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Editors: M. P. Fiodorov, W. Hogland and V. Yu. Rud’

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PHOTOMETRICAL APPROACH TO THE PROBLEM OF ESTIMATION OF PIGMENT ACTIVITY IN PLANT CELLS: UPDATE TECHNIQUE FOR BLACK MEDIC

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ABSTRACT

This work is aimed to study of the photosynthetic activity of plant sells by means of noninvasive method. We modify spectrophotometric technique of reflectance detection in leaves of black medic (*Medicago lupulina*) and test the response in the indices to different stress-factors: phosphorus fertilizer and inoculation of plants with endomycorrhizal fungus *Glomus intraradices*. The results indicate high responsiveness of noninvasive method. Two spectral photosynthetically active bands for leaves of black medic are localized and can be used for further update of reflectance techniques in agriculture, surface and aerospace monitoring landscape conditions.

INTRODUCTION

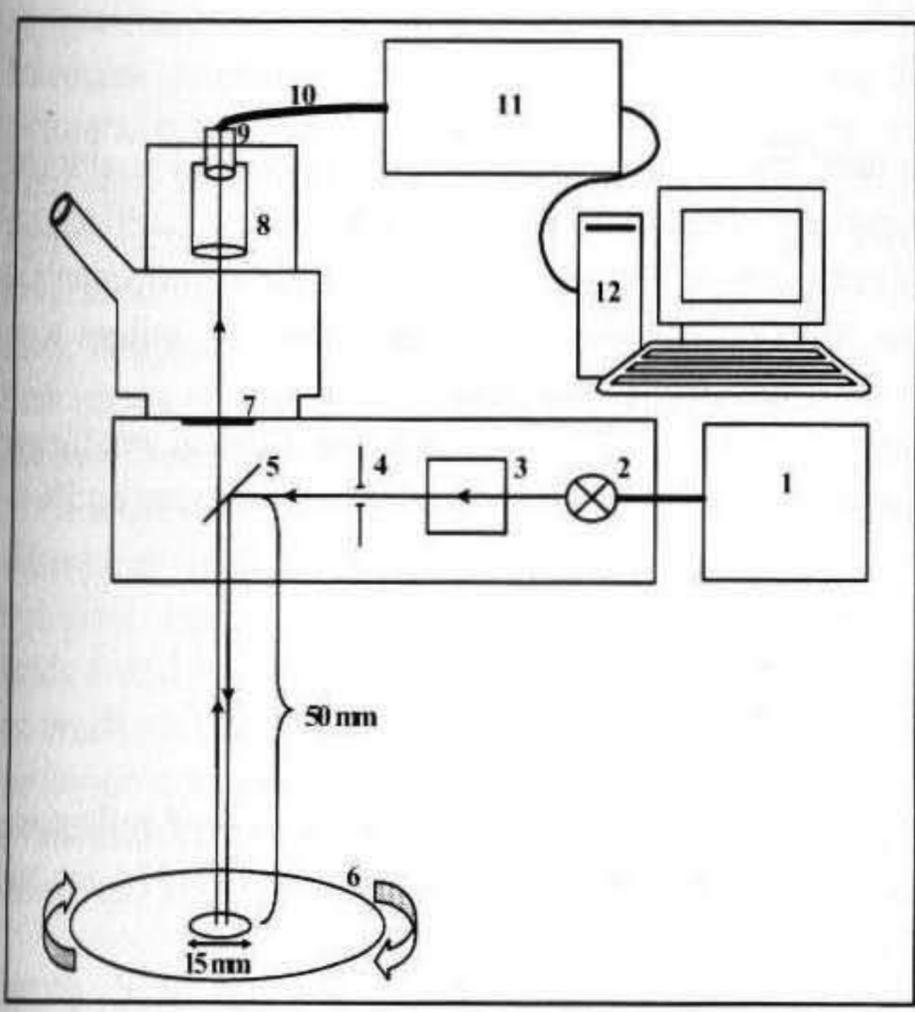
Analysis of reflectance spectrum of plant leaves is noninvasive optical method for assessment of absorbed photosynthetically active radiation. Reflectance measurement makes it possible to non-destructively estimate, *in situ*, the chlorophyll and carotenoid content and/or activity. Chlorophylls absorb solar light energy and provide mechanisms for its utilization in photosynthetic reaction. Carotenoids contribute to light-harvesting and also play a photo-protective role, preventing damage to the photosynthetic systems [1].

