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Any inquiry for subscriptions should be sent to:
Prof. Mukhambetkali Burkitbayev, al-Farabi Kazakh National University
al-Farabi ave., 71, 050040, Almaty, Republic of Kazakhstan
e-mail: Mukhambetkali Burkitbayev@kaznu.kz

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EDITORIAL

The most significant achievements in the field of natural sciences are reached in joint collaboration, where important roles are taken by biology and chemistry. Therefore publication of a Journal, displaying results of current studies in the field of biology and chemistry, facilitates highlighting of theoretical and practical issues and distribution of scientific discoveries.

One of the basic goals of the Journal is to promote the extensive exchange of information between the scientists from all over the world. We welcome publishing original papers and materials of biological and chemical conferences, held in different countries (after the process of their subsequent selection).

Creation of special International Journal of Biology and Chemistry is of great importance, because a great amount of scientists might publish their articles and it will help to widen the geography of future collaboration. We will be glad to publish also the papers of the scientists from the other continents.

The Journal aims to publish the results of the experimental and theoretical studies in the field of biology, biotechnology, chemistry and chemical technology. Among the emphasized subjects are: modern issues of technologies for organic synthesis; scientific basis of the production of physiologically active preparations; modern issues of technologies for processing of raw materials, production of new materials and technologies; study on chemical and physical properties and structure of oil and coal; theoretical and practical issues in processing of hydrocarbons; modern achievements in the field of nanotechnology; results of studies in the fields of biology, biotechnology, genetics, nanotechnology, etc.

We hope to receive papers from a number of scientific centers, which are involved in the application of the scientific principles of biology, biotechnology, chemistry and chemical technology on practice and carrying out research on the subject, whether it relates to the production of new materials, technology and ecological issues.

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[1]Gumarova L., [2]Cornelissen G.

[1]Al-Farabi Kazakh National University, Almaty, Kazakhstan,
[2]Halberg Chronobiology Center, University of Minnesota, Minneapolis, USA
*E-mail: gumarova.lyazzat@gmail.com

Terrestrial and solar magnetism' influence to diphtheria pandemics

Abstract. The number of physicochemical factors of the environment is extremely high. The geophysical components of the environment are under the influence of cosmic forces. The sun, the moon, the planets and the stars are connected with the Earth by invisible bonds. Gravitation, radiation, electromagnetic fields and corpuscular ceilings of the Sun and stars are far from a complete list of the cosmic forces acting on the Earth. Changes in the activity of the Sun are reflected in the biosphere directly through photosynthesis of plants, and indirectly through changes in climatic conditions on the Earth, which affects various biological processes. One of the first scientists, who paid attention to the dependence of pandemics with solar cycles, was Alexander Chizhevsky.

In this study, we perform a meta-analysis of statistical data on diphtheria, based on the data published by Chizhevsky in the 20th century. We here add for the association of terrestrial and solar magnetism with these infectious diseases that were common in the past, using nonlinear analysis, cosinor-analysis and cross-wavelet coherence. We showed the geographic differences hidden in Chizhevsky's data on diphtheria. Wavelets of Wolf numbers and of the antipodal geomagnetic index show maxima corresponding to the anticipated ~11.7-year cycle, also seen from the spectra plotted vertically next to the color key. The numbers indicate the period length (in years) corresponding to local maxima in amplitudes; but the color code matters most. The wavelet also reveals the presence of an about 22.1-year component differing from the smaller about 19.7-year peaklet observed for Wolf numbers. Both these peaks are less prominent than the about 29.5-year peak found for diphtheria.

Key words: Chizhevsky, diphtheria, helio- and geomagnetism, cycles in communicable disease incidence

The number of physicochemical factors of the environment is extremely high. The geophysical components of the environment are under the influence of cosmic forces. The sun, the moon, the planets and the stars are connected with the Earth by invisible bonds. Gravitation, radiation, electromagnetic fields and corpuscular ceilings of the Sun and stars are far from a complete list of the cosmic forces acting on the Earth. Changes in the activity of the Sun are reflected in the biosphere directly through photosynthesis of plants, and indirectly through changes in climatic conditions on the Earth, which affects various biological processes. One of the first scientists, who paid attention to the dependence of pandemics with solar cycles, was Alexander Chizhevsky.

In this study, we made a meta-analysis of statistical data on diphtheria, based on the data published by Chizhevsky in the 20th century dating back to the span from 1860 to 1910 [1], we here add for the association of terrestrial and solar magnetism with these infectious diseases that were common in the past, using nonlinear analysis, cosinor-analysis and cross-wavelet coherence [2-6]. We showed the geographic differences hidden in Chizhevsky's data on diphtheria. Diphtheria is a bacterial infection caused by Corynebacterium diphtheria, transmitted from person to person through close physical and respiratory contact. It can cause infection of the nasopharynx, which may lead to breathing difficulties and death. WHO reports about 4530 cases of death for 2015 [7].

For the span from 1860 to 1912, Shostakovich in Irkutsk resolved two τs of 2.77 and 11.33 years for data from Denmark [1]. Our meta-analyses were on yearly data on the incidence of diphtheria in Denmark, Sweden, Switzerland, England and Wales, and Romania, that all ended in 1910 but started at different times for the different countries, namely 1860 (England and Wales, and Denmark, T=51 years), 1861 (Sweden, T=50 years), 1876 (Switzerland, T=35 years), and 1886 (Romania, T=25 years).The database is actually larger, also including data from Prussia, Scotland, Belgium, Holland, Ireland, Austria, Italy, and France, for which, however, a nonlinear validation of linear results failed in our hands.

First, the data from all countries were plotted in chronograms, with superposed curves for the different countries to check on similar trends as a function of time, and also separately for the 5 countries of interest wherein a nonlinear validation of a τ was possible. Polynomial fits examined the extent to which such should be included in analyses aimed at assessing any periodicities. By visual inspection, whereas no trend was apparently needed for data from Romania and Denmark, a linear trend seemed desirable and was included for data from Switzerland and Sweden, and a third-order polynomial for data from England and Wales.

Least squares spectra were computed using a fundamental τ of 51 years and a 0.2 harmonic increment. Average amplitude (phase-unweighted) spectra were computed, including results from all countries as well as from the 5 countries of interest here. As expected, amplitudes tended to be much larger at low frequencies, likely related to trends present for most time series. Differences are observed by the naked eye in the spectral structure of statistics from the 5 countries, with spectral peaks occurring at different frequencies in different geographic locations.

Overall, a few peaklets are found that correspond approximately to cycles with τs of about 17, 12, 10.2, 6, and 3.5 years. Focus was first placed on putative components with τs of about 17 and 12 years. Nonlinear analyses were performed, using a single component model with a trial τ of either 12.0 or 17.2 years. Analyses were carried out as such and with the addition of a linear trend or a third-order polynomial. At a trial τ of 12 years, in Switzerland the τ converges toward 10.63, 12.01 or 13.03 years depending on whether there is no trend, a linear trend or a third-order polynomial. The conservative CI for the amplitude does not overlap zero only in the case of the model including a linear trend. In this case, the τ is estimated as 12.01 (CI: 9.80, 14.21) years, with an amplitude of 8.52 (CI: 0.22, 16.81) (table 1). There is a large discrepancy in the estimate of the τ for this country depending on whether a linear trend is included in the model or not. In England and Wales, the τ converges to 12.61, 12.20 or 11.07 years, respectively, but the CI of the amplitude invariably covers zero. In Romania, the τ converges to 16.73, 16.96, or 16.24 years, respectively, but in the latter case (including a third-order polynomial), the model does not fit and a CI cannot be obtained for the τ. Without a trend, the τ is estimated as 16.73 (CI: 12.66, 20.80) years and the amplitude as 9.65 (CI: 2.86, 16.45). In Denmark, the τ converges to 12.03, 11.96, or 13.47 years, respectively, the CI of the amplitude invariably not covering the pole. As no trend seemed to be needed, the τ can be estimated as 12.03 (CI: 10.72, 13.33) years with an amplitude of 33.89 (CI: 7.09, 60.69). In Sweden, the τ converges to 12.58, 12.76, or 17.44 years, a CI of the amplitude not covering zero only when a third-order polynomial is included, in which case the τ estimate converges to a longer τ of 17.44 years.

At a trial τ of 17.2 years, in Switzerland the τ converges toward 16.99, 12.01 or 12.70 years depending on whether there is no trend, a linear trend or a third-order polynomial, the CI for the amplitude not overlapping zero in the case of the model including a linear trend or no trend. Note the large discrepancy in τ estimate depending on whether a linear trend is included or not in the model. Furthermore, when a linear trend is included in the model, the same results are obtained whether the trial τ is 12.0 or 17.2 years. In England and Wales, the τ converges to 17.46, 17.58 or 16.08 years, respectively, the CI of the amplitude covering zero in the latter case. Relying on a linear trend rather than on a third-order polynomial, the τ is estimated as 17.58 (CI: 14.28, 20.88) years, with an amplitude of 4.72 (CI: 0.18, 9.26). In Romania, the τ converges to 16.73, 16.96, or 16.24 years, respectively, but in the latter case (including a third-order polynomial), the model does not fit and a CI cannot be obtained for the τ. Note that these results are the same as those obtained with a trial τ of 12.0 years. As no trend seemed to be present, the τ can be estimated as 16.73 (12.66, 20.79) years and the amplitude as 9.65 (CI: 2.86, 16.45). In Sweden, the τ converges to 17.71, 18.02, or 17.44 years, the CI of the amplitude invariably not covering zero. As a linear trend appeared to be present, the τ can be estimated as 18.02 (CI: 15.23, 20.81) years and the amplitude as 19.69 (CI: 5.67, 33.70). In Denmark, the τ converges to 33.04, 36.98, or 24.88 years, respectively, the CI of the amplitude invariably not covering the pole. Note that these τ estimates are much longer than the trial τ of 17.2 years, two of which correspond to a Brückner-Egeson-Lockyer (BEL) paratridecadal defined only by its CI approaching (not necessarily covering) the 30-40-year range.

William J.S. Lockyer [8-10] associated the paratridecadal with the τ he found to characterize changes of the length of the variable circadecadal Horrebow-Schwabe cycle in relative sunspot numbers, as confirmed by Liznar [11], a topic also of interest in 1903 to William Lockyer's father Sir J. Norman Lockyer, the codiscoverer of helium and founder of the journal Nature [10]. We now recognize that this cycle's τ length is highly variable, on the average, longer than (i.e., beyond = trans) 30 years, hence originally dubbed transtridecadal.

The BEL cycle (acronym taken from the initials of Brückner, Egeson and Lockyer) was defined as a cycle with the CI of its period (τ) overlapping (this definition was liberalized to the CI approaching) the range of 30 to 40 years. This was prompted by point estimates of τ, close to, but outside the 30-40-year range of variables closely related to those with τ inside that range.

Table 1 – Geomagnetic/geographic differences among cycles with periods in the range of 5 – 32 years, characterizing the incidence of diphtheria

Site	Span	Period (y) (95% CI)	Amplitude (95 % CI)	A (% of MESOR)	P-value
Kherson province	1874-1908	19.38 (14.53, 24.22)	31.93 (9.71, 54.15)	46	<0.005
Kherson county	1874-1908	16.996 (13.83, 20.16)	12.54 (3.42, 21.67)	36	<0.001
Elizavetgrad county	1874-1908	20.53 (17.23, 25.05)	27.74 (9.92, 45.56)	65	<0.005
Denmark	1860-1910	31.78 (25.80, 37.76)	43.91 (19.35, 68.46)	66	<0.001
		11.99 (10.89, 13.09)	35.77 (12.18, 59.36)	54	<0.001
Prussia	1875-1910	9.10 (6.63, 11.58)	24.27 (22.14, 70.68)	23	<0.05
Switzerland	1876-1910	16.98 (12.76, 21.2)	13.04 (0.04, 26.04)	36	<0.001
		12.01 (9.80, 14.21)	8.52 (0.22, 16.81)	23	<0.001
Scotland	1860-1910	26.33 (18.17, 34.30)	11.96 (0.38, 24.30)	29	<0.001
		12.75 (10.07, 15.42)	8.6 (0.00, 21.73)	21	<0.05
		9.89 (8.56, 11.22)	9.94 (0.00, 23.03)	41	<0.05
Belgium	1870-1910	21.49 (10.02, 32.96)	12.69 (9.02, 34.41)	23	<0.001
		14.02 (9.27, 18.78)	12.71 (9.08, 34.50)	23	<0.001
Holland	1875-1910	14.02 (9.42, 18.61)	6.82 (0.00, 17.6)	27	<0.05
		9.35 (7.64, 11.07)	8.23 (0.00, 18.48)	33	<0.05
England and Wales	1860-1910	29.37 (23.15, 35.59)	8.89 (4.06, 13.73)	29	<0.001
		17.46 (14.66, 20.26)	7.04 (1.32, 12.76)	23	0.001
		12.58 (10.53, 14.64)	5.10 (0.00, 11.37)	17	<0.05
Ireland	1864-1910	24.16 (10.45, 37.87)	3.21 (2.92, 9.34)	14	<0.05
		17.27 (7.67, 26.86)	2.29 (4.04, 8.63)	10	<0.05
		12.40 (9.26, 15.53)	3.62 (2.5, 9.73)	15	<0.05
		7.84 (6.54, 9.15)	3.42 (2.76, 9.59)	14	<0.05
Romania	1886-1910	16.73 (12.66, 20.80)	9.65 (2.86, 16.45)	49	<0.001
Austria	1880-1910	16.09 (7.55, 24.64)	23.66 (17.58, 64.9)	25	<0.001
Italy	1887-1910	12.51 (2.66, 22.36)	9.57 (14.64, 33.77)	28	<0.001
France	1889-1910	11.26 (7.056, 15.52)	13.528 (0.00, 30.624)	51	<0.05
Sweden	1861-1910	17.71 (15.45, 19.98)	22.00 (8.72, 35.28)	42	<0.001
		12.58 (10.16, 14.99)	11.89 (0.00, 28.14)	23	<0.05

In turning to the comment that Shostakovich found components with periods of 2.77 and 11.33 years in Denmark, additional nonlinear analyses were performed to examine whether these results could be validated. First, a single component with a trial period of 2.8 years was used, but could not be detected with statistical significance. In view of the large spectral peak corresponding to a period of about 12 years, this component was added to the model. This 2-component model resembles that of Shostakovich, but only the 12-year component could be demonstrated, the CI of the amplitude only slightly overlapping zero when the 2.8-year component is included in the model. Examination of the least squares spectrum, and in keeping with the nonlinear results reported above, statistics in Denmark are characterized by two large spectral peaks, corresponding to τs of about 32 and 12 years with two other smaller peaks also present with τs of about 6.7 and 3.5 years. Using this 4-component model, nonlinear results can only validate the first two components. Replacing the trial τ of 3.5 years with one of 2.9 years, in keeping with the report by Shostakovich, also validates only the first two components. A three-component model, excluding the 3.5- or 2.9-year component, also cannot validate the 6.7-year cycle. But a two-component model with trial τs of 32 and 12 years fits. τs are estimated as 31.78 (CI: 25.80, 37.76) and 11.99 (CI: 10.89, 13.09) years with respective amplitudes of 43.91 (CI: 19.35, 68.46) and 35.77 (CI: 12.18, 59.36). Figure 1 shows that the results are roughly in agreement for England and Wales, Romania, Sweden and one country in Russia with about 16-18-year cycles, but in Switzerland and Denmark, dominant components have τs around 12 years; Denmark also has a BEL, and Russia has τs with a CI nearing or covering 21 years.

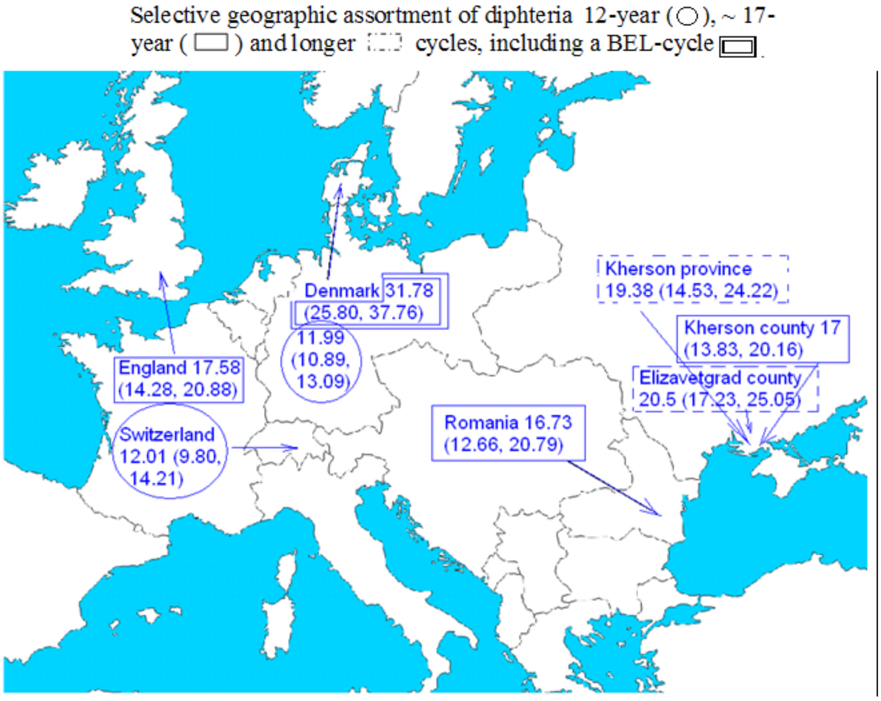

Figure 1 – Geographic map of infra-annual cycles characterizing the incidence of diphtheria

Data on diphtheria were also analyzed in some regions of what was formerly south Russia, now Ukraine: Herson county (uezd), Elizabet county and all of Herson province (guverny).

Figure 1 shows the dynamics of diphtheria in Russia (using a square root transformation of data): in Elizabet county the τ was 20.5 (17.2, 25) years with an amplitude of 27.74 (9.9, 45.56); in Herson county it was 17 (13.83, 20.16) years with an amplitude of 12.54 (3.37, 21.71), and in the larger Herson province, that included 7 other counties with Herson and Elizabet counties, nonlinear analysis shows a period of 19.38 (14.53, 24.22) years with an amplitude of 31.93 (9.71, 54.15).

We here show the presence of several cycles that may correspond to the Horrebow-Schwabe, the Hale, and the Brückner-Egeson-Lockyer cycles, among others. Such correspondence can only prompt a subtraction-addition approach. The use of special computer programs allows us to more accurately estimate the periods. A paradecadal 8.25-12.93-year cycle was found in Denmark, Belgium, Switzerland, Scotland, England (and Wales) and Ireland, whereas in Austria, Italy, Romania and Sweden, the period length of the most prominent cycle was between 15 and 18.8 years (Table 1). In the dynamics of diphtheria in the Kherson province (then South Russia, now Ukraine), statistically significant periodicities include a point estimate of 17 years, with others of 19.4 and 21 years, but with very wide overlapping CIs so that the periods cannot be separated. In Russia, only relapsing fever in Moscow had a prominent decadal period, Table 1 (bottom), a detail qualifying even a genius like Chizhevsky. If not an «echo» only to the sun, the biosphere and the noosphere it created are resonators to both the sun and the earth, among others, and the various environmental influences compete (wrangle) with one or the other dominating in different geographic regions at the same time. Figure 2 (top) shows a wavelet of the incidence pattern of diphtheria in Denmark from 1860–1910. An about 12.4-year component is seen to characterize the incidence pattern; it is within the cone of influence. Another component with a period of about 29.5 years is outside this cone. Both components are validated and are within the CIs (95% confidence intervals) of the nonlinearly extended cosinor, applied to the same time series on diphtheria.

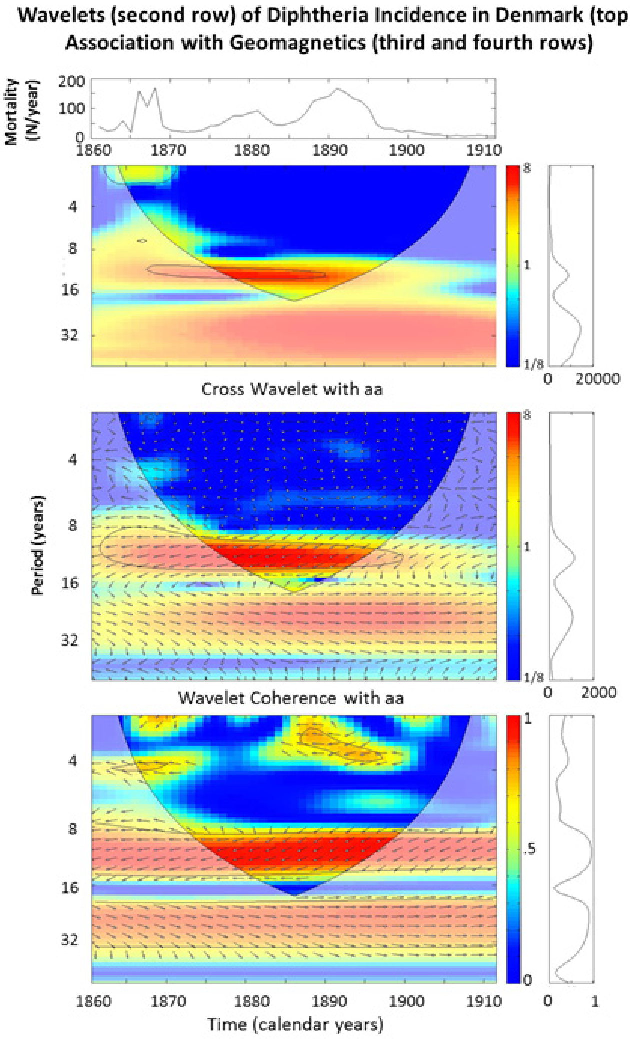

Figure 2 – Wavelet analyses of incidence pattern of two infectious diseases (pooled) centuries ago (top row), of Wolf sunspot numbers (middle), and of the antipodal geomagnetic index aa (bottom), with peaks indicated by color (key), and by numbers at the maxima in the spectrum (right, next to the color key) show a putative reflection of past and/or present solar variability

Wavelets of Wolf numbers, WN (Figure 2, middle), and of the antipodal geomagnetic index aa (Figure 2, bottom) show maxima corresponding to the anticipated ~11.7-year cycle, also seen from the spectra plotted vertically next to the color key. The numbers indicate the period length (in years) corresponding to local maxima in amplitudes; but the color code matters most. The wavelet of aa also reveals the presence of an about 22.1-year component differing from the smaller about 19.7-year peaklet observed for WN.

Both these peaks are less prominent than the about 29.5-year peak found for diphtheria.

Figure 3 shows cross-wavelet transforms (left) and coherence displays (right) of WN (top) and aa (bottom) with diphtheria. The 11.7-year period within the cone of influence stands out in association with both WN and aa in the cross-wavelets. An added large peak at 24.2 years is seen in association with aa. Peaklets are seen at ~23 and ~44 years in association with WN. Strong coherences around 10.4 (WN) or 11.0 (aa) years inside the cone of influence and additional strong coherences outside this cone around 23.4 (aa) or less intensely around 22.1 (WN) years are interesting. Added coherences around 5.5 years and at still shorter pperods in association with WN are intermittently statistically significant (as seen from black contours). Again, there is a difference in spectral location between WN and aa, the overall peak occurring around 3.5 years for aa.

Among para-annual components, solar wind speed, a measure of interplanetary magnetism, and aa share some frequencies but differ at others [12]. Here we find that, in the decadal range, both WN and aa, gauging solar and terrestrial magnetism, respectively, shared coherence (within the cone of influence) with the main cycle characterizing communicable diseases, such as diphtheria, in the past when they were pandemic. Just as helio-, inter- planetary or geomagnetism can influence sudden cardiac death [12, 13], they also influenced communicable diseases, probably via the host, whose steroidal defense shows a proven decadal cycle [12] and by the invading microorganism, whose mutations can also undergo a similar cycle mirroring that of sunspots [12]. Cycles in the sun's and the earth's magnetism are features of both communicable and noncommunicable disease etiology and, in both cases, are geo- graphically, selectively assorted, shown elsewhere [13, 14].

