumably able to transport simple ions or small molecules through the crystal along the \underline{z} axis. The tetra-n-propylammonium fluoride complexed with 18-crown-6 and hydrated [31] shows a layered structure in which the three-dimensional intermolecular bonding between water molecules is preserved only partially, i.e. within the layer formed by H₂O molecules.

When going from the cage structures to the layered one, fewer and fewer intermolecular water-water hydrogen bonds are seen and, at last, one can hardly identify any "host" or "guest" component in the structure. By inspection of the structures given in these Figures, and of the corresponding phase diagrams, of these systems, it may be concluded that the type of structure of the host-guest associates is strongly dependent upon the composition of the solution phase from which the solid crystallizes. If, for example, a layered structure is obtained for which less host material is necessary when compared to the cage type, then at higher guest concentrations in solution the crystallization of layered compounds is observed.

Hydrophobic and hydrophilic hydration do co-exist and cooperate. Bonding *via* hydrophobic hydration is an important contribution, in terms of attractive interactions, to overall stability of intermolecular compounds.

When the internal crown ether receptor site is blocked by complexation of anoty complexation of another species, (e.g. by an aminoacid or peptide moiety), then water-crown hydrogen bonding is no longer feasible. Regardless, high hydration of the complexes is possible as has been shown recently [32].

The examples shown above are intended as simple model illustrations of structural situations which may play, and presumably do, important role in biochemical systems. The structures which are most stable at given conditions and environment are the ones which represent the function of the system. Water is a predominant component in biosphere and its interactions with any other components are of major importance. What is taken as granted is that water is a hydrophilic molecule. It is, of course, true statement. However, this truth is incomplete. Water may, as shown on selected examples above, behave as a combination of hydrophilic and hydrophobic molecule. If hydrophilic functions (hydrogen bonds) are saturated by forming sufficient bonding to the surrounding hydrophilic species (other water molecules, e.g.), what remains is hydrophobic interactions geometrically situated in between the tetrahedrally arranged hydrogen bonds. These interactions are not negligible, in many cases these are the structure determining factor. This phenomenon is of great importance in biochemistry. There are many protein structures in which hydrophobic hydration, i.e. hydration without hydrogen bonds between water and the second component, plays decisive role and it may well be referred to smaller blocks of protein structures. Many details still await complete discovery but the role of 'hydrophobic water' in chemists' understanding of structure and function of biological systems is growing.

Conclusions

Hydrophobic hydration is the concept derived in the 1950s from thermodynamic studies from which it became clear that, in addition to hydrogen bonding, water has some extra ability to bind, without hydrophilic interactions, molecular species which are routinely considered hydrophobic. The outcome, e.g. in the form of stable compounds of water and hydrocarbons, is impressive. This binding is based upon structure effects, namely on aggregation of water molecules around a 'structure-directing agent' (as named by silica chemists) or 'template' (commonly used by organic synthetic chemists) or, simply, guest component. This is a typical collective phenomenon, not easily corresponding to the classical tools of chemists who prefer to rationalize interactions in terms of bonds between specified atoms. Hydrate solids are often non-stoichiometric, phenomenon which is certainly unknown in molecular chemistry while rather common in solid state sciences.

Hydrophobic hydration is a common phenomenon and is important from different viewpoints. Directly as a source of special type of material, which may be of practical importance. Also, indirectly, as a means of facilitating synthesis of organic and/or inorganic substances either in nature (as illustrated above on the example of porous silica materials) or in the laboratory.

Ecological aspects of hydrophobic hydration are manifold. Climate change due to stabilization /destabilization of huge deposits of natural gas in the form of 'soft' hydrate material seem the most important on large time scale whilst geohazards and resource of energy are important also on the short time scale. Common occurrence of hydrophobic hydrates on earth is a firm basis for further research of these complex systems, so important for biological life on our planet.

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ADVANCED WASTEWATER TREATMENT TECHNOLOGY FOR NITRITES REMOVAL

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Abstract: The paper discusses an innovative wastewater treatment technology for nitrites removal with hypochlorite produced during electrolysis.

Keywords: advanced technology, wastewater treatment, nitrite ions, the electrochemical cell, the methods of purification, sodium hypochlorite.

1. Introduction

The main reason for high concentration of nitrite ions in water is the existence of sources of industrial and agricultural pollution. Contamination of drinking water, juices, wine and other liquids of nitrite ions as a result of improper use of nitrogen fertilizers has an adverse effect on living organism, because under the influence of enzymes nitrite ions in living organisms form high carcinogenic nitrosamines, and the interaction of nitrite ions from blood hemoglobin causes such toxicity that leads to disease cyanosis [1]. Therefore removal of nitrite ions from water has received increased attention.

One effective way of removing nitrite ions from wastewater is the oxidation of hypochlorites [2], however, this method has a serious drawback in that time working solutions of sodium hypochlorite decompose and lose their activity. These working solutions must be stored protected from light in special containers equipped with air vents for discharge of free oxygen. In addition, sodium hypochlorite can form resistant to biochemical oxidation of toxic chlorine by-products, which is a limitation to its use. An alternative process water treatment with sodium hypochlorite neutralization process of nitrite-containing wastewater is applied to nitrite-containing wastewater by hydrogen peroxide [3]. Lack of water from the purification of nitrite ions by hydrogen peroxide, the same as the method using hypochlorite. Hydrogen peroxide tends to rapid self-destruction.

These drawbacks of methods of water purification from nitrite ions with sodium hypochlorite or hydrogen peroxide are absent in the electrochemical method of water purification of nitrite ions, considered by G. Duca, and co-workers [4].

In this method, water is treated in an electrochemical cell with an insoluble anode for 3 - 10 minutes to restore the nitrite ions. After this treatment the water does not contain the reduction products of nitrite ions and the concentration of nitrite ions themselves reduced to the requirements of the MPC.

Table 1

		03-) (NO)	2-) ·····8/··/ P···	.,.,	
N⁰	t, min.	C _(NO3-) , mg/l	C _(NO2-) , mg/l	Δ C _(NO2-) , mg/l	Reduction C _{(NO2-} , %
Initial	0	45,0	41,0		
1	3	45,0	13,2	27,8	67,8
2	5	45,8	2,4	38,6	94,1
3	7	46,8	0,5	40,5	98,8
4	8	47,0	0,2	40,8	99,5
5	9	47,2	0,1	40,9	99,8
6	10	47,7	0	41,0	100

Concentration of nitrite ions in the processing of natural water in an electrochemical cell with a	n insoluble
anode. $C_{max} = 45 \text{ mg/l}; C_{max} = 41 \text{ mg/l}; \text{pH} = 7.8; \text{I} = 1.2\text{A}.$	

The disadvantage of this method is the fact that, along with an insoluble anode (carbon electrode) used soluble cathode metal (Fe, Al or Mg), which in this case is a consumable item.

2. Experimental

We offer the technology of water purification of nitrite ions consisting in the oxidation of nitrite ions with sodium hypochlorite to give him direct in containers with purified water by electrolysis of sodium chloride solution with the formation of active sodium hypochlorite

 $NaCl + H_2O = NaOCl + H_2$.

Freshly NaOCl oxidizes immediately nitrite ions with the regeneration of sodium chloride

 $NaOCl + NaNO_2 = NaNO_3 + NaCl.$

Thus, it is possible to remove from the wastewater nitrite ions with little or no cost of consumables (NaCl).

3. Results and discussion

Waste water from chemical plants vary considerably in contamination of nitrite ions. For example, in electroplating effluent content of nitrite ions is about 180 mg /l. In some industries acid content of nitrite ions in the effluent up to 1000 mg/l. Therefore, we prepared a wide range of model solutions of sodium nitrite containing 300 mg /l, 500 mg/l and 800 mg /l and 1000 mg/l. In the electrolysis of chloride solutions there is no separation of electrode products. This method allows to obtain sodium hypochlorite of sodium chloride solutions. In a simplified technology and, therefore, less costly equipment operation is the main advantage of the proposed method for producing sodium hypochlorite for the removal of nitrite ions. The effectiveness of the method depends on two factors: the parameters of electrolysis and the electrode material. We used graphite electrodes. Such plants do not have the shortcomings of the electrolysis using diaphragms. In the formation of sodium hypochlorite is no stage of chlorine gas and does not generate by-products (alkali), which require disposal.

Table 2

Parameters of electrolysis model solutions					
Power supply frequency 50 Hz, V	220 + 22				
The power consumed from the network, W	130				
Constant current through the electrolytic cell, A	3+0,15 5+0,25				
The voltage on the electrolytic cell at which rum provided the nominal value DC, V					
mode 5A	до 6,5				
mode 3A	до 7,5				
Time of the solution preparation (session)	6 min + 10 s 20 min +10 s				
The nominal concentration of hypochlorite sodium, obtained in a given mode, mg / l					
3A, 6 min	350				
5A, 6 min	600				
3A, 20 min	870				
5A, 20 min	1200				

The resulting sodium hypochlorite reacts with sodium nitrite according to the equation

$$NaOCl + NaNO_2 = NaNO_3 + NaCl$$

Based on the fact that at 69 mg NaNO₂ consumed 74.5 mg of NaOCl, we can conclude that the former received 350 mg /l NaOCl for 6 minutes at a current of 3A (see table) is sufficient to neutralize the nitrite ions in the first model mixture (300 mg /l). In the second case 600 mg /l NaOCl for 6 minutes at a current of 5A is sufficient to neutralize the nitrite ions of the second model mixture (500 mg /l). In the third case 870 mg /l NaOCl for 20 minutes at a current of 3A is sufficient to neutralize the nitrite ions in the third model mixture (800 mg / l). In the fourth case 1200 mg /l NaOCl for 20 minutes at a current of 5A is sufficient to neutralize the nitrite ions in the third model mixture (800 mg / l). In the fourth case 1200 mg /l NaOCl for 20 minutes at a current of 5A is sufficient to neutralize the nitrite ions in the third model mixture (800 mg / l). In the fourth case 1200 mg /l NaOCl for 20 minutes at a current of 5A is sufficient to neutralize the nitrite ions in the third model mixture (800 mg / l). In the fourth case 1200 mg /l NaOCl for 20 minutes at a current of 5A is sufficient to neutralize the nitrite ions in the third model mixture (800 mg / l). In the fourth case 1200 mg /l NaOCl for 20 minutes at a current of 5A is sufficient to neutralize the nitrite ions in the fourth model mixture (1000 mg /l). As a result, the analysis of solutions after the electrolysis of model mixtures in any of the samples was not observed the presence of nitrite ions.

4. Conclusions

Our proposed method of wastewater treatment of nitrite ions favorably with previously used methods, such as safety and cost of consumables.

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4. References

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SYNTHESIS OF SPHERICAL SILICA PARTICLES WITH 3-MERCAPTOPROPYL GROUPS IN THEIR SURFACE LAYER

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Abstract: Spherical silica particles were synthesized by modified Stöber method using tetraethoxysilane and 3mercaptopropyltrimethoxysilane (MPTMS). It was shown that their size (500-760 nm) depends on the nature of the catalyst used in the prehydrolysis of MPTMS (at constant alkoxysilanes ratio). Elemental analysis, thermogravimetry and IR spectroscopy data indicate the presence of thiol groups (2.8 mmol/g) in the surface layer. It was established that obtained nanospherical silica particles can adsorb Ag(I) ions from their acidified aqueous solutions.

Keywords: silica spheres, modified Stöber method, 3-mercaptopropyl groups.

1. Introduction

Silica has been widely used in industry [1]. However, the increasing attention is recently drawn to micro-and nanoparticles of silicon oxide, primarily of spherical shape, which are used in many areas, such as ceramics production, chromatography, chemical sensory, biomedicine [2,3]. In addition, they increasingly began to be used as stabilizers, emulsifiers, binders, in creating coatings for glass, etc. [4]. Necessity to develop new ways to obtain these materials and more detailed study of their structure and properties has increased with the increasing demand on the silica particles for high-tech industries (such as pharmaceutics and biotech). Nano- and microparticles of silica with functional groups in the surface layer are of special interest. Functional groups endow the particles with new specific properties, which significantly expands the practical application of such materials [5-7].

Monodisperse silica spheres 50-1700 nm in size are usually obtained by Stöber method, i.e. hydrolytic polycondensation of tetraethoxysilane in the environment of low-molecular alcohol (catalyst - ammonium hydroxide) [8]. The same method in one- and two-stage version was used for the synthesis of silica spheres containing functional groups in the surface layer [9-11].

The aim of this work is to develop one-stage method of obtaining monodisperse silica spheres with complexing groups in the surface layer. The synthesis of silica particles with 3-mercaptopropyl groups \equiv Si(CH₂)₃SH is considered as the first example.

2. Experimental

The following reagents were used for the synthesis of spherical silica particles: tetraethoxysilane (TEOS), $Si(OC_2H_5)_4$ (99%, Aldrich); 3-mercaptopropyltrimethoxysilane (TMPTMS), $(CH_3O)_3Si(CH_2)_3SH$ (95%, Aldrich); ammonium hydroxide (25%, Khimlaborreaktyv, Ukraine); 0.1 M hydrochloric acid (Khimlaborreaktyv, Ukraine); NH₄F (98%, Fluka); absolutized ethanol.

Synthesis of silica spheres with 3-mercaptopropyl groups (TEOS/MPTMS = 3:1 (mol.)). Sample 1. 21 cm³ of 25% NH₄OH was dissolved in 40 cm³ of ethanol with stirring, after that 4.5 cm³ (0.3 mol) of TEOS was added. Opalescence appeared in 2 min. Suspension was stirred for 1 hour. Then 1.2 cm³ (0.1 mol) of MPTMS was added and stirring continued for 24 hs. In this case, there was further increase in opalescence. The resulting precipitate was centrifuged at 7000 rpm for 15 min. The filtrate was elutriated and a new portion of ethanol was added (25 cm³), treated with ultrasound for 3 min. and again centrifuged. The procedure was repeated two more times. The obtained white precipitate was dried in vacuum: 1 h – at room temperature, 1 h – at 50°C, 4 hs – at 100°C. The yield was 1.86 g.

Considering sample **2**, first 1.2 cm³ (0.1 mol) of MPTMS was dissolved in 4 cm³ of ethanol and then 2.5 cm³ of 0.1M HCl was added. After stirring for 1 h, the resulting solution was added to another solution – 4.5 cm³ (0.3 mol) of TEOS in 40 cm³ of ethanol and 21 cm³ of 25% NH₄OH, which previously also stirred for 1 h. Suspension was mixed 23 h more and further processed as described for sample **1**. The yield was 2.13 g.

In case of sample **3**, first 1.2 cm³ (0.1 mol) of MPTMS was dissolved in 4 cm³ of ethanol and the solution of NH_4F (5 µg NH_4F in 2.5 cm³ H_2O) was added. The obtained (within 2 min) clear solution was added to TEOS (which was also previously stirred for 1 h in alcohol with ammonia) and suspension was mixed for 23 h more. Further processing was carried as described for sample **1**. In this case, the formed precipitate featured curd-like consistency. The yield was 2.06 g.

Elemental analysis was performed in the certified analytical laboratory of the Institute of Organic Chemistry of NAS of Ukraine. IR spectra were recorded on Thermo Nicolet Nexus Fourier-transform infrared spectrometer in the 400–4000 cm⁻¹ range, working in "Nexus Smart Collector" mode and averaging 50 scans with a resolution of 8 cm⁻¹. The samples were previously ground with solid KBr ("Fluka", for IR spectroscopy) at a ratio of 1:30. The spectra of the initial alkoxysilanes were recorded in liquid form between the KRS plates.

The scanning-electron microphotographs were taken with Scanning Electron Microscope JSM 6060 LA (Jeol, Japan) using secondary electrons at an accelerated voltage of 30 kV. Samples were stuck on the objective table. Their surface was coated in vacuum with thin continuous layer of gold by cathodic sputtering to prevent the accumulation of surface charges and to receive a contrasting picture.

Thermogravimetric (TG) and differential thermal analyses (DTA) were performed on a Paulik-Paulik-Erde (Q-1500D) instrument operating in the 20–1000°C range, with a heating rate of 10°C/min. The sensitivity of thermal scales was ± 0.1 mg.

The measurement of low-temperature (-196°C) nitrogen adsorption and desorption isotherms was carried out on "Kelvin-1042" (Costech Microanalytical) analyzer. Sample outgassing was achieved at 100°C during 2 hs. Data were processed using BET equation [12].

Investigation of Ag⁺ sorption from nitrate solutions was carried out in static mode (m = 0.05 g (± 0.0005), V = 20 cm³, initial solution pH ~ 2, t = 20°C). The concentration of Ag⁺ in initial solution and filtrate were determined by atomic absorption method at the spectrophotometer C-115-M1.

3. Results and discussion

Stöber et al. in [8] showed that 2 hs mixing time is sufficient for obtaining spherical silica particles by hydrolytic polycondensation of TEOS in ammonium alkali medium. Moreover, they found that sometimes particles reach their final size during the first 15 min. In [13] during the synthesis of silica spheres with 3-aminopropyl groups in the surface layer, the suspension was stirred for 1 hour. For this reason, at first, this term was used for suspension stirring after MPTMS addition (synthesis of sample 1). Indeed, in this case the formation of spherical particles is observed (Fig. 1a), but their IR spectrum lacks the absorption band of valence v(SH) vibrations. It means, that thiol groups are practically absent in the surface layer of the particles. That is why suspension stirring time was increased to 24 hs for further syntheses. In addition, we established that using two-component systems (TEOS/MPTMS), the best results are obtained during successive addition of reacting components with prehydrolysis of trifunctional silane.







Figure 1. SEM microphotographs for: a – sample without thiol groups, b – sample 1, c – sample 2, d – sample 3.

Fig. 1 represents SEM microphotographs of synthesized samples. Note that Fig. 1a shows silica spheres without 3-mercaptopropyl groups. Their size is about 410 nm. Silica spheres with thiol groups always have greater diameter. So, according to Fig. 1b, the average diameter of silica spheres in sample 1 is 740 nm. In the case of samples 2 and 3 (Fig. 1c and 1d, respectively), there are some isolated spheres that differ in size significantly. If they are taken out of consideration, the average size of silica spheres is 760 nm for sample 2, and 510 nm for sample 3. Thus, varying the nature of catalyst used in the hydrolysis of MPTMS, one can obtain spherical silica particles with sizes in the range 500-760 nm.



Figure 2. Thermoanalytical curves for sample 3.

Estimation of specific surface area has shown that it is in the range of 7–9 m^2/g for synthesized hybrid materials.

The data of element analysis testified he presence of thiol groups in the obtained powdery materials. Thus, sulfur content is 9.06% for sample **2**. On this basis we can calculate the content of functional groups (2.8 mmol/g). It is close to the content, calculated from the molar ratio of reacting alkoxysilanes (3.6 mmol/g).

Thermogravimetry data indicate the presence of carbofunctional groups in the synthesized samples. Thus, the DTG curve of sample **3** (Fig. 2) has three exoeffects. The first centered around 120°C is associated with removal of adsorbed residual water (weight loss is ~5%). Second exoeffect on the DTG curve is centered around 280°C, which, likely, corresponds to the removing (or oxidation) of thiol-containing fragments $- H_2S$ and CH₃SH [14] (weight loss is 4%). The third exoeffect with a maximum at 350°C corresponds to more significant mass loss (19,1%), indicating the complete burnout of the organic residues. Therefore, endothermic effect is observed in the DTA curve in the range 350-450°C (Fig. 2). The total weight loss during the second and the third stages (3.1 mmol/g) agrees with the content of functional groups, calculated on the basis of elemental analysis (see above). TGA data (Fig. 2) make it easy to conclude that the destruction of the organic layer begins at 230°C. This is also typical for xerogels with thiol groups [15].

The presence of 3-mercaptopropyl groups in the surface layer is also confirmed by IR spectroscopy data. Thus, in the FT-IR spectra of all three samples (Fig. 3) the absorption band of medium intensity at 2555 cm⁻¹, attributable to v(SH), is clearly recorded, and a low-intense absorption band at ~685 cm⁻¹ is associated with valence vibrations of C-SH [16]. There is also a group of absorption bands at 2800-3000 cm⁻¹, which can be attributed to the valence vibration $v_{s,as}$ (CH) of the propyl chain. Its presence is also reflected in the presence of a group of bands of weak intensity in 1300-1490 cm⁻¹ region (Fig. 3). In addition, the IR spectra of obtained materials have the most intense absorption band at 1000-1200 cm⁻¹ with a high-frequency shoulder (Fig. 3). Its occurrence is associated with the existence of three-dimensional network of polysiloxane bonds, \equiv Si-O-Si \equiv [17]. Since all the synthesized samples contain water, there is a characteristic absorption band of deformation vibrations of H₂O molecules at ~1630 cm⁻¹, and a broad intense absorption band groups at ~3625 cm⁻¹. Thus, the obtained spherical particles are composed of polysiloxane network with attached carbofunctional groups. Besides, there also can be an insignificant number of surface alkoxygroups, which is indicated by the presence of additional low-intense absorption bands in the 1300-1490 cm⁻¹ and 2800-3000 cm⁻¹ regions (Fig.3).



Figure 3. IR spectra of initial alkoxysilanes and synthesized spherical materials.

Sorption properties of functionalized spherical silica particles to silver(I) ions were investigated for sample **2**. It was experimentally determined that 1.28 mmol/g of silver (I) ions can be adsorbed for 1 h. It means that the obtained materials could be perspective in the sorption of heavy metal ions from aqueous solutions.

4. Conclusions

The procedure of synthesis of silica spheres with 3-mercaptopropyl groups in their surface layer using twocomponent system (TEOS and MPTMS) was developed. SEM microphotographs showed the formation of spherical particles with sizes in the range 500-760 nm, which is determined by the nature of the catalyst used in the reaction of MPTMS hydrolysis. IR spectra indicate the presence of thiol groups and three-dimensional network of polysiloxane bonds in samples. The obtained silica spheres with 3-mercaptopropyl surface layer can sorb Ag(I) ions from their acidified aqueous solutions.

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INCREASE OF CROP YIELDS ON SEED TREATMENT BY ENVIRONMENTALLY SAFE COMPLEXONES IN SOLUTIONS

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Abstract: In the laboratory and plot experiments the effect of ethylenediaminedisuccinic acid (EDDS) and iminodisuccinic acid (IDS) on chlorophyll content in wheat germ leaves and adult plants was investigated. It was estimated that both complexons increased chlorophyll content in leaves and crop yield.

Keywords: chlorophyll a, complexones, ethylenediaminedisuccinic acid, iminodisuccinic acid.

Intensive chemicalization of agriculture in all developed countries leads (apart from the increase in agricultural production) to deterioration of its quality and slow but sure pollution of the environment [1].

Therefore methods of intensification of crop-producing power which do not induce ecological disturbance of agricultural lands and adjacent regions are of utmost interest.

One of the methods is pretreatment of seeds with substances showing biological activity at minimal application dose. Some of these preparations are biometal complexonates. Their effectiveness and the level of ecological safety depend on the nature of ligands to a large extend.

For this reason preparation and investigation of new complexonates of microelements as well as investigation of biological activity of ligands in their composition are of current interest. Such widely-applied complexonates as ethylenediaminoacid (EDTA) and nitrilotriacetic acid (NTA) and their effect on the metabolism in plants are relatively well-studied [2, 3] whereas complexonates – succinic acid derivatives have been investigated to a considerably lesser degree [4].

As the whole complex of vital manifestations of a green plant is closely connected with the process of photosynthesis and photosynthesis in its turn– with the level of photosynthetic pigment content, it is possible to estimate the nature of the effect of external and chemical agents on the basis of chlorophyll *a* content alteration.

In the context of investigation of complexonates succinic acid derivatives biological activity, laboratory and plot experiments were made to find out the capacity of these compounds to be absorbed and ingested by a plant body with further determination of chlorophyll content in plants and their yield level.