Traditionally, leaf extraction with organic solvents and spectrophotometric determination in solution is required for pigment analysis with wet chemical methods consuming a lot of time, effort and money. They require destruction of the measured leaves and thus do not permit measurement of changes in pigments over time in a single leaf. In contrast, spectral reflectance measurements provide a noninvasive, rapid technique that can be used at different spatial scales. By now a number of scientists devised different spectrophotometric indices for pigment activity, which characterize the photosynthetic plant activity and overall plant physiological status (*chlorophylls*: [2]; [3]; [4]; *carotenoids*: [5]; [6]; [7]).

To apply spectral indices it is necessary to update they in respect to diverse plant species. In this paper we localized a few spectral photosynthetically active bands for leaves of black medic. We tested the dynamics reflectance spectrum depending on different stress-factors: phosphorus (P) fertilizer and mycosymbiont.

BIOMATERIALS

The object model plant is black medic (*Medicago lupulina*), self-pollinating diploid species



characterized by broad ecological amplitude. Black medic is a grazing crop, planting filler and green fertilizer. Series of experiments were carried out on fast growing responsive plant line in cultivar-populations ARFI32. Seedlings were sowed in pots with soil containing low (1.7 mg P per 100 g soil) or middle (adding P fertilizer = 17.2 mg CaHPO₄ per 100 g soil) phosphorus level.

Arbuscular mycorrhizal fungus – highly efficient CIAM8 strain from the collection of the Laboratory of Biological Nitrogen at All-Russia Research Institute for Agricultural Microbiology was taken to inoculate the plants. According to the data of SSU rRNA analysis CIAM8 strain referred to *Glomus intraradices*. All experimental conditions were controlled to prevent the inoculation with rhizobia and other microorganisms.

We studied four experimental plant groups:

- 1) plants without arbuscular mycorrhiza (AM) under conditions of low phosphorus level in soil (M⁻P⁻);
- 2) plants without AM under conditions of middle phosphorus level in soil (M⁻P⁺);
- 3) plants with AM under conditions of low phosphorus level in soil (M⁺P⁻);

4) plants with AM under conditions of middle phosphorus level in soil (M⁺P⁺).

The leaves analyzed at different stages of plant development – at different dates after sowing (from 10 to 42 day).

MECHANO-OPTICAL SYSTEM

Spectral measurements of leaf reflectance were provided by using fiber-optic automatic spectrometer AVS-S2000 (Avantes B.V., Netherlands). We designed the measurement system basing on microscope LUMAM-KF, including different mechano-optical elements (Fig. 1).

Plant leaves (dozen) were set on object table as "all-over cover" ø 15 mm, which corresponded to the area of spot formed by falling light beam. Reflected light directed be dichroic mirror toward diaphragm, collecting lenses and optical fibre was detected by spectrometer AVS-S2000. Eight different position of the cover were analyzed by turning of the object table in steps of 45° for each measurement session.

REFLECTANCE REGISTRATION

Variations in leaf reflectance spectrum, induced by plant development shift, mycorrhization and/or phosphorus fertilizer, were recorded against Teflon as a standard, and also against control group M⁻P⁻. The reflectance spectrum was calculated as a ratio of leaf radiance to standard radiance at wavelength λ:

$$R_{\lambda} = \frac{[Sample_{\lambda}]}{[Teflon_{\lambda}]} \cdot 100 \tag{1}$$

$$P_{\lambda} = \frac{R_{\lambda}}{R_{\lambda c}} \cdot 100 \quad (2)$$

$[Sample_{\lambda}]$ – absolute value of reflectance from leaf cover at wavelength λ ; $[Teflon_{\lambda}]$ – absolute value of reflectance from Teflon cover at wavelength λ ; P_{λ} – relative characteristic of reflectance change by leaf pigments; R_{λ} – integrated value of reflectance from leaf cover of analyzed sample at limited wavelength range; $R_{\lambda c}$ – the same value for control sample. Calculated P_{λ} values were integrated at selected wavelength ranges enjoying different light absorption activity.

Biological replication averaged 12 plants for any one of plant groups, analytical coefficient was 8 (number of object table positions). In each tests the level of significance was chosen as 5% (*t*-test).