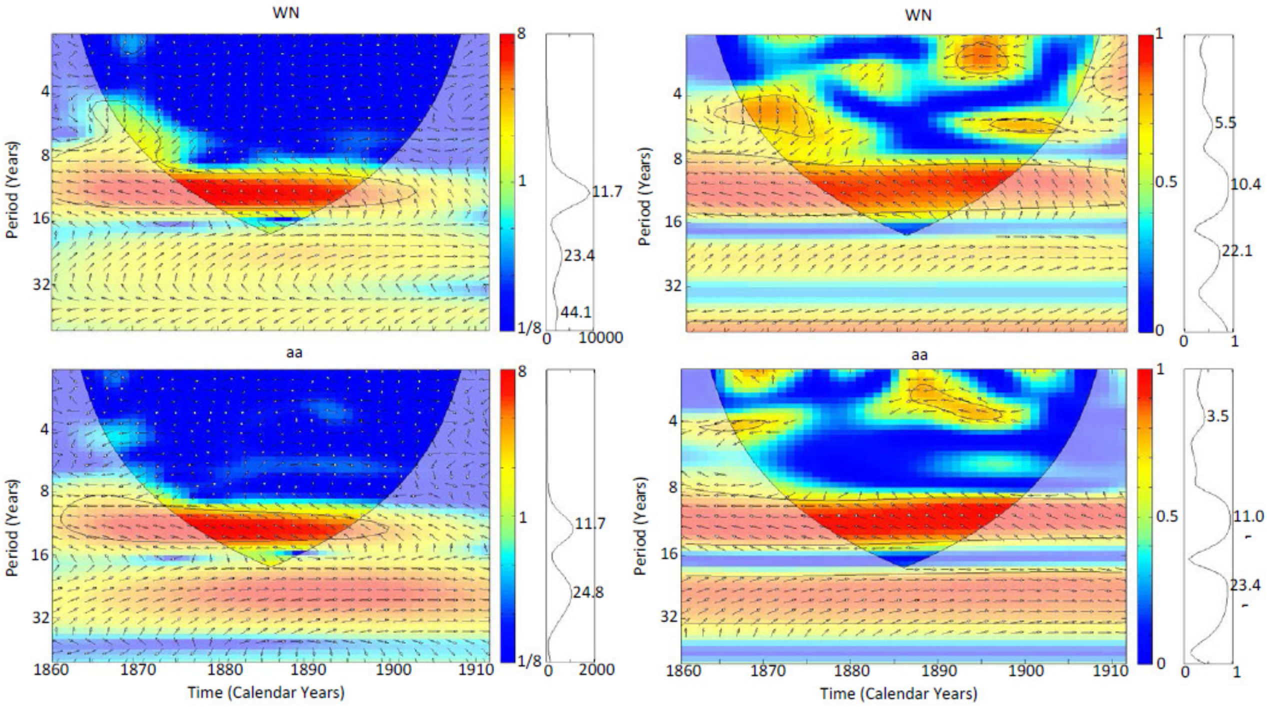

Figure 3 – Cross-wavelet transforms show a strong association of the incidence of two infectious diseases (pooled) with WN and aa at a period, t, of 11.7 years within the cone of influence. Coherence (right) is also found within the cone of influence at 10.4 and 11.0 years. These ts are also near those validated for the incidence of diphtheria by the extended nonlinear cosinor (not shown) that suggests the statistical significance of differences among the two aspects of solar and earth magnetism's association with rampant pandemics, some within the cone of influence.

References

1. Chizhevsky AL. The Terrestrial Echo of Solar Storms, 2nd ed. Moscow: Mysl, 1976, pp. 367.

2. Marquardt DW. An algorithm for least-squares estimation of nonlinear parameters. J Soc Indust Appl Math 11: 431-441, 1963.

3. Halberg F. Chronobiology: methodological problems. Acta med rom 18: 399-440, 1980

4. Cornélissen G, Halberg F. Chronomedicine. In: Armitage P, Colton T (Eds.) Encyclopedia of Biostatistics, 2nd ed. Chichester, UK: John Wiley & Sons Ltd; 2005. p. 796-812.

5. Refinetti R, Cornélissen G, Halberg F. Procedures for numerical analysis of circadian rhythms. Biological Rhythm Research 38 (4): 275-325, 2007.

6. Grinsted A, Moore JC, Jevrejeva S. Application of the cross wavelet transform and wavelet coherence to geo- physical time series. Nonlinear Processes in Geophysics 2004; 11: 561–566

7. WHO report: Immunization, Vaccines and Biologicals. Diphteria http://www.who.int/immunization/monitoring_surveillance/burden/diphtheria/en/ – Accessed December 16, 2016

8. Lockyer WJS. The solar activity 1833-1900. Proc Roy Soc Lond 1901; 68: 285-300.

9. Lockyer WJS. Die Sonnenthätigkeit 1833-1900. Meteorologische Zeitschrift 1902; 19: 59-71.

10. Lockyer N. Simultaneous solar and terrestrial changes. Science 1901; 18: 611-623.

11. Liznar J. Über die 33-jährige Periode der Sonnenflecken, Meteorologische Zeitschrift 1902; 19: 237-238.

12. Halberg F, Cornélissen G, Sothern RB, Czaplicki J, Schwartzkopff O. Thirty-five-year climatic cycle in heliogeo- physics, psychophysiology, military politics, and economics. Izvestiya, Atmospheric and Oceanic Physics 2010; 46 (7): 844–864.

13. Gumarova L., Cornelissen G., Hillman D., Halberg F. Geographically selective assortment of cycles in pandemics: Meta-analysis of data collected by Chizhevsky. // Epidemiology and Infection, Vol. 141, Issue 10, 2013, p. 2173-2184.

14. Gumarova L., Cornelissen G., Dimitrov B.D., Halberg F. Transyears competing with the seasons in tropical malaria incidence // in book: Current Topics in Malaria. – InTech, 2016, pp 39-52. – ISBN 978-953-51-2790-1, Print ISBN 978-953-51-2789-5, 504 p.

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[1]Habibi A., [1]Akmuhanova N.R., [1]Zayaadan B.K., [1]Sadvakasova A.K.,
[1]Baizhigitova A., [2*]Rahmatzai N.

[1]Al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Faculty of Agriculture, Baghlan University, Afghanistan
[*]E-mail: ainullah.habibi1@yahoo.com

The soil algal flora as bio-indicators of the soil condition in the Baghlan region (Afghanistan)

Abstract. Soil algae are present in all kinds of soils, both arable and virgin, in large amounts and in great diversity. They are photoautotrophic, aerobic organisms obtain CO_2 from atmosphere and energy from sunlight and synthesize their own nutrients. They are unicellular, filamentous and/or colonial. Soil algae are divided into four main phyla like *Cyanophyta, Chlorophyta, Xanthophyta, Bacillariophyta.* They play a crucial role in soil fertility. Particularly *Cyanophycae* members are known by their significant role in the nitrogen source of soils. There are numerous works about the soil algal flora as bio-indicators of the soil condition in the world but no research has been carried out on Baghlan region.

This article presents the results of the study on the species diversity of soil algal flora in Baghlan region (Afghanistan). In order to study the soil algal flora in the Baghlan region, soil sample were collected in August, 2016. Collected soil sample were cultured in standard medium and soil algae were observed thoroughly under research microscope and identified with the help of standard literature on algae. According to the results of the study, soil *algocenoses* in Baghlan province, Chashmaishir and Shamarq characterized by the predominance of *cyanobacteria.* We have identified 4 divisions, 8 classes, 18 orders, 24 families, 28 genera, 46 species. According to the analysis of certain kinds of saprophytic-indicator, the soil of the Baghlan region characterized as the *β-mesosaproprobic*. The saprobity index according to the method of Pantle – Buck was s 2.8. The main representatives of algae indicator in soils are *α, β- mesosaprobics* of microalgae.

Key words: soil aglal flora, soil algocenoses and α, β- mesosaproproic.

Introduction

Algae can be finding in most of the soils where humidity and sunshine are available. Their number in soil commonly ranges from 100 to 10,000 per gram of soil. They are photoautotrophic, aerobic organisms and acquire carbon dioxide from atmosphere and energy from sunlight and synthesize their own food [1].

They are unicellular, filamentous or colonial. Four different phyla of algae are recognized in soil: green (*Chlorophyta*), blue-green (*Cyanobacteria*), yellow-green (*Xanthophyta*), and diatoms or golden-brown algae (*Bacillariophyta*). Out of these four phyla, blue-green algae and grass-green algae are more abundant in the soil. The green-grass algae and diatoms are dominant in the soils of temperate region while blue-green algae predominate in tropical soils. Green-algae prefer acid soils while blue green algae are commonly found in neutral and alkaline soils. The most common genera of green algae found in soil are: *Chlorella, Chlamydomonas, Chlorococcum, Protosiphon* etc. and that of diatoms are *Navicula, Pinnularia. Synedra, Frangilaria.* Blue green algae are unicellular, photoautotrophic prokaryotes containing Phycocyanin pigment in addition to chlorophyll. They do not posses flagella and do not reproduce sexually. They are common in neutral to alkaline soils. The dominant genera of BGA in soil are: *Chrococcus, Phormidium, Anabaena, Aphanocapra, Oscillatoria* etc. Some BGA posses specialized cells known as «Heterocyst» which is the sites of nitrogen fixation. BGA fixes nitrogen (non-symbiotically) in puddle paddy/water logged paddy fields (20-30 kg/ha/season). There are certain BGA which possess the character of symbiotic nitrogen fixation in association with other organisms like fungi, mosses, liverworts and aquatic ferns *Azolla, eg Anabaena-Azolla* association fix nitrogen symbiotically in rice fields [2].

Soil algae are *photoautotrophs*. These species do not depend on the organic matter (carbon content) of the soil and play a role as primary colonizers. They produce large amounts of secreted polysaccharides that promote soil aggregation at the surface and they are capable of nitrogen fixation. Soil habitats are the most important non-aqueous ecosystems for microalgae where these organisms contribute to soil formation and stability In addition, microalgae contribute to energy and matter flux Green and blue green microalgae populations in upper topsoil can perform valuable services for soil ecosystems and agriculture One of the major benefits of microalgae is the generation of organic matter from inorganic substances In addition to providing a food source for other microorganisms, nematodes, and invertebrates, microalgae produce biologically active compounds such as enzymes and ions that can affect other components of soil communities, including plants [3-10].

In conditions of intensification of agriculture and a sharp increase of anthropogenic impact on the environment, particularly the soil cover, algae significantly increases the role of biological factors in extending the fertility of soils and their reclamation. A great help in this condition could be skillful use and development of soil biota, permanent and significant component of which are algae [11].

Soil algae have a various impact on the soil and its fertility, the most important aspect of the latter is the accumulation of organic matter. Filling the space unoccupied by higher plants, *algocenoses* serve as a factor of additional biomass [12].

Organic matter of algae easily available to many heterotrophic organisms. Therefore, their development stimulates the activity of many soil microorganisms. Assimilating the ash elements from the soil, they participate in small biological circulation of substances, as well as contribute to the preservation of moisture in the soil.

Soil algae absorbing various salts; affect the redistribution of elements in soil. Biological fixation of easily soluble salts can be a great value. Substances absorbed by the algae, or remain available to the roots or quickly return to the cycle of matter after their death. Many species of *Cyanobacteria* can fix atmospheric nitrogen, which is an additional source of nitrogen nutrition of higher plants.

Cyanobacteria compose active part of the soil *microbiota*. Due to the physiological and biochemical characteristics of protoplast, microscopic size, autotrophy, ability to fix atmospheric nitrogen and «*ephemerality*» of vegetation they can survive even in extremely unfavorable of existence conditions. As an explerents for life strategy, having low competitive power and high-energy reproduction, *Cyanobacteria* can explore space, especially in terrestrial ecosystems, where the limiting factors significantly limit the development of higher plants [13]. In this regard, the study of algal flora experiencing of different types and levels of anthropogenic impacts is extremely important, because it allows to identify the response of organisms to specific contaminants, establishes the degree of adaptation to the action of disturbing factors, determines the possibility of using certain species for assessment of soil condition, identifies the ways of a biological re-cultivation.

The blue green algae increase in soil pores with having filamentous structure and production of adhesive substances, excrete of growth-promoting substances such as hormones (*auxin, gibberellin*), vitamins, amino acids, increase in water holding capacity through their jelly structure, increase in soil biomass after their death and decomposition, decrease in soil salinity, prevent weeds growth, increase in soil phosphate by excretion of organic acids [14-18].

Cyanobacteria or Blue green algae (BGA) are a group of microorganisms that can fix the atmospheric nitrogen. BGA can adapt to various soil types and environment which has made it cosmopolitan in distribution. Efficient nitrogen fixing strains like *Nostoc linkia, Anabaena variabilis, Aulosira fertilisima, Calothrix sp., Tolypothrix sp., and Scytonema sp.* were identified from various agro-ecological regions and utilized for rice production [19]. Thus, the aim of this work is to study the species composition, structure of algal flora and anthropogenic-affected soils of Baghlan region.

Materials and methods

During the study, field collections and laboratory analyses were conducted, using methods generally accepted with algological practice. Algological collection of samples, the study of the taxonomy carried out in Chashmaishir and Shamarq areas of Baghlan province that located at 36°00'N latitude and 68°30'E longitude, altitude 531 m above sea level, minimum temperature during the winter 0-20°C and maximum temperature during the summer is 47°C, steppe climate, little rainfall about 282 mm of precipitation falls annually [20].

The physical and chemical properties of the soil were as follows: pH 8.2, containing of high calcium carbonate 10-12 percent [21], generally lack of nitrogen and phosphorus, soil characteristics alkaline and mineral soils containing 1-2% organic matter [22].

Algological samples collected in summer August, 2016. 12 mixed soil samples, were collected for analysis by algological classical methods. Determination of taxonomic composition of microalgae was carried out in the laboratory of phototrophic microorganisms at the Department of Biotechnology of al-Farabi KazNU. For identification of microalgae definitions from the «Determination of taxonomic composition of blue-green algae of Central Asia» were used [23,24,25].

To identify the species composition of the algae, culture methods were used: soil culture with glass fouling and agar cultures. ×50 field of view was observed on at least three preparations. The number of organisms was assessed by the frequency scale after the transfer to the ×100 field of view. The frequency of occurrences is recorded at 6 point system [26]. Types of microalgae indicators established by the atlas, *saprobity* index was calculated by the method of *Pantle – Buck* [27].

Results and their discussion

In the results of conducted research of the soil of Baghlan province in the area of Chashmaishir and Shamarq 46 species were detected belonging to 4 divisions, 8 classes, 18 forms, 24 families, 28 genera (Fig. 1) of algae and *cyanobacteria*. Taxonomic structure of algal flora was as follow: *Cyanobacteria – 21* species *(45%), Bacillariophyta – 5* species *(10%), Xanthophyta* species *– 5 (10%), and Chlorophyta –* 15 species (33%). Dominant position of algae groups occupied by the representatives of *Cyanobacteria* and *Chlorophyta*. However, the dominant role belongs to the *cyanobacteria*, typical representatives of soil ecosystems.

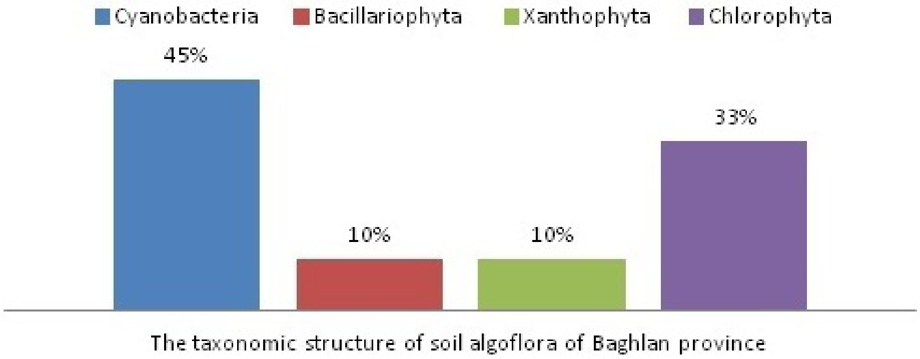


Figure 1 – Taxonomic structure of alga flora of Baghlan region

In the selected soils from the Chashmaishir area were revealed 44 species of microalgae and *cyanobacteria*. Five species are yellow-green, 19 species are cyanobacteria, 5 species are diatom and 14 species are green. Group which has the most frequently occurring types (frequency of 40 % and above) is represented by 6 types: *Chlorococcum sp., Phormidium autumnale, Nitzschia palea, Chlorella vulgaris* and *Nostoc linckia, Anabaena sp.* Most of the identified species (50% from the total species) had a low prevalence (below 10%), i.e., occurred in no more than 1-3 samples. The complex of dominant species is very rich and included representatives of the divisions of green algae and *cyanobacteria* (Fig. 2).

In algological samples obtained from soils of the Shamarq area identified 38 species and variations of microalgae and *cyanobacteria*. 17 species were *cyanobacteria*, i.e., blue-green – 3, green – 12, diatom – 4. In those sample dominated: from the blue-green – *Nostoc verricosum Vauch., Phormidium autumnale Cylindrospermum licheniforme, Nostoc commune.* From the diatom identified: *Navicula cohnii, Synedra ulna, Diatoma huemale, Cumbella ventricosa Kuetz.* From the yellow-green algae: *Botrydiopsis arhiza, Characiopsis anabaenae, Heterococcus caespitosus*. From the green dominated – *Chlamydomonas debaryana, Chlamydomonas minutissima, Chlorococcum infusionum, Scenedesmus obliquus, Desmococcus livaceus, Chlorella vulgaris Beijerinck, Chlorella ellipsoidea* (Fig. 3).

Algae are reliable indicators of the dynamics of pollutants in soil. They can indicate the presence of soil contaminants, quickly changing the soil algal flora. In the result of changes the species composition and abundance of soil algae, formed similar in composition *algocenoses* that reflects the level of soil contamination. In subsequently years, the range of their use as indicators has grown considerably. Large use of algae found in the study of fertility of soils and effects of chemicals, when determining the extent of urban loads on the soil and, especially for evaluating different types of anthropogenic and industrial pollution. Not so long ago, soil algae included in the monitoring associated with the problem of nature protection [28].

In the result of the analysis of certain types of microalgae and *cyanobacteria* revealed the presence of 10 species of indicator-*saprobic* species, including alpha-*mesosaprobic* (α)-2, *beta-mesosaprobic (β) – 3, polymesosaprobic (p)* – 2, *alpha-beta – mesosaprobic (α-β)-1*, *beta-alpha – mesosaprobic (β-α)-1*, *alpha-beta-mesosaprobic (β-p)-1* (Table 1).

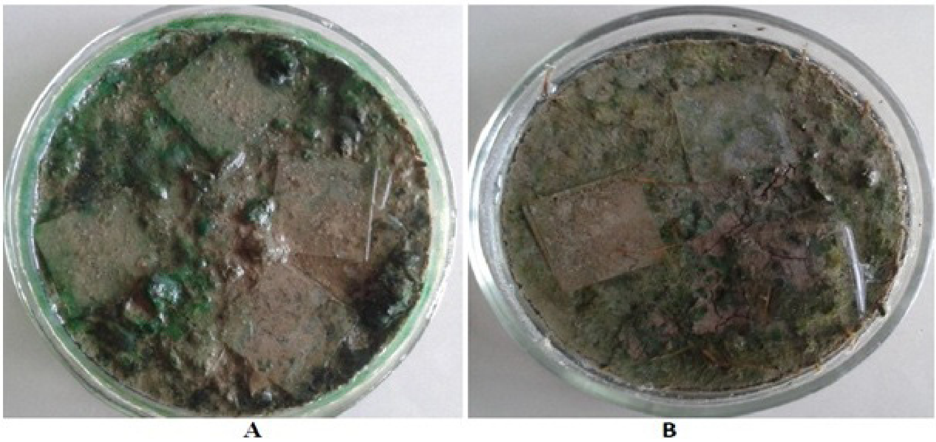
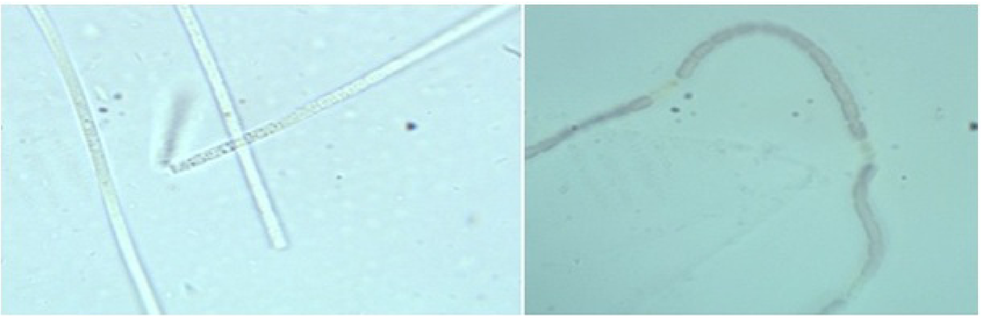


Figure 2 – Soil samples from Chashma-i-shir (A) and Shamarq (B)



A B

Figure 3 – Bacteria cell from Chashmaishir (A) and Shamarq (B) soils

Table 1 – Indicator-*saprobic* microalgae and *cyanobacteria* detected in the soils of Baghlan region

№	Species	*Saprobity*	S	Frequency, h	Sh
1	*Oscillatoria chlorina*	p	3.8	5	19
2	*Oscillatoria brevis*	α	2.8	9	25.2
3	*Pseudanabaena catenata*	α-p	3.6	7	25.2
4	*Phormidium uncinatum*	β-ά	2.6	5	13
5	*Nostoc pruniforme*	β	2	3	6
6	*Nitzschia palea*	α	2.75	5	13.75
7	*Navicula cuspidata*	α-β	2.6	5	13
8	*Pediastrum boryanum*	β	2	5	10
9	*Scenedesmus acutus var. quadricauda*	β	2	7	14
1	*Chlorella vulgaris*	p	3.6	7	25.2

So, part of the types of indicator organisms characterizes the studied soil as β-*mesosaprobicarea*. The *saprobity* index according to the method of *Pantle – Buck* S is 2.8.

From the literature it is known that the taxonomic structure of *algocenoses* simplified proportionally to the degree of human impact, in this case the changing the percentage of divisions to decrease the proportion of green and yellow-green algae and it has results of the negative impact of anthropogenic load. The high content of *cyanobacteria* indicates that the soil contained in the long-term «stress» [29].

Conclusion

The present investigations have revealed that algal flora of the soils of the Baghlan region is characterized by relatively high diversity and there are 46 species, forms and varieties, belonging to 4 divisions, 8 classes, 18 forms, 24 families, 28 genera. From certain species of microalgae and *cyanobacteria*, the *saprobity* index according to the method of *Pantle – Buck* S is 2.8. The soil is characterized as moderately polluted soil.