Methods

Seeds of winter wheat "Inna" were used for the experiment. Weighed portions of the seeds $(10,00 \pm 0,01 \text{ gr})$ were soaked in solutions of EDDS and IDS in concentration $1,5 \cdot 10^{-3}$ mole/l, volume – 100 ml.

Complexon solutions synthesized by the authors of the article were alkalinized with strengthened solution of NaOH to pH = 5,6 (pH-meter "pH-56M"). The control group included the seeds soaked in the same volume of distilled water.

In 24 hours the seeds were washed out with small portions of distilled water. The washings were added to the corresponding solutions left after soaking and the remaining content of complexons was investigated with the help of titrimetric analysis using solution of $Cu(NO_3)_2$ as titrant 0,002 M and murexide indicator at pH = 9,2 (ammonium buffer).

The absorbing capacity of both complexons was determined with the help of conventional method [5].

Imbibed washed seeds -50 pieces in each experimental sample were spread on wetted absorbent paper on Petri dishes and sprouted at daylight at t^o = 22 ± 1 C^o for 10 days. In 10 days leaf samples were taken to determine chlorophyll content. Pigments were estimated in acetone with the help of spectrophotometric method (photometer KFK- $3-01 \ll 30$ M3»). Statistical analysis of the results was made with standard methods [6].

In the second part of the experiment complexons succinic acid derivatives sorption by sprout root system was investigated.

For this purpose the root system of 5 three days' germs was dipped into solutions of EDDS and IDS in concentration 1,5·10⁻³mole/l, volume - 100 ml and distilled water, volume -100 ml (control group).

In 24 hours the germs were taken out of the solutions and the remaining content of the complexons in the sprouting solutions was estimated similar to the first part of the experiment.

At the same time the root system mass of the germs was determined and complexon sorption by the germ root

system estimated. To determine the effect of complexons succinic acid derivatives (absorbed by the root system) on photosynthetic pigments' content level three days' wheat germs were left to grow in solutions of EDDS and IDS. Later using the same methods [6] photosynthetic pigments' content in the germ leaves was determined.

Both parts of the experiment were reproduced 5 times.

For making plot experiment the seeds of winter wheat were soaked in EDDS and IDS in concentration $1,5 \cdot 10^{-3}$ mole/l and in distilled water (control group). In 24 hours the wheat germs were sown in the plot – 1 m² 4 times for each part of the experiment. At the stage of forming a stem the leaves were examined for chlorophyll content.

Results and discussions

Complexon succinic acid derivatives capacity to be absorbed by seeds and root system of wheat germs determined experimentally is shown in Table 1.

Table 1

Complexon sorption from water solutions in concentration $1,5 \cdot 10^{-3}$ mole/l at pH = 5,6, V = 100 ml for 24 hours (t° = $22 \pm 1^{\circ}$ C)

N₂	Sorbent	Sorbate	Sorption micromole/gr
1	Seeds	IDS	$6,8 \pm 0,6$
2	Seeds	EDDS	7,1 ± 0,5
3	Root system	IDS	$9,8 \pm 0,4$
4	Root system	EDDS	8,8 ± 0,4

As it is shown in the table both ligands are absorbed by the seeds almost equally.

Microquantities of complexons absorbed by the wheat germs cause a small increase in the total content of green pigments mainly due to chlorophyll *a*.

The suggestion that complexon sorption is brought into the chain of germ metabolic reactions is confirmed by the data of the second part of the experiment.

As IDS sorption by germ roots is better in comparison to EDDS sorption chlorophyll content in leaves increases to a greater degree. (Table 2).

Table 2

Chlorophyll content in the leaves of wheat germs (per 100 gr of green weight) using different methods of treatment with complexon solutions in concentration 1,5·10⁻³ mole/l and pH = 5,6

			Chlorophyll									
Nº	The object of	Option experience	a		b)	a + b					
	influence		m	% of control	m	% of control	m	% of control				
1	Seeds	H ₂ O dest (control)	69,4 ± 0,3	_	25,1 ± ±0,3	_	$94,4 \pm \pm 0,3$	_				
2	Seeds	IDS	$72,4 \pm 0,4$	104	$24,6 \pm \pm 0,3$	98	$96,9 \pm \pm 0,4$	103				
3	Seeds	EDDS	$76,6 \pm 1,0$	110	$24,0 \pm \pm 0,1$	96	$100,6 \pm \pm 1,0$	107				
4	Root system	H ₂ O dest (control)	93,4 ± 0,9	_	$20,3 \pm \pm 0,7$	_	$113,7 \pm \pm 0,9$	_				
5	Root system	IDS	$126,8 \pm \pm 0,4$	136	$18,9 \pm \pm 0,5$	93	$145,7 \pm \pm 0,5$	128				
6	Root system	EDDS	$116,5 \pm \pm 0,3$	125	$22,8 \pm \pm 0,5$	112	$139,3 \pm \pm 0,5$	123				

Judging by the results of quantitative analysis of pigments, complexons absorbed by the seeds or root system at the first stages of wheat plant development slightly effect on the level of chlorophyll b content but considerably increase chlorophyll a content – the main photosynthetic pigment.

Apparently in germ organisms both complexons (being aminoacids) undergo biochemical destruction forming carboxylic acids – common aminoacids and other products non-toxic for plants but capable of interacting in anabolic processes of plant development which is confirmed by an increase in the total number of green pigments in experimental plants in comparison to control group.

The results of the plot experiment are given in Table 3.

№	Option pretreatment of	Chloroph	yll content o raw leaves	n 100 gr the	Crop yield,	Increase in relation to the control, c/ha	
	seeus	a	b	a + b	c/na		
1	H ₂ O dest (control)	60 ± 2	139 ± 5	199 ± 5	11,78	-	
2	EDDS	76 ± 2	164 ± 6	240 ± 6	13,17	1,39	
3	IDS	66 ± 3	152 ± 2	218 ± 3	12,42	0,64	

Chlorophyll content in leaves and crop yield of winter wheat using different methods of seed pretreatment

Table 3

Seed pretreatment produced greater effect on adult plants than germs.

The investigation proved EDDS and IDS sorption capacity from the water environment in the form of atactic anions by plumping seeds and wheat germ root system as well as their action stimulating the formation of photosynthetic pigments in the leaves resulting in the increase of crop yield.

Therefore we came to the conclusion that EDDS and IDS show biological activity not only as parts of metal complexes [8, 9] but in their free state as well.

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SOIL DEGRADATION PROCESSES FROM POLLUTION

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Abstract: The Republic of Moldova is endowed with fertile soils. Soil pollution is a major concern since no effective treatment method exists to restore the natural qualities of the soils. Polluted soil can only be rehabilitated under the long-lasting influence of natural factors. A major source of anthropic impact on soils is the application of fertilizers and pesticides in agriculture. At the same time, manure and livestock excreta often are transported to communal dump sites, where they are mixed with other wastes, or are left in ravines, along the local roads and in other unauthorized places. Instead of improving the soil productivity the nutrients find their way to and pollute the surface and ground waters. At the same time, pollution of the soils in the vicinity of former pesticide storehouses remains a problem. More than 200 storehouses have been dismantled during the last years and some of the remaining are in a poor condition, unfenced, unguarded, without doors and windows, thus being sources of chemical pollution. Moldovan soils are prone to erosion and this is how agrichemicals can travel to the water environment thus endangering water life and limiting water uses. Investigations found HCH and DDT residuals in bottom sediments from several reservoirs and lakes as well as the main rivers, Nistru and Prut (concentrations ranged between 0.2 and 15.8 ppb). The concentration of PCBs in the topsoil collected beneath the capacitors battery at the Vulcănesti substation reached a level of 7100 ppm which is exceeding the MAC by five orders of magnitude (!). With no exception, allowable concentrations of PCBs in soil were exceeded also on the territory of other investigated substations, with peaks registered at the Briceni substation (2545 ppm) and the Orhei substation (1959 ppm). The degradation of soils implies the reduction or total loss of their biologic or economic productivity caused by anthropic or natural processes.

Keywords: soil degradation, erosion, contamination

Moldova is the world country that prossesses the most fertile soils – chernozems that occupy 75% of its area. But extensive soils' use led to intensive degradation of the ecological functions: energetic, biogheochemical, hidrological, gas-atmospheric, geological etc..

First of all, ecological territorial balance was disturbed. In the whole of the country, natural ecosystems (wood plantations, including forest shelter belts meadow, grassland, swamps and water systems) quota makes just 19 % and at some village it falls until 7 % of total territory's woodiness makes only 9,6 % and at some regions falls to 1,0 %. At the same time, agricultural lands' cultivation level has increased and rose up to 70 % in average, while in some villages exceeding 90 %.

The actual state of cultivated soil is no less alarming. Negative processes, which lead to soils' degradation and destruction, increase together with exploitation intensification. Soils' structure's damage, increasing compactable degree, dehumification, erosion, hydromorphism, lend slide processes, pollution are developing more intensely. Intense cultivation led to ploughing layer's natural structure's destruction, which worsened water and air penetrability along with other physical characteristics of soil. In consequences of applied tractors and other agrotechnique increasing weight a systematic increase of soil's density is observed. Moldova's chernozems and wood soils are shrinking and litterarum, which is also a negative influence on their characteristics.

Energetic function, reviewed as soil humus potential, plays an important role in soils' ecological function system, which is important not only for Moldova, but also for the other countries, where other energy source are limited. In early 60s Moldova's soils contained nearly 1 milliard ton of humus on the whole area in 1 meter layer, now it has 0,8-0,9 milliard ton. Two thirds of this huge loss in ecologically useless, because of erosion.

Water erosion in Molodva's condition is the most than wide-spread soil-destructing process. Averagely and strongly washed-off soils make more than 275 thousands of hectares. It should be noted that the more eroded soils is, the easier it is exposed to further washing off, because lower horizons have less anti-erosion resistance. Erosion processes intensity has particularly increased in last years as a consequence of wood protection belts' destruction, implementation of so-called intra-farm crops rotation, row crops' quota increasing in cultivated areas structure and their cultivation by industrial technologies. Applying herbicides to fight weeds excludes cultivation and also excludes possibility to realize appropriate agrotechnical anti-erosion measures: slitting, discrete furrowing, chiseling, and hollowing.

Erosion processes intensity on deep-ploughed soils has increased sharply because transitional chernozems horizons appear on the surface - less humified, with worse structure, water penetrability, and low anti-erosion resistance. Erosion processes are the reason of republic's annual losses of 50 thousands tones of winter grain crops, 100 thousands tones of maize, 25 thousands tones of sunflower seeds, 150 thousands tones of sugar beet. It was calculated that if we

take a full-profiled chernozems potential as 100, than weak wash- off one will have 75, averagely wash-off one - 60-65, strongly wash-off one - 34. Erosion completely disturbs soils" potential energy's accumulation process.

Thanks to their biogeochemical function, chernozems have accumulated in their profile carbon in organic form, nitrogen, phosphorus, some microelements. Every 100 ton erosion caused humus losses mean that also losing 5-6 tons of nitrogen, irretrievably almost half of phosphorus (which is the most scarce biofile in the world) quota, the low Clarke elements (manganese, boron, cuprum, zinc, molybdenum etc). In eroded chernozems these substances' losses make 25-75% from their initial proportion.

Moldova's soils fertility has decreased in half during the century; consequently cultures yield is decreasing accordingly.

In present for soils fertility restoration agrochemists recommend introducing 10-12 tons of manure in soil per hectare or 18-20 million of tons yearly.

Chernozems hydrological function operates with liquid effluent and solid elution. Liquid effluents may be powerful enough to cause erosion drought, and solid elution sharply disperses soils status as bio-inert carcass body. Soil's biota suffers severely too- the quantity of invertebrate, bacteria, fungi, their activity and specific diversity is reduced in 2-4 times, so on every part of washed-off chernozems the quantity of living beings is reduced, like a fragment of biosphere is dying.

The prognosis is quite imposing - all eroded agricultural lands will increase in area, and their growth in last decade makes more than 30% (tables 1-2).

Table 1

		0				1								
Zones	T. 4.1		Eroded soils:											
	area, hectares	total		wea	weakly-		averagely-		strongly-		averagely-+ strongly -			
		ha	%	ha	%	ha	%	ha	%	ha	%			
Northern	875176	296685	34,0	194340	22,2	73515	8,4	28881	3,4	102396	11,8			
Central	696345	314748	45,2	165730	23,8	102363	14,7	46655	6,7	149018	21,4			
Southern	732411	285640	39,0	157468	21,5	91551	12,5	36621	5,0	128172	17,5			
South-	234753	51880	22,1	36152	15,4	12442	5,3	3287	1,4	15729	6,7			
Eastern														
Total	2538685	949468	37,4	553433	21,8	279255	11,0	116780	4,6	396035	15,6			
Referring to														
Right bank	2274453	889481	39,1	511238	22,5	265486	11,7	112756	5,0	378242	16,7			
Left bank	264232	59987	22,7	42195	16,0	13769	5.2	4024	1,5	17793	6,7			

Eroded agricultural lands by zones in Repulic of Moldova'on 1.01.2002

Table 2

Plough soils' wash-off level on the slope with different steepness in 2001

	Total	Washed-off soils									
Zones	Iotai	Total:		weakly-		averagely-		strongly			
	Thousands hectares	Thousands hectares	%	Thousands hectares	%	Thousands hectares	%	Thousands hectares	%		
Total on slope with steepness > 1°											
Northern	596,5	226,7	38.0	148,4	24,9	56,2	9,4	22,1	3,7		
Central	374,7	208,8	55,7	109,9	29,3	67,9	18,1	31,0	8,3		
Southern	397,0	201,7	50,8	111,2	28,0	64,7	16,3	25,8	6,5		
South-Eastern	98,2	42,5	43,3	29,6	30,1	10,2	10,4	2,7	2.8		
Total	1466,5	679,7	46,3	399,1	27,2	199,0	13,6	81,6	5,6		
		Total on	slope v	vith steepnes	s 1 - 5°						
Northern	482,1	112,4	23,3	112,4	23,3	-	-	-			
Central	232.8	67,0	28,8	67,0	28,8	-	-	-			
Southern	293,1	97,8	33,4	97,8	33.4	-	-	-			
South-Eastem	85,3	29,6	34,7	29,6	34,7	-	-	-			
Total	1093,3	306.8	28.1	306.8	28.1	-	-	-	-		

Total on slope with steepness 5-8°										
Northern	89,6	89,6	100	36,0	40,2	53,6	59,8	-	-	
Central	104,4	104,4	100	42,9	42,1	61,5	58,9	-	-	
Southern	80,6	80,6	100	13,4	16,6	64,7	80,3	2,5	3,1	
South-Eastern	11,0	11,0	100	-	-	10,2	92,7	0,8	7,3	
Total	285,6	285,6	100	92,3	32,3	190,0	66,5	3,3	1,2	
		Tota	l on slope	e with steep	oness > 8°					
Northern	24,7	24,7	100	-	-	2,6.	10,5	22,1	89,5	
Central	37,4	37,4	100	-	-	6,4	17,1	31,0	82,9	
Southern	23,3	23,3	100	-	-	-	-	23,3	100,0	
South-Eastern	1,9	1,9	100	-	-	-	-	1,9	100,0	
Total	87,3	87,3	100	-	-	9,0	10,3	78,3	89,7	

Downhill and strongly wash-off lands ploughing is observed in 2001 in Stefan-voda, Anenii Noi and other regions. Chernozems' erosion should be minimized. Agronomic views on soils' erosion must give up to a more biosphereoriented and ecological view, as 1251.8 thousands of people became proprietors of more than 2 millions of hidelands at the end of 2000. While parting up land into hidelands, anti-erosion requirements of farm's territory organizing were not met, hidelands are often allotted along the hills, which significantly impedes implementation of anti-erosion measures to maintain soil cover.

In sum, erosion not only damages soil, but biosphere in whole, brings on negative social and demographic consequences.

Several measures - organization, agrotechnical, hydrotechnical, phytomeliorative and others should be taken in order to prevent Moldova's soils' ecological functions degradation. Their implementation should be complete, and not fragmented.

One of the first conditions is to change correlation between natural and anthropogenic, transformed landscapes in different regions. Ecological balance is off the question in conditions of complete lack of forests and natural gramineous vegetation in Belti Steppe and Southern Region. Consequently, there's a strong need in wood planting, hills tufting, flood plains, hollows, runnels, swamps and others natural destination's restoration. It is necessary to examine Moldova's flood plains' soils and to evaluate them.

The other condition is to create natural carcass, a green skeleton - protection belts situated on watersheds. They would serve to smooth climatic conditions; lessen wind's force and storms' destructive power. Anti-erosion, flow-regulating belts are strictly necessary in order to regulate surface flow on hills. These belts should be connected with areas of natural forests and wood plantings, created on ravine's and landslide's hills, with areas where natural gramineous vegetation is still preserved, and with protection zones along rivers and lakes. Natural carcass will provide a possibility to cultivate soil and plant cultures across the hills and by horizontals, to implement agrotechnical and anti-erosion measures. It would benefit to preserve local flora and fauna genetic fund.

The areas with mainly distribution of averagely and strongly washed-off soils should be taken out of ploughing fund and intense use; because they practically cannot be protected from erosion while being a part of crops rotation. These soils should be tufted and planted with multiyear herbs. Natural vegetation restoration in rivers' flood plains, cavins and hollows would regulate flow and clean surface waters. Thus, a considerable balance between natural and anthropogenic components of ecosystems will be created and chernozems' ecological functions will be preserved.

The problem of soils radioactive pollution becomes more and more actual and serious.

The application of radio nuclides, particularly Cs^{137} , for water erosion and sedimentation studies in Republic of Moldova has not been attempted so far. Consequently the investigations were made on reservoir sedimentation rates in an area subjected to wide range of land degradation by sheet-rill erosion.

The profile characteristics support the assumption that in most undisturbed sites there is a sharp decline in Cs^{137} activity along with increasing depth. Such an asymmetrical distribution of the Cs^{137} would suggest a standard pattern in the form of a cantilever. If the validity of this assumption is accepted it is possible to define two major types of Cs^{137} cantilever distribution: shallow and deep buried cantilever.

The main criterion in classifying these patterns lies generally in the shape of Cs^{137} depth profile and particularly in burial magnitude of Cs^{137} peak derived from Chernobyl.

Hills area has been impacted by greatest sedimentation. This regional differentiation is consistent with the decrease in clay and increase in sand content of substratum layers as moving from the North to the South.

Accumulation of Cs137 in reservoirs in the Republic of Moldova is mainly associated with the Chernobyl imputes and subsequently with Cs¹³⁷ derived from testing of nuclear weapons.

The pattern of shallow buried cantilever typifies those areas where Cs^{137} peak concentration exhibits in the upper part of the profile commonly at a depth 15 - 50 cm (a plant's main roots inhabited layer), especially in the northern and

southern part of Moldova, where rill - inter-rill erosion is the main sediment source.

Along with radioactive pollution, there's equally actual problem of waste pollution of soil. Around the localities by different ways, soil is polluted with solid waste. Even more dangerous consequences may have chemical pollution. Sulphur and nitrogen oxides diffuse through atmosphere, deposit on soil and in due course change its initial physical composition. Heavy metals get into soil together with wastewaters and their sediments (mercury, zinc, cuprum etc), from automobiles' exhaust gases (lead). Soils' chemical pollution takes larger scale together with agriculture's chemicalization increase. Ballast elements (fluorine, chlorine) are applied together with fertilizers, different elements which are non-characteristic for soil are introduced while spray treatment for diseases and pest control and particularly while introducing herbicides. As the areas of irrigated lands are widening, we may observe the increase in threat and processes' manifestation scales of gleyification, secondary water clogging, irrigational erosion, sodisation and salinization, particularly when alkaline and mineralized waters are used for irrigation.

That's why the state of land resources is stressed, and the state of soil cover is alarming. Systematic control over its usage is needed and in some special cases there's a need to take immediate action.

Apart from using soil, humankind has no other possibility to obtain food products and numerous materials necessary for existence. Soil as a source of material values, as a priceless natural wealth and the main environmental component must function perfectly and serve forever to us and our future generations.

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THE POWER OF KNOWLEDGE AND THE VALUE OF WISDOM

When you set out on your journey to Ithaca, pray that the road is long, full of adventure, full of knowledge...

Constantine P. Cavafy

Dear reader,

it is our favor to preface the current issue of "Chemistry Journal of Moldova" with a special dedication to the 60-th anniversary of Academician Gheorghe Duca, Editor-in-chief and our colleague who stands at the roots of the journal.

It was not easy to compile the thoughts connected to this prominent event. The role of Academician Duca to the appearance and development of Ecological Chemistry, as well as his contribution to the whole Moldovan scientific community is enormous. And a couple of journal pages can obviously not include even a brief narration. On the contrary, we decided to follow the journal tradition and to give the reader a chronological description of his carrier evolution as a chemist, manager and Man of Knowledge.

Gheorghe Duca is descendent of a intellectual family from the Northern Moldova. His parents Grigore and Nina Duca have been school teachers for their entire life. Therefore the young boy has acquired the thirst for knowledge directly from his beloved parents. In particular the passion to chemistry was due to his father, who taught him chemistry and encouraged his participation to different chemistry contests and Olympiads. And that was the beginning of his bold carrier in chemistry, when in the 8-th grade he becomes winner of the National Chemistry Olympiad. This event has deeply marked his following achievements: graduation of the secondary school with golden medal award (1969) and successful enrolment as a student of the Chemistry Department of Moldovan State University.

During his early activity in the University everybody remembers him as a open-minded colleague, able to combine social activity with deep and consciousness study. In the same time he has been always a good mixer and the hart of many "informal events" which make the student life so unique and unrepeatable. In 1974 he graduates with honors and is offered a position at the department of Physical Chemistry as a PhD student. At this point we shall mention his fortune to have as University mentors a myriad of distinguished scholars who shaped the modern Moldovan chemical community: professors Alexei Sytchiov, Boris Melnic, Isaak Bersuker, and many others who led him by their own example and provided high moral and scientific standards.

Duca's research during PhD studies was mainly concentrated on kinetics of redox processes of industrially relevant substrates. The doctoral thesis entitled "The catalysis of tartaric and dihydroxyfumaric acid oxidation" was finished in 1979 and defended successfully at the University Scientific Council. Following period witnessed a fruitful activity in the department of Physical Chemistry of the same Chisinau State University, dominated by successful implementation of theoretical findings in different industrial processes, relevant to Moldovan economical infrastructure. It is our assumption that during this closer contact with real chemical production resulted into the sprout of Duca's interest towards environmental chemistry and protection. Winged by awarding him the prestigious Youth State Award in Science and Technology (1983) he decides to embark on postdoctoral studies, which in fact resulted in his coining the field of Ecological Chemistry. That period was a tumultuous one, marked by Gorbachiov's Perestroika and dramatic changes in our mentality and life. These circumstances allowed the young and very active doctor in chemistry to say the things that previously was only allowed to think. Consequently, he joins the Moscow Institute of Chemical Physics of the Academy of Sciences of the Russian Federation (1985) as a postdoctoral fellow and successfully completes the doctor habilitate thesis "The mechanisms of the eco-chemical processes in the aquatic environment". It was defended in 1989 at the Scientific Council of Odessa University (specialty: 02.00.15, chemical kinetics and catalysis and 11.00.11, environmental protection).

Contact with the broader chemical community and his profound knowledge of the subject, provided him strong confidence to announce with the loud voice the paramount importance of Ecological chemistry for the wellbeing of the society, as a tool for both understanding environmental chemical processes as well as planning of corrective and preventing actions in order to minimize anthropogenic factor on the environment degradation. Due to his permanent strive and by his direct participation several Moldovan and foreign Universities have founded Departments of Ecological Chemistry, the closest to us being the Department of Industrial and Ecological Chemistry (Chisinau State University), to which Gheorghe Duca has served as a head at foundation and permanent and faithful collaborator allover its activity.