DYNAMICS IN LEAF REFLECTANCE SPECTRUM

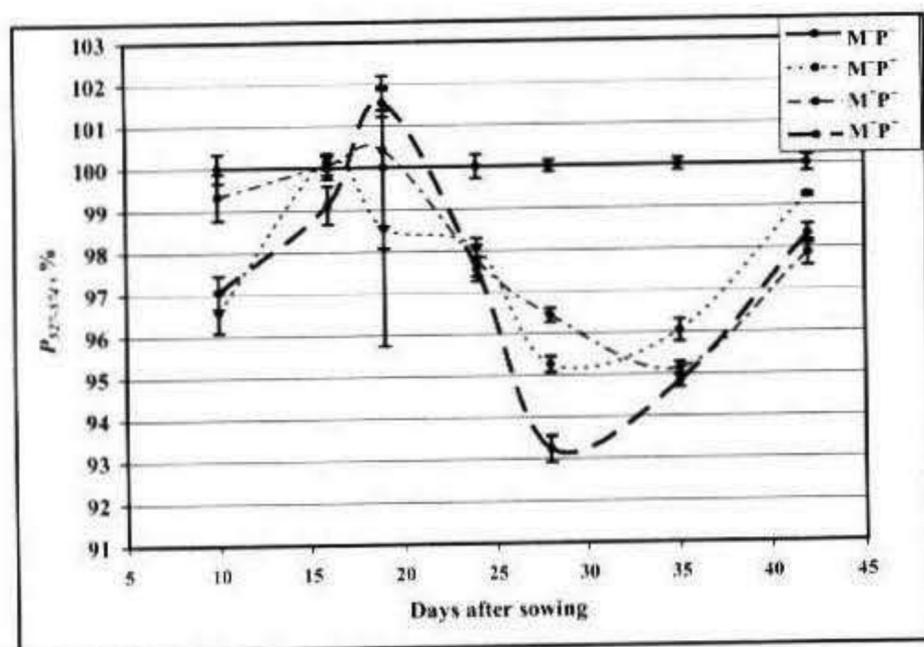


Fig. 2. Dynamics for relative characteristic of reflectance change ($P_{527-574}$) by pigments during black medic leaves development

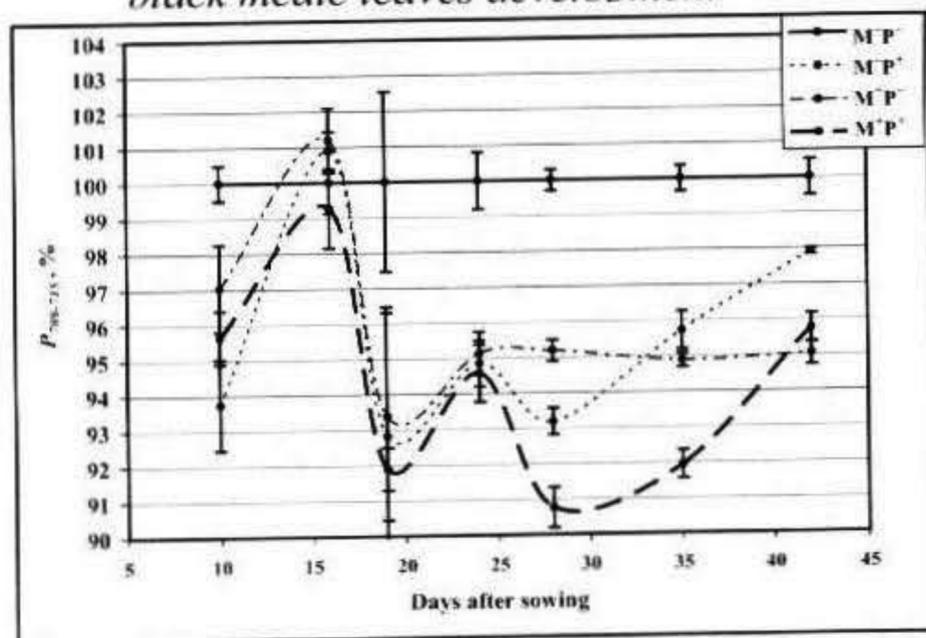


Fig. 3: Dynamics for relative characteristic of reflectance change ($P_{709-715}$) by pigments in black medic leaves

The most active ranges in leaf reflectance spectrum for black medic are 527-574 nm and 709-715 nm (Figs. 2-3).