References

1. Christopher J. (2015) Soil Microorganisms as Primary Drivers of Essential Ecological Processes. Northern Australia & land care. http://www.futuredirections.org.au/publication/under-our-feet-soil-microorganisms-as-primary-drivers-of-essential-ecological-processes/
2. http://agriinfo.in/default.aspx?page=topiclist&superid=5&catid=20
3. Hastings K.L., Smith L.E., Lindsey M.L. (2014) et al. Effect of microalgae application on soil algal species diversity, cation exchange capacity and organic matter after herbicide treatments [version 1; referees: 1 approved, 1 not approved] F1000Research 3:281 (doi: 10.12688/f1000research.4016.1)
4. Goyal S.K. (1995) Algae and the soil environment. Phykos. 1997; 36: 1–13.
5. Zenova GM, Shtina EA, Dedysh SN, et al.: Ecological relations of algae in biocenoses. Mikrobiologiya. 64(2): 121–133.
6. Metting B. (1981) The systematics and ecology of soil algae. Bot Rev. 47(2): 195–312.
7. Kuzyakhmetov G.G. (1998) Algological evaluation of the toxicity of copper compounds in grey forest soil and leached chernozem. Eurasian Soil Sci. 31(8): 877–882.
8. Starks T.L,. Shubert L.E., Trainor F.R. (1981) Ecology of soil algae: a review. Phycologia.20(1): 65–80.
9. Ruble R.W., Davis J.S. (1988) Soil algae from fallow potato fields in south Florida (USA) marl. Nova Hedwigia. 47(3–4): 403–414.
10. Alexander M. (1977) Introduction to Soil Microbiology. Wiley, NY. 467.
11. Novichkova – Ivanova L. N. (1977) About the role of soil algae and biocenosis // The development and significance of algae in soil non-Black earth zone: Materials of interuniversity conference. –Perm. – P. 62-64
12. Shtina Y. A. (1990) Soil algae as an ecological indicators //Botanical magazine. – ed.75. – № 4. – P. 441-453
13. Domracheva L. I. (2006) Algo-mycological complexes in soils upon their chemical pollution / L.I. Domracheva, Y.V. Dabakh, L.V. Kondakova, A.I. Varaksina // Eurasian Soil Science, – v. 39. – 591-597
14. D. Sahu, I. Priyadarshani, B. Rath. (2012) Cyanobacteria – as potential biofertilizer: CIBTech Journal of Microbiology. Vol. 1 (2-3). P. 20-26..
15. Roger PA, Reynaud PA (1982). Free-living Blue-green Algae in Tropical Soils. Martinus Nijh off Publisher, La Hague.
16. Rodriguez AA, Stella AA, Storni MM, Zulpa G, Zaccaro MC (2006) Effects of cyanobacterial extracelular products and gibberellic acid on salinity tolerance in Oryza sativa L. Saline System, 2: 7.
17. Saadatnia and Riahi (2009) Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants, Plant Soil and Environment 55(5) 207-212.
18. Wilson LT (2006). Cyanobacteria: A Potential Nitrogen Source in Rice Fields. Texas Rice 6 9–10.
19. Prasad R C and Prasad B N (2001) Cyanobacteria as a source Biofertilizer for sustainable agriculture in Nepal. Journal in Plant Science Botanica Orientalis 127-133.
20. http://en.climate-data.org/location/31392/. Retrieved 9 September 2016.
21. Salem, M.Z. and Hole, E.D. (1969), Soil Geography and Factors of Soil Formation in Afghanistan. Soil Science, Vol. 107, No.4.
22. UNDP/FAO T.A. Report No. 3016 – (1971), Soil fertility and fertilizer use in Afghanistan (based on work of Dr. Tamboli, FAO expert). FAO, Rome.
23. Kuzyakhmetov G. G., Dubovik I. Y. (2001) Methods of studying soil algae: the manual, Ufa, – 56 p.

24. Shtina Y. A. (1951) Methods of studying soil algae / Y. A. Shtina // coll. of articles «Microorganisms as a component biocenosis» M.: Science, 1984. P. 58-74.

25. Determinant of freshwater algae USSR / Executive editor M.M. Gollerbach. – L: Science, – ed. 1-14.

26. Muzafarov A.M., Yergashev A.Y., Khalilova S.Kh. (1988) Determinant of blue-green algae in Central Asia. –Tashkent: Fan, – ed.2. – p. 406-815.

27. Bachura Yu. M., (2011) Using of methods of soil and agar cultures in the study of soil algae / M.Yu. Bachura, O.M. Khramchenkova // Gomel State University. – № 4. – P.107-113.

28. Kabirov R.R. (1995) Algotesting and algoindication –Ufa: BGPI, -125 p.

29. Los D. A. (2007) How cyanobacteria feels stress? Chemistry and Life. № 7. 33 p.

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Murtazina A.S., Bishimbaeyva N.K., Mc Dougall G.

[1]Institute of Biology and Biotechnology, Almaty, Kazakhstan
[2]James Hutton Institute, Dundee, UK
*E-mail: gen_jan@mail.ru

Isolation of oligosaccharide fraction from wheat cell culture and determination of its monosaccharide composition

Abstract. Oligosaccharides, being a part of plant cell wall, can be secreted to the intercellular space and perform «signaling» activity on plant tissues – high physiological activity in very low concentrations. Oligosaccharide (OS) fraction was obtained from extracellular liquid wheat cell culture by size exclusion chromatography. Monosaccharide composition of extracellular OS has been determined by HPLC Ionic chromatography. It was shown that extracellular OS contain glucose, xylose, arabinose, galactose, fructose and rhamnose monosaccharide residues.

Key words: wheat cell culture, oligosaccharide, monosaccharides.

Introduction

Recently, attention of researchers is reasonably attracted by oligosaccharides – fragments of mushroom polysaccharides and plant cell wall. Oligosaccharide is any residue from polysaccharides linked by glycoside bonds. Some selected oligosaccharides can have a «signalling» effect on plant tissues at very low concentrations. Such oligosaccharides are called «oligosaccharines» [1]. To date, five groups of biologically active oligosaccharides (oligosaccharines – OS) are well known; oligoglucoside ß-glucan and oligomers of chitin (chitosan) of the mushroom cell wall, lipooligosaccharides produced by Rhizobium, and two groups of plant oligosaccharides – pectins and xyloglucans [2]. OS are characterized by high biological activity at very low concentrations: 2-3 times lower than that for the known phytohormones. They induce in plants a number of eliciting protective responses against plant pathogens: the formation of phytoalexins, proteinase inhibitors, ß-glikanase, chitinase, as well as the synthesis of callose and lignin [2,3]. Thus, pectin OS induce formation of flowers or plants vegetative shoots from explants in culture tobacco and inhibit root formation [4,5], and also inhibit stretching of etiolated pea stem segments induced by auxin [6]. Xyloglucan (XG) OS constitute the least studied OS group on biological activity. Antiauxin effect of nona- (XG9) and pentasaccharide xyloglucan (XG5) fragments of the cell wall have been showed in the segments Acerpaeudoplatarvus pea stalks [7] and in the culture of carrot isolated protoplasts [8]. Also, it is noted that oligosaccharines stimulate growth, basal metabolism, and division of plant cells [9]. Currently oligosaccharines are poorly investigated and of a great interest for further research of their role in signal transduction of plants [10]. The total content of oligosaccharides in plant tissues varies in a range of 10^{-7}-10^{-5} M [11]. Therefore, isolation and purification of oligosaccharides from native plant tissue extract is challenged by their low content and require a huge amount of plant material. Chemical and enzymatic hydrolysis of plant polysaccharides does not allow to obtain an exact replicate of biological active molecule's stereochemical characteristcs [12, 13]. In this regard, plant cell culture methods are known for their potential in producing biologically active substances, which make them suitable for search of OS in intercellular space of liquid plant cell cultures [14].

Materials and methods

Cell suspension culture was obtained from long-term wheat callus tissues. For this purpose callus tissues were placed in liquid culture medium [15] at a ratio 200-300 mg on 30-40 ml of media and cultured on a shaker at 140 rpm at 26 ± 2 °C and 16-hour photoperiod. Extracellular liquid was collected and filtered, then concentrated on rotary evaporator IKA «WERKE».

Isolation of oligosaccharides was carried out by ethanol precipitation [16,17] and centrifugation at 10,000 rpm 10 minutes at 8 °C. Total sugar amount was determined by Dubois method [18]. Oligosaccharide's fraction was obtained by size-exclusion column chromatography with Biogel P2 sorbent, calibrated with Blue Dextran (2000000 Da), maltoheptaose (1153 Da) and sucrose (342,3 Da). Monosaccharide composition was identified by the method of Ionic High Performance Liquid Chromatography (HPLC), by Dionex ICS 5000 chromatographic equipment (Thermo Scientific).

Results and discussion

Gel-filtration on Biogel P-2 sorbent is known to be effective for fractionation of low molecular weight compounds. Standard curve calibration has shown that Blue Dextran output takes place on 6-12 minute, maltoheptaose – on 12-18 minute, sucrose – on 18–25 minute. Two samples of extra-cellular liquid were fractionated by size-exclusion chromatography.

Retention time of oligosaccharide's peak of sample S6 was 13-18 minutes with the maximum on 16th minute, which corresponds to the retention time of maltoheptaose. Consequently, oligosaccharides molecular mass in sample S6 is around of 1153 Da, and the quantity of monosaccharides is approximately 7 monosaccharide residues.

Retention time of oligosaccharide's peak of sample S7 was 13-18 minutes with the maximum on 14th minute, which corresponds to the retention time of maltoheptaose (Figure 1). Consequently, oligosaccharides molecular mass in sample S7 was around of 1153 Da and higher, and the quantity of monosaccharides is approximately 7-10 monosaccharide residues.

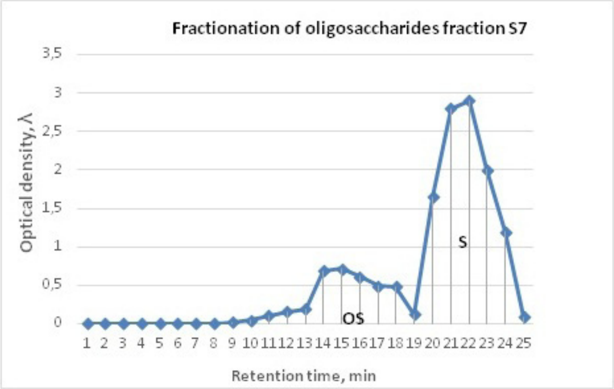

Figure 1 – Size-exclusion chromatography of S7-OS fraction; indicated OS-oligosaccharides, S – sucrose

Thus, two fractions of oligosaccharides have been obtained from extracellular liquid of wheat cell culture. These two fractions contain 7-10 monosaccharide residues. Study of these two oligosaccharide fractions on monosaccharide composition revealed almost similar characteristics. It was determined that in sample S6 oligosaccharide fraction is rich for glucose (50%) and contains the following monosaccharides: xylose (25%), rhamnose (18%), fructose (4%) and galactose (3%). Nevertheless, residues of arabinose, mannose, fucose, galacturonic and glucouronic acids were not identified in S6 fraction. Fraction S7 contains 50% glucose, 30% xylose, 11% fructose, 4% mannose and a small amount of arabinose (2%), galactose (2%), differed from the previous sample by the presence of arabinose, galactose and mannose, and a small amount of rhamnose (Table 1, Figure 2).

Table 1 – Monosaccharide composition of oligosaccharides from extracellular liquid of wheat cell culture, in %

Monosaccharide	S6 – fraction	S7 – fraction	% in both fractions
Arabinose	0	2	0-2%
Galactose	3	2	2-3%
Rhamnose	18	1	9,5%
Glucose	50	50	50%.
Xylose	25	30	27%
Mannose	0	4	0-4%
Fucose	0	0	0
Fructose	4	11	7,5%
Galacturonic acid	0	0	0
Glucorinic acid	0	0	0

Thus, results of high performance liquid chromatography of oligosaccharide fractions revealed, that in fraction S6 there are peaks of glucose, xylose, rhamnose, galactose and fructose. This S6 fraction, presumably, contain xyloglucan and ramnogalacturonan oligosaccharides with approximate 7 residues. S7 fraction conisists of glucose, xylose, fructose, mannose and galactose with arabinose. Consiquently, S7 fraction, could be represented by xyloglucan, glucomannan, arabinogalactan oligosaccharides, consists of approximate 7-10 monosaccharide residues.

On the basis of obtained results it can be presumed that oligosaccharides, obtained from wheat cell culture in the presence of 2,4 –D in medium, are mainly presented by xyloglucans, ramnogalactans, glucomannans, arabinogalactans, etc. and consist of 7-10 monomers. Further analysis of extracellular olygosaccharides will be targeted to study of their functional activity and structural study of physiologically active fractions.

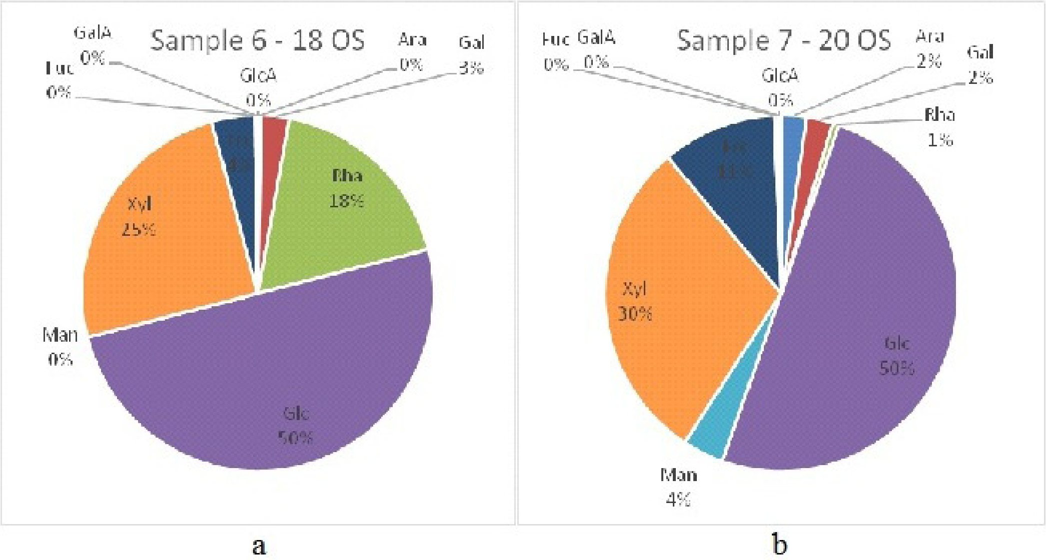

Figure 2 – Monosaccharide composition of oligosaccharide fractions, %; indicated samples 6 (a) and 7 (b)

References

1. Darvill A.G., Albersheim P. Phytoalexin and their elicitors – a defense against microbial infection in plants // Annual Review of Plant Physiology. – 1984. – Vol. 35. – P 243-275.
2. Fry S.C., Aldington S., Hetherington P.R., Aitken J. Oligosaccharides as Signals and Substrates in the Plant Cell Wall // Plant Physiol. – 1993. – Vol. 103. – P. 1-5.
3. Ryan C.A., An G. Molecular biology of wound-inducible proteinase inhibitors in plants // Plant Cell and Environment. – 1988. – Vol. 11. – P. 345-349.
4. Tran Than Van, K.; Toubart, P.; Cousson, A.; Darvill, A.G.; Gollin, D.J.; Chelf, P. & Albersheim, P. Manipulation Of The Morphogenetic Pathways Of Tobacco Explants By Oligosaccharins. Nature, 314:615-617, 1985.
5. Eberhard, S.; Doubrava, N.; Marfa, V.; Mohnen, D.; Southwiick, A.; Darvill, A. & Albersheim, P. Pectic cell wall fragments regulate tobacco thin-cell-layer explant morphogenesis. The Plant Cell, 1:747-755, 1989
6. Branca, C.; De Lorenzo, G. & Cervone, F. Competitive Inhibition Of The Auxin-Induced Elongation By A-D-Oligogalacturonides In Pea Stem Segments. Plant Physiology, 72:499-504, 1988.
7. Mcdougalll, G.J. & Fry, S.C. Inhibition Of Auxin-Stimulated Growth Of Pea Stem Segments By A Specific Nonasaccharide Of Xyloglucan. Planta, 175:412-416, 1988.
8. Emmerling, M. & Seitz, H.U. Influence Of A Specific Xyloglucan-Nonasaccharide Derived From Cell Walls Of Suspension Cultured Cells Of Daucus Carota L. On Regenerating Carrot Protoplasts. Planta, 182:174-180, 1990.
9. Alberto González, Jorge Castro, Jeannette Vera, Alejandra Moenne. Seaweed Oligosaccharides Stimulate Plant Growth … Journal of Plant Growth Regulation, 32 (2):443-448. 2013.
10. I. A. Larskaya and T. A. Gorshkova, Plant Oligosaccharides – Outsiders among Elicitors? Biochemistry (Moscow), 2015, Vol. 80, No. 7, pp. 881-900.
11. Melotto, E., Greve, L. C., and Labavitch, J. M. Cell wall metabolism in ripening fruit: biologically active pectin oligomers in ripening tomato (Lycopersicon esculentum Mill) fruits, Plant Physiol.,1994, 106, 575-581.
12. Liskova, O., Auxtova, O., Kakoniova, D., Kubackova, M., Karacsonyi, S., and Bilisics, L. Biological activity of galactoglucomannan-derived oligosaccharides, Planta, 1995, 196, 425-429.
13. Lorences, E. P., McDougall, G. J., and Fry, S. C. (1990) Xyloglucan − and cello-oligosaccharides: antogonists of the growth-promoting effect of H+ , Physiol. Plant., – 1990-, 80, 109-113/
14. Vanisree Mulabagal and Hsin-Sheng Tsay, Plant Cell Cultures – An Alternative and Efficient Source for the Production of Biologically Important Secondary Metabolites, International Journal of Applied Science and Engineering 2004. 2, 1: 29-48.

15. Murashige T., Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures // Phisiol. Plant. – 1962. – Vol. 15. – 473-497.

16. Gunther E.V., Popeiko O.V., Ovodov U.S. Production of polysaccharides by callus cultures of Lemna minor // Appl. Biochem. and microbiology (Rus.). 2008. Vol. 44. №1. P. 117-122.

17. McDougall G.J., Fry S.C. Structure-activity relationships for xyloglucan oligosaccharides with antiauxin activity // Plant Physiol. – 1989. – Vol. 89. – P. 883-887.

18. Dubois M., Gilles K.A, Hamilton J.K., Rebers P.A., Smith F. Colometric Method for Determination of Sugars and Related Substances // Analytical Chemistry. – 1956. – Vol. 28. – №3. – P. 350-356.

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[1]Myrzakhanov N., [2]Myrzakhanova M.N.

[1]Department of Tourism, Faculty of Tourism and Business, Turan-Astana University, Astana, Kazakhstan
[2]Department of Jurisprudence, Faculty of Jurisprudence, Kokshetau state university after named Sh.Ualikhanov
*E-mail: myrzahanova@mail.ru

Condition lymphatic flow and composition of lymph and blood in surgical interventions

Abstract. From various sources, we know that the science of spermatologii studied at various farm animals. The composition and properties of surface plasma membrane glycolipids determine mitotic phase cell activity and cell division. Demonstrated their exceptional role in cell-cell interactions: in the phenomena of «recognition» of cells to each other, adhesion, differentiation, growth, proliferation, contact inhibition of growth. In this article, we were examined lymph, lymph and blood composition, such as the volume of blood plasma in rams against the backdrop of one of the oldest forms of non-specific defense response – inflammation caused by surgical injury. Experiments were carried out on 14 rams Altai Merino. After a clinical examination of the animals, quickly create lymph venous anastomosis between the thoracic duct and jugular vein on the previously developed modifications N. Myrzakhanova, 1980. To prevent the lymph and blood clotting heparin was injected intravenously. A sample of the lymph and blood for analysis were taken from the thoracic duct and jugular vein. Elactica Kastrati morning before and after the conference. Analysis of morphological composition of blood and bodices, and total protein was carried out by conventional methods in hematology and hematocrit using microcapillary. Studies have shown that after elastica Kastrati observed very excited animal. There was an increase in heart rate of 66 to 81 beats per minute and respiration of 16 to 28 breaths per minute. Increased body temperature to the upper limit of normal (before 39.90) and inhibition of motor-evacuation activity of the rumen from 3.5 to 1.2 beats per minute. These changes most evident the first 1-2 hours (P<0, 01-0,001), and then restored to the original values. Dynamics of white blood cells in the lymph and blood in this phase were determined not only the formation and release them in the lymph and circulatory changes depended on. This is evidenced by the decline in the value of lymph flow and hematocrit. Thus, reduction in the number of blood leukocytes Incoming defined reduction in lymph circulation on the one hand and an increase of the plasma volume with the other. Physiological assessment of the above leads to the conclusion that post-traumatic reaction at elastica Kastrati proceed fully and relatively easily transferred animals.

Key words Hemo-lymph circulation, elastica Kastrati, lymph flow, protective reaction, reproduction.

Introduction

Currently only a few works are devoted to the study of the most important representatives of complex compounds containing lipids [1; 2, P.106; 3, P.90; 4, P.51]. Meanwhile, phospholipids and cholesterol are major structural elements inside membranes of any cells, not just nerve as previously believed. The composition and properties of surface plasma membrane glycolipids determine mitotic phase cell activity and cell division. Their exceptional role has been demonstrated in cell-cell interactions: phenomena in «recognition» of cells with each other, adhesion, differentiation, growth, proliferation, contact inhibition of growth, and others [5, P.8; 6, P.51; 7, P.45; 8, P.93].

Biochemical aspects of solving the problem of artificial insemination has not only theoretical value, but also is the most promising direction in the development of objective criteria for evaluating the quality of iaciat, the usefulness and viability of sperma, the best uses of sperma of male pigs [9, P.160; 10, P.32], and other farm animals [11, P.150; 12, P.38] stronge and preservation of sperma, forecasting and maintaining high fertilizing capacity of sperma [13, P.76; 14, P.73].

The study of lymph circulation rheology for various violations gemo-lymph circulation is an actual problem of modern physiology. This implies relevant primarily from the fact that there are no violations of regional gemo-lymph circulation which would leave intact lymphatic system. On the other hand, the lym-

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phatic system includes both non-specific mechanisms and specific defense of the organism. In this regard, the lymphatic system is evolutionarily the oldest, has a special role in ensuring and maintaining the stereotypical non-specific defense mechanisms. This is not only and not so much with the homeostatic function of the lymphatic system, but with the preservation of the dynamic balance of the internal environment, due to the movement of lymph – lymph-section [15, 16].

Materials and methods

Chronic experiments were performed on 14 young rams merino breed Altai six-month age-matched analogues principle. After a clinical examination of the animals surgically created lymph-venous anastomosis between the thoracic duct and jugular vein according to our earlier modification [17]. To prevent clotting of blood and lymph heparin intravenously injected at the rate of 1,500 units per 1 kg of animal body weight. Then, after one – two days, when the animals began to eat a full diet, started basic experiments. During the main experiment heparin was not performed. Surgery consisted of imposing animal rubber ring (elastica Kastrati) using special forceps at the base of the scrotum cervix (seven in animals) and in the same operation, but after infiltration anesthesia provisional neck scrotal tissue 100 ml of 0.5% novocaine solution (seven animals). A blood sample was taken for analysis of lymph from the breast duct and jugular vein in the morning before and after its elastica Kastrati of 1, 2, 3, 6, 24, 48, 72 and 96 hours. Analysis of the morphological structure of the lymph and blood and determination of the total protein level was carried out by conventional methods in hematology and hematocrit – using microcapillary. Of lymphatic thoracic duct measured in ml / min by collecting the lymph into a graduated test tube for 5 minutes.

Results

Studies have shown (Table 1), immediately after surgery, says very excited animal. This was manifested in increasing the duration of the active period of anxiety in an animal during the first hour (mean 49 minutes). Animals kicked, peering into the stomach, often lay down and immediately raised. There was an increase in heart rate of 66 to 81 beats per minute and breathing – from 16 to 28 breaths per minute, increase in body temperature to the upper limit of normal (up to 39.9^0 C) and breaking the motor-evacuation function of the scar from 3.5 to 2.1 beats per minute. These changes most evident the first hours, and then restored to their original values. It should be noted that the rams, the neck of the scrotum which previously subjected to tight circulatory infiltration anesthetic Novocain, drastic changes in behavior and autonomic responses did not show, and found fluctuations in rates of cardiac activity, respiration and rumination were not significant and, as a rule, are not diversified.

Analysis showed a lymph and blood (Tables 2 and 3), circulatory and morphological changes in internal environments occur in two phases. In the first 1-3 hours after elastica Kastrati marked decrease in the rate of lymph flow from the thoracic duct, an average of 24.1% ($P<0.01$), decrease in hematocrit level (12.8%) and the total protein in the blood (5.2%) and lymph (2.4%). There was also reduction in the number of white blood cells in the lymph and blood (Table 2). This shift in the content of white blood cells in the lymph is due to eosinophils, monocytes, lymphocytes, and subsequently, while the blood was caused mainly due to lymphocytes. Dynamics of white blood cells in the lymph and blood in this phase were determined not only the formation and release them in the lymph and circulatory changes depended on. This is evidenced by a decrease Volume of lymph flow and the value of hematocrit.