But it was not easy at all. The time after Soviet Union collapse was the most difficult included for Professor

Gheorghe Duca too. It was not easy because he had to swim a lot against the flow. We all know what the fate of the prophets is: to endure but to strive and say the truth. And he continued to actively play the game: either in research, political or social activities, either at home or abroad. His achievements are numerous and valuable, and we will try to mention some of them.

First of all, Academician Gheorghe Duca is the founder of the Scientific School of environmental chemistry. His disciples include 16 PhD and 2 habilitat doctors. His developed theory of redox phenomena in the environment helped elucidation of the redox mechanisms during the evolution of the quality of aquatic systems, including treatment of residual waters and waste recovery. He introduced new procedures and methods for chemical risk assessment, elaborated environmental audit methodology, formulated and proposed principles of environmental education. The research results were reflected in 1565 scientific works, of which 624 scientific papers, 139 patented inventions, 42 monographs, 25 textbooks and methodological works, including the first textbook of "Ecological Chemistry", published in three editions and translated into three languages.

He conducts also a prodigious teaching activity, delivering courses on Physical Chemistry, Chemical Technology, Ecological Chemistry, Processes and Appliances, Physical-chemical Methods of Research, Chemical Risk Assessment, Kinetics and Thermodynamics of Ecological Systems.

He founded the Industrial and Ecological Chemistry Department, MSU (1991), Centre for Research in Applied and Ecological Chemistry, MSU (1992), the Faculty of Ecology at the Free International University of Moldova (FIUM) (1992), the High School for gifted children of ASM (2007), the University of Academy of Sciences (2007), the non-governmental organization for environmental protection "Terra Nostra" (1992), the Association of Research and Development in Moldova – MRDA (2000), the magazines "Mediul Ambiant" ("The Environment") (2002), "Chemistry Journal of Moldova" (2005), Journal of Science, Innovation, Culture and Art "Akademos" (2005).

Besides, academician Gheorghe Duca's public activities are relevant, including positions of the Chairman of the Committee on Culture, Science, Education and Mass-media of Moldovan Parliament (1998-2001) and Minister of Environment and Territorial Development (2001-2004).

And finally there is an achievement we can not bypass: Academician Gheorghe Duca represents a personality to whom the whole scientific community of Moldova is greatly indebted. It is due to his permanent efforts to strengthen the image of the science in our society, to maintain the combative spirit of Moldovan researchers and to actively promote the knowledge based society principles. These are the precious results we greatly appreciate in his carrier of a diligent chemist and skilful manager of Moldovan research and innovation activities.

On the occasion of academician Gheorghe Duca's 60-th birthday, Moldovan Academy of Sciences will host the International Symposium on Ecological Chemistry – the field to which he devoted most of his brilliant scientific carrier. We have included in the current issue of the journal some of the representative Symposium presentations in the form of extended abstracts. It is our hope to provide a following impetus to the development of collaboration between Moldovan researchers and foreign colleagues in all fields of Ecological Chemistry and adjacent areas.

Dear academician Gheorghe Duca, distinguished scholar and precious colleague! On the occasion of Your 60-th anniversary, on behalf of the Editorial Board of the journal and of all Moldovan chemical community, we address You our best wishes of prosperity, health, welfare, new advances in your research and social activities.

Happy Anniversary!

Editorial Board Chemistry Journal of Moldova



The 5th International Conference ECOLOGICAL CHEMISTRY 2012 will serve as the main arena for discussion, experience and ideas exchange of recent achievements in the field related to investigation of mechanisms and chemical processes taking place in natural waters, atmosphere and soils under the influence of anthropogenic pollutants, the pollutants impact on the human health and habitat, as well as methods of environment pollution prevention and mitigation. The 5th International Conference ECOLOGICAL CHEMISTRY 2012 will contribute to:

- Short- and long-term planning of international cooperation in the field of ecological chemistry and related disciplines
- Involvement of young scientists in international research area of Ecological Chemistry
- Promotion of healthy life style
- Formulation of recommendations to reduce the impact of pollution on human health and environment and methods of their implementation
- Publication and wide dissemination of conference digest papers and young scientists' papers in the form of proceedings and book of abstracts

Topics:

- A. Ecological chemistry of water
- **B.** Ecological chemistry of atmosphere
- C. Ecological chemistry of soil
- D. Ecological chemistry and healthy style of life
- E. Ecological chemistry and sustainable development

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THE STUDY OF CADMIUM UPTAKE BY WATER HYACINTH (EICHHORNIA CRASSIPES) USING A NATURAL MODELLING APPROACH

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Abstract: The results of the investigation on the accumulation of cadmium by water hyacinth, depending on the conditions of pollutant exposure and the presence of various additives are discussed. The main specialty of this study is that all the experiments were carried out in natural conditions using the approach based on the application of the capacities called minicosms. It allowed estimating hit consequences of pollutant on ecosystem most really having made experiment in the conditions as much as possible close to the natural. In this article a very important problem of an accuracy and reliability of the results of trace elements determination in plants is also debated. As a result of carried investigations it was shown that the degree of cadmium extraction by hyacinth from contaminated natural water while maintaining the viability of the plants depends on the way of pollutant introducing into the reservoir and the maximum (about 79%) value is observed in the case of it's gradual entry.

Keywords: contamination, water purification, environmental analysis, bioaccumulation.

1. Introduction

It is known that the consequences of natural waters pollution for the person are diverse: the number of fresh water sources is reduced; danger of a poisoning increases at the use in food of hydrocoles from the polluted reservoir; the risk of various diseases acquisition raises at water use in the economic purposes, etc. In this regard phytoremediation became an effective and economic methods of environments clearing due to the plant's ability to accumulate the metals at concentrations thousands times higher than background level have been found out. Biological essence of this phenomenon up to the end is not clear and actively studied now. The floating macrophyte water hyacinth, *Eichhornia crassipes*, (EC) is applied most often to sewage treatment of the industrial enterprises. The plant is capable to clean the reservoirs from many toxic substances, acquiring the contaminants from waters as food elements, basically through root system using the products of decomposition in the course of the ability to live [1]. EC actively accumulates Pb, Cd, Cu, Fe, Mn, Zn, Cr and other elements and substances. The distinction reasons in sink ability of plants in relation to different metals by the present moment precisely are not known. Among the inorganic pollutants cadmium is one of the most toxic heavy metals for living organisms which negatively effects on the environment and human health being accumulated throughout the food chain. It is supposed that Cd is actively taken as on chemical behavior in plants it is close to Ca and Zn – to the important components of many biologically active substances, as, for example, arsenic is capable to replace phosphorus in live systems.

There are a lot of publications which concerns of an assessing and application of hyacinth ability to heavy metals accumulation [2-10]. Unfortunately, more often this data is received as a result of the experiments spent in essentially different from the real environmental conditions. At the same time the behavior of chemical substances in natural ecosystems is described by a set of very complicated chemical, physical and biological processes, which can't be taken into account in laboratory conditions where it is possible to study the influence of separate factors on these processes. However the full picture of the transformations of the substances in natural system may strongly differ from the model. Most really to estimate hit consequences of pollutant on ecosystem having made experiment in the conditions as much as possible close to the natural. It allows the method of natural modeling offered by Odum, 1984 [11] consisting in a statement of natural experiments with the use of mesocosms, established directly in a reservoir into which one enters the set portion of investigated substance and then supervises the dynamics of its concentration. This approach in minicosms version has been realized in present work.

Another deserves attention problem is concerned a reliability of the data on the assessment of the element's accumulation by plants. Most often trace elements determination in EC and other macrophytes carried out using modern instrumental techniques in line with guidelines on the analysis of plant samples without regard to their matrix composition. Such an attitude may lead to a systematic error of analysis, and, as a consequence, incorrect evaluation of the actual coefficient of the element's accumulation by the plant.
Thus, the goals of the present investigation were:

- to assess the correctness of the results of cadmium determination in samples of water hyacinth using independent analytical methods;

- to study the phytoremediation process in the presence of EC for the removal of cadmium from contaminated reservoir using minicosm approach;

- examine the dependence of cadmium accumulation by plants on the scheme of pollutant and additives introduction.

2. Experimental

2.1. Field experiments description

All experiments were carried out in summer 2009 in the field conditions on the river Yeltsovka (Novosibirsk region, Russia) served as a natural range. Young plants were grown in greenhouse (Institute of Cytology and Genetics of Siberian Branch of Russian Academy of Sciences) and in mid-June placed at disposal of field laboratory where were grown directly in the river close to the inflow to the reservoir of the Novosibirsk "artificial sea". The water reservoir where modeling experiments were realized refers to calcium-carbonate type with pH of 7.4-9.1 and the temperature range of 16-24° C in summer months [12].

Minicosm (M) represented a polyethylene capacity limited the allocated volume of 150-230 L of natural water from sides, whereas the top layers were in the same conditions as all reservoir, being treated thus to sunlight and atmosphere action, while the low layers had a contact with a sandy bottom included a little amount of silt. Six minicosms were arranged nearby the floating laboratory, in each of which three preliminary weighed water hyacinths were placed. Only mature plants with initial mass of 174-404 g were used in the experiments. One of these capacities (M6) with plants that were not exposed to metal served as a control, into other ones (M1-M5) the additives of cadmium and various fertilizers under the following scheme have been entered: M1: $Cd(NO_3)_2$, 100 µg L⁻¹, in a mode of additives on 20 µg L⁻¹ daily within 5 days; M2: $Cd(NO_3)_2$, 100 µg L⁻¹, at a time; M3: $CdSO_4$, 100 µg L⁻¹, at a time; M4: $CdSO_4$, 100 µg L⁻¹, at a time plus phosphate fertilizer as PO_4^{3-} anion (2 µg L⁻¹); M5: $CdSO_4$, 100 µg L⁻¹, at a time plus nitric fertilizers as NO_3^- anion (1 µg L⁻¹).

The pH, water and air temperature, cadmium and nitrate concentration in water were daily controlled in minicosms, the effect of weather factors was counted through the measuring of these parameters in the ambient river water. To control the level of the plant's vitality, the rate of their growth and the intensivity of nitrogen fixation as nitrate reductase activity (NRA) was measured every two-three days [13]. It has been established that during the whole experiment the concentration of nitrate ions varied in the range of 0.7-1.5 mg L⁻¹ for various capacities, indicating the normal provision of water hyacinth with one of the major nutrients, NRA of the leaves of EC was at the level of 0,10-0,15 μ M g⁻¹ h⁻¹, so the small variations in NRA suggest maintaining the viability of plants.

2.2. Materials and instrumentation *Reagents*

Cadmium nitrate $Cd(NO_3)_2$, cadmium sulfate $CdSO_4$, phosphoric fertilizer as Na_2HPO_4 , nitric fertilizer as $NaNO_3$ of analytical grade were obtained from "Reachem" (Moscow, Russia), high purity sodium chloride NaCl, nitric acid HNO₃, hydrochloric acid HCl, fluoric acid HF and boric acid H₃BO₃ were purchased from Merck (Germany). The multielemental water standard reference solutions MES-1 and MES-2 were obtain from the company "Skatt Ltd" (Novosibirsk, Russia).

Instruments

Field: the stripping voltammeter IVA-3 (Ekaterinburg, Russia) was applied to determine cadmium concentration in water samples. Nitrate-ion content in water was determined using ion-meter "Anion-410" (Novosibirsk, Russia) with ion-selective electrode ELIS-121 and a silver chloride electrode ESr-1021 as a reference one. Nitrate-reductasa activity was determined using *in vivo* method [14].

Laboratory: Atomic-emission spectrometer with direct current plasma source of excitation (DCP-arc-AES) PGS-2 (Carl Zeiss Jena, Germany) and atomic-emission spectrometer with inductively coupled plasma source of excitation (ICP-AES) iCAP 6000 (InterTechCorporation, USA) were used to determine the elements concentration in water hyacinth. For the digestion of the plants microwave accelerated reaction system Mars-5 (International Equipment Trading Ltd, USA) was used.

Analysis

To control the quality of the results of an evaluation of the metals content in plants the method of atomicemission spectrometry with two different sources of excitation was applied. The flame atomic-absorption spectrometry (FAAS) was used as a method for the intercomparison of the results of cadmium determination in the samples of water hyacinth by atomic emission spectrometry.

Sample preparation procedure for DCP-arc-AES analysis included the following steps: the blending of the sample with graphite powder (1:1); ash of the mixture at 450°C during 3 hours; cooling, homogenization, weighting and successive dilution with graphite powder supporting in each sub-sample a sodium chloride content at the level

of 4%. NaCl. This additive allows enhancing the analytical signals intensities of the trace element's and, on the other hand, to provide the constancy of temperature and electron concentration in plasma source [15]. A series of the powder subsamples with dilutions of 2, 4, 8, 16, 32 (64 and 128 for the roots) were prepared. The matrix effect considered eliminated if for two consecutive dilutions the distinctions between two values of detected concentration were inside of the confidential interval for the average value.

DCP-arc-AES analysis: graphite anode with a cavity of 4 mm in diameter and graphite cathode of conical form; slight width 20 μ m; direct current 13 A; spectra exposition time 18 sec. Spectra registration was performed using a solid state photodiode array. A unified series of reference samples on the base of graphite powder with sodium chloride (4%) as a strengthening additive containing elements under investigation at the level of 0.014 – 33.0 mg kg⁻¹ was used [15].

Sample preparation procedure for ICP-AES analysis was carried in microwave assisted digestion system using PFA-vessels where 0.1 g of the sample was mixed with 5 mL of nitric acid, 1 mL of hydrochloric acid and 0,4 mL of fluoric acid. After digestion and cooling 3 mL of boric acid solution (4%) was added into the vessel to destroy the insoluble fluoric compounds of calcium and aluminum, then the resulting solution was diluted to the volume of 25 mL. To eliminate the matrix effect scandium nitrate was added to the probe as internal standard at the level of 0.5 mg·L⁻¹. The working parameters of the microwave oven are given in Table 1.

Table 1

Stage	Temperature, 0C	Power, W per vessel	Duration, min.
Ι	90	120	10
II	150	120	30
III	180	120	5

Digestion program used for the sample preparation procedure in DCP-AES.

ICP-AES-analysis working parameters: power supply 1150 W; argon flows, L· min⁻¹ : 0.7 for the nebulizer, auxiliary -0.5, cooling -12. The data acquiescing and processing was provided by iTEVA (InterTech corporation) software. The calibration was done with the use of water solutions of the metal's salts mixture in the concentration range of 0.01- 10 mg·L⁻¹.

Sample preparation procedure for FAAS analysis was conducted using open vessels according to the scheme: 0.1-0.5 g of the sample was placed into the porcelain crucible and ashed in the muffle at 480-500°C, the ash was then dissolved in 5 mL of HNO₃ under the aging during 12 hours, heated until white vapor, then 5 mL of HF was added and the mixture was evaporated to the dry residue, which was then dissolved in 10 mL of water. The analysis was done using Perkin Elmer 3030B instrument.

3. Results and discussion

The results of cadmium determination in water hyacinth using two independent AES methods with different sources of excitation for all minicosms are given in Table 2. It is seen that the results of DCP-arc-AES method demonstrates the systematic understatement comparatively with ICP-AES analysis most pronounced for the roots; this discrepancy may be caused by two reasons: cadmium losses during dry ash procedure or incomplete elimination of the matrix effect using the above mentioned successive dilution approach. To clarify the question concerning sample pre-treatment procedure the residues of the roots, leaves and stems after dry ash were digested in the microwave oven and then the solutions were analyzed by ICP-AES. The data produced are shown in Fig.1 by the example of the samples from minicosm M3, it is seen that a volatilization of cadmium compounds when being heated at 450° C during ash is insignificant. It may be also suggested that under stating of the DCP-arc-AES this effect may result from incomplete accounting of the matrix effects using successive addition approach.



Fig. 1. The results of cadmium determination in various parts of the plant by ICP-AES using different sample preparation procedures.

To exclude the influence of organic matter on the results of DCP-arc-AES-analysis above mentioned microwave assisted samples preparation procedure was applied and the obtained solutions were analyzed using DCP-arc-AES being evaporated on graphite powder in the presence of 4% NaCl. As can be seen from Fig.2 in this case a significant differences in the results obtained by different methods is observed which is most pronounced for the roots. It can be assumed that the matrix effect is caused by the presence of major elements at the concentration level two or tree order of magnitudes higher than for the analyte . Unfortunately there are no published data concerning the mineral components content in water hyacinth. We evaluated the contents of Si, Mg, Fe, Na, K and Al in this plant using ICP-AES and compared them with those for other plants including the floating one which are produced as the standard reference materials (SRMs) at the Vinogradov Institute of Geochemistry (Irkutsk, Russia) (Table 3). It is clear that hyacinth in its mineral composition sufficiently differs from the other plants. Probably, the depressing influence on the analytical signal of cadmium is associated with the cumulative effect of the major elements: Si, Al, Na, K and others.

Table 2

Cd concentration (the average of three results)								
Part of the plant	DCP-arc-AES	ICP-AES						
Minicosm M1 (the rest Cd concentration in water 0.01±0.0008 mg·L ⁻¹)								
Roots	360±50	730±40						
Stems	72±9	71±5						
Leaves	13±3	20±2						
Minicosm M2 (the rest Cd concentration in water 0.005	±0.001 mg·L-1)						
Roots	240±30	500±20						
Stems	52±7	74±5						
Leaves	12±3	11±1						
Minicosm M3	(the rest Cd concentration in water 0.007	7±0.001 mg·L ⁻¹)						
Roots	100±20	520±20						
Stems	37±5	58±4						
Leaves	16±3	11±1						
Minicosm M4	(the rest Cd concentration in water 0.005	5±0.001 mg·L ⁻¹)						
Roots	240±40	420±20						
Stems	49±7	67±5						
Leaves	13±3	13±1						
Minicosm M5	(the rest Cd concentration in water 0.005	5±0.001 mg·L ⁻¹)						
Roots	220±30	350±20						
Stems	49±7	59±4						
Leaves	11±3	13±1						
Minicosm M	6 (the rest Cd concentration in water <0	.0001mg·L ⁻¹)						
Roots	7±4	5±0.7						
Stems	3.4±1.3	3.0±0.4						
Leaves	0.2±0.1	0.3±0.03						

Cadmium distribution between the parts of EC for the different minicosms
according to the methods of DCP-arc-AES and ICP-AES, mg ·kg-1(dry weight), P=0.95.
n=3 (the average of the results for three plants for each minicosm are presented).

Table 3

The major elements content in different types of the plants, mg·kg ⁻¹ .										
Plant Element	Si	Fe	Mg	К	Na	Al				
Water hyacinth	1.8 ± 0.1	0.4 ± 0.08	0.57 ± 0.08	5.5±04	4.9±0.2	$0.30{\pm}0.05$				
Elodea Canadensis	-	0.26±0.01	0.32 ± 0.02	3.22±0.16	0.69 ± 0.05	0.010 ± 0.01				
Birch leaves	0.40 ± 0.07	0.073±0.007	0.44 ± 0.03	0.71±0.04	0.018±0.003	0.083 ± 0.01				

Thus, the data obtained by ICP-AES technique were considered as the accurate results. To control the correctness of cadmium determination by ICP-AES in the samples of water hyacinth the inter-comparison with the results of independent method (FAAS) was applied. The results were compared with the use of t-criteria [16] calculated in accordance with the formulas:

$$\begin{split} S_0^{\ 2} &= ((n_1-1) \cdot S_1^{\ 2} + (n_2-1) \cdot S_2^{\ 2})/(n_1+n_2-2), \text{ where } S_1 \text{ and } S_2 \text{ -dispersions for two results } C_1 \text{ and } C_2 \text{ of independent } \\ \text{methods; } n_1 \text{ and } n_2 \text{ - the number of parallel results for two methods; } \\ t_{exp}^{\ 2} &= |C_1 - C_2| \cdot (n_1 \cdot n_2)^{0.5} / S_0 \cdot (n_1+n_2)^{0.5}. \end{split}$$

It is seen (Table 4) that the results of cadmium determination for the roots, stems and leaves are coinciding inside the confidence interval which confirms their reliability.

Table 4

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Cadmium concentration, mg·kg-1									
Sample	ICP-AES(1)	FAAS (2)	t _{experimental}						
	440±90	500±180	2.58						
Roots	750±130	730±230	0.59						
Leaves	21±3	21±5	0.0						
Stems	58±12	60±12	0.91						







Fig. 2. The results of cadmium determination in various parts of the plant after microwave digestion of the samples.

Fig. 3. Cadmium accumulation by the roots, shoots and leaves of the plants in different minicosms.

According the diagram (Fig. 3) it may be concluded that cadmium is mainly accumulated in roots that agree with the data of other researchers; the highest degree of cadmium uptake is observed in the plants of minicosm M1 with a droplet cadmium addition; moreover, it considerably exceeded these values for the other minicosms when the same Cd compounds were introduced of $100 \ \mu g \ L^{-1}$ at a time; cadmium accumulation by plants in minicosms with one time addition was lower in minicosm M3 (cadmium as sulphate salt) then in other two (cadmium as nitrate salt); the introduction of sodium hydrophosphate as fertilizer stimulated cadmium uptake in minicosm M4 while nitrate fertilizer (minicosm M5) was not effective.

The data on the accumulation of cadmium by water hyacinth dependently on pollutant introduction scheme and the presence of additives are summarized in Table 5. It is also seen that cadmium extraction by the plants from the water environment corresponds to the range of 50-79 %. As for the rest cadmium content in water to the end of experiment (8th day) it didn't exceed 20% of initial quantity. One may suggest that there are other channels of cadmium removal from water, among which the sorption on the suspended particles and an uptake by the water biota may supposed as the most essential.



Fig. 4. Cadmium uptake and hyacinth mass increase in minicosms.

Table 5

Mini- cosm number	Experimental conditions	Total amount of Cd introduced, mg	The rest amount of Cd in minicosm, mg	Cadmium concentration in whole plant, mg·kg ⁻¹	Total cadmium content in the plant, mg	The portion of Cd uptake by plant, %
M1	$Cd(NO_3)_2$: 20 µg·L ⁻¹ x 5	20.5±1.6	2.1±0.2	400±70	13±2	71±14
M2	$Cd(NO_3)_2$: 100 µg·L ⁻¹	23.0±1.5	1.2±0.02	400±70	11±1	50±4
M3	$Cd(SO_4)_2$: 100 µg·L ⁻¹	15.0±2.0	1.1±0.02	400±70	11±1	79±13
M4	$Cd(SO_4)_2$ 100 µg·L ⁻¹ + Na ₂ HPO ₄	20.0±1.5	1.1±0.02	400±70	13±2	69±10
M5	$\frac{\text{Cd(SO}_4)_2}{100 \ \mu\text{g} \cdot \text{L}^{-1} + \text{NaNO}_3}$	20.0±1.5	0.1±0.03	400±70	10±2	50±10

Accumulation of cadmium by a water hyacinth under different conditions. The results are presented for three plants for each minicosm.

The increase in weight of plants was observed in all minicosms. It means that the capacity of the hyacinth to absorb the pollutant is limited by their ability to self-preservation. Nevertheless, it appears (Fig. 4) that plant growth was depressed in all cases when pollutant was added into the minicosm at the level of 100 μ g·L⁻¹ at a time irrespective of the salt type and the presence of fertilizers. On the other hand the cadmium influence was practically not exposed in minicosm M1 with droplet addition of 20 μ g·L⁻¹ cadmium daily within 5 days. In accordance with the published data [17] a decrease of cadmium toxicity for some plants is caused due to the formation of the complexes with phytohelatins or flavonoids which have been discovered in water hyacinth [18]. Hence we can assume that because such transformation process takes place the regime of cadmium gradual addition is more sparing for the plant.