$P_{527-574}$ in M^+P^+ , M^+P^- and M^-P^+ groups (with phosphorus fertilizer and/or arbuscular mycorrhiza) don't have significant ($P < 0.05$) differences in comparison to uninoculated control (M^-P^-) at 10th and 16th day after sowing, but are lower in distinction from control group M^-P^- at 24th day after sowing. Then tested samples save lower reflectance in spectral range 527-574 nm.

$P_{527-574}$ in M^+P^+ , M^+P^- and M^-P^+ groups (with phosphorus fertilizer and/or arbuscular mycorrhiza) are lower in distinction from control group M^-P^- at 24th day after sowing, but extent of error is relatively high. These groups have significant ($P < 0.05$) differences in $P_{709-715}$ value in comparison to uninoculated control from 24th day after sowing.

The above will may show that after shooting at 24th day the photosynthetic activity of medic plants increases.

Thus it appears that used method of study of black medic responses to different stress-factors (as phosphorus fertilizer and mycorrhization) *de facto* are reasonable to apply in two active spectral range: 527-574 nm and 709-715 nm, which are near to ranges of light absorption by carotenoids and chlorophylls, respectively.

CONCLUSIONS

In our research we devised mechano-optical system for noninvasive monitoring of light sorption by photosynthesis pigments. We noted certain advantages of this method by the side of classic biochemical method for assessment of photosynthetic activity and pigment content. First of

all spectrometry *in vivo* is more adequate to analyze biological processes at real-time and one introduces minimum of artefacts. Secondly, by noninvasive methods we can define activity of pigments, containing in the native chloroplasts, which connected with cytoplasm of living cells. Apparently right in such conditions it's possible to detect active forms of pigments, functioning in light-collecting complex of photosystems. Moreover this method is more discriminating and hence more sensitive, then biochemical methods used in our recently made parallel study (unpublished data). Thirdly, in order to show differences between tested groups and control a few milligrams of fresh leaves and several minutes of measurements were enough against tens milligrams of leaf mass and hard biochemical analyses. At last the non-destructive method is inexpensive and quick.

It is very important that Spectrometric Reflectance Indices (such as *photochemical index* – PRI, *chlorophyll indices* – ChlRI, *carotenoid indices* – CRI, etc. [7-9]) widely used in different biological, ecological and agrotechnical spheres [3, 10] could be corrected for the spectral wave bands found in our study, at least for black medic. The test-system "*Medicago lupulina* – *Glomus intraradices*" may be used for further betterment of reflectance indices with a view to update noninvasive assessment of slight first response of plant pigments to disadvantageous transformation of environment, in particular this test-system may be used for bioindication of air pollution variations. They are subjects of our further investigation.