Therefore, reducing the number of leukocytes in blood was determined by a reduction in revenue lymph circulation, on the one hand, and the increase of plasma volume of the other. An indirect confirmation of this is the fact that lymph plasma protein ratio remains constant in both immediate and long-term periods after elastica Kastrati, indicating the absence of significant changes in exchange transcapillary state. In the second phase, covering the period from 6 to 96 hours after elastica Kastrati, there was a general trend towards a clinical recovery (Table 1) and lymph hematological parameters (Table 2). However, it should be noted that the reduction in full circulatory link covers only post-surgical intervention, whereas cell responses from lymph and blood remain, indicating recovery processes of tissue homeostasis.

As can be seen from Table 2 lymph flow and the value of hematocrit differed little from the original background. The increase in white blood cells in the lymph occurred, mainly due to basophils, eosinophils and neutrophils, whereas similar changes in blood were relative to the background of the changing nature of the total number of leukocytes in it.

Table 1 – Clinical parameters in rams in the surgical intervention

Indicators	Before	1	2	3	6	24	48	72	96
The heart rate (min^{-1})	66	81	72	70	68	66	69	68	68
Respiratory rate (min^{-1})	16	28	22	20	18	16	17	18	18
Body temperature (^{0}C)	38.5	39.9	39.9	38.6	38.6	39.0	38.8	38.6	38.6
rumination (min^{-1})	3.5	1.2	2.2	3.0	3.3	3.4	3.6	3.4	3.5

Table 2 – Condition lymph flow and composition of lymph and blood in rams during surgery

Indicators	Before	Hours, after surgical intervention							
		1	2	3	6	24	48	72	96
lymph flow thoracic lymphatic duct, ml/min of	3.3	2.7	2.4	2.4	3.3	3.3	3.0	3.0	3.2
leukocytes (10-9\L)									
lymph	16.9	13.6	10.6	20.1	24.6	25.6	23.4	23.1	20.9
blood	10.0	5.9	6.6	9.2	10.2	8.7	7.0	9.0	6.2
Leucoformula lymph(%)									
basophils	1.5	1.6	1.7	1.6	2.6	2.4	2.6	2.6	2.2
eosinophils	2.5	2.3	2.2	2.2	3.4	3.5	3.0	1.6	1.9
neutrophils	10.5	11.1	12.2	11.4	11.9	12.0	11.0	12.6	12.6
lymphocytes	84.5	84.5	83.8	84.3	81.8	81.7	83.0	82.8	82.8
monocytes	1.0	0.5	0.6	0.5	0.3	0.4	0.4	0.4	0.5
Leucoformula blood (%)									
basophils	0.7	0.9	1.1	1.2	1.9	1.3	1.0	1.0	1.0
eosinophils	7.0	6.8	6.8	6.9	8.0	8.2	8.4	6.6	6.9
neutrophils									
young	1.2	1.2	1.3	1.3	1.2	1.2	1.2	1.1	1.2
relating to stab	3.6	3.8	3.8	3.9	3.7	3.7	3.6	3.6	3.6
segmentonuclear	37.4	38.4	37.4	38.0	39.0	39.2	37.0	36.8	36.2
lymphocytes	49.0	45.9	46.6	45.8	43.4	43.6	45.0	48.0	48.2
monocytes	3.1	3.0	3.0	2.9	2.8	2.8	2.8	2.9	2.9
Hematocrit	42	38	36	36	40	40	41	40	40
Common blood protein (g\%)	5.98	5.86	5.78	5.68	5.98	6.02	6.02	6.02	5.98
Common lymph protein (g\%)	3.74	3.68	3.64	3.64	3.87	3.87	3.94	3.92	3.92
Lymph-plasma ratio of protein	0.64	0.62	0.63	0.64	0.64	0.65	0.64	0.65	0.65

The results of the study of lymph flow, the composition of lymph and blood, as well as some indicators lymph-gemodinamic in animals with preanesthesia neck scrotum Novocain solution showed that cellular and humoral changes in the lymph and blood of these animals were similar to those that have been found in animals which had not been held preliminary anesthesia of the cervix scrotal tissue (Tables 2 and 3), indicating that common mechanisms of these reactions. The results show that when local disturbances lymph-gemo circulation (elastica Kastrati) there are complex changes in the lymph, plasma volume, the number and ratio of formed elements in the blood and lymph. As the evidence in the initial phase after surgery marked reduction in lymph flow, which appears to be associated with reflex humoral influences on the lymph vessels and nodes. The subsequent normalization of lymph flow rate indicates re-adaptation lymph system and is aimed at restoring the initial state. In the first phase after surgery there is a decrease of leukocytes, primarily by monocytes and particularly lymphocytes, which are apparently also associated with reflex humoral influences. Thus, many researchers [18; 19] decrease for leukocytes found upon administration of corticosteroids and ACTH. In reducing, the number of lymphocytes in surgical trauma and sensitive denervation pointed out by other researchers [20; 21]. Studying the dynamics of white blood cells in the lymph and blood, especially their individual shapes in the second phase after surgery showed a characteristic increase in the content of basophils, eosinophils and neutrophils. According to modern concepts [18] neutrophils are not only involved in phagocytosis, but also secrete a number of substances stimulating tissue regeneration, vascular permeability while the increase in eosinophils and basophils are interrelated, namely, increased production of histamine by basophils is accompanied by eosinophilia. Eosinophilia, in turn, regulate the levels of biogenic amines in the tissues and has antihistaminic activity [22]. Thus, it becomes more clear the role of these cells in lymph hemodynamic disorders in connection with surgical intervention.

Table 3 – Condition lymph flow and composition of lymph and blood in rams during surgical intervention after Novocain infiltration anesthesia

Indicators	Before	Hours, after surgical intervention							
		1	2	3	6	24	48	72	96
lymph flow of thoracic lymphatic duct, ml/min	3.2	2.7	2.5	2.5	3.0	3.2	3.1	3.2	3.2
leukocytes (10-9\L)									
lymph	16.8	14.4	13.8	13.0	18.0	21.0	22.0	21.0	20.2
blood	10.1	6.4	6.2	9.8	9.9	8.9	8.4	7.0	7.1
Leucoformula lymph(%)									
basophils	1.4	1.6	1.6	1.6	2.8	2.4	2.1	2.6	2.1
eosinophils	2.3	2.0	2.1	2.9	2.6	3.4	3.3	2.2	1.9
neutrophils	10.4	11.6	11.2	11.1	11.1	11.9	11	11.8	12.3
lymphocytes	84.0	84.1	84.5	84.7	81.8	84.7	83.0	82.9	83.3
monocytes	0.9	0.7	0.6	0.6	0.7	0.6	0.6	0.5	0.4
Leucoformula blood (%)									
basophils	0.7	0.9	0.9	1.1	1.7	1.4	1	1.1	0.9
eosinophils	7.1	7	6.9	7.1	7.2	7.8	8.1	7.1	7.0
neutrophils									
young	1.3	1.2	1.3	1.3	1.2	1.3	1.2	1.1	1.2
relating to stab	3.6	3.9	3.8	3.8	3.7	3.7	3.6	3.5	3.6
segmentonuclear	36.2	39.0	37.8	38.3	37.8	37.4	36.5	36.9	37.1
lymphocytes	47	45.1	45.4	45.4	45.4	45.5	46.6	47.2	47.2
monocytes	3.1	2.9	2.9	3.0	3.0	2.9	3.0	2.9	3.0
Hematocrit	42	37.0	38.0	37.0	39.0	39.0	41.0	41.5	42.0
Common blood protein (g\%)	5.97	5.79	5.81	5.83	5.86	6.00	5.90	5.94	5.95
Common lymph protein (g\%)	3.68	3.59	3.57	3.56	3.86	3.90	3.91	3.92	3.92
Lymph-plasma ratio of protein	0.62	0.62	0.62	0.61	0.66	0.65	0.66	0.66	0.66

It is known that these forms of leukocytes mainly produced in the bone marrow and enter the bloodstream [22]. Increasing their content in the lymph, apparently due to the passage of the formed elements in the lymph nodes at level [20].

Conclusion

Experiments have shown that after a preliminary infiltration circular anaesthesia neck scrotum Novocain reactions associated animal's behavior changes weaken or even disappear, revealing the unreliability shifts both somatic and autonomic (pulse, breath, rumination, temperature) the nature, whereas the shifts taking place in the lymph and blood, as well as the cellular responses to these shifts were similar to those which have been found in animals without an anesthetic procaine. These data agree with the definition of Sherrington: «Bodily pains have a mental addition to the protective reflex» [23]. let's take the idea that pain and certain manifestations of the protective reflex is a reaction to the signals of the same sensory apparatus and was found to later [24, p.78], pain receptors, nociceptors presented H-structures, whereas tissue receptors – interoreceptor presented M-structures [25,26,27,28]. Consequently, subliminal receptors of internal organs responsible for the formation of the vegetative level of regulation of the internal environment, whereas high-threshold afferents form appropriate behavior [29, 30]. Thus, in our experiments, both mechanisms occurred reflex-humoral regulation of protective reflexes. Physiological assessment of the above leads to the conclusion that post-surgical intervention takes place fully and relatively easily transferred animals in the case of the intensity of the blockade nociceptive component of the body's protective reflexes.

References

1. Antonyuk B.C. Sperm and some biochemical parameters of bull semen with different sexual load: Diss. cand. Biol. Sciences. Minsk, 1969. – 160 p.

2. Bernshteyn A.D., Sokolova L.S. About lipoid capsule of spermatozoa. Animal husbandry Problems 1935, № 6, s.106-107.

3. Viha I.V., Privalenko M.E., Khorlin A.J. Determination of hyaluronidase activity in the blood serum in the presence of co-hyaluronidase, beta-glucuronidase, and beta-acetylglucosaminidase. Problems of Medical Chemistry, 1973, Vol. 19, Issue 1, p. 90-96.

4. Voloskova A.G. lipid composition of semen of domestic animals. Journal of Agricultural Science. Veterinary Medicine 1940, Issue 3, p. 51-94.

5. Komissarchik Y.J. The structure and chemical composition of the cell membranes. Proc.: Structural and functional organization of cell membranes. M.: Nauka, 1975, p. 8-25.

6. Kruglyak A.P. Topical issues of artificial insemination of farm animals. Agricultural abroad, 1983, № 2, p. 51-56.

7. Milovanov V., Koltsov E., and others. The chemical nature of antioxidants and their effect on freezing a ram semen. Animal Husbandry 1981, № 9, p. 45-46.

8. Roshkovan G. Prediction of reproductive qualities for enzyme tests. Proc.: animal husbandry technology in industrial complexes. Chisinau, 1978, p. 93-96.

9. Antonyuk B.C., Plyaschenko S.I. et al. The effect of climate on the physiological state and reproductive qualities of breeding boars. In the book. Scientific basis for livestock development in the Byelorussian SSR Screw: Uradzhay, 1983, Issue 13, p. 160-164.

10. Arhipovets A.I. Semen of boars of large white breed depending on age. Proc.: Physiology and biochemistry of farm animals. Republican interdepartmental thematic scientific collection. Kiev, 1970, Issue 12, p. 32-37.

11. Bocharov I.A., Pospelov A.I., Sokolov Z.A. To the question about the causes of the deterioration of sperm quality in bulls during the winter-spring period. In the book. Proceedings of the Leningrad Veterinary Institute, 1964, vol. 26, p. 150.

12. Burihanova S.I. Seasonal changes of chemical thermoregulation. In the book. Proceedings of Tashkent University 1966, Vol. 26. P.38-41.

13. Varaksa P.A. Seasonal fluctuations in semen of bulls and ways to eliminate them. Animal Husbandry, 1967, № 4, p. 76-78.

14. Georgiev G. Study of phospholipids in the semen of bulls-manufacturers. P. Phospholipids in spermatozoa and seminal plasma. -Veterinary-Medical sciences (Sofia), 1967, № 8, p. 73-76.

15. Ansell G. B., Hawthorne J.N. Phospholipids: Chemistry, Metobolism and Function //Am. Elsev. Publish. Comp. – N. Y., 1964. – 302 p.

16. Leninger A. Biochemistry. – M.: Mir, 1974. – 986 p.

17. Akkizov A.Y. Effect of carotene in the peripheral blood // Proc. Conf. young. researchers. – Collection, 2005 – P.4.

18. McDonald P., et al. Nutrition animals. – M.,1970. -221 p.

19. Solomatin N. The content of vitamin A in the blood of sheep, depending on diet and season//Proceedings of the Almaty veterinary Instituta. – 1964. – V.13. p. 44-56

20. Emelina I.T. et al. Vitamins in Animal Nutrition and birds. – M., 1970. – 109 p.

21. Alikaev V.A. Fundamentals of preventing non-communicable diseases of young farm animals // Prevention and treatment of diseases of young agricultural animals. – M., 1968. – 68-71 p.

22. Emilyanov L. On the question of the correlation of indicators of vitamin A and carotene in the blood serum of cattle // Proceedings of the Latvian. Agricultural Institut. – 1970. – Ed.30. – P. 21-23.

23. Goodwin T. Comparative Biochemistry karotinoidi. – M., 1954. – 243 p.

24. Ainzón H.H. Dependence of the content of carotene in the blood and lymph chickens on their the age, sex and breed // News of the Academy of Sciences Est. SSR. – 1962. – Vol.16. – P.78-82.

25. Aliev A.A.Operational research methods of agricultural animals,1974. – 680 p.

26. Kënig. A.I. Artificial insemination of pigs. Biology, technology, organization. – M.: Kolos, 1980. – 175 p.

27. Myrzakhanova M.N. The evolution of the nervous regulation of blood and lymph circulation in vertebrates //Monograph. LAP Lambert Academic Publishing. – Germany, 2013. – P. 62.

28. Myrzakhanov N. Laboratory studies of veterinary medicine. – Guidelines.-Semipalatinsk. 1981. – 23p.

29. Rokitsky P.F. Biological statistics. – Minsk, 1973. – 220 p.

30. Myrzakhanov N., Myrzakhanova M.N. On development of the lymph and blood of dogs in the protein composition under some effects on organisms. European scientific journal. Republic of Macedonia, 2013. – Vol. 9, No. 6, p.92-99.

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[1,2]*El-Sayed Negim, [4]Bekbayeva L., [5]Irmukhametova G.S.,
[3]Kuzhantayeva A., [1]Sultanova D., [1]Suleimenova A., [4]Yeligbayeva G., [5]Mun G.A.

[1]School of Chemical Engineering, Kazakh-British Technical University, Almaty, Kazakhstan
[2]National Research Centre, Polymer & Pigment Department, Giza, Egypt
[3]Kazakhstan Maritime Academy, Kazakh-British Technical University, Almaty, Kazakhstan
[4]Kazakh National Research Technical University named after K.I. Satpayev, Almaty, Kazakhstan
[5]Department of Chemistry & Technology of Organic Materials, Polymers and Natural Compounds,
al-Faraby Kazakh National University, Almaty, Kazakhstan
*E-mail: elashmawi5@yahoo.com

Utilization of styrene copolymer lattices (DBSS/PVA) as chemical admixture for mortar

Abstract. The physical and mechanical properties of mortar containing copolymer lattices as chemical admixtures were investigated. Copolymer lattices used based on styrene and butyl acrylate were prepared in presence of potassium persulfate/sodium metabisulfite (KPS/NaMBS) as redox initiation system and a coemulsifier that consists of dodecyl benzene sodium sulfonate and polyvinyl alcohol (DBSS/ PVA). The results showed that the addition of copolymer lattices to mortar improves the physical and chemical properties of mortar. However, as the dosage of lattices increases, W/C ratio as well as workability increases while compressive strength and chemically combined water content decrease.

Key words: copolymer, lattices, strength, workability

Introduction

Dispersions lattices are important polymers which used as admixtures to modify physical and mechanical properties of cement, mortar and concrete [1-5]. Polymer lattices are water-soluble polymer particles suspended in an aqueous solution. Many of researchers have investigated the effects of polymer lattices including acrylic, epoxy and polyurethane on the properties of cement materials. In our laboratory, it is of interest to study the effect of acrylic lattices on the properties of cement pastes and mortar including water/cement ratio, workability, water absorption, porosity as well as strength. Negim et al prepared copolymer lattices based on 2-hydroxy ethyl acrylate & 2-hydroxy ethylmethacrylate [6-8], 2-hydroxy ethyl acrylate & vinyl butyl ether [9], acrylic acid & butymethacylate [10] and styrene & methacrylate [3, 11-13] using radical and redox initiators technique. The results showed that the acrylic lattices improved the physical and mechanical properties of cement pastes and mortar. Lattices modification of cement mortar is affected by cement hydration and polymer film formation processes in binder phase. This effect increases with increasing dosage of polymer lattices to mortar mixes. Barluenga and Olivares [14] studied the dosage of styrene-butadiene rubber latex on the properties of mortar including setting time, consistency, porosity and compressive strength. The results showed that water/cement ratio, setting time, porosity decrease and compressive strength increases with increasing the concentration of latexes. This is attributed to that styrene-butadiene-rubber has excellent bond strength in the concrete, higher flexural strength and lower permeability [15]. Alexandre et al [16] reported that as the concentration of ethyl vinyl laurate-chlorine polymer lattice increases from 2.5 to 5%, density, compressive strength of mortar decreased. Negim et al [11] prepared copolymer emulsion lattices based on styrene and butyl acrylate in presence of potassium persulfate/ sodium metabisulfite (KPS/ NaMBS) as redox initiator system and a coemulsifier dodecyl benzene sodium sulfonate and polyvinyl alcohol (DBSS/PVA). The effect of copolymer lattices on physico-mechanical properties of cement pastes was investigated. The results showed that as the concentration of copolymer increases, water/cement ratio, setting time and porosity decreases while compressive strength of cement pastes increases. The work was further extended to include the application of the obtained copolymer lattices with different dosages to modify the properties of mortar.

Materials and methods

Dodecyl benzene sodium sulfonate (DBSS) was used as anionic surfactant with a molecular weight of 348.48g/mole. The nonionic surfactants used were polyvinyl alcohol (PVA) with a molecular weight of 13,000. The chemical structure of the various surfactants is shown in Table 1.

The raw materials used in the present study are Portland cement clinker (PCC) and raw gypsum (G). Each of those raw materials was separately ground in a steel ball mill until the surface area of respectively 3650 and 2800 cm^2/g was achieved. The chemical composition of the raw materials is shown in Table 2. The mineralogical composition of the PCC sample is C_3S, 58.79 %; β- C_2S, 17.68 %; C_3A, 8.08 %; C_4AF, 9.72 %. The Portland Cement (PC) was prepared by mixing 96 % PCC and 4 % G (by weight) in a porcelain ball mill for one hour using 3 balls to ensure complete homogeneity of the cement. The Blaine surface area of the cement sample was 3350 cm^2/g [17].

The fine aggregate used was sand with particle size ranging from 0.21mm to 0.53 mm and is free from organic or clay-like materials.

Table 1 – The chemical structure of surfactants

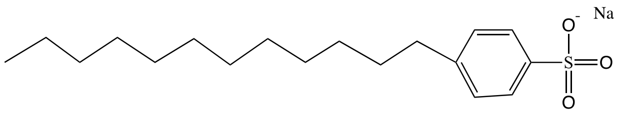
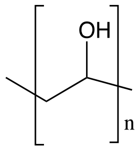
Surfactants	Symbol	Structure
Dodecyl benzene sodium sulfonate	DBSS	
Polyvinyl alcohol	PVA	

Table 2 – The chemical composition of the raw materials, mass %.

Oxides Materials	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	SO_3	Na_2O	K_2O	L.O.I
PCC	21.48	6.03	4.22	64.29	0.68	0.39	0.21	0.11	1.32
G	0.58	0.14	0.11	30.08	0.13	45.36	0.07	0.09	22.16

Synthesis and characterization of copolymers

Copolymer emulsion latexes based on styrene (St) with butyl acrylate (St/BuA) was synthesized with composition ratios (5: 5) using potassium persulfate/sodium metabisulfite (KPS/NaMBS) as redox initiation system in the presence of a co-emulsifier 2% dodecyl benzene sodium sulfonate with 1.5% polyvinyl alcohol (DBSS/PVA) The preparation of copolymers and the methods of analysis (^{1}H NMR, rheological and morphological techniques) have been previously described in a previous investigation [11].

Mixing and testing

Mortar specimens of size 70 mm cube were prepared in three groups. The control mix (M0) consists of Portland cement (PC), sand and water. The proportion of cement to sand was 1:3 (by weight). In mixes M1, M2 and M3, prepared lattices with dosage 0.25, 0.5 and 1.0 % was added. However, the mix M0 is the reference without lattices.

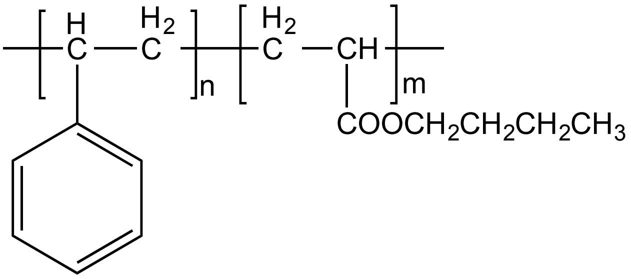
The cement and sand were intermixed until homogeneity was achieved. Then the prepared lattices were added to the mixing water. This was then added gradually to cement/sand mixture to determine the water of consistency using Vicat apparatus [18, 19].

The resulting mortar was directly placed into 70 mm cube stainless steel moulds. The moulds were manually agitated for 2 minutes and then on a vibrator for another 2 minutes. The moulds were kept in a humidity chamber at 100 % R. H and a constant room temperature overnight, then demoulded and cured under water till the time of testing. Testing included compressive strength, water absorption and combined water and was conducted at 1 day, 3, 7 and 28 days. The determination of water absorption as per the specifications of BS 1881: Part 122[20], compressive strength, water absorption and combined water were described in a previous investigation by the authors [21].

Results and discussion

Structure of Copolymers

The structure of the copolymers (St/BuA) is shown in Scheme 1 and further details about the synthesis and characterization have been previously reported by the authors [11].



Scheme 1 – The chemical structure of copolymer lattice [11].

W/C ratio

As shown in Fig. 1, styrene copolymer lattices reduce the W/C ratio of mortar compared to that of reference mix (M0). The reduction in W/C ratio is attributed to dispersion mechanism of styrene copolymer lattices on cement grain and coarse aggregate, this is in accordance with a previous study [6, 22]. However, the water /cement ratio of mortar increase with the addition of lattice (M3, 1%). Negim et al. [12, 13, 23, 5] found that styrene/methacrylate copolymers lattices reduced W/C ratio of mortar. Also, W/C ratio of mortar tends to change according to dosage of copolymer added to mortar during the mixing process (12, 13).

Workability

It is well known that the chemical admixtures have effective dispersing properties on the cement particles and improve the flow of mortar. Flow of mortar mixed with varied amount of styrene copolymer increased with increasing concentration from 0.25 to 1.0 %, as shown in Fig. 2. The increasing in workability of mortar is due to ball bearing action and dispersing effect of styrene copolymer particles among cement particles, there is a fluidity increase in the mortar mixture [12, 10]. In general, workability of mortar is affected by many factors such as composition, type, and functional groups of monomers in latexes [6]. When the concentrations of styrene copolymers are increased from 0.25 to 1.0%, the workability increased from 128 to 170 mm, due to the more water absorbed by the material. Negim et al., [6, 8, 10, 13] workability of the modified mortar increases with increasing latex concentration.

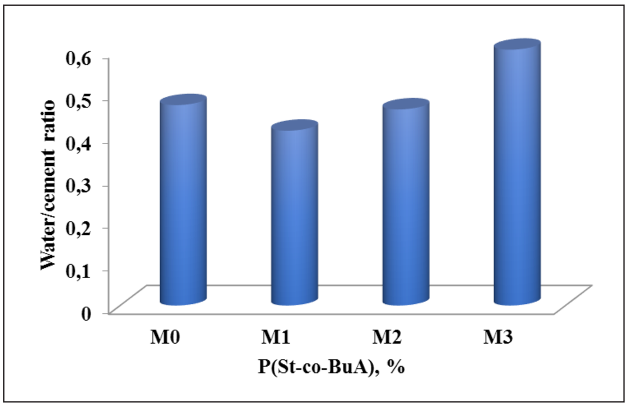


Figure 1 – The effect of copolymer lattices on the water/cement ratio of mortar.