4. Conclusion

The results of experiment carried out in frames of natural modeling approach allows us to conclude, that the water hyacinth capacity of growth and metal uptake strongly depend on the weather conditions, exposure mode and the presence of other constituents, herewith the basic part of heavy metal collects in the roots (about 70 % from absorbed). Much more active pollutant accumulation is observed when gradual additions mode used, where vital activity of a plant leads to higher gain of its biomass. It is noticed that the type of metal compound influences its accumulation by plant, however, to a lesser degree, than distinction in additive modes.

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GEOCHEMISTRY OF ATMOSPHERIC DUST ON THE TERRITORY OF THE CITY OF YEREVAN

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Abstract. This research is focused on the level of heavy metal contents in dust of a near-ground layer of atmosphere. The dust load level was evaluated as medial. Indicated was a quality composition of dominating dust pollutants. The level of summary load of metals was low.

Keywords: urban air pollution, dust load, heavy metals

Introduction

The air basin is a transit environment for diverse pollutants and particularly heavy metals in which they travel and disperse. Heavy dust load in cities in combination with high concentrations of heavy metals in the dust is a risk factor to public health.

The goal of this research was evaluation of both dust load on the territory of the city of Yerevan and the contents of harmful components – heavy metals – in the dust with help of snow survey data. The research was performed in 2011.

Yerevan is the capital city of Armenia. It covers an area of 227sq.km, its population is 1 117 800 people which makes over 24 % of the population of the republic. Yerevan is not only the administrative and economic center but also an essential transport junction of the country. The geological composition of the territory is dominated by volcanic lavas, tuffs and Quaternary sediments characterized by close-to-clarke contents of heavy metals. The rocks display development of brown and semi-desert soils. The soil profile is rich in carbonates, to the lower horizon the presence of gypsum is common, this evidencing a lack of chemical element washout and creating a good environment for heavy metal accumulation on soil profiles. The natural geochemical association is characterized by a weakly intensive series represented by Zn, Cu and Co. So, the pronounced geochemical anomalies of heavy metals in diverse environmental compartments emphasizing the atmospheric dust of Yerevan are of man-made origin.

Materials and methods

One of best informative indicators of dust pollution of the air basin in winter period is snow cover. Due to its good sorption property snow absorbs the major part of dust from the ambient air. The investigations were implemented in compliance with methods described in detail [1, 2].

Snow was sampled from a fixed site. Then the snow samples were transported to the lab and filtered and dried after melting. A dry residue was analyzed for concentrations of eleven elements (Hg, Cd, As, Pb, Cr, Ni, Co, Zn, Cu, Ag and Mo) on a PerkinElmer AAS AAnalist 800. Total 18 snow samples were collected and analyzed.

The dust load (P) was calculated by a formula $P = P_0 / S \cdot t$, where P_0 is the mass of filtered substance, S - a sampling site area, t - a fell snow layer formation time (in the considered case - 1 day) [1, 2]. The dust load level was assessed with help of a 4-grade scale suggested by N.S. Kasimov [3].

With a goal to identify heavy metal anomalies in dust, collation was done between the contents of heavy metals and a geochemical background for soils [7, 6]. To characterize quality and quantity indices of heavy metal anomalies in dust, arranged were ranked geochemical series of heavy metal contents in soils graded by concentration coefficients: $K_c = C_i / C_{background} K_c = C_i / C_{\phi\sigma\kappa}$, where C_i is the contents of *i* metal in dust, $C_{background}$ – its background contents in soils.

Results and Discussion

According to snow cover survey data for Yerevan for 2011, the major part of the territory displayed low levels of dust load (less than 250 kg/sq.km/day). However, against the background of a low dust level 28 % of the studied samples displayed a high dust load (varying 450-800 kg.sq.km/day), those values corresponding to a high level of danger. And finally 11 % of samples displayed a very high dust level (over 800 kg.sq./day), which corresponds to an extremely high level of danger [3].

Thus, Yerevan's air basin is characterized by a presence of sharp peaks against a background of low dust load values (Fig.1).



Fig.1. Dust load, summary load of heavy metals and summary contents of heavy metals in the dust of Yerevan.

A summary mass share of heavy metals in atmospheric dust varies within 2 orders: from 0,02-0,26 % and averages to 0,07 %. A summary load of heavy metals varies 0,03 to1,81 kg/sq.km/day and the value of this index for the city averages 0,28 kg/sq.km/day. Commonly, the value of summary load of heavy metals is directly proportional to the value of dust load (Fig.1). It is noteworthy that on the average 99 % of total sum of heavy metals in dust is made by six elements only, which include elements of 1st grade of danger – Pb and Zn and 2nd grade of danger - Cu, Mo, Ni and Cr. 1 % of the sum of metal falls on Co, Ag and three toxic elements: Hg, Cd and As (Fig.2).



Fig.2. Averaged shares of heavy metals in the dust of the snow cover in Yerevan.

Heavy metal concentrations in dust are given in Tab.1, their collation with the geochemical background of Yerevan's soils – in Fig.3.

neary metal contents and then geochemical series in atmospheric dust of rerevan.											
Index		I gi	ade of dan	ger		II grade of danger				-	
Index	Hg	Cd	As	Pb	Zn	Cr	Ni	Со	Cu	Mo	Ag
background	_	_	10	40	100	68	35	10	60,00	12	0,05
C _{min} ,	0,01	0,02	0,001	11,35	64,16	2,52	11,06	0,27	24,47	8,86	0,29
K _c	_	_	0,0001	0,28	0,64	0,04	0,32	0,03	0,41	0,74	5,78
					Ag _(5,8)						
$C_{\rm max}$, mg/kg	1,18	0,54	0,04	876,5	749,0	38,13	66,07	6,02	1853,5	216,9	4,13
K _c	_	_	0,004	21,91	7,49	0,56	1,89	0,60	30,89	18,08	82,54
$Ag_{(82,5)} - Cu_{(30,9)} - Pb_{(21,1)} - Mo_{(18,1)} > Zn_{(7,5)} - Ni_{(1,9)}$											
$C_{\rm avr}$, mg/kg	0,20	0,12	0,02	94,62	243,89	13,45	34,29	3,50	275,01	57,30	1,50
K _c	_	_	0,002	2,37	2,44	0,20	0,98	0,35	4,58	4,78	30,09
	$Ag_{(30,1)} > Mo_{(4,8)} - Cu_{(4,6)} - Zn, Pb_{(2,4)} - Ni_{(1,0)}$										

Heavy metal contents and their geochemical series in atmospheric dust of Yerevan.

Note: "–" – no data are available

Table 1

As seen from data given in Tab.1 and Fig.3, maximal excesses vs. geochemical series are established for Ag which is a dominating dust pollutant in Yerevan. K_c values for Ag show a one order excess as compared with those for an analogous index for other elements

The values of coefficients of concentrations of the elements of 2nd grade of danger Mo and Cu are similar; the noted elements are always found in the dust of the near-ground atmospheric layer of Yerevan. Moreover, the dust displays a stable presence of Ni in close-to-background concentrations.

 K_c values of the elements of 1st grade of danger are also similar. Only one sample displayed a single high concentration of Pb, but wholly K_c of this element is rather low. Arsenic (As) was indicated in 4 dust samples in concentrations which were well low vs. the geochemical background. Toxic elements Hg and Cd were indicated in all the collected samples in rather low concentrations. For Hg and Cd no geochemical background values have been calculated.



Fig. 3. Heavy metal concentrations in dust vs. the geochemical background for soils on Yerevan's territory

Qualitative and quantitative characteristics of atmospheric dust are clearly expressed in a geochemical series ranked by coefficients of concentrations. The averaged geochemical series is arranged as follows: $Ag_{(30,1)} > Mo_{(4,8)} - Cu_{(4,6)} - Pb, Zn_{(2,4)} - Ni_{(1,0)}$. The summary intensity of the averaged geochemical series of heavy metals in dust makes 45,3, which corresponds to the acceptable level of dust pollution with heavy metals [3].

Investigations of geochemical indices of dust migration flow on Yerevan's territory have a character of monitoring. Drastic quality and quantity changes in geochemical anomalies has been recorded since 2002. In period 1989-2001 geochemical series were dominated by Pb [7].

However, in 2001 the use of ethylated gasoline was banned, so Pb emissions into the atmosphere got reduced. In 2002 Pb was shifted from leading positions in geochemical series, and dominating dust pollutants has become Ag, Mo, Cu and Zn.

Besides, since 2002 the intensity of geochemical anomalies in the dust of Yerevan has been declining. Also, since 2002 the summary share of heavy metals in dust has reduced. Despite the fact, that between 2002 and 2011 a dust load level on the major part of the city showed insignificant variations, noteworthy is a sharp reduction of the share of heavy metal in dust and consequently the intensity of geochemical anomalies in dust depositions decreased by 2,6 times: from 122 in 2001 to 45,3 in 2011. So, the intensity of the averaged geochemical series in 2011 was by 2,6 times lower as compared with a similar index for 2002.

With a goal of providing a sanitary and hygienic assessment, concentrations of heavy metals in dust were collated with MAC for soils established for Armenia [1]. MAC exceeding values were indicated for five elements (Fig.4), including those of 1st (Pb, Zn) and 2nd (Cu, Co, Mo) grades of danger.



Fig. 4. Excessive heavy metal concentrations in dust vs. MAC for soils [1].

Conclusion

The performed investigations support the following conclusion. The major part of Yerevan displays low values of dust load: against such a background sharp peaks are recorded which corresponds to high and extremely high level of danger. The summary shares of heavy metals in dust vary 0,02 to 0,26 % and average to 0,07 %. The intensity of geochemical series of heavy metal anomalies is low, and against this background single peaks are detectable. 99 % of summary share of heavy metals falls on six elements, including those of the 1st (Pb, Zn) and 2nd (Cr, Cu, Mo, Ni) grades of danger. Dominating atmospheric dust pollutants on the territory of Yerevan were Ag, Mo and Cu. In a period 2002 to 2011 quality and quantity indices of geochemical anomalies of heavy metals in dust showed a steady inclination to reduction.

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THE IMPACT OF COMPOSITIONS FROM HYDROLYTIC RESIDUES ON **CHERNOSEM SOLONETS FERTILITY**

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Abstract: The results of different experimental modalities of residues from biochemical plants which function on the basis of hydrolysis (hydrolytic lignin, hydrolytic slime) as black soil solonets fertilizer and amendment are presented in the given paper. From the ecological and economic points of view, a new technology of chemical amelioration of alkaline soils with excessive administration of components from hydrolytic residues as soil ameliorators is founded.

Keywords: wastes, hydrolytic lignin, hydrolytic slime, chemical amelioration, solonets.

Introduction

An enormous quantity of residues is accumulated as result of any human activity. Being neutralized because of different reasons (psychological, economic, legal, technological, etc.) they cause an ecologic imbalance in nature, disturbing in such a way the normal functioning of the soil, of the atmosphere and of the aquatic resources. At the same time, the residues contain considerable quantities of elements necessary for the nutrition of the plans and for soil fertilization. In the present conditions, when the application of industrial fertilizers decreased to minimal quantities, the extensive utilization of residues, especially of organic ones is of especially significant importance for agriculture.

The rational production and utilization of ameliorators from hydrolytic residues might solve two important issues: the first is an ecological one: the diminishing of environment pollution with nutrients, and the second - the improvement of soil fertility.

Materials and methods

The research has been carried out by experiments, in vegetation vessels and in the field. The soil under experimentation is characterized as chernozem solonets possessing a moderate content of sodium (18-23 %), exchangeable magnesium (33-38%), sodium-sulfate salinity, and a clay texture.

For the experiments in vegetation vessels we used the ploughed layer (0-25) evacuated on a plot of the agricultural farm from the village Ciuciueni, Sangerei district. The vessel had a capacity of 28 dm3 and a bottom with holes. The preparation of the soil included the crumbling of the boulders having dimensions larger than 10 cm and homogeneity of the whole soil mass necessary for the experiment. The soil and the cultivated corn were provided with water from the atmospheric precipitations.

We made experiments on the residues from the biochemical plants that produce fodder yeasts (hydrolytic lignin and hydrolytic slime) separately and in in various combinations of sludge from urban waste water treatment and defecation mud from sugar mills (Table 1).

The indicators of residues used in experiments, % of dry mass										
Residue	Humidity	С	Ν	P_2O_5	K ₂ O	CaO	Ash			
Experiment in vegetal vessels										
Hydrolytic lignin (HL)*	58.7	37.4	0.46	0.14	0.11	1.0	18.0			
Hydrolytic slime (HS)	62.4	29.5	0.78	0.24	10.9	10.9	37.5			
Defecation mud (DM)	23.5	3.7	0.10	0.20	24.3	24.3	87.9			
Urban sludge (US)	38.1	26.4	2.50	1.77	7.4	7.4	55.7			
Bovine manure (BM)	78.0	33.9	1.22	1.20	6.2	6.2	31.4			
		Field	experiment							
Hydrolytic lignin (HL)	58.6	47.4	0.47	0.16	0.9	0.9	5.1			
Hydrolytic slime (HS)	59.4	46.8	1.52	0.49	14.2	14.2	33.7			
Defecation mud (DM)	28.5	4.0	0.25	0.18	20.9	20.9	87.5			
Urban sludge (US)	32.5	25.0	2.51	1.73	7.6	7.6	64.7			
Bovine manure (BM)	40.5	17.1	1.06	0.63	5.6	5.6	59.6			

*The abbreviations on the right will further stand for the given residue.

The hydrolytic residues and the obtained compositions were incorporated in a dosage of 60 g of wet mass per 1 kg of soil. The field experiment was carried out on a plot from the mentioned farm. The compositions from residues were incorporated in a dosage of 100t/ha. The total quantity of calcium in 50 tons of sludge is equal to the quantity of changeable sodium in the 0-60 cm layer attested in the most alkaline parts of the experimental field. Each variant was repeated four times. The surface of the plots was 8x20 m. The materials were applied only once during the autumn ploughing at the depth of 18-22 cm. Bovine manure + gypsum was used in the experiment as comparison, a traditional procedure that is used at present for the amelioration of soils affected by salinity.

Results and discussion

Because of the contents of organic matter such as calcium, nitrogen, phosphorus and other fertilizing elements, and of possession of exceptional physical properties, the researched residues influenced considerably the on the fertility of the chernozem solonets.

The obtained results in the vegetation vessels experiment demonstrate that the apparent density was reduced by 0.08-0.12 g/cm3 in the experimented variants (Table 2).

Table 2

Variant (dosage in g/kg soil)	Appa- rent density g/cm ³	Total porosi-ty %	Air porosi-ty for field capaci-ty, %	Saturated soil per- meabili-ty for water mm/ha	Field capaci-ty for water, %	Parch- ing coeffi- cient, %	Use-ful water capa- city, %	Penetra- tion resistan- ce, kg/cm ²			
	1987, the 6 th year of application										
Control without residues	1.31	50	14	0.6	27.3	16.1	11.2	11.6			
HL, 60	1.19	54	15	4.0	35.0	13.8	21.2	6.5			
HS, 60	1.21	53	14	2.4	32.0	14.0	18.0	8.5			
HS,15+US,45	1.23	52	15	3.0	30.4	14.9	15.5	8.9			
HS,15+US,45	1.20	55	20	2.9	29.4	13.8	15.6	7.1			
HL, 45+DM,15	1.21	53	11	3.7	34.5	14.1	20.4	6.9			
LD 5%	0,066	2.1	2.3	0.56	2.73	1.25	-	0.58			

The impact of hydrolytic residues and their compositions on the physical properties of chernozem solonets. Vegetation vessels experiment

The soil resistance to penetration decreased by 1.3-1.8 times. A tendency of increasing the total porosity was observed. The soil permeability increased by 4-7 times in comparison with the control soil. The residues contributed to the decrease of the parching coefficient by 2.0-2.3 % and to the increase of water capacity by 2.7-7.2 %. Simultaneously the range of soil capacity for useful water increased from 11.2 % for the control plot, which is characterized as average, to 15.2 - 21.2 % for the amended variants which is evaluated as high and very high for the agricultural fields [1].

It is known the fact that the improvement of physical properties is due, first of all, to the reduction of sodium contents and the enrichment of the absorptive complex with calcium. Even in the first year of application the hydrolytic residues and their compositions increased the contents of changeable calcium from 8.8 to 12.7-14.1 me/100 g of soil (Table 3).

Table 3

Impact of hydrolytic residues and their compositions on the changeable cations in the chernozem solonets. Vegetation experiment

Variant (dosage in g/kg soil)		m % 0	% of the total			
	Ca++	Mg++	Na+	total	Ca++	Na+
	1	1982, first ye	ear of application	on	11	
Control	8.8	9.1	4.6	22.5	39	21
HL,60	13.2	8.0	2.9	24.1	55	12
HS,60	13.3	8.0	2.5	23.8	56	10
HS,15 +US,30+DM,15	14.1	7.4	1.9	23.4	60	8
HS,15+US,45	13.4	6.6	1.5	21.5	62	7
HL,30+DM,30	12.7	6.1	1.2	20.9	64	6
LD, 5%	1.6	0.9	0.2	4.3	6	2

1987, the 6 th year of application								
Control	9.5	9.1	3.0	21.6	44	14		
HL,60	13.7	10.1	1.4	25.2	54	6		
HS,60	14.6	8.9	1.1	24.6	59	5		
HS,15 +US,30+DM,15	17.1	7.2	0.2	24.5	70	1		
HS,15+US,45	15.9	8.0	1.1	25.0	64	4		
HL,30+DM,30	16.2	7.8	0.9	24.9	71	4		
LD, 5%	1.6	0.9	0.1	2.7	6	0.4		

Continuation of the table 3

The concentration of sodium in the absorptive complex was reduced by 1.7-3.8 me/100 g. The sodium was substituted more intensively and more completely in the soil treated with comparative mixtures and the application of separate hydrolytic residues. In the sixth year of application, the sodium had 1% of the changeable capacity in the variant treated with a mixture of, for example, 15 g/kg of hydrolytic lignin + 30 g of urban sludge + 15 g of defecation mud. In the field experiment the impact of the compositions from residues was analogous to that from the vegetation vessels. Since the first year of application, a significant increase of calcium and a reduction of sodium in the absorptive complex have been observed in all the experimental variants (Table 4).

Table 4

Modifications in the contents of changeable cations in the chernozem solonets at the application of various hydrolytic residue compositions. Field experiment

Variant	Depth,		me		% of the total		
(residue dosage, t/ha)	cm		1				
		Ca++	Mg++	Na+	Total	Ca++	Na+
		5 th year	of application	n		-	
Control without residues	0-20	8.3	9.8	4.7	22.8	36	21
	20-40	7.2	10.7	6.1	24.0	30	25
	40-60	5.8	10.9	6.5	23.2	25	28
Average content	0-60	7.1	10.5	5.8	23.3	30	25
	0-20	14.1	9.0	1.2	24.3	58	5
BM, 100+ gypsum,10	20-40	10.3	10.0	2.8	23.1	45	12
	40-60	6.1	13.4	5.3	24.8	25	21
Average content	0-60	10.2	10.8	3.1	24.1	43	13
	0-20	11.2	9.3	2.8	23.3	48	12
HL,50 + HS, 50	20-40	8.3	10.2	3.6	22.4	37	16
	40-60	5.5	12.7	6.1	24.3	23	25
Average content	0-60	8.3	10.7	4.2	23.3	36	18
	0-20	14.5	8.9	1.1	24.5	59	5
HL,50+HS 50+, gypsum 10	20-40	10.2	11.6	2.5	24.3	42	10
	40-60	6.9	13.1	5.1	25.1	28	20
Average content	0-60	10.5	11.2	2.9	24.6	43	12
	0-20	13.2	7.2	0.8	21.2	62	4
HL,25+HS,25+US,50	20-40	11.7	6.7	1.4	19.8	59	7
	40-60	12.6	8.8	2.1	23.5	54	9
Average content	0-60	12.5	7.6	1.4	21.5	58	6
	0-20	17.2	7.8	0.7	25.7	67	3
HL,25+HS,25+DM,50	20-40	12.6	7.7	1.8	22.1	57	8
	40-60	9.0	10.9	3.6	23.5	38	15
Average content	0-60	12.9	8.8	2.0	23.8	54	9
	0-20	16.9	7.6	0.7	25.2	67	3
HL,10+H5,10+U5,30+	20-40	12.3	8.8	1.7	22.8	54	7
	40-60	9.4	10.2	3.5	23.1	41	15
Average content	0-60	12.9	8.9	2.0	23.7	54	

Though the residue mixtures were incorporated in the 0-20 cm layer, the substitution of sodium in the absorptive complex by calcium spread to the lower layers as well. The mixtures that contained in their composition urban sludge

or defecation mud manifested themselves by a more pronounced amelioration effect. The mixtures containing bovine manure + gypsum and hydrolytic lignin, 50 + hydrolytic slime, 50 + gypsum, 10 t/ha had the same useful effect on the absorptive complex. A less pronounced ameliorative effect was observed in the variant treated with hydrolytic lignin, 50 + hydrolytic slime, 50 + hydrolyti

We suppose that the ameliorative impact on the deep layers is done by the soluble compounds or the ones found in suspension and transferred with water from the administrated residues.

In the first year of residues application, a part of the substituted sodium from the absorptive complex remained in the soil solution. In the vegetation vessels experiment the concentration of soluble sodium on the control variant was of 3.46 me/100 g of soil, while on the treated variants it significantly exceeded this value (Table 5).

Table 5

Variant	NCO ₃	Cl	SO4	CA++	Mg++	Na+	K+	Total residue, %		
1982, the 1 st year of application										
Control	1.08	0.12	3.28	0.32	0.34	3.46	0.02	0.30		
HL,60	0.52	0.16	4.14	0.56	0.52	3.70	0.04	0.34		
HS,60	0.76	0.20	4.83	1.16	0.76	3.81	0.06	0.41		
HS,15+US,30+DM,15	0.92	0.20	4.85	1.00	0.88	4.02	0.07	0.42		
HS,15+US,45	1.20	0.18	3.43	0.56	0.46	3.75	0.04	0.31		
HL,30+DM,30	1.16	0.08	3.28	0.60	0.40	3.48	0.04	0.33		
LD,5%	0.17	0.04	1.02	0.28	0.18	0.69	0.01	0.09		
	19	87, the 6^{t}	^h year of app	lication						
Control	1.04	0.04	2.43	0.36	0.16	2.96	0.03	0.26		
HL,60	0.50	0.20	0.47	0.40	0.10	0.65	0.02	0.08		
HS,60	0.30	0.10	0.70	0.50	0.20	0.37	0.03	0.08		
HS,15+US,30+DM,15	0.60	0.10	0.62	0.50	0.30	0.51	0.01	0.09		
HS,15+US,45	0.60	0.30	0.11	0.20	0.20	0.57	0.04	0.07		
HL,30+DM,30	0.80	0.20	0.15	0.30	0.30	0.54	0.01	0.08		
LD,5%	0.05	0.02	0.06	0.05	0.03	0.08	0.004	0.02		

Impact of hydrolytic residues and their compositions on the contents of soluble salts in the chernozem solonets me/100 g of soil. Vegetation vessels experiment

In enrichment of the liquid phase with salts is also due to the incorporated residues. In all the experimented variants the concentration of Ca⁺⁺ and SO4—ions increased. Probably, this fact is due to the contents and the mobility of these elements in the corresponding residues. Simultaneously, the increase of soluble ions concentration in the first year of application conditioned the increase of total residue in all the experimented variants compared to the control variant. The amelioration of the solonets is considered complete when the salts are removed from the profile. In the following years the hydro-physical properties of the solonets improved. More precipitations fell and gradually the salts were washed, thus desalinizing the soil. In the sixth year, the total residue in the experimental variants was reduced by 2.9 -3.7 times compared to the control one. Among the experimented compositions in the field experiment, the hydrolytic lignin, 10 t + hydrolytic slime, 10 t + urban sludge, 30 t + defecation mud, 50 t/ha and hydrolytic lignin, 25 t + hydrolytic slime, 25 t + urban sludge, 50 t/ha had a more advantageous impact on the desalinization.