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TABLE OF CONTENT

PLENARY SECTION	15
Assessment of environmental risks of landfills by means of GIS databases <i>H. Adwiraah, R. Vankevych, M. Ritzkowski, R. Stegmann</i>	16
Fires in storages of organic materials <i>William Hogland and Marcia Marques</i>	24
Fabrication and properties of cadmium-free thin-film Cu(In,Ga)Se₂/(In₂S₃) heterophotoelements for solar energetics <i>Yuri Rud', Valeriy Gremenok, Vasiliy Rud'</i>	33
Economic-mathematical model of combined autonomy energy system with including renewable energy machines <i>Ivan Kuznetsov</i>	39
Technological, economic and social problems of wasteless production <i>V. Goryunov</i>	44
 CONFERENCE 1. ENVIRONMENTAL PROTECTION AND ECOLOGICAL NATURE MANAGEMENT	 47
Changes of spetiation of organic matter during sewage treatment in hybrid wetland systems <i>Agnieszka Tuszyńska, Hanna Obarska-Pempkowiak</i>	48
Mechanical milling study of waste-chip magnesium: effects on specific surface area and microstructure <i>Aysel Kanturk, Muge Sari, Ozgul Dere, Sabriye Piskin</i>	56
Effectiveness of wildlife under crossings. The study of the high way Vilnius – Kaunas, Lithuania <i>Diana Padvelskytė, Gytautas Ignatavičius</i>	61
The effect of slowly biodegradable substrate on the oxygen uptake rate in activated sludge systems <i>Jakub Drewnowski</i>	69
Restoration of drained peatlands in Finland <i>Marja Kurkela, Kirsi Kurko, Taru Palviainen</i>	77
Bioenvironmental advantages of pyrite ashes pellets reduction for iron production <i>Mehmet Burçin Pişkin, Nurcan Tugrul, Emek Moroydor Derun, Ahmet Ekerim</i>	82
The effect of thermal treatment on chemical and physical properties of galvanic sludge: prevent ecological impact on bioenvironment <i>Ozgul Dere , Mehmet Burçin Piskin, Müge Sari, Aysel Kanturk</i>	89
Revealing the periodicities and methodology of long range forecast of lakes water level (Ladoga lake and Vettern lake as a case study) <i>A.V. Babkin</i>	94
Natural biological degradation of oil hydrocarbons in various soil types of the North-West <i>M.V. Chugunova, L.G. Bakina, T.V. Bardina, A.O. Gerasimov</i>	99
Recent problems of urban forestry in Ukraine <i>Nadiia Yu. Basos, Yuriy I. Vergeles</i>	103
Estimation of the reservoir's trophic condition for engineering problems decision <i>I.P. Bulgakov, A.I. Shishkin</i>	106

Meiobenthos of Baltic sea and its use as indicator of ecological state of marine ecosystems <i>Valentina Galtsova, Denis Alexeev</i>	109
Coagulation treatment peculiarity for pulp and paper plants' wastewaters <i>D.A. Sergeeva, M.P. Shestakova, A.B. Dyagileva, Yu. M. Chernoberezhskii, E.V. Evdokimova</i>	114
Edafic characteristic of alluvial soils of Gulf of Finland coast <i>L.P. Kapelkina, V.V. Chasovskaya</i>	118
The impact of radiation factor on the low molecular weight antioxidants content in <i>Melandrium album</i> and <i>Bromopsis inermis</i> <i>E.M. Karimullina</i>	121
Investigation and control of biodegradation processes of municipal solid waste (MSW) model samples of Almaty city <i>A.V. Cheremisin, E.Yu. Negulyaeva, S.A. Basov, D.V. Molodtsov, V.V. Korablev, V.I. Maslikov, A.N. Chusov, A.S. Nurkeyev, S.S. Nurkeyev, Ritzkowski Marco</i>	125
Laboratory investigation of biodegradation processes of municipal solid waste (MSW) model samples of Ashgabat city <i>A.V. Cheremisin, E.Yu. Negulyaeva, D.V. Molodtsov, M.P. Fedorov, V.V. Korablev, V.I. Maslikov, A.N. Chusov, Gurbangeldi Allaberdiyev, Elena Allaberdiyeva, Orazgeldi Kurbanmuradov, Ritzkowski Marco</i>	128
Environmental risk assessment when polluting atmospheric air by MSW landfills emissions of Central Asia states <i>E.Yu. Negulyaeva, Z.V. Naumova, V.V. Korablev, V.I. Maslikov, A.N. Chusov, V.V. Petukhov, Gurbangeldi Allaberdiyev, Elena Allaberdiyeva, Orazgeldi Kurbanmuradov, Maksat Anarbaev</i>	132
The impact of meliorated land use intense on nutrient run off to small rivers of Baltic sea catchment area <i>Nadegda Nemchinova, Vera Klubova</i>	136
Mathematical modelling of biogenes pollutions of small rivers in the nonchernozem zone of the Russian Federation <i>S.N. Kovalenko</i>	140
Eco-toxical assessment of oily soil on the basis of biotesting <i>N.V. Majachkina</i>	142
Analyses of risks and uncertainty in the sphere of nature management <i>Liubou Minniyeva</i>	146
Anthropogenous composition of microelements of group IIb and their distribution in disturbed ecosystems of Karasaj landfill of municipal solid waste <i>S.S. Nurkeev, G.A. Jamalova, A.S. Nurkeev, G.V. Kurbanova, R. Adyrbaikyzy</i>	150
Calculation model of leachate composition at Karasaj landfill of municipal solid waste of Almaty city <i>A.S. Nurkeev, A.K. Kazbekova</i>	153
Research of pollution processes on urban areas using walnut (<i>Juglans regia</i>) <i>Olga Khandogina, Lidya Svirenko, Pavel Karnozhicki</i>	155
Photometrical approach to the problem of estimation of pigment activity in plant sells: update technique for black medic <i>A.P. Yurkov, D.G. Semenov</i>	160
Purification of off-gases of the fluoropolymer heat treatment furnaces of fluorine hydride by natural zeolites <i>A.V. Chechevichkin, N.I. Vatin, V.N. Chechevichkin</i>	164
Mode of soil and subsoil waters in parks of St.-Petersburg <i>V.V. Chasovskaya, T.V. Izotova</i>	169