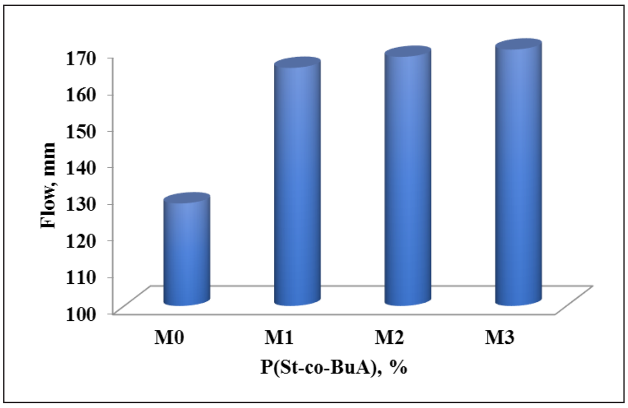


Figure 2 – The effect of copolymer lattices on the workability of mortar.

Compressive strength

Polymer lattices are admixtures that increased workability of mortar and produce high strength mortar. Fig. 3 shows the effect of dosage of copolymer lattices on the compressive strength of mortar. The compressive strength of mortar increases with curing time up to 28 days as shown in Fig. 3. This is attributed to the continual formation of hydration products, which tend to deposit into the pore structure of mortar cubes. The compressive strength of mortar tends to decrease with an increase in dosage of copolymer lattices. The highest compressive strength of mortar mixed with lattice (M1, 0.25%) while the lowest strength of mortar mixed with lattice (M3, 1.0%). It is well that the compressive strength of mortar is affected by dosage of the copolymer lattices [24, 25].

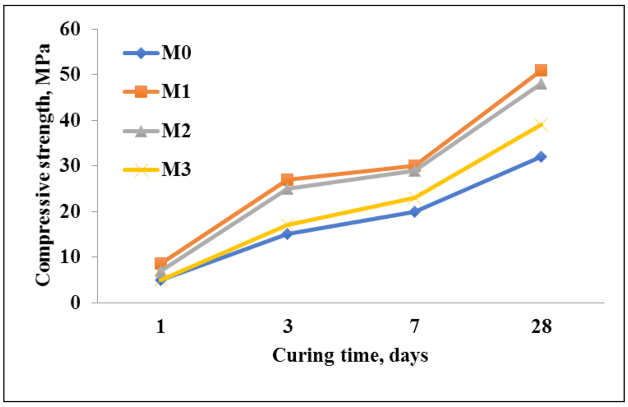


Figure 3 – The effect of copolymer lattices on the compressive strength of mortar.

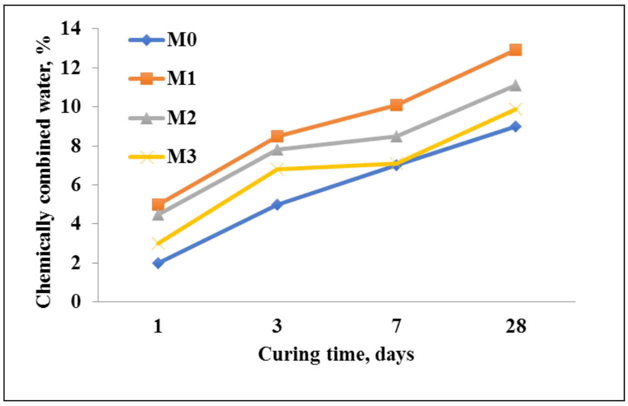


Figure 4 – The effect of copolymer lattices on the chemically combined water content of mortar.

Chemically combined water content

The chemically combined water contents of the mortar (M_0) and premixed with varying concentration of lattices are plotted as a function of curing time up to 28 days in Fig. 4. The results show that the combined water content of mortar increases with the increase of curing time. It is attributed to that the cement hydration process generally precedes the polymer film formation process by the coalescence of polymer particles in copolymer. As shown in Fig. 4, the addition of polymer lattices increases the chemically combined water content. However, the chemically combined water decreases with increasing dosage of lattices from 0.25 % to 1.0 %. The increasing in the chemically combined water content is attributed to the formation films of polymers around the cement grains or crystallizes inside the pore structure of the hardened cement pastes.

Conclusions

Mixing the mortar with the copolymers lattices enhance most of the specific characteristics of the mortar. The obtained results confirmed the important role of W/C-ratio on the effectiveness of the added copolymer so that their optimum values at W/C = 0.41. However, W/C ratio increased with increasing dosage of copolymer lattices in mortar mixes. There is evidently an improvement in the compressive strength of premixed mortar with copolymer for all experimentally at all curing ages of hydration compared with those of the pure cement pastes (M0) and good workability was achieved by mixing mortar with M3 (1% w/w lattices). There is an increase in the chemically combined water content of mortar premixed with 0.25% lattices (M1) compared with those mortar without polymer (M0), M2 (0.5 % lattices) and M3 (1.0 % lattices).

References

1. Y. Ohama, Handbook of Polymer-Modified Concrete and Mortars. Park Ridge, New Jersey, USA, 1995.
2. O. Xinping, G. Yongxia, Q. Xueqing, Constr. Build. Mater. – 2008. – 22. – P.1774–1779.
3. MMH Ayoub, MM El-Awady, HE Nasr, SM Negim, Polymer-Plastics Technology and Engineering. – 2003. – 42(5). – P.863-881.
4. MMH Ayoub, HE Nasr, MHH Darweesh, SM Negim, Polymer-Plastics Technology and Engineering. – 2005. – 44(2) – P.305-319.
5. ESM Negim, M Ramli, SE Mansour, B Saad, MI Saleh, Middle-East Journal of Scientific Research. – 2010. – 6(2). – P.99-107.
6. ESM Negim, J Khatib, MM Mohammed, SK Kerimbaevna, World Applied Sciences Journal. – 2012. – 19(6). – P.832-83.
7. ESM Negim, J Khatib, KA Mutairi, R Raikhan, MA Grigoriy, Middle- East Journal of Scientific Research. – 2012. – 11(8). P. 1131-1139.
8. ESM Negim, L Kozhamzharova, Y Gulzhakhan, J Khatib, L Bekbayeva, C Williams, The Scientific World Journal. – 2014. – 11. – P.235-241.
9. ESM Negim, J Khatib, M Ramli, B Saad, MI Saleh, World Applied Sciences Journal. 2010. – 10(6). – P.685-694.

10. ESM Negim, J Khatib, NO Inkarbekov, World Applied Sciences Journal. – 2013. – 23(4). – P.549-553.

11. SM Negim, MMH Ayoub, GM Enany, GA Mun, Eurasian ChemTech Journal. – 2006. -8(3). – P.243-252.

12. ESM Negim, M Ramli, B Saad, L Bekbayeva, MI Saleh, J. Polymer-Plastics Technology and Engineering. – 2011. – 50. – P.941 – 946.

13. ESM Negim, L Kozhamzharova, J Khatib, L Bekbayeva, C Williams, The Scientific World Journal. – 2014. – 10. – P.235-242.

14. G. Barluenga, F.H. Olivares, Cement and Concrete Research. – 2004. – 34. – P.527-535.

15. MA Bhutta, Y. Ohama, Concrete Research Letters. – 2010. – 1(4). – P.25-130.

16. M Alexandre, G Philippe, RR Humberto, Effect of EVLC latex polymer on masonry mortar properties and microstructure, 12th International BRICK/BLOCK Masonry Conference, Madrid-Spain, 25-28 June, 2000, – P.707-717.

17. ASTM C204-82, Standards Test Method, 1993.

18. ASTM C187-86, American Standard Test Method, 1993.

19. ASTM C191-92, American Standard Test Method, 1993.

20. BS 1881: Part 122 Testing concrete. Method for determination of water absorption, 1983.

21. ASTM C170-90, American Standard Test Method, 1993.

22. ESM Negim, N Rimma, L Bekbayeva, U Akmaral, BM Herki, N Merey, I Rinat, G Yeligbayeva. Oriental Journal of Chemistry. – 2017. – 33(1). P.450-457.

23. S.M. Negim, HY Cho, MMH Ayoub, SH Han, Effects of emulsifiers on the properties of styrene/methacrylate –type superplasticizer in cement paste. 4th International Symposium & Plasmo Chemistry. – Almaty, Kazakhstan, 12-14 September, 2007, P.135-139.

24. ESM Negim, G.Zh. Yeligbayeva, R. Niyazbekova, R. Rakhmetullayeva, A.A. Mamutova, R Iskakov, M Sakhy, GA Mun, International Journal of Basic and Applied Sciences. – 2015. – 4(3). – P.297-302.

25. ESM Negim, AMB Aisha, U. Yessimkanova , A. Kurmanbekova, K. Tyazhina, P.I. Urkimbaeva, R.K. Rakhmetullayeva, E. Shatabayeva, G. Irmukhametova, G.A. Mun, G.Zh. Yeligbayeva, JM Khatib, International Journal of Basic and Applied Sciences. – 2015. – 4(1). – P.10-18.

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Moldabergenova A.K., Litvinenko Yu.A., Akhtayeva N.Z.,
Kiekbayeva l.N., Ross S.A.

Al-Farabi Kazakh National University, Kazakhstan, Almaty
E-mail: akmaral-moldaber@mail.ru

Amino and fatty acid composition of the aerial parts of *Echinops albicaulis*, growing in Kazakhstan

Abstract. The article discusses the results of amino and fatty acid analysis of the aerial part of Echinops albicaulis , collected during mass flowering in Malaysary area in 2015 Almaty region. The research was conducted at the laboratories of the Faculty of Chemistry and Chemical Technology of Al-Farabi Kazakh National University t and Kazakh Academy of Nutrition. During research we determined the qualitative component composition anJd quantitative content of various amino and fatty acids in the aerial part of Echinops albicaulis of the Asteraceae. The research showed the predominate of proline, alanine, glutamic and aspartic acid in the elevated part of the amino acid, oleic and linoleic acid from fatty acids.

Key words: quantification, amino and fatty acids, Echinops Albicaulis, Asteroideae.

Introduction

There are different groups of biologically active substances in plants. It is important to know in what quantities they should be used for the medicine preparation.

To study the chemical composition Echinops albicaulis, the plant of Asreraceae family which is spend in the flora of Kazakhstan, was chosen by us. Echinops albicaulis – is a rather large genus of perennial, rarely one-biennial, spiny herbaceous plants, including about 190 species [1].

This species belongs to the of Asteraceae plants which is not well-studied, the study of qualitative and quantitative composition of Echinops albicaulis and the development of different preparations based on this plant is a burning issue. Amino acids – is the class of organic compounds containing amino and carboxyl groups. It has the properties of acids and bases, playing important roles in biological processes of the plant [4].

Among the amino acids, ten of them are essential and very necessary for the normal functioning of organism. The deficiency of amino acids disturbs the protein synthesis leading to different diseases. The article discusses the research results of the problems of qualitative composition and quantitative content of amino and fatty acids in Echinops albicaulis. Saturated and unsaturated higher fatty acids play an important role in nature. They are the part of glycerides which form the basis for cell membranes, so they should be classified as biologically important compounds. The greatest biological activity was found to be shown by not individual lipids but the whole set of lipids. Although each class of lipid fraction show biological activity. The fats are always in a liquid state in the living plant. Plants accumulate the fats of many families, especially Asteraceae, cabbage family, celery, Rosaceae, Euphorbiaceae, Papaveraceae, Lamiaceae.

The process of creation and accumulation of fats depend on environmental factors and genetic features of species and varieties. Therefore, study of fatty acids of Echinops albicaulis is necessary. We made a comparing analysis of amino and fatty components composition in the aerial part of this plant.

Materials and Methods

Research material is the aboveground part Echinops albicaulis family Asteraceae, collected during flowering was determined in Malaysary areas of Almaty region.

The qualitative composition of amino acids set by chromatography (on paper and in a thin layer) in the presence of substances standards [2], and quantitative composition- by GLC method [3].

Chromatographic conditions:

- Temperature of the flame ionization detector – 300 ° C
- Evaporator temperature – 250 ° C
- Initial column temperature – 110 ° C

- Final column temperature – 250 ° C

- Column temperature programming ranged from 110 ° C to 185 ° C – 6 ° C a minute; from 185 ° C to 250 ° C – 32 ° C a minute. On reaching the column temperature of 250 ° C it should remain the same temperature until the full yield of all amino acids.

To separate amino acids we used a stainless steel column, of 400 to 3mm ,which filled with a mixture of polar% from carbowax 0.31 m 20 0.28 5% Silar and 0.06% on Lexan hromasorbe WA-W-120-140 mesh . Counting of chromatogram is conducted by external Altex comany.

Determining the number of amino acids was performed by GLC [5].

1 g of the analyte was hydrolyzed in 5 mL of 6N hydrochloric acid at 105 ° C for 24 hours in vessels under an argon stream. The resulting hydrolyzate was evaporated three times until dried on a rotary evaporator at 40-50 ° C and a pressure of 1 atmosphere. The resulting precipitate was dissolved in 5 ml of sulfosalicylic acid. After centrifugation (1500 rev / min) for 5 minutes, the supernatant was passed through a column of ion exchange resin by Dauks 50, H-8, 200-400 mesh, at a rate of 1 drop per second. The resin is then washed with 2.1 ml of deionized water and 2 ml of 0.5 N acetic acid; then the resin is washed until neutral pH.

For Amino acid elution from 3 ml 6 N NH4OH solution was passed through column at 2 drops per second. The eluate is collected in a round bottom flask together with distilled water, which is used to wash column to neutral pH. The flask content is evaporated until dried on a rotary evaporator under a pressure of 1 atm. and a temperature of 40-50 ° C.

After adding to the flask, 1 drop of a freshly prepared 1.5% SnCl2 solution, 1 drop of 2, 2-dimethoxypropane and 2.1 ml of propanol saturated with hydrochloric acid, it was heated to 110 ° C, maintaining this temperature for 20 minutes and then the content was again evaporated in the flask on the rotary evaporator.

The next stage is the introduct on 1 ml of freshly prepared acylating reagent (1 volume of acetic anhydride, triethylamine, 2 volumes and 5 volumes of acetone) into the flask and it was heated at 60 ° C for 1.5-2 min. Then the sample is evaporated until dried on a rotary evaporator and were added into the flask 2 ml of ethyl acetate and 1 ml of saturated NaCl solution. The flask contents was well stirred as while two liquid layers were forming . Then we took upper (ethyl acetate) layer for the gas chromatographic analysis, which was performed on «Carlo-Erba-4200» (US-Italy) a gas-liquid chromatograph.

The results are shown in the Figure 1 and Table 1.

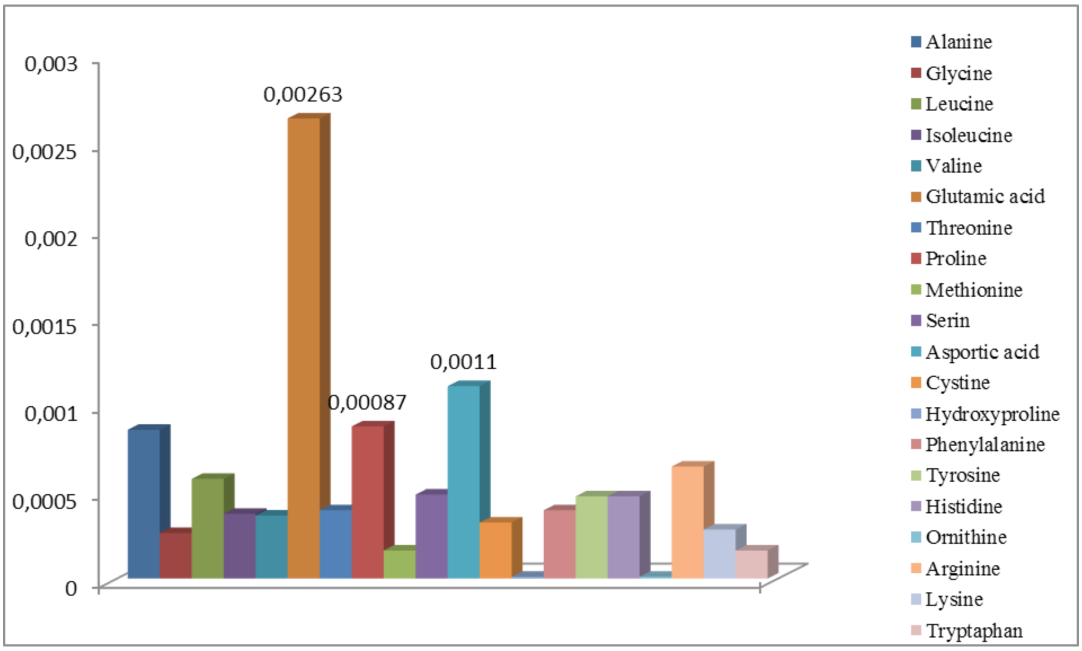

Figure 1 – The quantitative content of individual amino acids in the aerial part of Echinops albicaulis, in%

Table 1 – The quantitative content of individual amino acids in the aerial part of Echinops albicaulis, in%

The name	Ratio,%	Name	Ratio,%
Alanine	0,85	Asportic acid	1,1
Glycine	0,26	Cystine	0,32
Leucine	0,57	Hydroxyproline	0,01
Isoleucine	0,37	Phenylalanine	0,39
Valine	0,36	Tyrosine	0,47
Glutamic acid	2,63	Histidine	0,47
Threonine	0,39	Ornithine	0,01
Proline	0,87	Arginine	0,64
Methionine	0,16	Lysine	0,28
Serin	0,48	Tryptaphan	0,16

According to the results shown in the Table 1 and Figure 1, it can be concluded that the essential amino acids from the aboveground parts of Echinops albicaulis, contain large amounts of alanine, glutamic and aspartic acids.

It should be noted that the amino acids in the aerial part of Echinops albicaulis contains all the essential amino acids.

The results are shown in Table 2 and Figure 2

Determination of fatty acids was performed by GLC [5].

1 volume of sample is extracted by 20 times volume of chloroform-methanol (2: 1) for 5 minutes. Then the content is filtered through a paper filter to obtain a clear extract which is evaporated in a round bottom flask on a rotary evaporator at a bath temperature of 30-40 ° C until dried. Then, the flask is was filled whith 10 mL of methanol and 2-3 drops of acetyl chloride and methylated at 60-70 ° C in a special system for 30 minutes. Then methanol is evaporated on a rotary evaporator and the sample is extracted from the flasks and 5 ml of hexane was injected into the gas chromatograph. Experiments were carried out on the device «Carlo Erbo-4200» (USA, Italy).

Conditions of chromatographic:

- Injector temperature – 188 ° C, temp. Detector – 230 ° C;
- Furnace temperature – 188 ° C, Analysis time – 1 hr;
- The contents of the column: polyetyleneglycoladipinat (20%) on the zeolite -545.

The results are shown in the Figure 2 and Table 2.

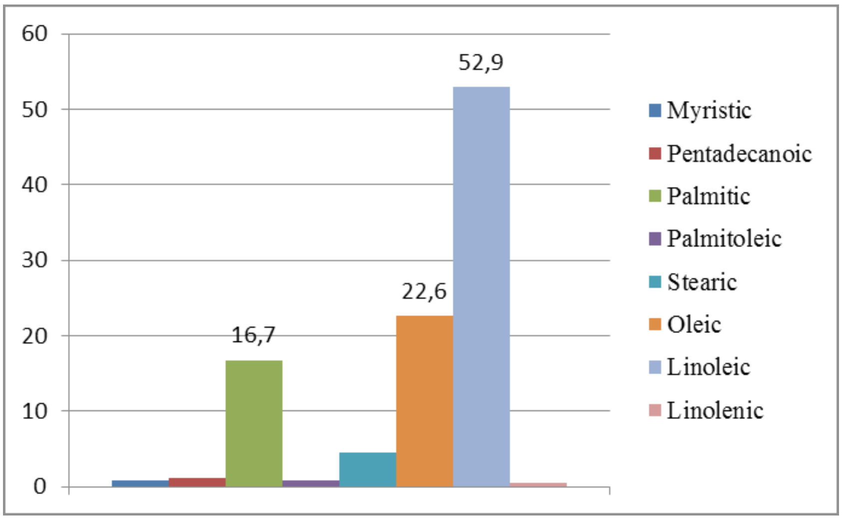

Figure 2 – The quantitative content of the individual individual fatty acids in the aerial part of Echinops albicaulis, in%

Table 2 – The content of individual fatty acids in the aerial part of Echinops albicaulis, in%

The name of acid	Symbol of acid	Ratio,%
Myristic	$C_{14:0}$	0,84
Pentadecanoic	$C_{15:0}$	1,1
Palmitic	**$C_{16:0}$**	**16,7**
Palmitoleic	$C_{16:1}$	0,9
Stearic	$C_{18:0}$	4,5
Oleic	**$C_{18:1}$**	**22,6**
Linoleic	**$C_{18:2}$**	**52,9**
Linolenic	$C_{18:3}$	0,5

The results of the research, showed that from the fatty acids in the aerial part of Echinops albicaulis dominate by quantitative content: linoleic, oleic, palmitoleic and palmitic acid, also an essential omega-6 linoleic acid, an amount of which is more than 50 %.

Conclusion

1. The amino and fatty acid composition were studied in the aerial part of Echinops abilcaulis by GLC (Gas Liquid Chromatography), which arecollected near Malaysary in Almaty region;

2. The aboveground part of Echinops abilcaulis is a source of many essential compounds, such as amino acids and fatty acids;

3. It is concluded that the aboveground part of Echinops abilcaulis, can be used as a medicinal plant for the production of new medicine

References

1. Bobrov EG 1567. Echinops // Flora of the USSR. V. 30 / Begun under the leadership of the principal, and under the editorship of acad. Komarov; Editors of volume are B.K. Shishkin and EG Bobrov. – M.-L .: Publishing House of the USSR Academy of Sciences, 1962. – V. XXVII. – P. 2-54. – 653 P.
2. Mashkovsky M.D. Medicianl plants-M., 1987.-V.2. –P.328
3. Kiselyova T.L, Samilina I.A The composition of free amino acids medications from leaves, flowers and fruits of Crataegus // grow. resources. – 1989 – V.25.- Pub. 4. – P.546-551.
4. Sheluto V.L., Buzuk G.N., Konoplyova M.M., Lovchinovsky J.O. Pharmacognosy – M.: Vitebsk. 2003. – 203-490.
5. Adams R. Determination of aminoacids profiles biological samples by gas chromatography // J. Chromatography.1974. – Vol. 95, № 2. – P. 188-212.

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Nyssanbayeva G., Tursunbek S., Kudaibergenov K.,
Ongarbayev Ye., Mansurov Z., Lubchik S.

Al-Farabi Kazakh National University, Almaty, Kazakhstan
E-mail: kenes19851911@gmail.com

Synthesis and study of physical-chemical properties of expanded graphite

Abstract. One a of the most urgent problems in the field of ecological status assessment is developing of a single integral approach to the problem of environmental quality and, in particular, of water as well as the evaluation criteria of their quality.

In this study, natural graphite is converted to adsorbent, intended for the use in getting rid of oil stains. The microstructure and morphology of expanded graphite made of antitermal natural graphite were investigated by Raman spectroscopy and scanning electron microscope (SEM).

Innovative terminative process was discovered and investigated in the systems «graphite – reactant-oxidant» – it is a direct oxidative conversion of graphite in TWG going through the sig formation of as unstable intermediate reactions thermolysis.

The study of graphite's as sorbents of oil in Kazakh science and technology is very relevant and has will be used in future.

Key words: expanded graphite, compounds of graphite intercalcination, thermal destruction, carbon plane.

Introduction

Compounds of intern calcination of graphite (SIG) obtained in the reactions of graphite with acids and oxidizers have been attracting attention of scientists and practitioners for over 150 years [1-3]. This growing interest is caused by the unique ability of SIG in to increase its volume hundreds of times when heated as well aspractical value of the obtained product – thermografenit (TWG) [1,2]. High heat resistance, chemical inertness and incombustibility as well as the ability to be compressed without a binder in the carbon products of any form have led to the widespread use of TRH and its products in fire-prevention defense, metallurgy, power industry, aerospace engineering and automotive industry worldwide [4-7].