In the treated variants the reaction of the solonets improved. The average values of pH for the three years in the 0-20 cm layer were of 0.4-0.6 units smaller than on the control variant (Table 6).

By increasing the hydrogen ions activity, the organic residues increase simultaneously the tamponing capacity of the solonets [2]. In the ploughed layer, where the compositions of hydrolytic residues, which contained defecation mud, were incorporated a moderate increase (0.6 - 1.0%) of the calcium carbon was observed. There exists the hypothesis that a moderate content of CaCo3 positively influences the structure and the tamponing capacity and maintains the soil reaction in the limits of the average values [3].

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SPECTROSCOPIC STUDIES OF AMINOACIDS COMPLEXES WITH BIOMETALS

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Abstract: The $[Cu(L)_2] \cdot H_2O$, $[Co(L)_2] \cdot 2H_2O$, $[Zn(L)_2] \cdot H_2O$ complexes with methionine (L) as ligand, were synthesized in water solution and analyzed by means of: elemental analysis, atomic absorption spectroscopy, thermogravimetry, FT-IR, UV-VIS and EPR spectroscopies.

The atomic absorption spectroscopy and elemental measurements confirm the ratio 1:2 metal ion: methionine composition for the synthesised compounds. The IR spectra show that amino acids act as bidentate ligands with coordination involving the carboxylic oxygen and the nitrogen atom of the amino group. Spectral UV-VIS data confirmed the covalent metal-ligand bonds, the pseudotetrahedral symmetry around the copper and zinc ions and the octahedral environment for the cobalt ion. Powder ESR spectra at room temperature are typically for monomeric species.

Key words: biometals complexes, methionine, DSC, spectroscopies

1. Introduction

Minerals such as zinc, copper, iron and others can chemically bond to amino acids resulting chelates. Amino acids are ideal chelators or ligands from both chemical [1] and nutritional [2] points of view. Metal amino acid chelates resemble these compounds which allow the minerals to be carried in with the amino acids during absorption. Finally, the amino acids, once released from the metal, can be used to build proteins or provide energy.

The metabolism of sulfur amino acids, methionine has been linked to several key aspects of human health and cellular function [2]. **Methionine** (Fig.1) is the only sulfur-containing amino acid that is essential for mammals and must therefore be derived entirely from the diet. In contrast, methionine is synthesized by plants and most microorganisms after the initial steps of inorganic sulfate assimilation and cysteine or homocysteine syntheses [3-6]. A preliminary study has suggested that methionine (6 grams per day) may improve memory recall in people with AIDS-related nervous system degeneration [7] and also may help treat some symptoms of Parkinson's disease [8].



Fig.1. Methionine structural formula

2. Experimental

2.1 Physical-Chemical Measurements

The Vario El device allows the quantitative determination of the carbon, nitrogen, hydrogen, sulphur and oxygen in various operating modes. Atomic absorption measurements were realized with an AAS-1 device at λ =320nm wavelength. The Differential scanning calorimetry measurements were carried out with a Mettler Toledo device, with 10°C/minute heating rate until a 500°C temperature was reached. The samples weights were between 1 and 2.5 mg. FT-IR spectra were taken with a Perkin-Elmer FT-IR 1730 spectrophotometer over KBr solid samples in 4000-400 cm⁻¹ range. UV and visible electronic spectra were recorded in the λ =190-1100 nm range in aqueous solution (10⁻⁵M) for threonine, copper and cobalt complexes and in ethanol solution for the zinc complex, using a standard Jasco V-530 spectrophotometer.

Powder EPR measurements were performed at room temperature at 9.56 GHz (X band) using a standard JEOL-JES-3B equipment.

2.2 Synthesis of the complexes

The purpose of the study was to obtain neutral complexes of $ML_2 \cdot nH_2O$ type (M= Cu, Co, Zn) at pH=8-10, in the presence of a strong basis (NaOH) to obtain the ionisation conditions of the amino acid.

The complexes were prepared following the next procedure: 2mmol of the methionine (0,286g) were dissolved in 5 ml warm distilled water. For the deprotonation of the amino acid 0.33 ml 30% NaOH was added.

For all the complexes the precipitation was instantaneous, and a grey-blue precipitate was obtained (η = 99.8 %) for 1, a pink one for 2 (η =72.7%) and a white one for 3 (η = 75.7%). The complexes were filtered, washed with ethanol and dried in desiccators under P₄O₁₀. Then, the complexes were recrystallized on methanol, dried and weight to establish the percent of complexation.

3. Results and Discussions

3.1 Elemental analysis

The elemental analysis results for the synthesized complexes confirm the 1:2 ratio metal/ methionine. The elemental analysis data of the metal-methionine complexes are pointed up in Table 2.

Elemental analysis results the complexes									
Symbolic formula	Molecular	%C		%H		%N		%Metal	
	weight	Calc.	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.	Meas.
$[Cu(L)_2] \cdot H_2O$	357.5	33.56	31.50	5.5	5.72	7.8	7.27	17.9	17.26
$[Co(L)_2] \cdot 2H_2O$	353	33.59	30.75	5.66	6.21	7.93	7.41	18.13	17.76
$[Zn(L)_{2}]\cdot H_{2}O$	359.6	33.39	33.14	5.56	6.33	7.69	6.74	17.81	16.19

3.2 Atomic absorption spectroscopy

The atomic absorption results for the synthesized complexes are in concordance to those theoretical obtained (Table 2).

Complex	Metal concentration	Metal concentration from the complex (%)						
	Calc.	Meas.						
1	17.44	17.43						
2	16.05	15.77						
3	15.66	16.47						

Metal concentrations obtained by means of atomic spectroscopy

3.3 Differential Scanning Calorimetry

The differential scanning calorimetry advantages are owed to the direct measurement of the intrinsec thermal properties of the sample and are not invasive and do not need chemical conversion [10].

Differential scanning calorimetry (DSC) was used for a qualitative interpretation of the synthesized complexes.

The ligand trace indicate an endothermic reaction at 275 °C specific to pure substances and an exothermic reaction between 310 to 360°C attributed to the decomposition and combustion of organic matter components. The thermogram of complex 1 indicates a melting process fallowed by decomposition between 230 to 270 °C and the several exothermic peaks between 270 to 420 °C are attributable to the oxidation of the decomposing product. The complete combustion took place subsequent to 500 °C. The complex 2 thermogram indicates a more complex plot with peaks owing to dehydration, decomposition, oxidation and combustion processes. Between 80 to 100 °C two successive endothermic peaks appeared and were attributed to dehydration processes. After 100 °C a two step decomposition processes took place between 130 to 200°C and 150 to 170°C, and an intermediary product was formed. The exothermic from 380°C and 400°C were associated to the oxidation of the decomposing products. The complete combustion took place subsequent to 480 °C. The DSC trace of complex 3 indicates an exothermic reaction between 130 to 150 °C attributed to the crystallization process. The melting point appears at lower temperature (220 °C) than the one for the ligand (275°C), which profs the complex formation. The complete combustion took place subsequent to 450 °C. The DSC trace of complex 3 indicates an exothermic reaction between 130 to 250 °C.



Fig 2. DSC thermograms of L (a), 1 (b), 2 (c) and 3(d)

141

Table 1

Table 2

1.4 FT-IR spectroscopy

In the figure (Fig.3) the main parts of the IR spectra are presented and most important absorption bands and their assignments are shown in Table 3.

In the ligand spectra the v(N-H) stretching vibration appears at 3146cm⁻¹ and is shifted at 3229 cm⁻¹, 3172 cm⁻¹ and 3450 cm⁻¹ in the Cu(II), Co(II) and Zn(II) spectra proving the involvement of the $-NH_2$ - group in the complex formation [11-13]. The CH₂-S and CH₃-S stretching vibrations appears as a sharp band at 2915 cm⁻¹ in the ligand spectrum and are insignificant shifted in the complexes spectra confirming the non involvement to the coordination. The absorption band from 1610 cm⁻¹ in the ligand spectrum was attributed to the v(C=O) stretching vibration and appears to be shifted toward higher wave numbers in the complexes **1** and **2** spectra, which involves the carboxylic group in the covalent bonding to the metal ion [14]. The v (OH) stretching vibrations do not emerge in the ligand spectrum, but they appear in the complexes spectra at values between 3383 to 3449 cm⁻¹, suggesting the presence of the crystallisation water within these complexes.



				Table 3
Band	L	1	2	3
v(N-H)	3146	3229	3172	3450
v(O-H)	-	3449	3419	3383
v(C=O)	1610	1640	1640	1586
δ _s (N-H)	1580 1563 1508	1568 1616	1584	1502 1558

Fig. 3. FT-IR spectra of L (a), 1 (b), 2 (c) and 3 (d)

3.4 UV-VIS Spectroscopy

The local symmetry around the metallic ions was determined comparing the amino acid and metallic complexes UV-VIS spectra [14].

The $n \rightarrow \pi^*$ characteristic band in the UV spectra assigned to the C=O bond appear at 267 nm for threonine (Fig.3.a) and is shifted toward higher wave lengths with 8 nm, 7 nm and 2 nm for 1, 2 respectively 3 confirming the presence of the ligand in the complex [15] and the covalent nature of the metal-ligand bond. In the visible domain (Fig.4) a d-d transition appears between at 625 nm in the copper complex spectrum assigned to the ${}^2T_{2g} \rightarrow {}^2E_g$ transition, specific for Cu (II) complexes with tetragonal distortion owing to the Jahn-Teller effect. In the visible domain, the Co-L spectrum shows a band at 512nm attributed to the d-d transition of cobalt electrons. Based on the d⁷ diagram in Oh field the fallowing attribution were maid: $v_1 = 8100 \text{ cm}^{-14} \text{ T}_{2g}(\text{F}) \rightarrow {}^4\text{ T}_{1g}(\text{F})$, $v_2 = 16000 \text{ cm}^{-1} {}^4\text{ A}_{2g}(\text{F}) \rightarrow {}^4\text{ T}_{1g}(\text{F})$, $v_3 = 19400 \text{ cm}^{-1} {}^4\text{ T}_{1g}(\text{F}) \rightarrow {}^4\text{ T}_{1g}(\text{F})$. The last band within the spectrum was assigned to the ${}^4\text{ T}_{1g}(\text{P}) \rightarrow {}^4\text{ T}_{1g}(\text{F})$ transition, expected for an octahedral symmetry of cobalt ion.





Fig. 4. UV and VIS spectra of L (a), 1 (b), 2 (c) and 3 (d)

3.5. EPR Spectroscopy

Powder EPR spectrum at room temperature are typically for pseudotetrahedral symmetry around the copper ion with the g tensor value: g=2.094 corresponding to a CuN₂O₂ cromophore [16,17]. The Co-L powder EPR spectrum revealed the presence of monomeric compounds, with octahedral symmetry around the cobalt ion, the g tensor value is g=2.201[18].



Fig. 5. Powder ESR spectrum of complex 1

4. Conclusions

Three new metallic complexes with methionine as ligand were synthesized and analyzed by means of: elemental analysis, atomic absorption, IR, UV-VIS and EPR spectroscopies.

The study reason was to compare the complexation capacity of the amino acids with the copper ion in similar synthesized conditions to choose those complexes which are easy to synthesize, with high yield, to use it as a method of extraction and separation of the amino acids from mixtures.

The atomic absorption spectroscopy and elemental analysis confirms the stoichiometry of the compounds, the composition corresponded to a metal-ligand ratio of 1:2. The IR spectra show that amino acids act as bidentate ligands with the coordination involving the carbonyl oxygen and the nitrogen atom of amino group. The EPR spectra confirm the pseudotetrahedral local symmetry for copper ion and octahedral symmetry for cobalt ion.

The obtained structural data allow us to propose the molecular formulas for the studied metal complexes which are shown in Figure 6.



Fig. 6. Structural formulas proposed for the synthesized complexes

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ENVIRONMENTAL ASSESSMENT OF HEAVY METALS CONTENT IN SOIL-PLANT SYSTEM

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Abstract. The content of heavy metals (Ag, Cd, Sn, Cs, Ba, La, Rb, Sr, Mn, Fe, Cu, Zn) has been determinate in the soil-plant system: cambic chernozem - winter wheat. It was found that the amount of Ag, Sb, Sn and Ba are accumulating in soil, exceeds the admissible concentration limit. In winter wheat, the content of Cd, Fe and Zn exceed the maximum admissible concentrations. The Rb, Mn, Zn accumulates in the winter wheat grains, and Fe and Cu – in the vegetal organs of wheat.

Keywords: cambic chernozem, winter wheat, heavy metals, concentration, environmental pollution.

Introduction

Heavy metals are considered hazardous environmental pollutants. Their high concentration in plants causes inactivity of enzymes, disturbing processes of photosynthesis, respiration, transpiration, water regime, and absorption, nutrients movement in plants. Accumulation in great amounts in plants and penetrating trophic cycles, heavy metals causing various toxic diseases in human and animal organisms.

The main local sources of environmental pollution with heavy metals are industrial and municipal wastes, gas and smoke exhaust of the electric power station, uncontrolled application of fertilizers and pesticides [1]. A significant source of environmental pollution has transport that gives annually about 500 tons of harmful substances, including some heavy metals. Exhaust emissions in rural areas consist 36%, in urban area – 80%. The exhaust gases contain Pb, which share the environment pollution can be up to two thirds. In processes of tire vulcanization is used Zn and Cd, the grinding or burning these conducts to dispersed elements in the environment. As a result of research on the content of heavy metals (Pb, Cd, Zn, Cu, Mn) in soils was found a high concentration in the vicinity along roads and auto streets, on both sides [2]. In many cases the concentration of heavy metals in soils exceeds the maximum permissible concentration (MPC) in three times. Plants around of the auto roads are also polluted with heavy metals [3].

In addition to local pollutants, the Republic of Moldova is affected by smoke gas flow entering with air masses from neighboring countries, brought by winds. This impact becomes more intense: in the 80s the acid rains consisted 1/3 from total amount of rain fall, in the present – about 70% [4,5]. The accumulation of heavy metals in plants from smoke and exhaust gas takes place both by root uptake from soil and foliar from air.

According to the research, most soils of republic are weakly and moderate polluted with heavy metals [6]. To soil pollution with heavy metals contribute the exaggerate administration of fertilizes, which are contained in the composition chemical elements as ballast or added specially (superphosphate ennobled with zinc), soil fertilization with wastes, application of pesticides. Calculations show that mineral fertilizers administrated in the soil in dose of $(NPK)_{100}$, contain in g/ha: Pb-10, Ni-2, Zn-50, Cu-36, Cr-59, Cd -2 [7]. During three decades the carbonate chernozem was fertilized annually with high doses of fertilizers, the total quantity of Zn was 143 mg/kg [8]. Some guidelines show the MPC of Zn in soils – 100 mg/kg [9].

Material and method

Research has been conducted at the Experimental station "Ivancea" of Institute of Pedology, Agrochemistry and Soil Protection "Nicolae Dimo" on the Cambic chernozem. As organic fertilizer was used sludge from wastewater treatment. Were determinate heavy metals content in soil and plants which are studied and insufucient studied in Moldova. The total content of elements (Ag, Cd, Sn, Cs, Ba, La, Rb, Sr, Mn, Fe, Cu, Zn, Br) in soils and plants was determinate in Laboratory of Nuclear Reactions "Gh. N. Flerov", Institute for Nuclear Research Unit (Dubna), X-ray spectrometric method of determination, Camberra 1000.

Results and discussion

1. Heavy metals in soils

Silver (Ag) clark in lithosphere is 0,7 mg/kg, in pedosphere - 0,1 mg/kg [10, 11]. The average content of Ag in the parental rocks is 0,35 mg/kg, in soils – 0,5 mg/kg. Optimal amount of Ag varies from 0,1 to 1,8 mg/kg [12]. The technophility (ratio of element mass produced to his clark content) of Ag is >10¹⁰ [13]. According to the results amount of Ag in soils of Moldova varies from 0,4 to 2,0 mg/kg, the avarage content is 1,2 mg/kg [14, 15]. Clear regularity in

the distribution of Ag in soil profile is not observed. According to gradation the quantity of Ag in soil can be assessed as medium and high, MPC of Ag is 1 mg\kg [16]. From this criteria, should be mentioned that in most soils of Moldova the Ag concentration is higher than MPC. In the arable layer of cambic chernozem Ag content varies within 1,0-1,3 mg/kg and is evaluated as high (table 1).

Table 1

Variant and d	epth, cm	Ag	Cs	Ba	La	Ce	Nd
Control,	0-20	1,3±0,1	11±2	510±30	50±5	110±20	40±5
N ₁₂₀ P _{4,5} K ₆₀ ,	0-20	1,0±0,1	12±1	490±30	50±5	120±20	40±5
Sludge, 80 t/ha,	0-20	1,3 ±0,1	13±2	480±30	40±5	110±20	40±5
Sludge, 80 t/ha,	20-40	1,3±0,1	18±2	500±30	50±5	110±20	40±5

Heavy metals in Cambic Chernozem clay-loamy, mg/kg

Cadmium (Cd) – pollutant, which refers to substances excessively dangerous, I class [17]. Clark's in the litoshre is 0,13 mg Cd/kg, in pedosphere – 0,5 mg/kg [10, 11]. The average content of Cd varies from 0,1-0,6 mg/kg in parental rocks, to 0,2-0,84 mg/kg in soils, averaging 0,4 mg/kg [24]. According to the previously results obtained by the spectrographic metod Cd content in soils varies in limits 1,2-1,5 mg/kg [8].

The soil samples analyzed in the years 1960- 1965, a period without intensive application of chemical fertilizers and possible pollution of soils, the average amount of Cd was 0,41 mg/kg, the limit of variation 0,18-0,84 mg/kg [18]. The other sources mentioned that the amount of Cd in soils varies within 0,2-1,2 mg/kg [19]. Other authors marked concentrations within 0,76-1,76 mg/kg [20]. Technophility of Cd is 10⁸ [13]. Annualy with fertilizers are incorporate in soil 3-4 g Cd/ha [21,22]. In the arable horizons of fertilizers soil the amount of Cd execcted MPC - 2 mg/kg. In the analyzed soil samples of cambic chernozem the amount of Cd consists 1,2-3,1 mg/kg, the average 1,9 mg/kg [14,15].

Stanium (Sn), the clark in lithosphere is 2,5 mg/kg, in pedosphere – 10 mg/kg [10, 11]. The content of Sn in rocks varies in limits 1-10 mg/kg, average 5,4 mg/kg [12]. Technophility - Sn >10⁸ [13]. MPC of Sn is 4,5 mg/kg, according to gradation, Sn amount is higher - 8-15 mg/kg [16]. MPC value of Sn 4,5 mg/kg in 2,5 times lower than the pedosphere (10 mg/kg), which considerd the restriction underestimated. In this case is proposed a concentration 20 mg/kg of Sn in soils [16]. In the arabile cambic chernoziom Sn content consists 13 mg/kg and be considered high for soils of Moldova.

Stibium (Sb) as a polluting substance refers to II class moderately hazardous [17]. Clark Sb in litisphere consists - 0,5 mg/kg, in pedosphere – 1,0 mg/kg [10, 11]. The average content of Sb in parental rocks is 0,5-3,0 mg/kg, in soils – 1-5 mg/kg, averaging 2,0 mg/kg [12]. Technophility Sb >10⁸ [13]. According to results the investigated soil contain Sb in limits 4,3-9,0 mg/kg, averaging 6,6 mg/kg [14, 15]. In conformity with gradation 4,1 mg/kg this content in soil is considered very high – MPC of Sb in soil is 4,5 mg/kg, for soils of Moldova is proposed 5,0 mg/kg [16]. Mention that Sb concentration in soil samples exceeded the MPC and incadred within the low polluted degree. Arable soil layer contains 7 mg/kg Sb and is considered to be weakly polluted.

Cesium (Cs) in the lithosphere is 3,7 mg/kg, in pedosphere - 5 mg/kg [10, 11]. The content of this element in rocks and soils of Moldova varies in limits 1-14 mg/kg, averaging - 5 mg/kg [12]. Technophility Cs >10⁶ [13]. The results show that the content of Cs in soils varies within 8-21 mg/kg, averaging 14,1 mg/kg [14, 15]. Increasing the Cs content was detected in fertilized soils with sludge in dose 80 t/ha. In this variant was found a tendency to increase the amount of Mn (1300-1590 mg/kg), Cr (100-151 mg/kg), Cu (28-34 mg/kg [8, 22]. The urbam sludge contain Mn - 160-870 mg/kg, averaging - 300 mg/kg; Cr - 70-830 mg/kg, averaging -364 mg/kg; Cu - 59-1347 mg/kg, averaging - 486 mg/kg [12]. The fertilizer increased the content of elements in soil.

Bariun (Ba) – refers to substances polluting excessively dangerous I class. Clark's in the lithosphere is 650 mg/kg, in pedosphere - 500 mg/kg [10, 11]. Ba content in rocks is 60-490 mg/kg, averaging - 360 mg/kg; in soils - 140-640 mg/kg, averaging - 460 mg/kg [12]. According to results the Ba content in soils varies within 310-540 mg/kg, averaging 452 mg/kg [14,15]. The MPC of Ba in soils is relatively small - 100 mg/kg, in this context is proposed for soil of Moldova – 1200 mg/kg [12]. In the experimental samples of cambic chernozem is founded 480-510 mg/kg, being in the moderate class of toxicity.

Strontiun (Sr) – referes to substances polluting excessively dangerous, I class. Clark Sr in the lithosphere is 340 mg/kg, in pedosphere – 300 mg/kg [10, 11]. Sr content in rocks varies within 40-800 mg/kg, averaging – 210 mg/kg, in soils – 50-400 mg/kg, averaging – 240 mg/kg [12]. MPC of Sr in soils consists – 600 mg/kg [16]. In the cambic chernoziom Sr consists in arable stratum 99 mg/kg and considered very low [8, 16].

Manganese (Mn) concerns as substances polluting excessively dangerous I class. Clark Mn in lithosphere is 1000 mg/kg, in pedosphere – 850 mg/kg [10,11]. Mn content in the rocks is 180-900 mg/kg, averaging 610 mg/kg, in soils – 150-2250 mg/kg, averaging 790 mg/kg [12]. MPC of Mn in sois is 1500 mg/kg [16]. In arable horizon of cambic chernoziom Mn content is 1320 mg/kg and considered highly toxic.

Copper (Cu) as a moderate pollutant refers to substances hazardous of II class. Clark's in the lithosphere is 47 mg/kg, in pedosphere – 20 mg/kg [10,11]. The Cu content in rocks varies within 2-34 mg/kg, averaging 22 mg/kg, in soils 2-400 mg\kg, averaging - 32 mg/kg [12]. In the arable horizon of cambic chernoziom content of Cu is 28 mg/kg, considerate moderately toxic. MPC of Cu in soils is 60 mg/kg, but it is proposed for soils of Moldovs – 150 mg/kg [16].

Zinc (Zn) as a pollutant refers to excessively dangerous substances, I class. Clark in the lithosphere is 83 mg Zn/kg, in pedosphere – 50 mg Zn/kg [10,11]. Zn content in the rocks varies within 5-162 mg/kg, averaging 65 mg/kg, in the soils - 10-166 mg/kg, averaging 71 mg/kg [12]. In the arable layer of cambic chernozem Zn amount is 39 mg/kg is considered low. MPC of Zn in soil consist 300 mg/kg, it is proposed - 250 mg/kg [16].