The International Youth Science Environmental Forum "ECOBALTICA-2008"

Aggregate stability and coagulation of sulphate (kraft) lignin water dispersions – one of the main impurities of cellulose plants wastewaters	
<i>Yu. Moreva, I. Rudakova, L. Molodkina, A. Dyagileva, Yu. Chernoberezhskii</i>	173
The hydroengineering constructions for continuous sorbent sewage treatment	
<i>Nikolay Vatin, Natalia Golovkova, Victor Chechevichkin</i>	177
Recommendations for improvement of waste management systems in the NIS of Central Asia	
<i>K. Kholmatov, D. Khashimova</i>	181
Assessment of dynamic properties of municipal landfill in Uzbekistan	
<i>K. Kholmatov, D. Khashimova</i>	186
Assessment of the waste logistics in Central Asia	
<i>M. Musaev</i>	191
Radar-based SeaDarQ system for environmental and hydrographical applications	
<i>Geert Mosterdijk, Elena Ananyeva, Jan Kleijweg</i>	197
Freon refrigerants - recovery methods	
<i>Paweł Powierża, Carer Franciszek Świtła</i>	203
Spatio-temporal dynamics microbiota, distributed on range landfill Karasai c. Almaty	
<i>G.A. Jamalova</i>	204
Assessment of passive remediation approach for treatment of oil-polluted subsurface	
<i>Hiie Nõlvak, Jaak Truu, Mait Kriipsalu</i>	207
Some aspects of the account, planning and regulation of hunting resources of Latvia	
<i>Boris Yarinovsky</i>	208
The influence of illumination on the workplace's atmosphere	
<i>Piotr Świtła, Carer Franciszek Świtła</i>	208
Assessing waste management and landfills in Central Asia	
<i>M. Ritzkowski and H. Adwiraah</i>	209
Dynamic metal speciation analysis for the risk assessment of an industrial polluted site	
<i>Daniela Sani, Maria Letizia Ruello, Miriam Sileno</i>	209
Control of NO_x by injection of aqueous urea in a 150 kW pilot scale facility at PIEAS	
<i>Muhammad Tayyeb Javed, Naseem Irfan, Muhammad Asim Ibrahim, Asif Mahmood, Adnan Hamid, and Khalid Waheed</i>	210
Mycological problems in buildings of various age	
<i>Albinas Lugauskas, Bronius Jaskelvičius</i>	210
Benefiting from co-composting of municipal sludge and wastes	
<i>Piret Värnomasing, Mait Kriipsalu, Jüri Haller</i>	211
Influence of sewage on species diversity of the Pissa and the Pregolya rivers zoobentos and zooplankton	
<i>Maria Shibaeva, Evgeniya Pronkina</i>	212
Adsorption properties of schungite III related to humate of sodium	
<i>A.V. Mikhaylova, A.V. Lorentsson</i>	212
Laser application for conservation of monuments from destruction due to environmental influence	
<i>A.N. Kylosova, V.A. Parfenov</i>	213
Newtectonic structures on the platform	
<i>A.V. Karpunin, A.I. Klimova</i>	214
Toxicity assessment of natural waters in Kharkiv region, Ukraine, with biotesting	
<i>Tatyana Biryukova, Valeriy Yakovlev, Svetlana Matsuk</i>	214