Practical orientation to the TWG and its products production led to the preferential study of the properties of TRH and improving of its technical characteristics. At the same time, issues connected with kinetics and specific mechanisms of thermal destruction of and formation of TWG have not been studied. The main routes of the thermal destruction of SIG are considered on a qualitative level, quantitative description of the thermal destruction process is performed for the most simple systems.

This situation has led to the fact that the process of obtaining of the TWG is considered only technologically two-staged (obtaining of an intermediate product SIG and final product – TWG), the management of this process is transferred to the area of synthesis of SIG. When the fruitfulness of this approach excludes the question of technologically one-step receipt of TWG and thus greatly restricts the use of thermolysis reactions in systems of «graphite – reactant-oxidant» for the synthesis of TRH having desired properties.

It is obvious that the processes of thermolysis are far from exhausted, and new theoretical and practical approaches to the process of obtaining of TWG may be offered on the basis of data about the mechanism of conversion of graphite into systems «graphite – reactant-oxidant» [3-5]. The performed studies are an attempt to mut the specified lack, and to consider the synthesis process of SIG and its thermal destruction to the TWG as a single process of the direct oxidative conversion of graphite in TWG.

The objective of the work is synthesis and study of physico-chemical properties of expanded graphite.

Printed in Kazakhstan

Experimental part

Physical-chemical properties of the samples are determined in the laboratory of physical and chemical research, as well as in the a National nanotechnological laboratory of an open type. al-Farabi: KazNU their macrostructure is defined by various methods of physical chemical analysis.

In order to make the study the original substances and some inorganic salts were used: such as natural graphite, barium nitrate and iron chloride. Natural flake graphite brand,such as GL-1 and GT-2were used. The part of graphite was demineralized in order to eliminate the influence of mineral components on the thermal processes at elevated temperatures.

Demineralization included processing of 5% HCl (heated for 1.5 h in boiling water bath at a ratio of 4 dm3 HCl:1kg of graphite), filtration, washing out with 0.5% of HC1, processing of 20% HF (60°C, 3 h). filtration, washing out with water til neutral reaction and bringing to air dry condilion the at room temperature. The ash content of the demineralized sample is < 0.5%.

The entering of salts was carried out by the dry method: mechanical mixture of graphite with salts in a molar ratio of graphite:salt -62,5:1; 33,3:1; 19:1; 6,4:1; 2,7:1. and method of impregnation: 0.6 g of graphite were moistened to 0.8 cm3 by aqueous solutions of different salt concentrations: 0,41; 1.24; 2,07; 3,33; 4,6 (M) and brought to air dry state at room temperature and of was periodically stirring. Then the samples were dried for 2 h at 105 ± 2°C. The Samples were systems «graphite – salt» in molar ratio 119:1; 54:1; 30:1; 18:1; 12:1

Kinetic studies of thermal destruction of SIG in isothermal mode were performed in thermoweightl installation (Fig. 1), which is running on gas, the reactor (1) has the evaporator coil (2). The reactor with the evaporator are arranged in the air chamber of the thermostat (3) (accuracy of temperature control ±1°C).

To create a uniform upward flow of gases and vapors inside the reactor (1) a porous glass septum is soldered (4). Measurement of loss of mass was performed using a torsion balance (5) with measuring range of 0 to 250 mg.

Hitch SIG (50-100 mg) was placed into a specially designed crucible (6) with a cover (d=12 mm, height 10 mm), suspended to the yoke of weights using a Teflon thread. Measurement error of the mass does not exceed of ± 2×10-4 g. Measure ment of the mass loss of the samples was performed in air medium.

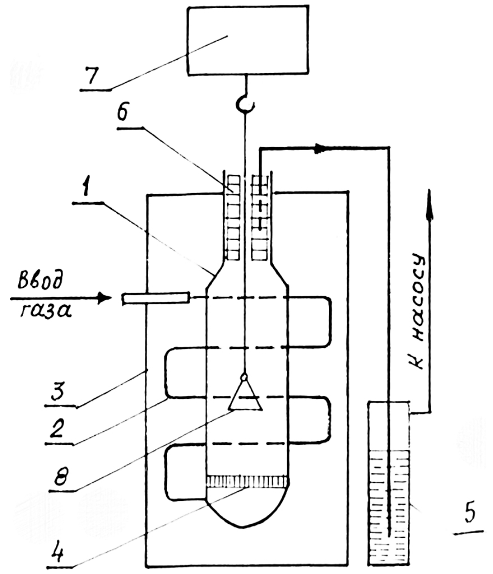


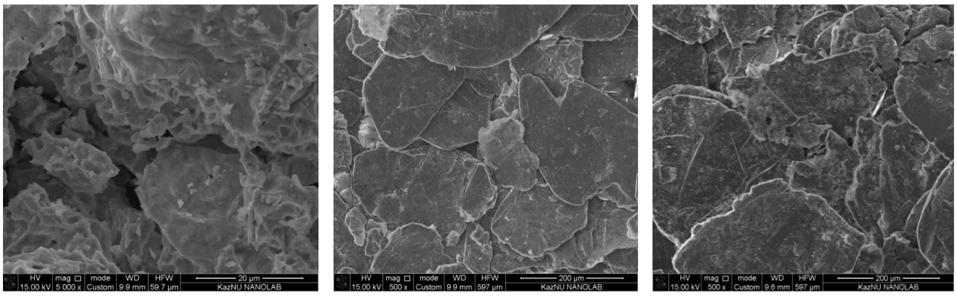
Figure 1 – Schematic diagram thermovisual installation 1) glass reactor; 2) the evaporator coil; 3) thermostat; 4) porous glass partition; 5) converter of gases; 6) the mixer; 7) torsion scales; 8) measuring cell

Results and their Discussion

From the results of electron-microscopic examination, one can see that the natural graphite is composed of thin layers of a thickness of 80 nm. These layers have a high degree of crystallinity.

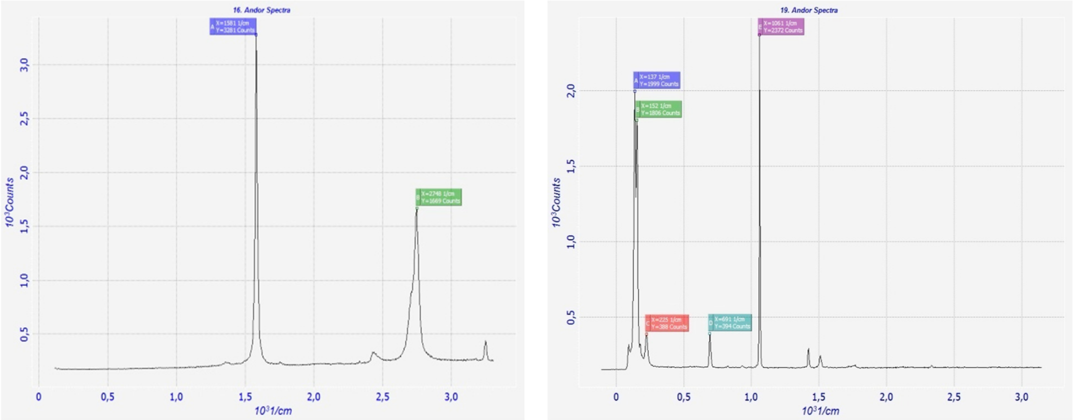
Electronic microscopic analysis showed that the natural graphite impregnated with a solution of barium nitrate has macropores (Figure 2). Metal containing phase is unevenly distributed on the surface expanded graphite matrix. The shape of the particles GHG is determined by the layered structure of graphite and foaming occurs in one direction only, namely, perpendicularly to the stacking of layers, particle penografia becomes of a worm-shaped form. Color of expanded graphite is from ash-grey to black without typical for initial graphite metallic shine. The chemical composition of expanded graphite represents the carbon like the initial graphite, however, the level of impurities in PG is much higher than for the original material. As one of the aims of the present work was the doping of penografia by metalic compounds; then IG, modified by nitrates of metals (IG(IU)), is to be the best precursor. Getting of penografia, doped by metal oxides, will be already possible at 850°C, i.e. at the temperature of decomposition of barium nitrate.

Produced samples were also investigated by Raman spectroscopy. We can see from the spectra that all samples have carbon structure.



a) b) c)

Figure 2 – SEM images: (a) natural graphite, (b) graphite impregnated with salts $FeCl_3$; (c) modified graphite with barium nitrate



a) b)

Figure 3 – Raman spectra of: (a) natural graphite, (b) graphite impregnated with salts of $BaNO_3$

Further analysis of the sample the structure of the original and heat-treated graphite was performed by the method of Raman scattering (RS). For the excitation of spectra a semiconductor laser emitting a wavelength of 532 nm was used. The study was carried out at the room temperature. The analysis of spectra of Raman scattering of graphite sample revealed the presence of several lines of cattle; Line A of about 1581 cm-1, peak B of about 2748 1/cm. Lines of about of 1581 cm-1, as belonging to the defects on the edges of the plots ideal carbon planes.

In the spectra of heat-treated graphite several different peaks appeared, which indicate different planes. Line A-1371 cm-1, peak B-1521 cm-1, peak C-225 cm-1, peak D-691 1/cm, and the line E 1061-cm-1. Carbon is linked in flat sheets, similar to the planes in graphite. Such a conclusion can be made on the basis of the complete absence of a peak-1350 cm1 in the spectrum, in the presence of intense and narrow line of about1575 cm-1. It says that the vast majority of carbon atoms are in the sp2 state and form well-shaped flat structures. The presence of lines C and D is characteristic for graphite having a high degree of order.

Thus the obtained results show that heat-treated graphite has an almost perfect crystalline structure of graphite.

For the formation of intercalation compounds of graphite with the chlorides of metals mechanism involving the direct participation of chlorine in the process of internalrevenue is suggested.

In the gas phase chlorine molecules are adsorbed on graphite, then the chlorine is transferred to the atomic state and oxidizes the graphite matrix to form microcation CP. Electroneutrality is supported by adsorbed SG. Molecules of the metal chloride diffuse in-charged graphite matrix, to capture the anions of chlorine give the compound of electroneutrality, and an embedded layer in ISH with chlorides of metals represents a chlorine anions surrounded by neutral molecules of the metal chloride.

The phase composition of GHGs regardless of the temperature of foaming represented by graphite-at the interlayer distance of 3.36 Aº. The yield of a solid product is within the limits of 80-94 % and shows once again that the chemical processing with the subsequent foaming is accompanied by the partial transfer of carbon to carbon dioxide. The yield of carbon at 900°C in the process of obtaining chemically-modified expanded graphite is 92-95 %.

Table 1 shows the basic characteristics of natural graphite of grade GL-1 and GT-2. As the table shows, the specific surface of natural graphite of grade GL-1 and GT-2 is 6,382 and 9,546 m2/g, respectively. The highest amount of carbon is~92% for natural graphite.

Table 1 – Main characteristics of natural graphite

Samples	Carbon content, %	Size of particles, mm	Mass fraction of moisture, %	Specific surface, m^2/g	Mass of ash, %
Natural graphite of GL-1 brand	~92	~ 0,5	1,0	6,382	10
Natural graphite of GL-2 brand	~95	~ 0,5	0,8	4,546	7

For the formation of intercalation compounds of graphite with chlorides of metals a mechanism involving the direct participation of chlorine in the process of inter calcinations is suggested.

The thermodynamic conditions of the process of direct oxidative conversion of graphite in TWG considered. Its place in a number of known processes for the synthesis of SIG and its transformation in TWG for «graphite – reactant-oxidant»system is determined.

References

1. Wbellode A.R., Lewis F.A., Graphite and his crystalline Connections, M.: Mir, 1965, 265 p.(in Russ)

2. Enoki T., Suzuki M., and Endo M., Graphite Intercalation Compounds and Applications Book, Oxford University Press (USA), 27 March 2003, 456 Pages ISBN:9780195128277.(in English)

3. Fischer J.E., Thompson T.E., 2008, Doi: http://dx.doi.org/10.1063/1.2995104. (in English)

4. Yoon G., Seo D., Ku K., Kim J., Jeon S., and Kang K., Chem. Mater., 2015, pp 2067–2073; DOI: 10.1021/cm504511b. (in English)

5. Rangappa D., Sone K., Wang M., Gautam U., Gilberg D., Iton H., Ichihara M., Honma I., A European Journal, Vol 16,N 22, 2010, pp. 6488–6494. (in English)

6. Cai M., Thorpe D., Adamson D.H., and Schniepp H. C., J.Am. Chem Mater, 2012,Vol. 22, pp. 24992-25002; DOI:10.1039/C2JM34517J. (in English)

7. Parvez K., Wu Z.S., Li R., Liu X., Graf R., Feng X. and Müllen K. J.Am. Chem. Soc, 2014, Vol. 136 , N16, pp 6083–6091; DOI: 10.1021/ja50171567(in English)

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[1]Otzhan U.N., [1]Kurmansitova A.K., [1*]Sassykova L.R., [1]Serikkanov A.A.,
[1]Kenzhebekov A.S., [2]Starikov E.B.

[1]Faculty of Chemistry and Chemical technology, Al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Department of Physical Chemistry, Chalmers University of Technology, Sweden
*E-mail: larissa.rav@mail.ru

Catalysts for selective hydrogenation of aromatic nitrocompounds and hydrocarbons

Abstract. The liquid-phase hydrogenation of nitrocompounds and aromatic hydrocarbons at the elevated pressure of hydrogen on the mono- and bimetallic supported catalysts was studied. The optimum technological parameters of reactions for obtaining a high yield of target products were determined. It was shown that the mechanism of transforming aromatic nitro compounds – hydrogenation and it was identical for all solvents. It was studied the influence of the added hydrogenation reaction product (p-aminophenol) during p-nitrophenol hydrogenation for determination the cause of reducing of velocity of hydrogenation. It was revealed that the monometallic Rh/Al_2O_3 or bimetallic catalysts of $Rh\text{-}Pt/Al_2O_3$ composite are the most active catalysts for hydrogenation of aromatic hydrocarbons. The catalysts applied on aluminum oxide are more active and selective, than the catalysts applied on a silica gel.

Key words: catalyst, hydrogenation, aromatic nitrocompounds, aromatic hydrocarbons.

Introduction

Aromatic mono- and polyamines are applied in the synthetic dyes production for wools, clothes, furs and synthetic fibres (aniline, nitroaniline, phenylendiamines, aminophenols). Liquid-phase catalytic reduction of nitrocompounds for producing amines allows to carry out process at rather low temperatures that leads to the considerable decrease in expenses of the electric power and potential of explosion of system [1, 2]. At application different solvents the reaction performs in milder conditions, than at a steam method. Such method of amines synthesis is more pollution-free and amines are obtained with rather high yields [3, 4]. Processes of hydrogenation of aromatic hydrocarbons are one of important among large-tonnage processes of oil processing and organic chemistry [5], for example, from synthesizable benzene worldwide about 20% are applied further for cyclohexane producing. Process of catalytic hydrogenation of aromatic hydrocarbons gives the possibility not only to change the chemical structure of hydrocarbons in the important direction and to reach the high yields of target products but also to improve production characteristics of oil fuels, oils and raw materials for petrochemical processing [6-8]. It is well known that the processes of reduction of nitrocompounds and aromatic hydrocarbons are very difficult and multistage. These reactions require careful selection of active, selective and enough stable catalysts, and also process conditions: solvent, temperature and hydrogen pressure. Thus the development catalysts for selective hydrogenation of aromatic nitrocompounds (on nitrogroup or a benzene ring) and aromatic hydrocarbons is a very important and perspective problem.

The purpose of work was a creation of the put (supported) catalysts on the basis of platinum metals; studying their activity in reactions of hydrogenation of nitrogroup in nitrocompounds, and also at hydrogenation of aromatic hydrocarbons (benzene, ethylbenzene, toluene, cumene) at elevated hydrogen pressure.

Materials and Methods

The equipment, applied reagents, compounds analysis

In the work the put (supported) mono – and bimetallic catalysts on the basis of platinum metals and on the basis of Pd and Pt with the modifying additive – Cu were prepared. Content of the active metals was varied within 0.5-5.0 wt.%. For synthesis of catalysts $PdCl_2$ salts of «pure» brand and $RhCl_3 \cdot 3H_2O$, $H_2PtCl_6 \cdot 6H_2O$, $RuOHCl_3$, $CuSO_4 \cdot 5H_2O$ salts of «chemically pure» brand were applied. Chromatographically pure nitrobenzol (NB), para-, ortho- nitrophenols (p-NP, o-NP), benzene (B), ethyl benzene (EB), toluene (T), cumene, which were prepared by vacuum distillation or a recrystallization, were used in this work. The physical and chemical properties of former to be hydrogenated compounds corresponded to references [9]. Reactions were carried out as with solvents (distilled water, alcohols C2-C5 of «chemically pure» brand) as without solvents. Hydrogenation was researched with using of the electrolytic hydrogen from cylinder (99.8%) and for gas-liquid chromatography (GLC) helium from a cylinder (99.992%) was applied. For carrying out of the experiments on a liquid-phase hydrogenation the kinetic installation of high pressure (KIHP) consisting of the advanced autoclave of Vishnevsky with intensive hashing and a measuring part was used. The main part of KIHP – the advanced autoclave of Vishnevsky from a titanium of VT-3 (total amount – 60.0 ml) with the hermetic electric drive, turbine stirrer (rotation speed – 2.800 rev./min, stirring intensity -40.000 Re). The experiments were carried out in the isobaric-isothermal mode by a method developed by authors of this article in a laboratory [10-12]. For the analysis of initial compounds and reaction products the methods of gas-liquid chromatography (GLC), diazometric titration and IR-analysis were used [13, 14]. In the work physical and chemical research techniques of catalysts: X-ray diffraction, IR-analysis method on «Specord JR-75» and «UR-20» were also applied. The synthesized catalysts were investigated by techniques a electron microscopy (scanning and penetration), BET, porosimetry, BET (by nitrogen adsorption on the apparatus «Accusorb»), porosimetry.

Preparation of catalysts

The catalysts for the hydrogenation of gasoline fractions were synthesized by impregnating a carrier (alumina γ-Al_2O_3, silica-SiO_2, activated carbon) with aqueous solutions of the active metal salts. Beforehand carriers were crushed to fractions 0.1 and 0.2 of mm, washed out by distilled water at 80°C and dried at 100°C. For carrying out a stage of impregnation was prepared aqueous 1.0% solution with the compound containing the active component. Two-component catalysts (based on Pd-Pt, Rh-Pt, Pd-Rh) were prepared by joint impregnation of the carrier solutions of the corresponding compounds. Some samples of catalysts were reduced in hydrogen current at 200°C in the quartz furnace. If in the reduction time of the catalysts samples even slight quantities of HCl were noticed, the procedure of washing of the catalyst from Cl-1 ions was repeated. The prepared catalysts were cooled to room temperature and stored in sealed containers in desiccators. Before each experiment, the catalysts in the conditions required for the experiment, in a solvent medium was reduced in hydrogen for 30 min.

Results and Discussion

Reduction of nitrogroup in aromatic nitrocompounds

Reduction of aromatic nitrocompounds has begun with selection of solvent. Among the solvents used in work (distilled water, C_2-C_5 alcohols) isopropanol is the most suitable for hydrogenation of NP on Pd-catalyst, and on Pd-Pt, Pd-Cu – catalysts – ethanol. These solvents were also used for studying the process of hydrogenation under various pressure and temperatures. The products analysis during reaction showed that the mechanism of transformation of aromatic nitrocompounds is identical to all solvents and it is a hydrogenation mechanism. There were the least yields of arylamines when using distilled water and C_4-C_5 alcohols for reduction of nitrocompounds on Pd-Pt-catalysts. When using isopropanol for these catalysts the yield of target amines decreased due to the side reactions (reduction by a benzene ring). So, at hydrogenation of NB in the final sample, except aniline, already at 20-25°C there was a cyclohexylamine-hydrogenate of a benzene ring (4-6%) and at further increase in temperature the content of cyclohexylamine increased to 8-10%. At hydrogenation of NB and NP on the Pd-containing catalysts hydrogenation of only nitrogroups was noted, irrespective of solvents and the applied experimental conditions. Herewith the yield of aniline made 94-99%, p-phenylenediamine – 97-98%, p-aminophenol – 89-97% and o-aminophenol – 83-94%.

At research of NP reduction in comparison with NB in identical experimental conditions the reduction rate of NP is slower, than NB recovery rate. The form of kinetic curves at change of a structure of aromatic nitro compound practically does not change. Herewith the reaction rate, the yield of aminophenol (AP) for p-NP is significantly higher, than in a case of o-NP. The studied nitro compounds on decrease in initial rate of hydrogenation on the synthesized catalysts form a row: NB>p-NP>o-NP. Emergence of OH-group in a molecule of aromatic nitrocompound in p- and, especially, in o-position, reduces adsorption of nitro compound on the surface of the catalyst. Due to this the ratio of reaction components (hydrogen and nitrocompounds) on the catalyst surface is broken, the reaction rate and AP yield are reduced. Relatively low speed of o-NP reduction, apparently, is associated with a manifestation of ortho-effect [15, 16]. The ortho-effect is a set of all types of the spatial and stereoelectronic interactions of close located substituent and reaction center of a molecule. The substituent creates space barriers which prevent approaching of reagent to the reaction center and its solvating in solvent. Due to violation of coplanarity to an aromatic core, the substituent or reactionary at ortho arrangement there is a stereoelectronic braking of reaction. Ortho substituents are close enough to the reaction center that considerable vicinal effect could occur.

p-AP was added to the reaction medium in equivalent amount during p-nitrophenol hydrogenation for determination the cause of reducing of velocity of hydrogenation. Addition of the reaction product reduces the reduction rate and the amount of absorbed hydrogen (see Tab.). Possibly it was due to the blocking effect of the surface of the catalyst reaction product – p-AP.

Table – Investigation reduction of n-NP and p-NP (calculated on 400 cm3 of hydrogen) in the mixture with n-AP in an equivalent amount at 0.5 MPa, T = 30°C, catalyst- Pd-Cu/γ-Al2O3 (0.05 g)

#	The volume of absorbed hydrogen from the gas phase, cm^3	W, sm^3/min.		The ratio of the adsorption coefficient $b_{1/}b_2$
		p-NP	p-NP+p-AP	
1	100	85.0	20.0	0.23
2	150	72.0	16.0	0.22
3	180	65.0	13.5	0.21
4	250	56.0	12.0	0.21
5	300	34.0	7.7	0.22

Ratio b_1/b_2 indicates stronger adsorption of n-AP compared to n-NP. p-NP has fewer opportunities for access to the catalyst surface, as a surface of the catalyst in the first seconds of the reaction occupied by molecules of p-AP, which are readily adsorbed on the catalyst surface. Speed reaction hydrogenating the n-NP sharply reduced in the presence of p-AP, as catalyst surface poisoned by the reaction product. The calculation of the rate constants based on adsorption coefficients shows that the reaction rate constant, confirming the assumption of zero orders the reduction of p-NP on the substrate.

The data, which is obtained in this work, suggests reduction mechanism of investigated nitrocompounds. In the hydrogenation of o- and p-NP, apparently, at first is formed corresponding hydroxylamine derivative which is immediately rearranged into quinoneimine. For this reason, it is difficult to register particle by gas-liquid chromatography. Then quinoid group quite easily rearranges to the corresponding aminophenol. This reaction is substantially easier than the transformation of NO_2-group into NH_2-group.

Hydrogenation of aromatics

It was revealed that the most active catalysts in the hydrogenation of aromatics – monometallic Rh/Al_2O_3 (%Rh – 4-5% wt.) or bimetallic – Rh-Pt/Al_2O_3 (atomic ratio of active metal – 3:7, 2:8, 1:9, total % – the content of metals: 5%). It is not required increase in temperature at hydrogenation of benzene in the absence of solvent; the process was carried out at 2.8-3.0 MPa. Hydrogenation of EB was carried out at heating the reaction system (not higher 50ºC), and at room temperature – 20-25ºC, with hydrogen pressures – 3.0-3.3 MPa.