Lanthanum (La) clark in pedosphere is 40 mg/kg [10,11]. The amount of La in soils varies within 30-60 mg/kg, averaging 44 mg/kg [14,15]. The experimental soil contains the 40-50 mg La /kg (tab.1).

Cerium (Ce) clark in pedpsphere is 50 mg/kg [10,11]. The concentration in soils varies in limits 70-130 mg/kg, averaging 99 mg/kg. The experimental soil samples contain Ce within 110-120 mg/kg.

Neodium (Nd) content in soils varies within 30-50 mg/kg, averaging - 35 mg/kg [14,15]. In the experimental soil is founded 40-50 mg Nd/kg.

2. Heavy metals in plants

Silver (Ag) content in cereal grains varies within 0,01-0,1 mg/kg, in vegetal organs of wheat - 0,05-0,2 mg/kg [12]. According to researches, the amount of Ag in grains of winter wheat is 0,044 mg/kg, in vegetal organs - 0,026-0,035 mg/kg. The content of Ag in cereal grains in condition of Moldova less than 0,01 mg/kg is considered deficient, more than 1,0 mg/kg considered excess toxical [12]. So, the amount of Ag in winter wheat in the ecological aspects is considerate as optimal (tab.2).

Table 2

Variant	Organ	Ag	Ba	Rb	Sr	Mn	Fe	Cu	Zn	Br
Control	Grain	0,044	12	8,0	5,15	11,7	150	9	70	15,0
Control	Chaff	0,044	5	6,0	4,45	12,4	130	5	70	2,1
	Straw	0,044	8	5,6	11,68	20,1	130	5	70	3,5
$N_{120}P_{4,5}K_{60}$	Grain	0,044	12	8,0	6,43	11,7	140	9	70	15,0
	Chaff	0,032	7	3,0	3,03	14,9	140	5	70	6,0
	Straw	0,026	21	3,8	8,03	40,0	210	5	70	13,0
Sludge,	Grain	0,044	11	3,4	7,10	85,0	280	8	66	15,0
	Chaff	0,035	7	5,6	2,83	34,5	160	5	66	9,0
outina	Straw	0,035	9	2,7	13,7	81,0	81	6	66	9,0

Heavy metals in winter wheat, mg/kg of dry mass

Cadmium (Cd) content in cereal grains varies in limits 0,01-0,5 mg / kg, in vegetative organs - 0,01-0,8 mg / kg [12]. According investigation, in the variant with sludge application, 80 t/ha the quantity of Cd in grains is 0,9 mg/kg, in vegetative organs - 0,2-0,3 mg / kg. In condition of Moldova the quantity of Cd in winter wheat is consider weakly toxic, and >12 mg / kg – excessive toxic. Maximum allowable level of Cd in grains is 0,3 mg/kg, in bakery products - 0,02 mg/kg [12].

Stanium (St) content in winter wheat chaff and straw varies in limits 0,05-0,14 mg/kg.

Cesium (Cs) content in wheat grains is 0,19 mg/kg, in leaves - <0,1 mg/kg, in straws - 0,14 mg/kg.

Barium (Ba) content in wheat grains is 1-10 mg/kg, in chaff and straw - 10-50 mg/kg [12]. According to researches the amount of Ba in wheat grains is 11-12 mg/kg, chaff - 5-7 mg/kg, straw - 8-22 mg/kg, leaves - 18-20 mg/kg. Ba amount in grains exceeds the normal content. In condition of Moldova Ba content in cereal grains <1 mg/kg

considered weakly dangerous and >200 - surplus of content [12].

Lanthanum (La) content in wheat grains is 0,6 mg/kg, in leaves - 0,4 mg/kg, in the stems - 0,6 mg/kg. As mentioned above, the amount of this element in soil and plants is studied insufficiently.

Rubidium (Rb) content in cereal grains varies within 1-5 mg/kg [12]. In wheat grains investigated Rb amount varies in limits 3,4-9,9 mg/kg, chaff – 3-6 mg/kg, leaves – 4,0-4,8 mg/kg, strain - 2,7-5,6 mg/kg. The small amount of Rb was detected in grains on variant with sludge application.

Strontium (Sr) content in cereal grains is 1-15 mg/kg, in vegetal organs – 30-140 mg/kg [12]. According investigations the quantity of Sr in winter wheat grains consists – 15,5 mg/kg, pulp - 13 mg/kg, in chaff - 2,83-4,45 mg/kg, leaves - 17,6-25,6 mg/kg, stems - 8,03-13,7 mg/kg. The quantity of Sr in wheat grains <10 considered poor and >200 – toxical [12].

Conclusion

The concentration of Ag, Sb, Sn, Ba in the soil exceeds the MPC. High content of Cd in the arable layer of

cambic chernoziom can be explained by intensive management of mineral fertilizers containing Cd as ballast. In the variant with sludge (80 t/ha) the content of Cs increased from 11 to 18 mg/kg, Mn - 1230 - 1590 mg/kg, Cr - 100 - 151 mg/kg, Cu - 28 - 34 mg/kg. The quantity of Cd, Fe, Zn, Ba in wheat grains exceeds MAC. In grains accumulation of Br, Rb, Mn, Zn, Fe, Cu is optimal.

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ADEQUATE UV EXPOSURES FOR HEALTHY LIFE: IN SITU MONITORING AND MODEL CALCULATION OF THE VITAMIN-D-SYNTHETIC CAPACITY OF SUNLIGHT

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Abstract: Vitamin D which is formed upon UV solar radiation in human skin is essential in many physiological functions. To estimate beneficial vitamin-D-synthetic capacity of sunlight a bio-equivalent UV dosimeter that is based on the same molecular photochemistry from which vitamin D is photosynthesized in human skin has been developed. The examples of an *in situ* monitoring of the vitamin-D-synthetic capacity of sunlight using an *in vitro* model of vitamin D synthesis are presented, and various operational principles of the UV biodosimeter are discussed. In addition, reliable algorithm is presented for direct calculation of previtamin D₃ accumulation using the photoreaction mathematical model with solar UV spectra as input data. Critical dependence of previtamin D₃ accumulation on cloudiness and aerosols is demonstrated.

Keywords: Solar UV radiation, vitamin D synthesis, UV monitoring, UV biodosimeter, mathematical model.

Introduction

It is well understood now that the appropriate doses of UV sunlight are beneficial for humans initiating vitamin D^{1*} synthesis in skin as opposed to excessive UV exposures that are generally associated with acute and chronic health effects (erythema, skin cancer, immune system suppression, cataract, etc.). Natural exposure to sunlight is responsible for maintaining adequate vitamin D nutrition for most of the population in the world, and the importance of sunshine as a therapeutic measure for preventing the bone deforming diseases is now appreciated. Moreover, in addition to the well-established effects of vitamin D on maintenance of mineral homeostasis, recent evidences suggest a wider biologic role of vitamin D not primarily related to mineral metabolism [2-4]. The findings of recent epidemiological studies [5] demonstrate that vitamin D can significantly reduce the risk for several types of cancer (breast, colon, kidney, myeloid leukemia, ovary, pancreas, prostate), and the cancer mortality rates are correlated inversely with local solar UV-B doses. The most likely mechanism whereby solar UV-B radiation provides protection against cancer is natural production of vitamin D.

Sunlight has long been recognized as the main source of vitamin D_3 for humans, and all higher vertebrates as well as most plants, zooplankton, avian species and aquatic mammals have an endogenous mechanism involving solar UV radiation that affects the synthesis of vitamin D from its precursor [6].

These facts testify that an *in situ* control of solar UV radiation with respect to its ability to initiate vitamin D synthesis demands particular care, especially in view of dramatic effect of seasonal and latitudinal changes of the UV-B intensity on the vitamin D synthetic capacity of sunlight [7]. (There are numerous observations that people previously living near equator have severe vitamin D deficiency and the associated health problems when moving to the North). It is just sharp decrease in provitamin D absorbance spectrum from its maximum at 282 nm to the longer wavelengths that implies selective sensitivity of vitamin D synthesis solely to the UV-B part (280 – 315 nm) of solar spectrum which is very changeable (Figure 1). The solar zenith angle change (daily, seasonal and latitudinal) is the governing factor, but clouds, aerosols, albedo and air pollution also affect the UV-B irradiance at the Earth surface. Just adverse effects of air pollution include primary health effects, environmental acidification, and possible long-term changes in the global climate and in UV radiation levels. As one can see from Figure 1, the absorption spectrum of SO₂ overlaps UV-B region and therefore increase in its level due to volcanic eruptions can inhibit the vitamin D synthesis.

¹ * Vitamin D is represented by cholecalciferol (vitamin D3) and ergocalciferol (vitamin D2) that are structurally similar secosteroids derived from the UV irradiation of provitamin D sterols. (Secosteroids are steroids in which one of the rings has broken). In vertebrates vitamin D3 is produced *in vivo* by the action of sunlight on 7-dehydrocholesterol (7-DHC) in the skin. Vitamin D2 is produced in plants, fungi and yeasts by the solar irradiation of ergosterol.

Vitamin D2 and vitamin D3 differ structurally only in the C-17 side-chain, which in vitamin D2 has a double-bond and an additional methyl group. Irradiation of parent steroid results in breakage of the B-ring at the 9,10-carbon bond, resulting in the conjugated triene system of double bonds in. previtamin D molecule [1].



Figure 1. Absorption cross section of 7-DHC (provitamin D3) molecule *versus* solar UV spectra calculated by FASTRT program [8] (<u>http://nadir.nilu.no/~olaeng/fastrt/</u>) for Kiev (50°27'N, 30°31'E), June, 22 and December, 22, 10:00 GMT, total ozone column 300 DU, clouds- and aerosol-free sky (a), and absorption cross section of gaseous molecules SO, and NO₂ [9] (<u>www.atmosphere.mpg.de/spectral-atlas-mainz</u>) (b).

Methodical part

Prediction of biologic effects of solar radiation on human health, in particular, involving terrestrial level of solar UV radiation, requires development of adequate methodological strategy.

But as far as excessive UV exposures cause negative health effects, in most cases biological activity of solar UV radiation is calculated by weighting solar UV spectra with CIE erythema action spectrum [10]. Yet, the beneficial vitamin D synthetic capacity of sunlight cannot be correctly estimated by this way in view of significant difference between the erythemic and the vitamin D synthesis action spectra [11, 12]. With due regard to the essential role of vitamin D_3 for human health we have developed a bio-equivalent UV dosimeter that is based on the same molecular photochemistry from which vitamin D is photosynthesized in human skin, i.e. 7-dehydrocholesterol (provitamin D_3) photoconversion [13-17].

The complex network of vitamin D synthesis consists in the two stages of monomolecular isomerizations [16]. At the first stage UV irradiation of provitamin D within its absorption band (240-315 nm) yields previtamin D, which further is converted into vitamin D (Figure 2) and just amount of previtamin D accumulated during an UV exposure is a measure of biologically active UV dose.

However, upon UV-B radiation previtamin D itself undergoes a number of side photoconversions. As a result, UV irradiation of initial provitamin D gives rise to formation of multicomponent photoisomer mixture which composition is highly dependent on the irradiation wavelength [19, 20].

1. Original spectrophotometric (SPM) analysis which takes into account irreversible photodegradation has been designed to follow the photoreaction course in real time as alternative to the time-consuming HPLC analysis [15]. This opened the way to an *in situ* UV monitoring using the photochemical stage of vitamin D synthesis (*in vitro* model).



Figure 2. Simplified scheme of vitamin D3 synthesis from initial 7-Dehydrocholesterol.

With this aim solution of 7-dehydrocholesterol (provitamin D_3) in ethanol is exposed to sunlight in rectangular quartz cuvette, and the solution absorption spectrum is recorded before and after an exposure with UV spectrophotometer. The previtamin D concentration (accumulated 'antirachitic' UV dose) is determined by computer processing of the recorded spectra using specially designed computer program which allows deriving the photoisomer concentrations based on the photoisomer individual absorption spectra [21]. An important point is that the ambiguity in the solution of the over determined system of equations requires expertise in pursuance of concentration analysis. Therefore, for general use, the simplified method of UV dosimetry was introduced [17] similar to a number of chemical UV dosimeters as, for instance, polysulphone film, that measured an accepted UV dose by the absorbance decrease at the fixed wavelength [22].

2. Previous data on transformation of provitamin D absorption spectrum during the summer day exposure to sunlight in Greece [14] suggest that the 'antirachitic' UV dosimetry can be carried out by recording the absorbance decrease at $\lambda = 282$ nm as a function of exposure time after a calibration procedure. As was shown in [17], the absorbance decrease plotted as a function of the previtamin D concentrations determined by the spectrophotometric analysis was described rather well by a linear approximation Y = A + Bx: (A = 0.995, B = -0.0096, R = -0.996, SD = 0.0069, R = -0.0069, R = -0.0

correlation coefficient, SD – standard deviation). Therefore the previtamin D concentration ($C_{Pre}(\%)$ can be calculated

from the absorbance A at $\lambda = 282$ nm before (A_{282}^0) and after (A_{282}^t) UV irradiation using the formula:

$$C_{\rm Pre}(\%) = (1 - A_{282}^t / A_{282}^0) / 0.01 \tag{1}$$

Although comparison of calculated previtamin D concentrations during long term monitoring of 'antirachitic' solar UV radiation in Kiev (50°23'N, 30°32'E) showed larger data scatter, nevertheless, the correlation coefficient R (between previtamin D concentrations determined by means of the optical density decrease at 282 nm and those ones determined by spectrophotometric analysis) was rather high (R = 0.77) within upper and lower 90% prediction limits [17].

3. These results provided a basis for the new method of *in situ* determination of a vitamin D synthesizing amount of natural and artificial UV irradiation [23]. The method comprises: (1) exposing a polymer film doped with 7-dehydrocholesterol to an UV radiation the vitamin-D synthesizing amount of which is to be determined; (2) measuring change of absorbance of the film before and after an exposition to the UV radiation using specially elaborated opto-electronic device (or UV spectrophotometer); and (3) correlating the measured change of the absorbance to a previtamin D synthesizing amount.

4. Finally, to visualize the process of previtamin D photosynthesis and provide the easiest detection of previtamin D synthesis the optically active 7-DHC (provitamin D_3) molecules were dissolved in liquid crystalline (LC) matrix [24]. It was assumed that alteration of molecular geometry of provitamin D by the photoinduced conversion into previtamin D should affect the selective reflection and, as a result, the LC cell color [25, 26]. This could provide the easiest detection of previtamin D synthesis and evaluation of the accumulated UV dose *in situ* by comparison of the LC cell color with the calibration scale (like litmus paper measures a solution pH).

5. Additionally, the vitamin D synthetic capacity of sunlight can be directly calculated using adequate mathematical model which enables calculation of the photoreaction kinetics for any UV radiation source with known spectral irradiance, and the results of the laboratory and field tests lent validity of both the model and the spectrophotometric analysis [14, 17].

The kinetics of provitamin D photoisomerization is described by the following system of rate equations (2) [14]:

$$\frac{dC_1}{dt} = \int d\lambda I^*(\lambda) \left[-\varepsilon_1(\lambda)C_1\phi_{12} + \varepsilon_2(\lambda)C_2\phi_{21} \right]$$

$$\frac{dC_2}{dt} = \int d\lambda I^*(\lambda) \left[-\varepsilon_2(\lambda)C_2\phi_{21} + \varepsilon_1(\lambda)C_1\phi_{12} - \varepsilon_2(\lambda)C_2\phi_{23} + \varepsilon_3(\lambda)C_3\phi_{32} - \varepsilon_2(\lambda)C_2\phi_{24} + \varepsilon_4(\lambda)C_4\phi_{42} - \varepsilon_2(\lambda)C_2\phi_{25} \right]$$

$$\frac{dC_3}{dt} = \int d\lambda I^*(\lambda) \left[-\varepsilon_3(\lambda)C_3\phi_{32} + \varepsilon_2(\lambda)C_2\phi_{23} \right]$$

$$\frac{dC_4}{dt} = \int d\lambda I^*(\lambda) \left[-\varepsilon_4(\lambda)C_4\phi_{42} + \varepsilon_2(\lambda)C_2\phi_{24} \right]$$

$$\frac{dC_5}{dt} = \int d\lambda I^*(\lambda) \left[\varepsilon_2(\lambda)C_2\phi_{25} \right]$$
(2)

where C_i are the photoisomers concentrations, $\varepsilon_i(\lambda)$ the molar absorption coefficients and ϕ_{ij} the quantum yields of the photoreaction in the channel $i \Rightarrow j$. Photoisomers *Provitamin D*, *Previtamin D*, *Tachysterol*, *Lumisterol* and irreversible photoproducts *Toxisterols* are designated by the numbers 1, 2, 3, 4, 5 respectively.

 $I^*(\lambda)$ is co-called photokinetics factor: $I^*(\lambda) = I_0(\lambda) \frac{1 - 10^{-A(\lambda)}}{A(\lambda)}$, (3)

where $I_0(\lambda)$ is the intensity of the incident light at the wavelength λ , and $A(\lambda)$ is the solution absorbance:

$$A(\lambda) = l \sum_{i=1}^{5} \varepsilon_{i}(\lambda) C_{i}$$
(4)

The initial conditions for the concentrations are $C_1 = 100\%$ and $C_2 = C_3 = C_4 = C_5 = 0$.

The system of rate equations (2) can be numerically solved for any monochromatic or/and polychromatic light source with the intensity distribution $I_0(\lambda)$. To estimate the vitamin D synthetic capacity of sunlight at any latitude the solar spectra (either calculated using a radiative transfer model or measured by a spectroradiometer) might be used at the model input.

Results and Discussion

The two cuvettes of 0.5cm thickness with 7-DHC ethanol solution (C = 20 mkg/ml) were exposed at the roof of the Institute of Physics building in Kiev during 3 hours around noon (from 11:30 to 14:30 local time). Solar rays were kept normal to the cuvette surface by using a specially constructed mechanism for automatic tracking of the solar zenith angle. Further, the change of optical density at $\lambda = 282$ nm was determined for each measurement and concentrations of previtamin D were calculated using equation (1). The examples of the 7-DHC spectrum changes as a result of exposure to sunlight in summer and in autumn are shown in Figure 3.



Figure 3. Absorption spectra of 7-DHC in ethanol before (solid line) and after (dash line) exposure to sunlight during 3 hours in early July (clear sky) (a) and September (scattered clouds) (b), and (c) concentrations of previtamin D calculated from the spectra using formula (1).

It is seen from Figure 3c that two previtamin D concentrations occur just within $\pm 90\%$ prediction limit. The examples of calculated kinetics of previtamin D accumulation using the system (2) and FASTRT program [8] for the three hours exposure to sunlight depending on the atmosphere properties (cloudiness and aerosol content) are shown in Figure 4.



Figure 4. (a) Calculated kinetics of previtamin D photosynthesis *in vitro* on June 22 in Kiev (SZA = $27^{0}52^{\circ}$, ozone 300 DU depending on cloudiness (cloud liquid water column = 400 gm-2 for clouds fractions 25-100%, aerosol free sky, albedo 0, exposure time 180 min), (b) calculated effect of aerosol content on previtamin D photosynthesis *in vitro* (β = aerosol optical thickness at 1 µm) [8].

It is apparent that effect of air pollution on previtamin D synthesis can be also estimated using the equations system (2) with solar spectra corrected for the absorption of gaseous air pollutants.

Conclusion

Initiation of endogenous synthesis of vitamin D in human skin is important biological function of solar UV-B radiation. Air pollution and the increase in tropospheric ozone hinder penetration of solar UVB to the Earth surface. Nowadays emerging research indicates that Vitamin D is more important to human health than previously thought. Vitamin D is recognized as a critical hormone that helps regulate the health of more than 30 different tissues, from the brain to the prostate [4].

Many ecologic, cohort and case control studies have shown that cancer mortality and in some cases cancer incidence depend inversely on ground level UV radiation. Prostate, colorectal, and breast cancers are most studied, although new data has shown that non-Hodgkin lymphoma [27, 28] and even survival with melanoma [20] are also inversely associated with sun exposure.

It is suggested that the major reason of a protective effect of sun exposure in the development of cancer and improved survival is just vitamin D synthesis which is a critical component of cellular networks that inhibit cellular proliferation and encourage apoptosis [30]. Therefore for validating the hypothesis many researchers today are focused on the measurements of sun exposure, serum vitamin D levels (and associated metabolites), and genetic variants that may affect vitamin D synthesis.

Although there are both beneficial and detrimental UV effects, it is an increase in the detrimental effects, associated with climate change, attract most attention. Concerns range from human health (increases in the incidence of skin cancer [31] to agriculture (reduction in crop yields), and to aquatic ecosystems (changes in the world's oceans) [32].

Recent findings on Europe's darker atmosphere in the solar UV-B [33] are in line with anticipated direct health consequences of climate change that include increases in air pollution-related illness. The studies in Canada and the United States indicated a high incidence of vitamin D deficiency in almost all populations, and chronic diseases associated with vitamin D deficiency are 25 to 50 percent more frequent in northern climates than among people living closer to equator [5, 34].

We think that global UV mapping as well as annual and daily forecasts should consider both erythemal and 'antirachitic' solar indices, and presented methods provide a means for direct monitoring of the vitamin D synthetic capacity of sunlight and the presented algorithm is useful for predictions of climate change effects on the vitamin D synthesis

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ENVIRONMENTALLY FRIENDLY COMPLEXONES. THE THERMODYNAMIC CHARACTERISTICS OF THE FORMATION OF Al³⁺ ION COMPLEXES WITH ETHYLENEDIAMINEDISUCCINIC ACID IN AQUEOUS SOLUTIONS

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Abstract: Complex formation between Al³⁺ and ethylenediamine - N,N'-disuccinic acid (H₄L) was studied at 25°C against the background of 0.1, 0.5, 1.0 N solutions of KNO₃ by potentiometry and mathematical modeling. The extrapolation of concentration constants to zero ionic strength was used to calculate the thermodynamic constants of the formation of the AlL⁻, AlHL complexes using an equation with one individual parameter (log β^0 = 16.27 ± 0.07, 9.19 ± 0.2 respectively).

Keywords: aluminum, ethylenediaminedisuccinic acid, derivatives of ethylenediaminedisuccinic acid, complex formation, complexones, thermodynamic constants, concentration constants.

1. Introduction

Chelating derivatives of succinic acid - iminodisuccinic acid (IDS) ethylendiamindisuccinic acid (EDDS), have been created by the Department of Inorganic and Analytical Chemistry, Tver State University, are biologically active compounds and open up entirely new opportunities for process control in wildlife. The growing interest in coordination compounds of metals, having the biologically active properties, due to their high activity as the means against chlorosis of plants, as catalysts of metabolic reactions, as stabilizers of different degree of oxidation of metals, as the vehicle of physiologically important molecules, atoms and cations across membranes cells.

With increasing environmental problems is increasing interest in biologically active coordination compounds with new chelator-containing fragments of natural aminoacids. These include ethylenediamine - N,N'-disuccinic acid (H₄L, EDDS), which contains in its molecule of succinic residues and aspartic acids.



Earlier [1], data on the stability of medium EDDS complexes with aluminum were studied $lg\beta = 13,5$. However, the paper does not mention the possible existence of protonated complexes. It is also not considered the effect of ionic strength on the complexation. To identify the concentration dependence of the stability constants, we carried out studies of EDDS - Al³⁺ - water at different ionic strength and calculated thermodynamic stability constants of aluminum etilendiamindisuccinates.

2. Experimental

To calculate the values $lg\beta$ the method of potentiometric titration at various ionic strengths in a KNO₃ medium at 298.2 K was used.