Problems of collection of payments for pollution of the surrounding environment and their direction in the budget <i>E.E. Prynichnikova, A.I. Klimova</i>	215
Problem of an estimation of actions on ecological design of rooms in children's preschool establishments <i>O. Kudriavtsev, K. Vorobjev, V. Zamaraeva</i>	215
Creation of the laboratory plant for the estimation of biogas deep cleaning parameters <i>D.V. Molodtsov, A.N. Chusov, M.P. Fedorov</i>	216
Comparative study of efficiency of biological preparations for oil-contaminated soils cleaning in the course of field experiment <i>A.O. Gerasimov, L.G. Bakina, M.V. Chugunova</i>	217
Regulation of electrosurface properties of microcrystalline cellulose in management of wastes in paper production <i>P.M. Mosur, Yu.M. Chernoberezhskii, A.V. Lorentsson</i>	217
Algorithm of interaction schemotechnical modelling with geoinformation systems <i>N.V. Gorbunov, I.S. Popova</i>	218
Concept of reduction and recycling of emissions and flare gases <i>V.I. Firsov, N.V. Voronov</i>	219
Execution of construction projects in compliance with environmental requirements <i>Alexander Kholodnov</i>	219
Math models of multi commodity flows and applying them in scheduling and managing industrial process of paper manufacturing <i>V.A. Kusnetsov, I.G. Sidorenko</i>	220
Crushed rock as concrete ballast in stead of natural gravel <i>Susanne Skyllerstedt and Tommy Claesson</i>	221
К вопросу о правовых аспектах безопасности гидротехнических сооружений в контексте национальной безопасности государства <i>А.Б. Качинский, Д.В. Стефанишин, Д.Э. Бенатов</i>	221
 CONFERENCE 2. UNTRADITIONAL ENERGY SOURCES AND THEIR INFLUENCE ON ENVIRONMENT (DEVICES, MATERIALS, NEW METHODS OF TRANSFORMATION, ACCUMULATION AND TRANSFER OF ENERGY) 	
Preparation of kernite based borosilicate glass as a starting material for NaBH₄ production <i>Muge Sarə, Aysel Kanturk, Ozgul Dere, Sabriye Piskin</i>	226
Optical constants of Cu(In_{1-x}Ga_x)₃Se₅ solid solutions <i>G. Gurieva, S. Levchenko</i>	230
The use of biological and hydroenergetic resources in the conditions of northwest of Russia <i>M.Yu. Korol</i>	235
The prospects of wind-power engineering development in Russia <i>Yu.S. Chernenko</i>	239
Research of solid phase lithium accumulator <i>S.S. Smirnov, I.A. Putsylov, A.N. Savostyanov, K.S. Smirnov</i>	244
Making ecologically clean nanocomposed materials with the high dielectric constant on the basis of artificial porous matrixes <i>A.V. Filimonov, E.Yu. Koroleva, S.B. Vakhrushev, A. Naberezhnov</i>	247
Pellet heating system in Sweden	

The International Youth Science Environmental Forum "ECOBALTICA-2008"