It is revealed, that the hydrogenation of cumene was carried out only at elevated temperatures (50 to 70-80 ºC) and hydrogen pressure – 3.2-3.3 MPa.

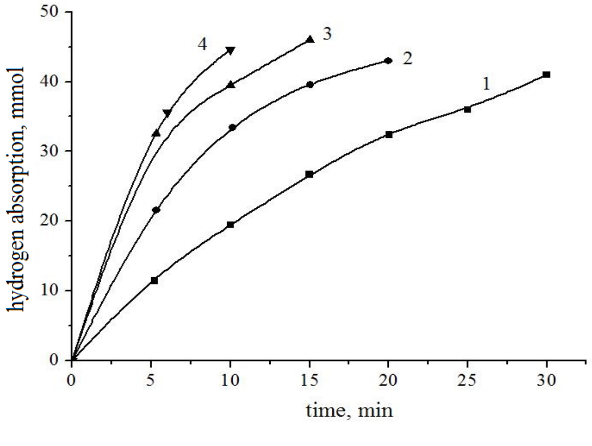
The reaction rate and the catalyst activity decreases in the series of catalysts: Rh-Pt>Rh-Pd>>Pd-Ru>Rh (fig.1). Bimetallic catalysts are more active and selective than monometallic. The maximum values of benzene and toluene conversion (85.0-93.0%) and yield of the corresponding hydrogenation products of the aromatic ring were found on the bimetallic catalysts Pd-Pt/Al_2O_3 and Rh-Pt/Al_2O_3. Catalysts supported on alumina were more active and selective than the catalysts supported on silica. It was found that the reaction rate decreases with increasing complexity of the structure of compounds in the series: benzene>>ethylbenzene>cumene. These data are consistent with published data. Thus, according to [18-20], the rate of hydrogenation of benzene is higher than the rate of its homologues hydrogenation.

This is probably due to the presence of unsaturated side chains in aromatic hydrocarbons. GLC analysis and IR spectroscopy data showed the high yields of the desired products (up to 98-99%).

Hydrogenation of aromatic hydrocarbons on Pd-Pt-catalysts on various carriers was carried out. On catalysts supported on zeolites, in the reaction products the cracking products were also present in minor amounts (to 5.0%). There is a partial reduction of benzene to cyclohexene (10-18%) on Ru catalysts, the yield of cyclohexane – not higher than 30-46%. Optimum catalysts for the hydrogenation of benzene and toluene – the catalysts with ratio Pt:Pd = 3-7; 2-8; 1-9.

Physico-chemical properties of the synthesized catalysts

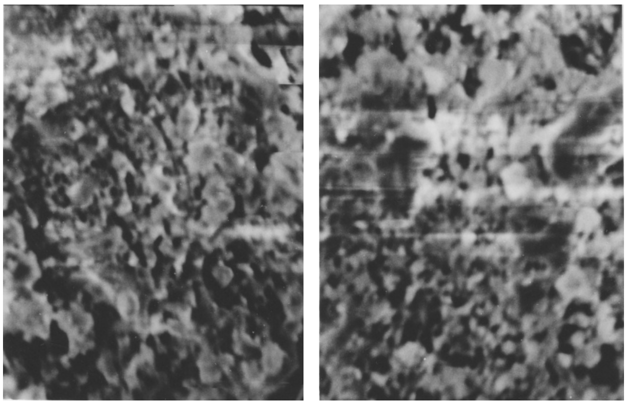
Specific surface area of the developed catalysts on the basis of various platinum metals is within values 175.0-290.0 m^2/g. Addition of the second metal slightly reduces the specific area. The synthesized catalysts possess a developed surface and have high volume of pores. The volume of the pores of catalysts represents: Pt-Pd(1:1)/Al_2O_3 S_w=318.33 ml/g; Rh-Pd(1:1)/Al_2O_3 – S_w=282.25 ml/g; Rh-Pt(9:1)/Al_2O_3 S_w=313.28 ml/g; Rh-Pd(9:1)/Al_2O_3 – S_w=213.39 ml/g; Pd-Ru(1:1)/Al_2O_3 – S_w=297.09 ml/g.



1-Rh, 2-Rh-Pd (1:1), 3-Rh-Pd(9:1), 4-Rh-Pt

Figure 1 – The hydrogenation of benzene in an autoclave without solvent at different catalysts supported on Al_2O_3, P_{H2}=2.5 MPa, T=120°C

The used carriers and catalysts deposited on them were investigated by Scanning Electron Microscopy (SEM). Applying metals as mono- Pd/Al_2O_3 (fig.2 a), and as bimetallic forms (in fig. 2b) produces a specific structure on the surface carrier. It was found that structure of Al_2O_3 carrier consists of acicular crystalline structures. The initial sample of HZSM-5 is resented by predominantly extensive congestions of dispersible particles, which are located on the support surface. The dispersed particle size of ~ 5.0 nm occupies the large swathes of the carrier surface.



a b

a – 2%Pd/Al_2O_3, c – Pd-Pt(1:1)/Al_2O_3

Figure 2 – SEM-images

Microdiffraction patterns of catalyst with Pd/Al_2O_3 composition corresponds to metallic Pd, moreover, there are in a small amount (4-5%) the particles of PdO. X-ray phase analysis of the catalyst of composition Pd/Al_2O_3 showed the presence of the metallic phase Pd peak – ICDD No.87-0653, d = 2.25, 1.95, 1.38, and phase γ-Al_2O_3 (2u = 458 d -1.99, 2u = 36.18 d-2.46) [21]. It was revealed the formation of solid solutions and the presence of traces of free Pd for systems Pd-Pt-catalyst. X-ray analysis of the reduced mixed Pd-Pt-catalysts showed that palladium indicated distinct midline intensity on the diffraction patterns; parameter of lattice didn't differ from the known in the literature. There are lines of Pd and unreduced oxide PdO on the diffractogramm, and its amount is close to 10%. The results of temperature-programmed reduction (TPR) show that all Pd-containing catalysts possibly contain palladium in at least in two forms: PdO particles and Pd oxide species stabilized on a surface.

For studying the behavior of NP at hydrogenation in the liquid phase at hydrogen pressure it was synthesized supported catalysts based on Pd and Pt with builder – Cu. In the preparation of catalysts as the carriers were used γ-Al_2O_3 and activated carbon (C). As a result of physico-chemical studies of catalysts it was found that catalysts supported on a surface area C is almost 2 times greater than the surface of catalysts supported on γ-Al_2O_3. Pores of catalysts with γ-Al_2O_3 carrier have the shape of cylinders, which radius are within the range 20-22 Å. According to XPS, palladium on C is fully reduced to the zero-valent state, whereas γ-Al_2O_3, palladium is not fully reduced. Electron binding energy Pd° $3d_{5/2}$ Pd/C is 336.5 eV, corresponding to Pd^{2+}. Modification of the catalyst with copper ions does not change the binding energy of the electron Pd $3d_{5/2}$. By XRP-method was revealed that in the copper-modified catalysts based on Pd (Pd-Cu) – Pd is in the zero valence state, and the state of copper Cu is characterized by the binding energy of $2p_{3/2}$ electrons, equals to 932.7 eV, corresponding to Cu +, so it's possible to suggest that Cu catalysts are in the form of Cu_2O. It is found that in addition to the zero valent Pd, there is also oxidized form of palladium (PdO_2) on the surface.

Conclusion

The reactions of the liquid-phase hydrogenation of the nitro group in nitro compounds and the hydrogenation of aromatic hydrocarbons (benzene, ethylbenzene, toluene) at different temperatures and hydrogen pressures were studied. The effective selective catalysts on the basis of the platinum metals applied on various carriers were created. Chromatographic analysis showed that the conversion mechanism of aromatic nitro compounds is hydrogenated and it is identical to all solvents. The shape of the kinetic curves at changing the structure of aromatic nitro compounds are not virtually changed. The sharp decrease in the hydrogenation rate of aromatic nitrophenol, compared with nitrobenzene is probably associated with decrease in the adsorption capacity of o- and p-NP, especially in the case of o-NP. It is obvious that the presence of groups OH-substituents in the molecule in the case of NP reduces the rate of hydrogenation of the compounds. It was studied the influence of the added hydrogenation reaction product (p-aminophenol) during p-nitrophenol hydrogenation for determination the cause of reducing of velocity of hydrogenation. Addition of the reaction product reduces the reduction rate and the amount of absorbed hydrogen. Hydrogenation of aromatic hydrocarbons at elevated pressure of hydrogen was studied in the solvents and in the absence of solvents. Rh-Pt-catalysts supported on γ-Al_2O_3 are the most active catalysts in the process. The reaction rate and selectivity of the catalysts decreases in the series: Rh-Pt>Rh-Pd >>Pd-Ru>Rh.

References

1. Sangeetha P., Shanthi K., Rama Rao K.S., Viswanathan B., Selvam P., Appl.Catal,A, 353, 160–165(2009).

2. Obraztsova I.I., Eremenko N.K., Simenyuk G.Y., Solid Fuel Chem., 46, 364(2012).

3. Turakova M., Salmi T., Eranen K., Warna J., Murzin D.Yu., Kralik M., Appl.Catal, A, 499, 66–76(2015).

4. Sasykova L.R., Bizhanov Ph.B., Masenova A.T., Catalytic reduction of o-nitrophenols on 4%Pd/Al_2O_3 catalyst in the liquid phase, News of National Academy of RK, 1, 50-54(1995).

5. Orozco J.M., Webb G., The adsorption and hydrogenation of benzene and toluene on alumina and silica- supported palladium and platinum catalysts, J.Applied Catalysis, 1, 67 (1983). doi:10.1016/0166-9834(83)80189-4.

6. Poondi D., Vannice M.A., Competitive hydrogenation of benzene and toluene on palladium and platinum Catalysts, Journal of Catalysis, 2, 742 (1996). doi:10.1006/jcat.1996.0236.

7. Frolova O., Massenova A.T., Sassykova L.R., Basheva Zh.T., Baytazin E., Ussenov A., Int. J. Chem. Sci., 12, 625-634. (2014).

8. Mary G., Esmaeili A., Chaouki J., Int. J.Chem. React.Eng, 14, 859-874(2016).

9. Rabinovich V.A., Havin Z.Ya., Brief chemical reference book, L., Chemistry, 1978.-392 p.

10. Baiseitov D.A., Gabdrashova Sh.E., Magazova A.N., Dalelkhanuly O., Kudyarova Zh. B., Tulepov M. I., Sassykova L. R., Mansurov Z.A., Int. J. Chem. Sci., 14, 244-250(2016).

11. Baiseitov D.A., Gabdrashova Sh.E., Akylbai A.K., Dalelkhanuly O., Kudyarova Zh.B., Sassykova L.R., Tulepov M.I., Mansurov Z.A., Int. J. Chem. Sci., 14, 261-268(2016)

12. Massenova A.T., Sassykova L.R., Eur.Chem. Techn.J., 2, 101-105 (2000).

13. Sasykova L.R., Kasenova D.Sh., Masenova A.T., Bizhanov F.B., Russ.J.Appl.Chem., 71, 1401-1403(1998).

14. Guben-Veyl, Methods of organic chemistry,V.2, M., Khimiya, 1967, 1032 p.

15. Stradyn J.P., Polarography of organic nitrocompounds, Riga, Acad Latv.SSR, 68(1961).

16. Remick A., Electronic submission in organic chemistry, M., Inostr.liter., 1950, 550p.

17. Tomkins P., E.Gebauer-Henke, Leitner W., Muller Th.E., ACS Catal., 5, 203–209(2015).

18. Kalmykov P.A., Magdalinova N.A., Klyuev M.V., Pet. Chem., 55, 63(2015).

19. G.U.Huizi, X.U.Xiangsheng, C.Aoang, Y.Xinhuan, Chin.J.Cat., 33, 1423-1426(2012).

20. R.J.Bertolacini, Nature, 92, 1179-1180(1961).

UDC 541.128, 547.261, 665.612.3,
662.767, 66.023:088.8, 66.093.673

[1]Aubakirov Y.A., [1]Sassykova L.R., [2]Starikov E.B.

[1]Faculty of Chemistry and Chemical technology, Al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Department of Physical Chemistry, Chalmers University of Technology, Sweden
*E-mail: larissa.rav@mail.ru

Researching effective catalysts on metal blocks for neutralization of exhaust gases of vehicles

Abstract. Catalysts on metal block carriers were prepared and used in laboratory tests and in the conditions of actual operation. Stability of the carrier and the active phase of the catalysts to poisoning by water vapour was examined. Such poisoning of catalysts promoted with Pt is reversible and the catalysts are again activated by heating at T = 300°C under a stream of dry air for 4 h. The experiments by defining of possibility of interaction of NO with the soot supported on the block catalysts was investigated. Full-size catalyst samples were tested at the diesel engine – generator of Kama Automobile Plant truck running on diesel fuel and at the stand on the basis of diesel generator capacity of 4 Kv. Application the catalytic neutralizer of the fulfilled gases resulted in decrease in harmful emissions in comparison with the engine without neutralizer: in case of Kama Automobile Plant on a mode of the minimal idling (800 rev./min.) efficiency of neutralizer has made on NO_x-59.0 %, C_xH_y-86.0%, CO-99.0%; in case of a stand a degree of cleaning from CO was 100%, hydrocarbons – 99.0%, nitrogen oxides – 61.9%.

Key words: catalyst, exhaust gases, metal block carriers.

Introduction

Protection of the environment from industrial and transport pollution poses to humanity demands to improve the synthesis methods of neutralizing catalyst and purification of gas emissions from harmful [1-4]. The most effective means of purifying of the exhaust gases of internal combustion engines of automobiles is a catalytic method [5, 6]. It is possible to use a metal wire, a steel foil, a grid from stainless steel or from bronze, the granulouse carrier – in the form of balls or extrudates, the ceramic carrier from a spodumen, oxide or zirconium nitride, etc. as a primary catalyst carrier for neutralization of exhaust gases of motor transport and the industry. However not all of them are capable to maintain the loadings received in use in actual practice operation (on the car and in the production conditions), and also to correspond requirements imposed to catalytic converters (thermal stability, mechanical strength, gas-dynamic resistance, stable catalytic activity throughout the progressive time of operation). The monolithic ceramic or metallic blocks are the most suitable carriers of the catalysts used for the solution of environmental issues thanks to their principal specifications: to a developed surface, a low pressure drop, high thermal and mechanical stability, ease of an arrangement in the reactor and a possibility of their use as a substrate for a secondary catalyst carrier [7-9]. The development of compositions and methods of catalysts preparation with low content of platinum group metals for complex purification of exhaust gases of motor transport becomes more relevant in the world due to the deteriorating state of the atmosphere, especially in the industrial cities, and the tightening of environmental standards [10, 11].

The work purpose – synthesis of efficient and stable catalysts of neutralization of harmful emissions of the industry and exhaust gases of motor transport on metal block carriers and their testing in the laboratory and in actual application on the diesel engine of Kama Automobile Plant (KamAP) and at the stand on the basis of diesel generator.

Materials and Methods

Preparation of laboratory and full-size examples is carried out by earlier developed technique on the experienced and experimental base where there is a necessary equipment [12]. The primary carrier of catalysts is made of heat-resistant steel foil (corrugated and rolled into a block) brands H23Yu5, H15Yu5 containing about 5% Al, of 50.0 microns thick. The ends of a foil are cut off and welded on the cylindrical block by means of contact welding. The washcoat is a suspension of aluminum salt or of aluminium salt with additives, its quantitative and qualitative compositions are controlled on pH, viscosity and the content of a solid phase. Metal block carriers are completely immersed in suspension till the ending of appearing of air bubbles, then are placed in the centrifuge where surplus of suspension is removed. The promoting metals are applied on the prepared carrier by impregnation from aqueous solutions of salts: cobalt, manganese, nickel, etc. (on a moisture capacity). The active phase based on compounds Mn, Ni, Co, Fe, prepared from acetates and formiates and the platinum group metals converted into colloidal state was used. In fig.1 full-size samples of catalysts for test in the conditions of actual operation are shown.

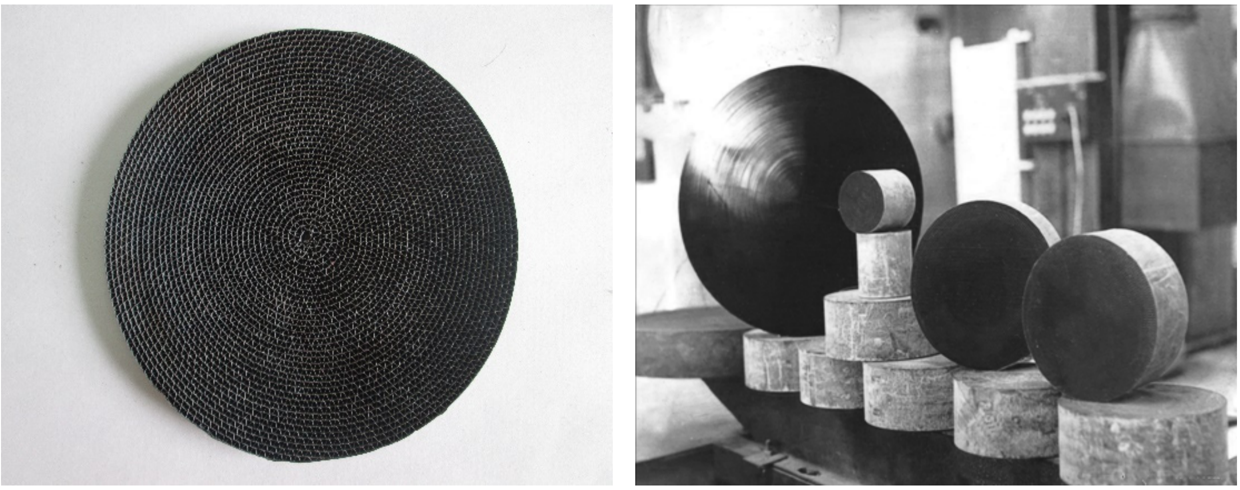


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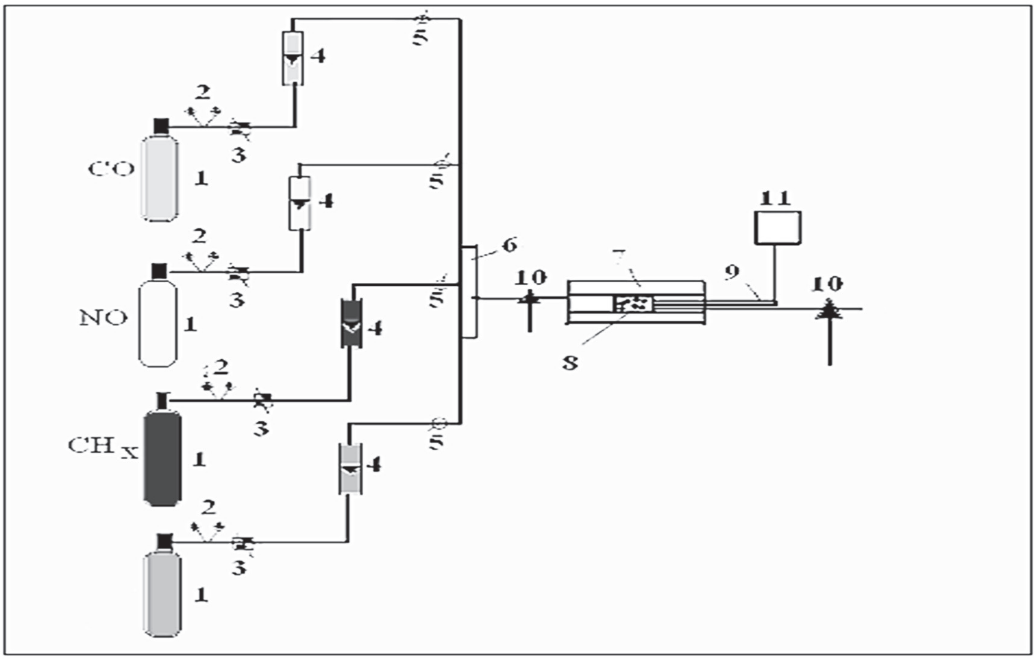
Figure 1 – The full-size samples of catalysts on the metal block: a-cross-section, b-a general view



1 – A gas bag; 2 – Manometer; 3 – Ventile of thin regulation; 4 – Rotameter; 5 – Crane; 6 – Mixer; 7 – Heating system; 8 – Catalyst; 9 – Thermocouple; 10 – Selection of tests before and after the catalyst; 11 – IRT

Figure 2 – Scheme of the flowing installation.

For test of catalysts in the laboratory the flowing catalytic installation with the tubular reactor of integrated type (fig.2) was used.

The gas mixture was prepared by feeding of hydrocarbons from container and the compressed air from the line into the mixer. The hydrocarbon content of the mixture was about 0.5%. The oxygen concentration was varied from 2.0 to 10.0 vol.%. The gas mixture was analyzed by GLC and OPTOGAZ gas analyzer before and after the reaction. Crystal 2000M and Chrom 3700 chromatographs with the flame ionization detector are used. Duration of analysis-20-30 min. Previously the catalyst was calcinated at 500°C within 4 h. on air in the muffle furnace. The activity of the catalysts was determined at temperatures of 150-500°C. When designing the optimal compositions and methods of preparation of colloid metals were varied its dispersion, the active metals content, their relative proportions and a temperature of the preliminary heat treatment.

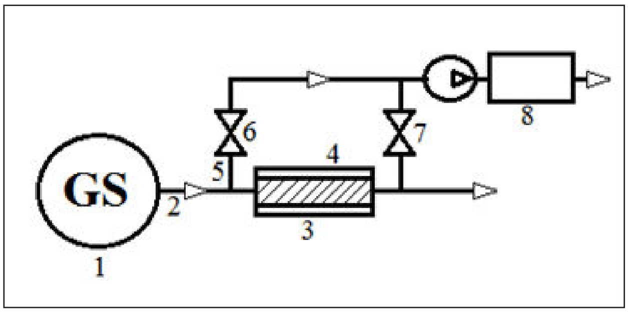
Characteristic of activity of the catalyst was the degree of conversion (α) of initial reagent (hydrocarbon, carbon monoxide, nitric oxide), defined by the formula:

$$\alpha = C\ init.\text{-}Cfin.\cdot Cinit.\cdot 100\ \%,$$

where $C_{init.}$ and $C_{fin.}$ – are the initial and final concentrations of a reagent in volume of a test.

For test the full-size samples of catalysts a stand on the basis of diesel generator (fig.3) was made [13]. As the loading device the rheostats served. Tests before and after the catalyst were selected on all operating modes of the diesel engine (from idle motion up to 4 Kv) directly from an exhaust branch pipe by a gas analyzer «OPTOGAS-500.3». Researches were carried out in a range of loadings from 0 up to 4.0 Kv. The temperature in a zone of work of the catalyst was measured by chromele-alumele thermocouple.

Also as the object of test was served a diesel engine – generator of Kama Automobile Plant (KamAP) of model 820.52-260- is completed with pistons of model 820.52-1004015-40 CB with the chamber of combustion in diameter of 80.0 mm, depth of 25.0 mm, cylinders heads of model 7406.1003040, turbo kompressors «Schweitzer» S2B/7624TAE with cases of turbines with A/R=1.0 and the complete set of the catalytic neutralizers which were elaborated by authors of this article. The neutralizer consists of 2 block catalysts on the metal carrier in diameter of 220 mm and height 90.0 mm everyone with the honey comb structure of channels. 0.1 weight. % Pt was used as an active phase. Neutralizer was tested on the engine working on the diesel fuel with the characteristics: cetane number, not less – 49, density at 150°C-820-860, concentration of sulfur, not more than-500 ppm. In system of greasing oil «Lukoil Super» SAE15W40, APICF4 was used. As cooling liquid (freezing) water was applied. Definition of concentration of gaseous harmful emissions in the waste gases, including, nitrogen oxides (NO_x), total hydrocarbons (C_xH_y), carbon oxide (CO), was made by a multicomponent gas analyzer «Autotest – 02.03» of the I-st class. Calculation of specific emissions was carried out in view of power consumption at n=1500 rev/min., power consumption – 3.5 kVA, at n=2,200 rev./min, power consumption – 11.2 kVA. Definition of harmful emissions in the exhaust gases on a minimal idle motion was carried out on the preliminary preheated engine in a mode of rated power.