EDDS was synthesized by the interaction of ethylenediamine with maleic acid [2]. Working solutions (0.005 M) were prepared gravimetrically from dry complex one at the day of measurments. The concentration of solutions was controlled by potentiometric and complexonometric titration. of aluminum nitrate were prepared from weighed amounts of the salt of kh. ch. (chemically pure) grade, their concentration was determined by complexonometry [3]. Solutions of potassium nitrate were prepared gravimetrically from KNO₃ of kh. ch. grade. The salt was doubly recrystallized. Carbonate free 0.05 M solutions of NaOH were prepared from a 50% solution of NaOH by dissolving its necessary amount in preliminarily boiled water. The solutions were standardized by titration of preliminarily prepared sodium hydrophthalate. To broaden the working range of pH values, a standard solution of nitric acid prepared from concentrated HNO₃ and standardized against sodium tetraborate was used.

Potentiometric measurements were performed with an accuracy of ± 0.05 pH units on an Akvalon pH-410 pHmeter-millivoltmeter using an ESLK - 01.7 combined pH electrode. The system was calibrated against standard buffer solutions with pH values of 1.68 and 9.18. Titration was performed in a temperature controlled cell at 298.2 K. The ionic strength value was changed by adding the necessary amount of potassium nitrate. The number of parallel measurements was no less than 4. The experimental data were processed using the AUTOEQUIL universal computer program [4]. Its algorithm allows significant complex forms to be determined from a general sample of admissible forms and the corresponding formation constants to be calculated under the conditions of an automatic search for the model correctly describing pH-metric data.

3. Results and discussion

The ratio between the concentrations of Al^{3+} and H_4L in the solutions studied was 1:1. The titration curves of the $c_{Al}: c_{EDDS} = 1:1$ systems (Fig. 1, curve 2) were different from the titration curve of pure H_4L acid (Fig. 1, curve 1) already at the initial pH values; that is, along with the step dissociation of H_4L , complex formation processes occurred in the system studied. We also titrated the $c_{Al}: c_{EDDS} = 1:2$ system. The character of the curves coincided; that is, the conclusion could be drawn that the system contained no biligand complexes. All the subsequent measurements were therefore performed only at a $c_{Al}: c_{EDDS} = 1:1$ ratio.



Fig. 1. Potentiometric titration curves of (1) H_4L (0,0010 mol/l) and (2) Al^{3+} : $H_4L = 1$: 1. Titrant 0,0510 M NaOH (T = 298.2 K, ionic strength 0,5 (KNO₂)). Open circles are measurement results, lines correspond to calculations.

The resulting titration curves can be divided into two areas. In an acidic medium and the pH was observed the formation of protonated and medium-sized complexes. In the alkaline pH region is clearly flow processes to form various hydroxocomplexes, but to get the model adequately describes this region of the titration curve was not possible. Therefore, the calculation was carried out at pH less than 7.

Were used H^+ , L^{4^-} , Al $^{3+}$ as basis particles in the calculations. The following equilibria were taken into account:

$H_2O = H^+ + OH^-,$	(1)
$H_4^{-}L \longrightarrow H^+ + H_3L^-,$	(2)
$H_1L^- = H^+ + H_2L^{2-},$	(3)
$H_2L^2 \longrightarrow H^+ + HL^3$,	(4)
$HL^{3-} = H^+ + L^{4-},$	(5)
$Al^{3+} + OH^{-} = AlOH^{2+},$	(6)
$AlOH^{2+} + OH^{-} = Al(OH)_{2}^{+},$	(7)
$Al(OH)_{2}^{+} + OH^{-} = Al(OH)_{3}^{+},$	(8)
$Al(OH)_3 + OH^- = Al(OH)_4^-$.	(9)

We used the constants of the dissociation of H4L and aluminum hydrolysis obtained at various solution ionic strengths under the conditions indentical to those used in studies of aluminum interaction with EDDS. The values obtained closely agreed with each other and the literature data [5-8]. The results of this study are presented in the table.

The determination of aluminum hydrolysis constants showed that the concentration of $AlOH^{2+}$ formed in reaction (6) under the experimental conditions was negligibly low. Therefore, when calculating the stability constants of complexes of this equilibrium is not taken into account.

Logarithms of stability constants lgß at 298.2 K							
Doutiele							
Farucie	0	0.1	0.5	1,0			
$AlOH^{2+} + OH^{-} \implies Al(OH)_{2}^{+}$	9.0* [5]	9.13 ± 0.10	8.99 ± 0.20	8.93 ± 0.10			
$Al(OH)_2^+ + OH^- \implies Al(OH)_3$	8.5* [5]	7.94 ± 0.10	7.37 ± 0.10	7.36 ± 0.10			
$Al(OH)_3 + OH^- \implies Al(OH)_4^-$	4.6* [5]	4.63 ± 0.10	4.58 ± 0.20	4.58 ± 0.20			
L ⁴⁻ + H ⁺ + HL ³⁻	10.73 ± 0.16	10.02 ± 0.02	0.65 + 0.02	$10,03 \pm 0.03$			
	11,12 [6]	9,82 [7]	$9,05 \pm 0.02$	10.10 [6]			
	7.23 ± 0.07	$6,70 \pm 0.02$	6.57 + 0.02	$6,89 \pm 0.04$			
$HL^3 + H^+ - H_2L^2$	7,52 [6]	6,83 [7]	$0,37 \pm 0.03$	6.65 [6]			
	4.01 ± 0.09	3.68 ± 0.03	2.57 ± 0.02	$3,88 \pm 0.05$			
$H_2L^2 + H^+ - H_3L$	4.28 [6]	3.86 [7]	3.37 ± 0.03	3.81 [6]			
····	2.68 ± 0.2	2.68 ± 0.05	2.50 ± 0.00	3.03 ± 0.07			
$H_{3}L + H^{+} - H_{4}L$	3.45 [6]	2,40 [7]	2.30 ± 0.09	3.28 [6]			
$Al^{3+} + HL^{3-} \implies AlHL$	9.19 ± 0.2	7.29 ± 0.2	7.38 ± 0.07	7.41 ± 0.2			
$Al^{3+} + L^{4-} \implies AlL^{-}$	16.27 ± 0.07	$13,86 \pm 0.1$ 13,52 [1]	13.15 ± 0.06	13,39 ± 0.1			

The values found for reactions of $lg\beta$ etilendiamindisuktsinatov aluminum at 298.2 K and I = 0,1; 0,5; 1,0 (KNO₃) are presented in the Table.

* Values recalculated from hydrolysis constants

According to the survey were diagrams of the distribution of various forms of complex Al^{3+} with H_4L (Fig. 2).



Fig. 2. The concentration diagram of complex forms of Al^{3+} with H_4L distribution

Concentration equilibrium constants depend on experimental conditions. Of considerable interest are thermodynamic constants β_0 , which are more objective characteristics of ionic equilibria and are independent of the nature and concentration of solvents. They were calculated by the extrapolation of the data obtained (fig.3) at fixed ionic strength values to zero ionic strength using the equation with one individual parameter [9]:

$$\lg \beta_c - \frac{\Delta Z^2 A_{\gamma} I^{1/2}}{1 + 1.16 I^{1/2}} = \lg \beta_0 + bI$$

where β_c and β_0 are the concentration and thermodynamic stability constants, respectively; ΔZ^2 is the

difference of the squares of charges of reaction products; A_{γ} is the limiting Debye law constant (0.5108 [10] at 298.2 K), I is the ionic strength of the solution, and b is the empirical coefficient.



Fig. 3. Graphical determination of the thermodynamic stability constants of complexes at 298.2 K: 1 – AlL, 2 – AlHL.

The resulting thermodynamic stability constants (Table) can be used for reliable interpretation of the results of calorimetric studies of complex formation involving ethylenediaminedisuccinic acid.

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HEAVY METALS IN THE ECOSYSTEM COMPONENTS AT "DEGELEN" TESTING GROUND OF THE FORMER SEMIPALATINSK TEST SITE

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Abstract. The ecological situation in the former Semipalatinsk test site is characterized by a combination of both radiative and "nonradiative" factors. There were investigated near-portal areas of the tunnels with water seepage at "Degelen" site. All the tunnel waters are characterized by higher concentrations of uranium, beryllium, and molybdenum. The watercourse of the tunnel # 504 is unique for its elemental composition, in particular, the content of rare earth elements, whose concentration in the water is in the range $n*10^{-5} - n*10^{-7}$ %. Of all the rare earth elements in the samples were found 13, the concentrations of aluminum, manganese, zinc are comparable to the concentrations of macro-components. Concentration of ²³⁸U in the studied waters lie in the range of $n*10^{-4} - n*10^{-6}$ %, which suggests the influence of uranium, not only as a toxic element, but its significance as the radiation factor. The analysis of complex data obtained showed that the elevated concentrations of heavy metals in the soils of the areas under study, as a rule, are a consequence of the carry-over of these metals by water flows and their subsequent deposition in the sediments.

Keywords: tunnel, lanthanides, element, STS, Degelen.

Introduction

The Semipalatinsk test site is one of the three largest testing sites in the world. The nuclear tests were conducted not throughout the range, but on the testing grounds. A total of 456 nuclear tests (616 nuclear explosions) were carried out. The total area of the STS is 18 500 km². "Degelen" site was used for nuclear testing of medium and small capacity. The site is located on the same mountain range, which is a dome-shaped elevation with the size of 17-18 km in diameter; total area is about 300 km². The underground nuclear tests were conducted in tunnels. A tunnel is a horizontal excavation in length from a few hundred meters to 2 km, with a hole diameter of about 3 meters. Total for the period 1961-1989 209 nuclear tests was conducted in 181 tunnels. From 1996 to 1998 at "Degelen" site operations on the closure of the tunnels were carried out [1].

For many years, the Semipalatinsk test site has been thought to be a source of radiation hazard, excluding the effect of other factors on the environment. However, there is evidence of high concentrations of heavy metals in soil and water [2].

The areas adjacent to the mountain range "Degelen" were investigated during 2 years, more than 40 chemical elements were studied. Abnormally high contents of a number of elements were revealed. Areal distribution of the elements by their concentrations is differentiated, expressed by local spots of irregular shape in all areas [2].

In addition, in the STS there is evidence of finding the deposits of minerals such as tungsten, beryllium, and molybdenum. There are zirconium, antimony, and tin. The western slope of the Degelen Mountains has a group of greisen bodies with a tungsten mineralization forming the ore field. The greatest number of greisen bodies enclosing rare-metal mineralization is concentrated on Southern and Northern parts. The ores contain wolframite, hematite, pyrite, sphalerite, galena, scheelite, molybdenite, beryl and other minerals. Thus, the study of heavy metals in the components of the ecosystem, having a negative impact on the natural environment, is necessary [2].

Many of the tunnels at "Degelen" site are characterized by having water seepage. The watercourses are observed in more than 50 tunnels. The watercourses are seasonal and occur in autumn and spring seasons. Continuous water seepage is observed in 8 tunnels: # 104, 165, 176, 177, 504, 506, 511, 609, which were selected as objects of study (Figure 1) [1].

The goal of this paper is to investigate the contamination levels of the ecosystem components with heavy metal and mechanisms of its formation.

To achieve the goal there were investigated the vegetation and soil, made geobotanical description of the near-portal areas of tunnels with water seepage at "Degelen" site, determination of communities and dominant plant species growing in the area adjacent to the near-portal areas and stretching all the way along the stream. The chemical composition of tunnel water, the content of heavy metals in soil and vegetation, as well as the contamination mechanism of the ecosystem components with heavy metal were investigated. To assess the mechanisms for contamination levels of soil and vegetation with heavy metal there were analyzed samples of soils, plants, taken from the profiles of watercourses perpendicularly crossing the bed from where water flows out to the surface and to the end of the channel.



Figure 1. Location of the tunnels with water seepage at "Degelen" Mountains Massif

Experimental Part

The tunnel water, soil and plants were selected as subject of research.

Sampling of water. To assess the content and changes in the concentration of heavy metals in the water the water samples were collected where the watercourse flows out to the surface. Each month, a different amount of water samples were taken due to changes in water flow rate of the watercourse at each tunnel.

In order to assess changes in the concentration of heavy and toxic elements in water down-stream in the period 2010 - 2011 water samples were taken at the point where the watercourse flows out to the surface and every 50 meters watercourse downstream to a distance of 300 m.

The water was sampled in accordance with GOST 17.1.5.05-85 "General requirements for sampling of surface and marine waters, ice and atmosphere". The main activities at water sampling were: water filtration to remove mechanical impurities through a paper filter "white ribbon" (lit. "belaya lenta"), conservation of samples by adding concentrated "highly pure" nitric acid (HNO₃) at a rate of 3 ml of HNO3 to 1 liter of water sample. Water samples were collected in clean polypropylene 1 litre bottles with screw-cap. Conservation and filtration of the samples were conducted at sampling site. The conserved samples were stored prior to analysis in a cool dark place for 2-7 days [1].

Soil sampling. To determine the level of soil contamination at the estuarine areas of the tunnels at Degelen mountain massif the soil at tunnel \mathbb{N} 177, 504 was sampled. Sampling was carried out as follows: on the profiles (each tunnel had 7 profiles, each profile had 5 points) perpendicularly to the direction of a stream bed, the distance between the profiles is 50 m, the sampling step is 10 m. The central sampling point was laid directly in the stream bed. Samples were collected by puncture with a metal soil sampler. The depth of sampling is 0-5 cm and sapling area is 100 cm².

To determine the boundaries of soil contamination near the tunnel #504 the soil samples were collected along the streambed with a broader area of the point locations than in 2010, since according to the last year data the area of contamination at the near-portal area was much higher than expected. Thus, sampling was carried out on the profiles (3 profiles, 5 points at each profile) perpendicularly to the direction of a streambed, the distance between the profiles is 200 m, the sampling step is 100 m. Samples were collected in accordance with GOST 17.4.3.01-83 "The Nature Conservancy. The soil. General requirements for sampling". There were taken 20 soil samples. Soil sampling was carried out by puncture with a depth of 0-5cm [3, 1]

Sampling of plants. To study the migration of heavy metals in the system "soil - plant" several near-port sites were investigated. The vegetation cover was described, the communities and dominant species were identified [1,2]. In the territory under study the following plants were sampled: bush grass (*Calamagróstis epigéjos*), southern reed (*Phragmites austrális*), naked-leaved briar (*Rosa glabrifolia*), weeping birch (*Bétula péndula*), gray willow (*Salix cenerea*). The plants were collected at different sites, conventionally separated by contents of heavy metals and radionuclides in the soil. The plants were taken according to profiles previously laid for the soil sampling (7 profiles, 5 points at each profile). Each profile crossed the stream bed perpendicularly to flow. The profiles for plant sampling were placed in the same points as for the soil samples. The number of points in the profiles for the plant samples depended on species diversity. The number of sampling points in each profile ranged from 2 to 5, depending on the species grown. Sine qua non was the discovery of at least one recurring species for all the profiles for comparative analysis for heavy and toxic elements as distance from the center of the bed and the tunnel portal. All the requirements were met by such plant species as bush grass (*Calamagróstis epigéjos*). The plant samples were collected from 1 m².

Preparation of water samples for analysis. Preparation of the water samples directly before the analysis was as follows: 20 ml aliquot of solution was taken, diluted in a ratio of 1:5 and analyzed for the content of elements interested.

Preparation of soil samples for analysis. The soil samples preparation consisted of drying the soil, grinding and acid leaching. The soil samples were dried in an oven at 70°C for 5 hours. Then, for grinding purpose a 100 g weighed sample was taken from the dried sample by the method of quartering. The grinding was carried out manually by the abrasion of the sample in a porcelain dish to a particle size ≤ 250 mesh.

Leaching was conducted in accordance with "Methods of sample preparation in analytical autoclave NPVF "Ancon-AT-2. The soil. Biological objects of analysis" (MI 2339-95, VNIIMS, Moscow).

A 200 mg weighed sample was placed in a Teflon liner and was added in small portions of 10 cm³ 7M nitric acid. Then the Teflon liner was inserted in a Teflon "bomb" and autoclave decomposition was made for 2.5 h at 150 C. After autoclaving, cooled sample was transferred to a centrifuge tube and centrifuged for 10 min at a frequency of rotation of 4000 rpm, then the centrifugate was transferred in a 15 cm³ measuring tube, and the precipitate was washed with 5 cm³ 7M nitric acid and again centrifuged. The centrifugate and wash solution was combined and brought to a volume of 15 cm³ with 7M nitric acid. Thus obtained stock solution and diluted 1:10 was analyzed for chemical elements [3,4].

Preparation of plant samples for analysis. Preparation of plant samples consisted of weighing (green weight), washing of each sample with distilled water, drying, grinding, homogenization, determination of dry weight, sifting through a sieve with a diameter of 0.200 mm. Next up to 5 g weighed samples were taken, then weighed on an analytical balance at 2 replications with mass of 0.5-1, and decomposed by the acid autoclave decomposition.

Acid decomposition of plant samples for elemental analysis was as follows:

A weighed sample of 0.5 g of dried (grinded) analytical sample was placed into a reaction glass, wetted with bidistilled water and then nitric acid in an amount of 5 ml was added. The weighed sample was placed in an autoclave and heated in a laboratory oven during 30 minutes. After that, the weighed sample in a collection container (glass) was placed in a drying oven heated to a temperature of $160 \pm 2^{\circ}$ C and was heated for 2.5 hours. For the control experiment (blank) another reaction glass was placed a 6 cm³ of concentrated nitric acid without addition of sample and 1 ml bidistilled water. At the end of the decomposition process the containers were cooled to room temperature. After that the weighed sample was added 1.5 cm³ of hydrogen peroxide. The blank sample underwent the same procedure. Then the weighed samples were again dried in the drying oven for 1.5 hours. Further the cooled to room temperature weighed sample were transferred to a measuring flask and was adjusted to a volume of 15 cm³ with 1% solution of nitric acid and packed in polypropylene tubes with screw caps (vials).

If there was a precipitate the solution was centrifuged, the supernatant fluid was transferred into a measuring tube, and then the volume was adjusted to 15 cm³ with 1% solution of nitric acid, was stirred and transferred to vials.

The metals were determined in the sample, diluted (1:5) by bidistilled water according to ISO 17294-2 method "Water quality - Application of mass spectrometry with inductively coupled plasma. Part 2: Determination of 62 elements". The samples were diluted using dispenser with disposable tips and polypropylene tubes with screw caps.

The analytical work. The heavy metals and toxic elements were measured by ICP-MS and ICP-ES, using a quadrupole mass spectrometer Elan 9000 manufactured by «Perkin Elmer SCIEX», complete with a computer and specialized software. The device is equipped with a standard spray chamber Scott with the cross-stream spray and single-collector two-segment detector of ions (without the dynamic reaction cell) or by using an optical atomic emission
spectrometer iCAP 6300 DUO made by Thermo Scientific complete with a computer and specialized software. The device is equipped with a cyclone spray chamber with a cross-stream spray and CID semiconductor detector [3].

To construct the calibration diagrams there were used multi-elemental reference standards, registered in the RK State System for Ensuring Uniform Measurement № KZ.03.02.00902-2010, KZ. 03.02.00901-2010. The measurement quality control was carried out by measuring the calibration solution after every 10 samples. If unsatisfactory calibration results occurred (deviation of calibration curve by 8-10%) the device was subjected to re-calibration.

The analysis was conducted to determine the elements according to the method ISO 17294-2:2003 (E) (state registration number 022/10505 from 27.12.05).

Thus, there were analyzed samples of water, plants and soil leached solutions, determined the content of the following elements Na, Mg, K, Ca, Al, Li, Be, Sc, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Mo, Ag, Cd, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Re, Tl, Bi, U. The analysis was performed according to the method ISO 17294-2:2003 (E) (state registration number 022/10505 dated 27.12.05). Macro components (Na, K, Mg, Ca) were analyzed in solutions obtained by diluting the stock solutions 10 and 100 times.

Results and discussion

Tunnel waters. Table 1 lists the average concentrations of microelements in the tunnel waters at "Degelen" site for 2010-2011. Additionally it shows the values of maximal allowable concentrations for water in Kazakhstan. Sampling in 2010 and 2011 was conducted in the same locations (at the exit point of the watercourse to the surface), about the same time of year (Table 1) [3,4].

It should be noted that the contents in water of such elements such As, Se, Ag, Tl are at levels below the detection limit, which were for As - 0,006 mg/L, for Se - 0,06 mg/l, for Ag - 0,0005 mg/l, and for Tl - 0,0003 mg/l. Thus, for the past 2 years, the chemical composition of the tunnel waters has unchanged, despite fluctuations in the concentrations of some elements. No evident about the elements previously detected in the tunnel waters have been reported - for example, for rare-earth elements in the tunnels 104, 165, 176, 177, 506, 511, 609 the concentrations remain at levels below the detection limit. The concentrations of such elements as uranium, beryllium, and molybdenum remain high.

Concentrations of beryllium in some cases (for tunnels 104, 176, 504) increase by 20-30% compared to 2010 and decrease by 10-20% (in the tunnels 511, 609). Content of in lithium and molybdenum in the tunnel waters increased slightly compared with 2010, from a few to tens of percent in some cases. However, the concentrations remain at the same level as in 2010, and such changes are natural and are associated with changes in the chemical composition of groundwater under the influence of different factors over time.

The concentration of uranium markedly decreased in the tunnel waters compared with 2010, an average of almost 3.5 times. Probably this change is due to seasonal groundwater recharge from atmospheric precipitations, which make the reaction of groundwater medium [9,3,6,7,4] more neutral and, therefore, the leaching of uranium is less - it is likely that groundwater recharge by atmospheric precipitation in 2011 was higher than in 2010.

The analysis of the water in the tunnel # 504 confirms the presence of lanthanum elements, anomalous content of manganese, aluminum, and zinc. Based on data from 2010 and 2011, we can assume that the causes of the elevated concentrations of these elements could be such factors as the collapse of the rock in the tunnel area. The watercourse, washing the contaminated fragments of rocks, takes the contaminants and lanthanides to the surface.