<i>Semie Sama Memuna</i>	252
Atomic force microscopy of fuel cell components	
<i>A.V. Ankudinov, E.V. Guschina, E.E. Terukova</i>	259
Thin film solar cells based on semiconductor materials	
<i>V.F. Gremenok, V.Yu. Rud'</i>	265
The research of PtIr catalysts for unitised regenerative fuel cell with solid polymer electrolyte	
<i>A.V. Ladovsky, N.N. Kabalina</i>	265
Wastewater is a source of electricity	
<i>I.A. Samarukha</i>	266
The wind power generators prototype	
<i>S.S. Timofeyev</i>	266
Energy wood potential, supply systems and costs in Tihvin and Boksitogorsk districts of the Leningrad region	
<i>Ján Ilavský, Vadim Goltsev, Timo Karjalainen, Yuri Gerasimov and Timo Tahvanainen</i>	267
The research of sodium borohydride hydrolysis and electrooxidation of hydrogen obtained during hydrolysis	
<i>A.V. Ladovsky</i>	267
The analysis of carbon balance of Republic Kareliya and role in it of a biomass	
<i>G.I. Sidorenko, A.G. Gutaev</i>	268
To a question on environmental protection by produce of agricultural production for biofuel	
<i>S.S. Vinokurov</i>	268
Prospects of liquid hydrogen application as a fuel for aircrafts and as a coolant for airplane motors and generators	
<i>D.A. Volkov</i>	269
Ecologically friendly thermoelectrics for the waste heat utilization	
<i>I.S. Eremin</i>	270
 CONFERENCE 3. ENVIRONMENTAL SAFETY PROVISION	 271
Electron-beam flue gas treatment modelling	
<i>A.A. Bogdanov, M.I. Petrov, A.M. Polyansky, V.A. Polyansky</i>	272
The treatment process of flue gases with SO₂ content up to 30%	
<i>A.A. Bogdanov, M.I. Petrov, A.M. Polyansky, V.A. Polyansky</i>	276
The influence of cottage building on the ecological environment	
<i>E.V. Golovanova, M.S. Petrova, N.A. Leonova</i>	281
Water supply crisis and reservation of drinking water: a tactical and strategic approach	
<i>Olena Sierikova, Valerij V. Jakovljev</i>	285
The influence of hydrological changes to the technical and economical basis of arctic industry	
<i>E.V. Shevnina</i>	290
Fractal diagnostics of evaporation data on European part of Russian federation	
<i>D.V. Chistyakov, E.V. Gaydukova</i>	296
Climate risks estimation for power generation and building industry	
<i>E.M. Akentyeva, E.V. Ivanova</i>	300
Estimation of water quality in the lake Sestroretsky Razliv flood on hydrochemical parameters on the basis of geoinformation technologies	
<i>A. Shishkin, A. Epifanov, L. Kochubey</i>	303
Appraisal of risk of Karasay polygon functioning	
<i>S.S. Nurkeev, N.I. Utegulov, G.B. Kezembraeva</i>	308

Biodisplay kinds of microorganisms at an estimation of a condition of the closed municipal solid waste landfill of Astana <i>S.S. Nurkeev, G.A. Jamalova, G.M. Bitimshina, K. Musabekova</i>	314
Process of formation of biogas at municipal solid waste landfill Karasai of Almaty <i>A.S. Nurkeev</i>	317
Experience of practical use of satellite SAR and passive microwave data for ecomonitor ice cover parameters and optimization ice navigation in the Finish Gulf <i>S.V. Zelenetsky, V.V. Melentyev</i>	321
Object of destruction of chemical weapon Leonidovskogo of the arsenal <i>J.A. Jurtaeva</i>	326
Estimation of influence of municipal solid waste landfill of Astana city on the environment <i>S.S. Nurkeev, Z.M. Zhapparova, M.B. Oralbaeva</i>	326
Mandatory insurance liability of owners of hazardous production facilities: importance, reliability and timeliness <i>T.I. Yakimenko</i>	328
Compared characteristic of the methods of the complex estimation of the Suzdal Lakes's condition on various integrated parameters <i>D. Sharenkov, A. Shishkin</i>	329
Information support of decision acceptance for water bioresources protection field <i>S. Shibaev, A. Aldushin</i>	329
Social-ecological problems of urbanized territories, by the example of Saint-Petersburg <i>Lubov Volkova</i>	330
<i>CONFERENCE 4. SOCIAL-ECOLOGICAL AND ECONOMICAL PROBLEMS OF GLOBALIZATION: BALTIC DIMENSION</i>	331
Application mathematical models for operative short-range forecasts of the river runoff <i>N.V. Viktorova, A.V. Poljakova</i>	332
Ilya Prigogine and the postmodernity <i>Frédéric Malaval</i>	336
Influence of the market challenges on the ecological approach at the timber industry complex in Russian Federation <i>G.V. Michailova, K.L. Michailov</i>	339
Scientific-methodical bases of training to use ecological terminology with the support of its three-level semantic organization <i>A.V. Dolgoplov, A.V. Cheremisin</i>	342
Education and professional training of sustainable development manager <i>Andrey A. Avramenko, Anastasia Burtseva</i>	344
Physical bases of geodetic devices <i>A.I. Azarov, S.A. Rulev, D.G. Bokuchava, L.V. Volkova, I.V. Smirnov</i>	348
The importance of complex method of studying anthropocosystem for sustained development <i>I.S. Vostryakova</i>	352
Little sea — big problems <i>Ekaterina Ermolina</i>	353

The International Youth Science Environmental Forum "ECOBALTICA-2008"

Ecological crime

D.G. Nikulin

353

Pedagogical designing of process of training of ecologists

A.V. Dolgoplov, A.V. Cheremisin

354