1 – Diesel generator; 2 – exhaust pipe; 3 – catalytic reactor; 4 – catalyst sample; 5 – gas operated probe; 6, 7 – sampling valves before and after the catalyst; 8 – a gas analyzer.

Figure 3 – A test bench (a stand) on the basis of the diesel-generator

Results and Discussion

The test of the laboratory samples of catalysts

The laboratory test were carried out at the mounted laboratory installation. In exhaust gases of motor transport in the course of combustion of fuel the water vapor is formed therefore in this work the researches of stability of the synthesized catalysts to catalytic poison – water vapor which content was 5% in nitrogen were carried out. Adding to the gas mixture of water vapor was carried out by a saturator, it kept at 50°C in a thermostat and calibrated on changing of volume of water at a constant feed rate of nitrogen giving through it. By adding water vapor, some catalysts sharply reduced its activity (Table 1).

In the presence of water vapor at 400°C the activity of the tested catalysts became unstable. For example, at 300°C on the catalysts which were not promoted Pt, nitrogen oxide degree of conversion was reduced to zero, while in the absence of water vapor on these catalysts degree of nitrogen oxide conversion reached 30.0-38.0%. Co-Mn and Ni-Mn-catalysts promoted with 0.1% Pt, retained a high activity, which is not reduced during the 50 h. experiment. The experimental results show that poisoning by water vapor is reversibly and catalysts are activated by heating again at a temperature of 300^{o}C in a stream of dry air for 4 h.

Table 1 – Change of activity of the catalysts processed by water vapor and in absence of water vapor in reaction of nitrogen oxide reduction by a propylene at 5% the content of oxygen

Samples of catalysts	The degree of conversion of NO at different temperatures		
	300^{o}C	400^{o}C	500^{o}C
Ni+Mn+Pt	70.0	80.0	73.0
Ni+Mn+Pt (H_2O)	64.0	71.0	61.0
Co+Mn+Pt	65.0	77.0	74.0
Co+Mn+Pt (H_2O)	54.0	58.0	49.0
Fe+Mn+Pt	38.0	52.0	50.0
Fe+Mn+Pt (H_2O)	30.0	47.0	40.0
Co+Mn	32.0	54.0	47.0
Co+Mn (H_2O)	28.0	29.0	22.0
Ni+Mn	38.0	53.0	50.0
Ni+Mn (H_2O)	32.0	51.0	48.0
Fe+Mn	25.0	42.0	40.0
Fe+Mn (H_2O)	0.0	10.0	4.0

It is known that soot and nitrogen oxides $(NO)_x$ are combustion by-products on air of any carboniferous material polluting the atmosphere, are capable to chemical interaction with each other and the environment [6, 9, 15].

Main products of interaction of soot with NO_2 is CO_2 and NO:

$$C + 2\ NO_2 = CO_2 + 2\ NO$$

In this regard were made the experiments in which the possibility of interaction of NO with the soot supported on the block catalysts was investigated. Soot was selected by means of the filter from exhaust gases of buses and supported on the surface of the block from alcohol suspension followed by drying at 40°C. The catalyst with the put on it soot was tested in the medium NO + Ar and NO + O2 + Ar at temperatures 100 – 500°C and volume velocity (Vv) = 25•103 h-1.

IR data confirm the presence of residual of organic and sulfur-containing compounds in soot of the exhaust gases. At interaction of the catalyst with gas mixture NO+Ar with temperature increase from 100 to 500°C was observed a decrease of content of NO from 195 ppm to 113 ppm probably due to interaction of NO with the supported soot. In reaction products there were also a trace amount of carbon monoxide formed during the oxidation of carbon in the presence of insufficient oxygen. When passing the gas mixture containing NO+O_2+Ar through the carboniferous metal block with temperature increase from 100 to 300°C excess of amount of NO over initial (from 260 ppm of an initial mixture to 293 ppm at 100-300°C) was observed, and then with increase of temperature from 300 to 500°C – a decrease in content of NO took place. The observed excess, apparently due to the fact that the formed nitrogen dioxide (NO_2) in admixture with NO by passing through the catalyst and soot at 100 – 300°C is first reduced to NO, resulting is observed excess NO content over the initial amount but with further increase in temperature from 300 to 500°C there is the reduction to N_2.

The tests of full-size samples of catalysts

Results of tests of the diesel engine of KamAP (Table 2) equipped with the catalytic neutralizer of fulfilled gases which was elaborated by the authors of this article, show, that application the catalytic neutralizer of the fulfilled gases resulted in decrease in harmful emissions in comparison with the engine without neutralizer: on NO_x-33.0 %, C_xH_y -82.0 %, CO-98.0 % (2,200 rev./min.). On a mode of the minimal idling (800 rev./min.) efficiency of neutralizer has made on NO_x-59.0 %, C_xH_y-86.0 %, CO-99.0 %.

Table 2 – Values of specific toxic emissions of engine KamAP without neutralizer and with neutralizer of the fulfilled gases

Number of revolutions, rev./min.	CO, ppm			C_xH_y, ppm			NO_x, ppm		
	without neutralizer	with neutralizer	degree of cleaning, %	with out neutralizer	with neutralizer	degree of cleaning, %	without neutralizer	with neutra lizer	degree of cleaning, %
800	123	1.3	99	3182	445	86	128	75.25	59
2,200	733	8	98	3728	671	82	118	79.06	33

Full-size samples of catalysts on the basis of 0.1% of Pt were tested at the stand on the basis of the diesel generator of 4.0 Kv. The block catalysts on the metal carrier were manufactured with the following dimensions: diameter – 30 mm, height – 90 mm, volume – 63.4 mm^3. According to the accepted methods [2,10, 14] the tests on the toxicity of vehicles, including a catalytic converter envisages the beginning of measurement from a cold condition of the car. In this case for a phase of warming up 80-90% of harmful emissions are allocated. For this reason, the catalysts must have the high degree of conversion at low temperatures. In the case of the developed catalysts in this work the full-sized sample at the tests on the mode «idling» showed efficiency of cleaning from CO even at 20°C – 90.6%, the degree of purification from nitrogen oxides in these conditions was low (3.0%). Effectiveness of the catalyst at 267°C is already more noticeable: degree of transformation of CO was equal to 99.6%, hydrocarbons – 80.7%, nitrogen oxides – 44.4%. Results of experiment show that cleaning from CO begins already at low temperatures while for cleaning from hydrocarbons and nitrogen oxides higher temperatures are required. With increase of engine capacity up to 3-4 Kv the high activity on NO_x (61.1-61.9%) is observed (Table 3). At the power consumption of 4 Kv at 427°C degree of cleaning of CO was amounted to 100%, hydrocarbons – 99.0%, nitrogen oxides – 61.9%. Heat stability of full-size samples of catalysts was defined by determination of activity of the initial catalyst on the diesel generator working under loading in 3.0 Kv with the subsequent fractional calcination of neutralizers at 600°C with an interval of 5 h. in a muffle furnace. During 100-h test the high heat stability of catalysts is revealed.

Table 3 – The test data of the full-size block catalyst on the basis of 0.1% of Pt in the bifunctional mode at the various loadings of the engine operation

The consumed capacity, Kv	Temperature of the exhaust gases, °C	Degree of the exhaust gases cleaning, %		
		CO	CH_x	NO_x
Idle motion	20	90.6	21.5	3.0
2	267	99.6	80.7	44.4
3	308	100	95.6	61.1
4	427	100	99.0	61.9

Conclusions

Thus, the catalysts supported on metal block carriers for cleaning of exhaust gases of motor transport were developed. Laboratory tests and tests in real operating conditions of vehicles of motor transport were carried out. Stability of the carrier and the active phase of the catalysts supported on metal blocks to poisoning by water vapor was researched. At poisoning by water vapor of catalysts promoted with Pt they are activated again under heating at T=300^{o}C under a stream of dry air for 4 h. In the presence of steams degree of transformation of nitrogen oxide on the catalysts which are not promoted by Pt decreases to zero whereas the catalysts promoted by 0.1% of Pt (wt.) kept higher activity during 50 h of an experiment. Full-size catalyst samples were tested on truck of KamAP running on diesel fuel and at the stand on the basis of diesel generator capacity of 4 Kv. Thanks to the use the catalytic converter exhaust emissions are reduced (in comparison with the engine without catalyst), for example, in case of KamAP – on NO_x-33.0%, C_xH_y-82%, CO-98% (at 2,200 rev/min). At a minimum idling mode (800 rev/min.) the efficiency of the neutralizer was on: NO_x-59%, C_xH_y-86%, CO-99%. As for the stand on the basis of diesel generator at the power consumption of 4 Kv at 427°C degree of cleaning from CO was 100%, hydrocarbons – 99.0%, nitrogen oxides – 61.9%.

References

1. O'Neill B.C., J. Science, 2002; 5575 : 1971. DOI 10.1126/science.1071238.

2. Kołodziej A., Łojewska J. chapter in: New and Future Developments in Catalysis, 2013; pp. 257. DOI 10.1016/b978-0-444-53870-3.00010-1.

3. Lee B.Y., Inoue Y., Yasimori I., Bull. Chem. Soc. Jpn., 1981; 54: 3711. DOI 10.1246/bcsj.54.3711.

4. McGrath M. Four major cities move to ban diesel vehicles by 2025. http://www.bbc.com/news/science-environment-38170794.

5. Lucena P., Vadillo J.M., Joserna J.J. Compositional mapping of poisoning elements in automobile three-way catalytic converters by using laser-induced breakdown spectrometry, J.Appl.Spectrosc., 2001, V.55.-N3.-P.267-272.

6. Thomson J., Anstice P.C.J., Price R.D., chapter in : Catalysis in Application, 2003; pp 63-69. DOI 10.1039/9781847550347-00063. ISBN: 978-0-85404-608-9.

7. Rauch S., Harold H.F., Barbante C., Masanori O., Morrison G.M., Peucker-Ehrenbrink B., Wass U. , J. Environ. Sci. Technol., 2005; 1, : 8156. DOI 10.1021/es050784m.

8. Sassykova L.R., Ussenov A., Massenova A.T., Gil'mundinov Sh.A., Rakhmetova K.S., Bunin V.N., Basheva Zh.T. and Kalykberdiyev M.K.. Creation of high effective nanostructured catalysts on base of Pt, Pd for neutralization of motor transport exhaust, Int. J. Chem. Sci., 14(1), 2016, 206-212.

9. Kramer M., Schmidt T., Stowe K., Maier W.F., J. Applied Catalysis A: General, 2006; 302 : 257–263. DOI: http://dx.doi.org/10.1016/j.

10. Val'dberg A.Yu., Kosogorova T.O., Tsedilin A.N., Pokrovskii D.D., Yakimychev A.A., Chemical and Petroleum Engineering, 2007; 5-6: 287.DOI 10.1007/s10556-007-0051-7.

11. Burke N.R., Trimm P.L., Howe R. Cold start vehicle emission control using trapping and catalyst technology. Proceedings. Pt.B. 12thInt.Congress on Catalysis, Granada, 2000, July 9-14: Elsevier 2000, P.1451-1456.

12. Sassykova L.R., Gil'mundinov Sh., Nalibayeva A.&Bogdanova I.O.Catalytic systems on metal block carriers for neutralization of exhaust gases of motor transport, Rev. Roum.Chim, 2017, 62(2) – in print.

13. Sassykova L.R., Massenova A.T., Gilmundinov Sh.A., Bunin V.N., Rakhmetova K.S.,

14. Gilmundinov Sh.A., Sassykova L.R., Nalibayeva A.M., Proceeding of International Symposium on Metastable and Nano Materials, ISMANAM , Corfu, Greece, 2007: 168.

15. Sassykova L.R., Massenova A.T., Gilmundinov Sh.A., Bunin V.N., Rakhmetova K.S., The Effective Catalytic Systems on the Base of Colloid Platinum Metals for Oxidation of CO, Methane and Decomposition of Nitrogen Oxides, DGMK, Tagungsbericht, 3, 181-188 (2014)

16. Burdeinaya T.N., Matyshak V.A., Tret'yakov V.F., Glebov L.S., Zakirova A.G., Carvajal G, M.A., Villanueva A.M.E., J.Applied Catalysis B: Environmental , 2007, 1-4:128. DOI 10.1016/j.apcatb.2005.11.027.

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[1*]Seitimova G.A., [1]Alzhanbayeva A.M., [1]Burasheva G.Sh.,
[1]Yeskaliyeva B.K., [2]Choudhary M.I.

[1]Al-Farabi Kazakh National University, Faculty of Chemistry and Chemical Technology, Almaty, Kazakhstan
[2]H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences,
University of Karachi, Karachi-75270, Pakistan
*E-mail: sitigulnaz@mail.ru

Phytochemical study of *Kochia prostrata*

Abstract. In this study, complete comparative analysis of the component composition of plant of the genus *Kochia prostrata* were conducted for the first time. The data for quantitative determination of biologically active compounds and the study of amino, fatty and phenolic acids composition of plant of the genus *Kochia prostrata* family *Chenopodiaceae* were presented. 20 amino acids, 8 known fatty acids and 4 phenolic acids have been identified in the studied objects; their quantitative composition has been established. The major amino acids were glutamic acid (23.46%), aspartic acid (1.88%) and alanine (5.82%), while the major fatty acids were oleic (64.9%) and linoleic (22.0%), palmetic (5.3%) acids. Moreover, a sufficient amount of protocatechuic, vanillic, isovanillic and p-coumaric acids have been found.

Key words: *Kochia prostrata*, amino acids, fatty acids, phenolic acids.

Introduction

Kochia prostrata Schard belongs to the family *Chenopodiaceae*, a family comprising of probably about 100 genera and 1400 species. It mostly comprises perennial herbs or shrubs mostly xerophytic or halophytic.

The genus *Kochia* is similar to *Bassia* but flowers are not hidden in hairs. *Kochia* is found in arid areas, deserts, and costal and saline habitats of Central Asia, North and South Africa, Europe, Russia. There are 10 species of the plant genus *Kochia*; 9 of these are indigenous to Kazakhstan [1,2].

The genus *Kochia* has been used in traditional Chinese medicine to treat diuresis and skin diseases. This is also used for making brooms. It has also been used for the treatment of pain in micturition, rubella, eczema and cutaneous pruritus in traditional Chinese preparations. The cardiotonic and diuretic activities of the plant have already been reported [3-6].

One of the important tasks of modern pharmaceutical science is the search for domestic sources of biologically active substances from plants to create on their basis of drugs of different pharmacological direction. These plants include the family *Chenopodiaceae.*

Object of current study is the aerial parts of *Kochia prostrata* Schard. The aim of our study was to investigate amino and fatty acids composition of aerial parts of *Kochia prostrata* Schard by gas chromatography. The isolation and identification of amino, fatty and phenolic acids composition from the aerial part of *Kochia prostrata* was the first ever to be reported from this plant.

Materials and methods

Analysis of amino acids. To determine the amino acid in 1 g of substance, it hydrolyzed in 5 ml of six normal (N) hydrochloric acid at 105ºC for 24 hours in vials sealed under a stream of argon. The hydrolyzate evaporated to dryness three times on a rotary evaporator at a temperature of 40-50ºC and a pressure of one atmosphere. The resulting precipitate dissolved in 5 ml of sulfosalicylic acid. After centrifugation (1500 rev / min) for 5 minutes, the supernatant passed through a column of ion exchange resin Dowex 50, H-8, and 200-400 mesh, at a rate of 1 drop per second. Thereafter, the resin washed with 1-2 mL of deionized water and 2 ml of 0.5 N acetic acid; Resin washed to neutral pH deionized water.

Amino elution from the column passed there through 3 ml six N NH4OH solution at 2 drops per second. The eluate collected in a round bottom flask with deionized water used for washing the column to neutral pH. The flask contents evaporated to dryness on a rotary evaporator under a pressure of one atm. and a temperature of 40-50ºC.

After addition to the flask of 1 drop of a freshly prepared 1.5% solution of SnCl2, 1 drop of 2,2-dimethoxypropane and 1-2 ml of saturated hydrochloric acid-propanol, heated to 1100S, maintaining this temperature for 20 minutes and then the contents of the flask was evaporated again on a rotary evaporator.

In the next step in the flask, 1 ml of freshly prepared acylating reagent (1 volume of acetic anhydride, triethylamine, 2 volumes and 5 volumes of acetone) and heated at 600 ° C for 1.5-2 min. The sample re-evaporated to dryness on a rotary evaporator and added to the flask 2 mL of ethyl acetate and 1 ml of a saturated solution of NaCl. The flask contents mixed thoroughly and as two layers of liquids clearly formed – take the upper (ethyl acetate) for gas chromatography analysis, which conducted on gas-liquid chromatography «Carlo-Erba-4200» (Italy-USA).

The conditions of chromatography:

- Temperature of the flame ionization detector – 300^{0}C
- Evaporator temperature – 250^{0}C
- The initial temperature of the column – 110^{0}C
- The final column temperature – 250^{0}C
- Column temperature programming speed: from 110^{0}C to 185^{0}C – 60^{0}C in the minutes; from 185^{0}C to 250^{0}C – 32^{0}C min. When the temperature reaches 250^{0}C column it should remain until the full release of all amino acids.

For the separation of amino acids a stainless steel column, size 400 mm 3 filled with a polar mixture of 0.31% Carbowax® 20M, 0.28% Silar® 5CP, and 0.06% on the *Lexan*® on *Chromosorb*® *W AW*, *120-140* mesh were used . Counting carried out on the chromatogram external standard of company Altex [7,8].

Analysis of fatty acids. Fatty acids were analyzed as methyl esters in in a Chrom-42 chromatograph using Cellite 545 adsorbent on WAW Chromosorb, He carrier gas, flame ionization detector, carrier gas flow rate 30 ml/min, detector temperature 188 ˚C and oven temperature 230 ˚C, Acids were methylated by NaOMe at 60–70°C [9].

Analysis of phenolic acids. The ethyl acetate extract of Kochia *prostrata* studied in phenolic content by paper chromatography in solvent systems:

I – benzene – acetic acid – water (6: 7: 3);

II – Sodium formate – formic acid – water (10: 1: 200) [10].

Plant material

The aerial parts of the plant were collected on September, 2015 from South Kazakhstan region of Republic of Kazakhstan. The plant material was taxonomically identified, authenticated by professors of botany at Institute of Botany and Phytointroduction, Almaty. The aerial parts of the plant were air dried, powdered to particle size in the range 6.0-8.0 mm, according to regulatory documents, sieved and weighed.

Results and discussion

The moisture content, total ash, qualitative and quantitative contents of biologically active constituents of *Kochia prostrata* were determined according to methods reported in the State Pharmacopoeia XI edition techniques [11].

The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, environment etc. The constituents of the ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant and is useful in determining authenticity and purity of sample and these values are important qualitative standards. The ash content is a measure of the total amount of minerals present within a plant, whereas the mineral contents are a measure of the amount of specific inorganic components present within it.

Moisture content is an important factor because appearance and stability of dried plants depends on the amount of water they contain and the propensity of microorganisms to grow depends on their water content.

The data quantitative determination of are shown in Table 1. From Table 1 it should be noted the predominance of saponins, tannins and flavonoids in *Kochia prostrata.*

The study revealed that *Kochia prostrata* contains 20 amino acids and 8 known fatty acids but differ in their quantitative contents (Tables 2 and 3). The major amino acids were glutamic acid (23.46%), aspartic acid (1.88%) and alanine (5.82%), while the major fatty acids were oleic (64.9%) and linoleic (22.0%), palmetic (5.3%) acids.

Eight phenolic acids were identified in *Kochia prostrata*. Of these, protocatechuic, vanillic, isovanillic and p-coumaric acids were determined by using authentic phenolic acid samples. These acids are identified for the first time from the plant genus *Kochia prostrata*.

Thus, the study of amino, fatty and phenolic acids composition of plant the genus *Kochia prostrata* is of great scientific and practical interest.

The results based for a deeper study of plants as a kind of source natural biologically active compounds.

Table 1 – Qualitative and quantitative screening of the powdered aerial parts of *Kochia prostrata*

Plant	Contents, %								
	Moisture content	Ash	Extractives substance 70% – aqueous alcohol	Flavonoids	Coumarins	Saponins	Alkaloids	Tannins	Organic acids
Kochia prostrata	7.12	8.62	37.40	1.8	0.004	2.39	0.31	2.17	0.22

Table 2 – Composition of the saturated and unsaturated carboxylic acids (fatty acids) in *Kochia prostrata, %*

Fatty acids	Relative percentage %	Fatty acids	Relative percentage %
$C_{14:0}$	1,5	$C_{18:0}$	2,8
$C_{15:0}$	2,1	$C_{18:1}$	64,9
$C_{16:0}$	5,3	$C_{18:2}$	22,0
$C_{16:1}$	1,0	$C_{18:3}$	0,4

Table 3 – Composition of the amino acids contents in *Kochia prostrata, %*

Amino acids	Relative percentage %	Amino acids	Relative percentage %
Tryptophan	0.52	Serine	2.00
Lysine	2.70	Methionine	0.48
Arginine	3.32	Proline	3.35
Ornithine	0.01	Threonine	1.41
Histidine	1.65	Glutamic acid	23.46
Tyrosine	2.49	Valine	1.72
Phenyl alanine	2.31	Isoleucine	3.03
Oxyproline	0.01	Leucine	3.20
Cysteine	0.15	Glycine	2.11
Aspartic acid	1.88	Alanine	5.82

Conclusions

- A phytochemical study of *Kochia prostrata* was carried out. The qualitative composition of amino, fatty and phenolic acids of the plant genus *Kochia prostrata* has been studied by using method of paper chromatography (PC) and *thin-layer chromatography* (TLC), their quantitative composition has been identified by gas chromatography.

– A comparative analysis of plants of the genus *Kochia prostrata* has been carried out. The major amino acids were glutamic acid (23.46%), aspartic acid (1.88%) and alanine (5.82%), while the major fatty acids were oleic (64.9%) and linoleic (22.0%), palmetic (5.3%) acids.

– By method of paper chromatography (PC) with authentic phenolic acid samples: protocatechuic, vanillic, isovanillic and p-coumaric acids have been determined from the plant genus *Kochia prostrata*.

References

1. Flora Kazakhstana. – A.: AN Kaz SSR, 1960. – T.3. – S.231-234

2. Flora SSSR pod red. Komorova. – M-L., 1936. – T.6. – S.127-134

3. G.P. Phondke. The wealth of India. – Council of Scientific and Industrial Research: New Delhi, 1959. – Vol 5. – P. 323

4. Jiangsu New Medical College, Dictionary of Chinese crude drugs, Shanghai Scientific Technologic Press, Shanghai, 1979. – P. 816

5. K. Z. Zhao, F. Z. Li. Chinese Halophyte Resources, 1st Edition, Science Press, Beijing, 1999. – P. 146

6. Ali S.I., Qaiser M. Flora of Pakistan, No. 204. Chenopodiaceae. – Department of Botany, University of Karachi, 2001. – P.85.

7. Adams R. J. Chromatography. – 1974, 95 (2). – P.188-212.

8. I. M. Skurikhin and V. A. Tutel`yan (eds.), *Handbook of Analytical Methods for Food Product Quality and Safety* [in Russian], Meditsina, Moscow, 1998.

9. Vegetable Oil. *Method for determining fatty-acid composition*. – Izd. Standartov Minsk, – 1997 [in Russian].

10. Seitimova G.A., Eskalieva B.K., Bauyrzhanov K.B., Choudhary I.M., Burasheva G.Sh. Amino-, zhirno-, phenolokislotnyi sostav nekotorykh vidov rasteniy roda Klimakoptera *(Climacoptera)* // Izvestiya nauchno-tekhnicheskogo obshchestva «Kakhak», Almaty, 2013, №1(40), стр.83-88.

11. State pharmacopoeia of the Union of Soviet Socialist Republics: General methods of analysis. – Moscow; Medicine, 1991. – Vol 2. – P. 400.