Table I

Average concentration 104 165 176	Average concentration 104 165 176	Average concentration 165	Werage concentration 165 176	ncentration 176	ion 176	s of mic	croeleme	ents in tun 77	nel waters 504	of Degel	en site fo	or the 201 506	0 and 20	11, mg/l		609	ндК для
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67 117 64 65 34 42	117 64 65 34 42	64 65 34 42	65 34 42	34 42	42		54	88	125	295	10	17	70	90	51	54	30
5,7 8 0,56 0,52 0,52 0,64	8 0.56 0.52 0.52 0.64	0,56 0,52 0,52 0,64	0,52 0,52 0,64	0,52 0,64	0,64		1,3	1,4	270	384	0,75	0,7	87	70	3	2,5	0,2
<0,2 <0,2 <0,2 <0,2 <0,2 <0,2 <0,2 <0,2	<0,2 <0,2 <0,2 <0,2 <0,2 <0,2	<0.2 <0.2 <0.2 <0.2 <0.2	<0,2 <0,2 <0,2	<0,2 <0,2	<0,2		<0,2	0,7	<0,2	<0,2	0,77	0,45	<0,2	<0,2	<0,2	<0,2	0,1
1,8 <0.2 2,8 1,3 2 <0.2	<0.2 2.8 1.3 2 <0.2	2.8 1.3 2 <0.2	1,3 2 <0,2	2 <0,2	<0,2		2	1,5	0,45	<0,2	2,4	<0,2	<0,2	<0,2	3	<0,2	500
41 5 1,2 0,8 1,5 1,2	5 1,2 0,8 1,5 1,2	1,2 0,8 1,5 1,2	0,8 1,5 1,2	1,5 1,2	1,2		<0,2	2,5	$0,11*10^{6}$	$>2*10^{6}$	182	150	10180	6700	146	37	500
<0.5 4 <0.5 6 <0.5 <0.5	4 < <0.5 6 < 0.5 < 0.5	<0.5 <6 <0.5 <0.5	6 < 0,5 < 0,5	<0,5 <0,5	<0,5		<0,5	24	<0,5	96	<0,5	23	<0,5	27	<0,5	334	1000
<0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2 <0.2	<0,2 <0,2 <0,2 <0,2 <0,2 <0,2	<0.2 <0.2 <0.2 <0.2 <0.2	<0,2 <0,2 <0,2	<0,2 <0,2	<0,2		<0,2	<0,2	12	18	0,35	0.54	0,6	0,37	<0,2	<0,2	100
<0.5 0.6 <0.5 0.7 <0.5 0.39	$0.6 < < 0.5 \\ 0.7 < 0.5 \\ 0.39$	<0,5 0,7 <0,5 0,39	0,7 < < 0,5 $0,39$	<0,5 0,39	0,39		<0,5	1,2	9	14	0,52	3,3	1.5	1,7	<0,5	<0,5	100
<0.3 1.6 <0.3 2.2 <0.3 1.5	$1,6 < <0.3 \\ 2.2 < <0.3 \\ 1.5$	<0.3 2.2 <0.3 1.5	2.2 < 0.3 1.5	<0,3 1,5	1.5		<0,3	2	18	24	<0,3	5	<0,3	1.5	10	2,2	1000
40 25 32 28 30 37	25 32 28 30 37	32 28 30 37	28 30 37	30 37	37		49	52	11000	10700	11	42	3770	1950	7	15	5000
480 421 317 324 156 164	421 317 324 156 164	317 324 156 164	324 156 164	156 164	164		380	470	760	800	690	670	385	306	170	175	7000
<0,01 <0,01 <0,01 <0,01 <0,01 <0,01 <0,01	<0,01 <0,01 <0,01 <0,01 <0,01 <0,01	<0,01 <0,01 <0,01 <0,01 <0,01	<0,01 <0,01 <0,01	<0,01 <0,01	<0,01		<0,01	<0,01	385	342	<0,01	<0,01	<0,01	2,8	<0,01	<0,01	ı
550 880 65 95 175 220	880 65 95 175 220	<u>65 95 175 220</u>	95 175 220	175 220	220		660	678	<0,3	2,5	10	10	0,82	2	165	204	250
1.25 1.7 <0.3 <0.3 1 <0.3	1,7 < <0,3 < 0,3 1 < <0,3	<0.3 <0.3 1 <0.3	<0,3 1 <0,3	1 < 0,3	<0,3		<0,3	1,3	34	32	<0,3	<0,3	6	9	<0,3	<0,3	
1,4 2,4 1,4 01.май 1 0,8	2,4 1,4 01.maŭ 1 0,8	1,4 01.май 1 0,8	01.май 1 0,8	1 0,8	0,8		0,15	0,1	6,5	7	<0,05	<0,05	3,5	3,8	1,2	1,3	,
10 10 10 12 3 4	10 10 12 3 4	10 12 3 4	12 3 4	3 4	4		13	20	8	6	20	24	5,5	4,2	1	2,7	100
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05 <0,05 <0,05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	455	320	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	
<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05	<0,05 <0,05	<0,05		<0,05	<0,05	890	583	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	
<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	85	68	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	
<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	280	235	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	65	55	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	24
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0,05 <0,05 <0,05 <0,05 <0,05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	100	80	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	16	14	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	95	80	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	·
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0,05 $<0,05$ $<0,05$ $<0,05$ $<0,05$ $<0,05$	<0.05 <0.05 <0.05 <0.05 <0.05	<0,05 $<0,05$ $<0,05$ $<0,05$	<0,05 $<0,05$	<0,05		<0,05	<0,05	19	16	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	ı
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05 <0.05 <0.05 <0.05 <0.05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	56	42	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	ı
<0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	<0.05	<0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	8	6	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	,
<0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05 < 0,05	<0.05 <0.05 <0.05 <0.05 <0.05 <0.05	 <0,05 <	<0,05 $<0,05$ $<0,05$ $<0,05$	< 0.05 < 0.05	<0,05		<0,05	<0,05	53	35	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	
<0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,05 <0,0	<0,05 <0,05 <0,05 <0,05 <0,05 <0,05	<0.05	<0,05 <0,05 <0,05 <0,05	<0,05 <0,05	<0,05		<0,05	<0,05	8	5	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	ı
<0,06 <0,06 <0,06 <0,06 <0,06 <0,06 <0,06	<0,06 <0,06 <0,06 <0,06 <0,06	<0,06 <0,06 <0,06 <0,06 <0,06	<0,06 <0,06 <0,06	<0,06 <0,06	<0,06		<0,06	<0,06	2	1	<0,06	<0,06	<0,06	<0,06	<0,06	<0,06	I
<0,01 0,14 <0,01 0,1 <0,01 0,25	0,14 < < 0,01 0,1 < < 0,01 0,25	<0,01 0,1 <0,01 0,25	0,1 < 0,01 = 0,01 = 0,25	<0,01 0,25	0,25		<0,01	0,16	<0,01	77	<0,01	0,35	<0,01	1,2	<0,01	0,45	30
<0.05 <0.05 <0.05 <0.05 <0.05 <0.05 0.52	<0,05 <0,05 <0,05 <0,05 <0,05 0,52	<0.05 <0.05 <0.05 0.52	<0,05 $<0,05$ $0,52$	<0,05 0,52	0.52		<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	<0,05	2,2	100
2025 878 2565 753 1075 310	878 2565 753 1075 310	2565 753 1075 310	753 1075 310	1075 310	310		1960	690	4535	800	76	17	555	144	1040	317	100

Assessment of changes in the content of heavy metals in the water during the season

Figure 2 shows the diagrams of changes in the concentration of elements in time at the exit point of the watercourse to the surface (Figure 2).



Figure 2. Change in concentrations of elements in the sampling period at the exit point of the watercourse to the surface: a) the tunnel 177, b) tunnel 504, c) tunnel 504

The results presented here suggest that the concentration of the detected elements during the observation period varies slightly, from 5 to 15%. Most streams are characterized by decreasing the concentration of elements in the water in August, possibly due to seasonal changes in flow rate of the watercourse.

However, in the water of tunnel 165 the content of heavy metals during the period of sampling almost does not change. It is also important to note that the maximal content of lanthanides in the water of the tunnel 504 is observed in August, it is also typical beryllium in water of the tunnel 177.

Investigation of variations in the content of heavy metals in the water along the watercourse bed

Figure 3 shows the diagrams of the variations in the concentrations of some elements in water, depending on the distance from the exit point of water to the surface (Figure 3).





Figure 3. Variations in the concentration of elements in water, depending on the distance from the exit point of water to the surface: a) tunnel 177, b) tunnel 504, a) tunnel 504

When assessing the results it should be noted that:

- 1) The content of lanthanides in the water of the tunnel 504 is reduced to almost zero (limit of detection equipment) at a distance of 200 m;
- 2) Beryllium in the water of all watercourses, except for the tunnels # 177, # 506, is detected at a maximal distance from the exit point of water to the surface, which is also associated with the migration properties of this element.

Concentrations of heavy metals in soils of estuarine areas of the tunnel # 504 and # 177

Low "Degelen" mountains are situated within the sub-zone of the zonal light-chestnut soils with desert steppes. The study area is located in the valleys of the streams in the array, where there are intrazonal meadow soils, and very rarely, meadow marsh. The meadow soils were subjected to the greatest contamination. It should be noted that the near-portal areas, where there is maximal pollution, are characterized by artificial substrates, both brought from other sections and collected from this place, mixed with soils. We can assume that here there is an initial stage of soil formation, and this is confirmed by the presence of very small thickness of the humus horizon.

Table 2 shows the average concentration of chemical elements in soil leached solutions, on the sampling profiles, collected along the banks of the watercourse of the tunnel # 504 (Table 2).

Table 2

Profile #	1	2	3	4	5	6	7	Clarke by Vinogradov
				mg/g				
Mn	4,0±0,4	3,9±0,4	12,0±1,0	5,1±0,5	$14,0{\pm}1,0$	17,6±1,5	13,7±1,3	1,0
La	1,0±0,1	1,3±0,1	2,2±0,2	2,3±0,2	5,2±0,5	6,3±0,6	2,1±0,2	0,049
Ce	5,8±0,5	6,9±0,7	11,5±1,0	10,0±1,0	19,8±2,0	19,7±2,0	5,0±0,5	0,070
U	2,2±0,2	2,8±0,2	6,2±0,6	3,8±0,3	4,7±0,4	4,5±0,4	1,0±0,1	0,4
Y	2,5±0,2	3,0±0,3	5,5±0,5	5,2±0,5	11,7±1,0	12,5±1,0	4,3±0,4	0,029
Nd	1,3±0,1	1,3±0,1	2,3±0,2	2,0±0,2	3,5±0,3	3,1±0,3	0,75±0,01	0,037
mcg/g								
Be	20±2	32±3	76±7	63±6	141±14	134±13	90±9	3,8
Sc	835±85	680±70	1230±125	800±80	950±95	1000 ± 100	420±45	10
Li	35±3	28±2	15±1	30±3	17±1	20±2	32±3	32
V	33±3	21±2	12±1	27±2	10±1	13±1	19±2	90
Cr	23±2	15±1	9±1	19±2	8,0±0,7	7,0±0,6	10±1	83
Со	8,0±0,7	6,0±0,6	5,0±0,4	7,0±0,6	6,0±0,5	13,0±1,0	18,0±1,5	18
Ni	35±3	32±3	18±1	18±1	17±1	23±2	50±4	58
Cu	1200±100	66±6	180±15	140±10	160±15	115±10	45±4	47
Zn	720±70	530±50	1000±80	980±80	2350±230	3120±300	2360±230	83
As	7,0±0,7	5,5±0,5	4,5±0,4	6,0±0,6	5,0±0,5	5,5±0,5	8,0±0,8	1,7

Average concentration of elements in the soil leched solutions of the tunnel # 504

Sr	37±3	46±4	135±15	85±8	94±9	82±8	135±15	340
Cd	2,0±0,2	2,0±0,2	2,0±0,2	3,0±0,3	5,0±0,5	9,0±1,0	6,0±0,5	0,13
Cs	5,0±0,5	5,0±0,5	8,0±0,7	3,0±0,3	6,0±0,5	5,0±0,4	3,0±0,2	3,7
Ba	140±10	120±10	120±10	114±10	118±10	200±15	230±15	650
Pr	320±30	370±35	660±65	580±60	1150±100	1000±100	240±25	9
Sm	360±35	380±35	680±65	430±45	820±80	580±55	150±15	
Gd	360±35	400±40	765±75	600±60	1200±100	1000±100	280±25	
Tb	56±5	63±6	130±15	93±8	180±15	160±15	44±4	
Dy	245±25	285±25	560±55	440±40	880±85	780±75	217±20	5
Но	60±6	65±6	130±10	110±10	220±20	200±20	49±5	1,7
Er	174±15	200±20	390±40	324±35	620±60	578±55	160±15	3,3
Tm	24±2	31±3	55±5	41±4	78±7	77±7	21±2	
Yb	180±15	200±20	370±35	250±25	490±50	370±35	100±10	3,3
Lu	27±2	30±3	55±5	36±4	70±7	50±5	12±1	0,8
Pb	765±75	320±30	100±10	40±4	53±5	29±3	32±3	16

To identify the most important elements – the main pollutants, a method was used to compare these results with an average content of elements in the soil (Clarke), although this is not correct with respect to the acid digest, as Clarke implies total content of elements in the soil. The comparison revealed that the major pollutants of soil for the tunnel #504 are such elements as Be, U and rare earth elements (REE).

For the elements that are present in abnormal amounts, maps, diagrams of the spatial distribution of elements were constructed (Figure 4).





Figure 4: Spatial pattern of distribution of some elements in the soil at the near-portal areas of thetunnel #504: a) uranium, b) beryllium, c) scandium, d) yttrium, e) cerium

Analysis of data on the spatial distribution showed that such elements as uranium, beryllium, and lanthanides are concentrated in the immediate vicinity of the watercourse, and the points with a maximal content are confined to the stream bed.

For a more visual representation Figure 5 shows the typical distribution of the concentration of lanthanum and yttrium elements in soil leached solution on the perpendicular profile (Figure 5).



Figure 5. Typical distribution of elements in soil leached solutions of the tunnel #504 on the sampling profile

An analysis of the results suggests that the points with the highest concentration of elements are found in the streambed, the levels of heavy metals in the soil at a distance of 20 m from the channel are reduced 10 times.

This suggests that contamination of the near-portal areas is caused by carry-over of lanthanides by tunnel waters and is not related to their content in the soil itself of the near-portal area.

Table 3 presents the concentrations of chemical elements in soil leached solutions on sampling profiles at the tunnel # 177 (Table 3).

Profile #	1	2	3	4	5	6	7	Clarke by Vinogradov
				mcg/g	<u> </u>			
Li	25±2	20±2	21±2	20±2	26±2	23±2	25±2	32
Be	14±1	7,0±0,7	4,0±0,4	10±1	3,0±0,3	5,0±0,5	3,0±0,3	3,8
Sc	260±25	170±15	180±15	200±20	270±25	230±20	230±20	10
V	35±3	28±2	30±3	34±3	44±4	39±4	40±4	90
Cr	31±3	22±2	21±2	25±2	30±3	27±2	27±2	83
Mn	450±45	650±60	710±70	920±90	970±90	1280±120	800±80	1000
Со	5,0±0,4	5,0±0,4	5,0±0,4	5,0±0,4	7,0±0,6	7,0±0,6	7,0±0,6	18
Ni	<0,5	6,0±0,5	23±2	<0,5	14±1	<0,5	<0,5	58
Cu	53±4	54±4	33±3	40±4	29±3	28±3	24±2	47
Zn	210±20	160±15	145±15	220±20	105±10	156±14	100±8	83
Sr	127±10	127±10	78±7	79±7	100±8	63±5	55±4	340
Y	1400 ± 140	720±70	670±65	750±70	830±80	780±75	700±65	29
Cd	2,0±0,2	<0,6	<0,6	2,0±0,2	1,0±0,1	1,0±0,1	<0,6	0,13
Cs	4,0±0,3	3,0±0,2	2,0±0,2	2,0±0,2	3,0±0,3	3,0±0,3	3,0±0,3	3,7
Ba	165±15	150±15	130±10	140±10	260±20	220±20	170±15	650
La	510±50	390±35	420±40	440±40	550±50	530±50	510±50	49
Ce	2000±200	1700 ± 150	2200±200	2100±200	3200±300	3100±300	2800±250	70
Pr	120±10	90±9	100±10	100±10	135±12	130±12	120±10	9
Nd	510±50	370±35	400±40	400±40	470±45	460±45	450±45	37
Sm	100±10	70±7	71±7	74±7	96±8	91±8	87±8	
Gd	120±10	76±7	73±7	78±7	106±10	99±10	97±9	
Tb	13,0±1,0	6,5±0,6	6,0±0,6	6,5±0,6	9,0±0,8	7,5±0,7	8,5±0,8	
Dy	72±7	40±4	39±4	43±4	56±5	53±5	50±5	5
Но	16,0±1,5	8,0±0,8	7,5±0,7	8,5±0,8	10,0±1,0	10,0±1,0	9,0±0,8	1,7
Er	55±5	28±2	27±2	29±2	39±3	35±3	32±3	3,3
Tm	5,0±0,5	2,0±0,2	1,0±0,1	1,0±0,1	2,0±0,2	1,0±0,1	1,0±0,1	
Yb	50±5	25±2	24±2	27±2	33±3	30±3	26±2	3,3
Lu	5,0±0,5	3,0±0,3	1,0±0,1	1,0±0,1	2,0±0,2	2,0±0,2	1,0±0,1	0,8
Pb	25±2	33±3	22±2	29±3	33±3	39±3	27±3	16
U	271±25	48±4	132±15	106±10	42±4	70±7	47±4	

Average concentration of elements in the soil leached solutions of the tunnel # 177

Applying the already used method of comparison with Clarke identified the following a series of elements which are considered as components of the major pollutants: Be, Y, U, Ce, Pr, Cd. Maps of the spatial distribution of these elements were built (Figure 6).



Table 3



Figure 6. Spatial pattern of distribution of some elements in the soil at the near-portal areas of the tunnel #177: a) beryllium, b) uranium, c) yttrium, d) cadmium, e) praseodymium, f) cerium

The soil of the tunnels also had subgroups of lanthanum elements, but in the water of the watercourse they are not found. Probably the highest concentrations of these elements are not the consequence of their carry-over by tunnel waters, but due to the geochemical features of the studied area. This fact is confirmed by the spatial distribution of the identified elements, the maximal contents of the studied elements are not in the streambed (Figure 7).



Figure 7: Typical distribution of elements in soil leached solutions at the tunnels # 177 on the sampling profile

Concentrations of heavy metals in the plants at the estuarine areas of the tunnel #504

The vegetation of the valleys of streams, are mostly represented by marshy (*Phragmites australis, Ligularia macrophilla*), natural (*Calamagrostis epigeios, Glycyrrhiza uralensis*), steppificated (*Poa trivialis, Galatella biflora*), halophytic (*Achnatherum splendens, Liminium gmelini*i) variants of meadows, bushland of *Salix cinerea, Rosa glabrifolia* is distributed piecewise.

During the reconnaissance the vegetation was described not only to identify the communities and the dominant species, but also provide a complete picture of the flora of the investigated area. Thus, such species as bush grass (*Calamagróstis epigéjos*), southern reed south (*Phrágmites austrális*), sedge grass (*Carex supina*), weeping birch (*Bétula péndula*), gray willow (*Salix cinerea*), naked-leaved briar (*Rosa glabrifolia*), black-fruited cotoneaster (*Cotoneaster melanocarpus Fisch*), tutsan-leaved meadowsweet (*Spiraea hypericifolia*) fescue (*Festuca valesiaca*), feather capilata (*Stipa capillata*), Austrian wormwood (*Artemisia austriaca*), estragon (*Artemisia dracunculus*), narrow-leaved wild rye (*Leymus angustus*), highland wheat (*Orostachys spinosa*), greater burnet (*Sanguisórba officinális*).

As mentioned earlier, the soil cover at the near-portal areas are characterized by artificial substrate with sparse vegetation, represented mostly by weedy species. They grow on such areas by the type of primary succession. It consists of several stages. The first – colony of individual plants with the differentiation by substrate and microrelief. The formation rate of secondary vegetation is directly dependent on the particle size (no growth at very rocky areas of the near portal-areas). Firstly weedy and adventive species are settled: Amaranthus retroflexus, Ceratocarpus arenaria, Artemisia scoparia, Kochia scoparia, Acroptilon repens, Fumaria vailantii. Further there is a distribution of vegetation in altitudinal belts and microrelief. The following stages are sparse groupings dominated by species of the genus Artemisia, meadow grasses - Medicago falcata, Galatella, Achillea asiatica - grasses: Stipa capillata, Psathyrostachys juncea, Festuca valesiaca, Agropiron cristatum.

Table 4 presents the average concentrations of chemical elements in the sampling profiles in the samples of plants, selected on the bed of the watercourse and the territory adjacent to the tunnel #504 (Table 4).

as concentration of claments in plant complete

Table 4

		Average conc		ements in plai	it samples		
Profile #	1	2	3	4	5	6	7
				mcg/g			
Al	300,0	324,7	491,8	231,3	167,8	144,3	289,5
Cr	5,9	4,0	2,9	2,5	5,3	4,9	5,0
Zn	133,0	145,6	470,2	506,4	105,1	64,4	62,9
Cd	0,9	0,5	0,8	0,7	0,4	0,4	0,4

Pb	7,3	5,6	2,8	2,4	1,7	4,1	2,8
Sr	48,9	25,0	30,5	42,7	16,1	10,9	24,5
U	5,0	2,6	4,0	0,8	1,2	1,9	9,6
Cu	22,8	17,4	8,7	7,1	5,0	6,2	2,8
Mn	1250,5	1790,3	5302,4	7059,0	1562,7	1738,7	2096,5
Be	2,8	3,6	10,8	7,3	3,5	3,3	3,6
Li	13,7	6,9	5,5	5,1	14,6	4,9	3,3
Со	0,4	0,4	0,4	0,4	0,4	0,4	0,4
Ni	4,0	2,9	3,9	2,8	3,3	3,2	3,4
Rb	5,1	12,8	3,4	4,3	13,5	16,2	5,8
Y	1,7	1,9	2,0	0,3	0,9	0,9	5,6
La	1,7	1,8	1,9	0,4	0,9	1,0	5,0
Ce	2,8	3,0	3,1	0,6	1,2	1,4	7,2
Nd	1,2	1,3	1,2	0,2	0,4	0,5	2,6
Sm	0,3	0,3	0,3	0,1	0,1	0,1	0,5
Fe	264,0	322,3	262,6	248,1	172,2	169,4	173,0
Gd	0,4	0,4	0,5	0,1	0,2	0,2	1,0

Figure 8 shows the diagrams of the variations in the concentrations of some elements in plant samples



Figure 5. Variations in the concentration of elements in plant samples of the tunnel #504 on the sampling profile

Analysis of the results suggests that the point with the highest concentration of elements such as Al, Zn, Mn located at a distance of 100-150 m (2.3 profile) from the exit site of the watercourse to the surface.

The concentration of lanthanides in plant samples is reduced to almost zero at a distance of 150 m and increases sharply at a distance of 300 m from the exit site of the watercourse to the surface. The uneven distribution of elements of the subgroup of lanthanum due primarily to the fact that the flow of the lanthanides in plants is carried out by the removal of these elements shtolnevymi waters, rather than their content in the soil itself, on which grow the studied species.

Penetration of heavy metals in plants through the root system depends primarily on the amount of these metals in the soil. The correlation coefficients between the content of metals in plants and environments under different conditions (soil type, moisture, acidity, etc.) can be quite high - in some cases exceeds the value 0.80. There are both linear and nonlinear increases of metal content with increasing their concentration in solution or culture media.

The contents of excessive amounts of heavy metals in the plant mass may vary during the growing season. One of the reasons of this is the inability of the stream coming from the soil into plants evenly throughout the growing season to saturate biomass growth with heavy metals, which in mid-summer reaches the peak, and although the rate of their income is more or less uniform, there is a so-called "dilution effect".

Conclusion

The above experimental data allow us to consider the environmental situation in the STS, particularly at Degelen site as a complex system of radiative and "nonradiative" factors. The main conclusions of this work can be considered as follows:

- all the investigated tunnel waters are characterized by higher concentrations of uranium, beryllium, and molybdenum, at that, molybdenum level in the water of some tunnels (tunnel #104) exceeds the typical content in the water for the climate up to 800 times;
- water of the tunnel #504 is absolutely unique for its elemental composition, in particular, for the content of lanthanum elements subgroup (total REE ~4 mg/l), as well as the content of aluminum, manganese, zinc, the concentrations of which are comparable to the concentrations of macro-components;
- high content in plants such elements as aluminum, zinc and manganese as well as in water is comparable to the concentrations of macro-components;
- analysis of the results on the content comparable by radiotoxicity α-nuclides of the natural isotope ²³⁸U and plutonium isotopes ²³⁹⁺²⁴⁰Pu showed greater significance of natural uranium for the studied waters in terms of radiation hazards and potential dose loads. In this connection, it is necessary to consider this factor when assessing the radiation hazard of the STS facilities;
- elevated concentrations of rare-earth metals in the soil and plants at estuarine area of the tunnel #504 are associated with the carry-over of heavy metals by water from a watercourse of the tunnel. Soil of the tunnel #177 also has high amounts of REM, but the reason for this is the geochemical characteristics of the area.

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