IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-2, 2019, pp369-376
http://www.bipublication.com

Research Article

Fractal Analysis of Flavonoids in Composition HPLC-Fingerprint Extracts of *Oxycoccus palustris* Pers. *(ERICACEAE)* in Oligotrophic Swamps of Western Siberia

[1, 2]Usmanov I. Yu., [3]Ivanov V. B.,
[4]Abdrakhimova G. S., [5]Shcherbakov A. V.,
[6]Yumagulova E. R. and [7]Aleksandrova V. V.

[1]Doctor of Biological Sciences (Advanced Doctor), Professor of Ecology of the Nizhnevartovsk State University, Lenina St., 56, Nizhnevartovsk, Russia, 628605,
[2]Doctor of Biological Sciences (Advanced Doctor), Professor of Economic and Turizm of the – Ufa State Petroleum Technological University, Cosmonauts St., 1, Ufa, Russia, 450062,
[3]Candidate of Pedagogical Sciences (Ph.D.), Associate Professor of Ecology of the Nizhnevartovsk State University, Lenina St., 56, Nizhnevartovsk, Russia, 628605,
[4]Candidate of Chimical Sciences (Ph.D.), Leading Researcher of the FSBI Ufa Institute of Chemistry STC of the RAS, Davletkildeev Boulevard St., 5/2, Ufa, Russia, 4540098,
[5]Candidate of Biological Sciences (Ph.D.), Leading Researcher of the Research and innovation enterprise Bachincom, Karla Marksa St., 37, Ufa, Russia, 450015,
[6]Candidate of Biological Sciences (Ph.D.), Associate Professor of Ecology of the Nizhnevartovsk State University, Lenina St., 56, Nizhnevartovsk, Russia, 628605,
[7]Candidate of Biological Sciences (Ph.D.), Associate Professor of Ecologyl of the Nizhnevartovsk State University, Lenina St., 56, Nizhnevartovsk, Russia, 628605,

[*]**Corresponding author:** Ivanov V.B., Candidate of Pedagogical Sciences, Nizhnevartovsk State University, Russia, Tel: +79129385128; E-mail: karatazh@mail.ru

[Received: 04/04/2019; Accepted: 01/05/2019; Published: 09/05/2019]

ABSTRACT.

The HPLC – high performance liquid chromatography of *Oxycoccus palustris* Pers. leaf extracts from natural populations in oligotrophic swamps of the Middle Ob area (Western Siberia) was investigated. The samples to carry out the HPLC – high performance liquid chromatography analyses were taken in ecologically equivalent swamp areas. Chromatograms differed in the number of peaks, the time of their release, and the area of particular peaks. To emphasize the individuality of such chromatograms, the term "fingerprint" was taken from criminalistics. The hierarchy constructing procedure from single up to "combined" chromatograms, where peaks from 1, 2, 3, 5, and 9 chromatograms were placed on the common time scale of the peaks release, was carried out for fractal analysis. The number of peaks varied from 21-30 in particular chromatograms up to 76 peaks in a "generalized" united chromatogram. The complex of calculation procedures has shown that all chromatograms obtained from *Oxycoccus palustris* plants are stochastic fractals, where the independent variables are the number, the size (area) and release time of each particular peak. In the presence of the fractal properties of the flavonoid system, it is necessary to analyze the possibility of implementing both neutral and deterministic variants of the synthesis of this or that flavonoid.

Keywords: stochastic fractals, flavonoids, *Oxycoccus palustris*, HPLC – High performance liquid chromatography, oligotrophic swamps, Western Siberia.

INTRODUCTION

In plants from natural ecosystems, combinations of recorded traits or compounds often show the signs of a stochastic process, with weak dependencies on external conditions [1]. The previous results indicate the fractal nature of the formation of morphological and physiological complexes of *Oxycoccus palustris* Pers. plants [2, 3].

Flavonoids are considered the group of polyphenols with numerous physiologically active properties – various antioxidants, antibacterial, photo- and cryo- protectors, pigments involved in photosynthesis, etc. The biological diversity of flavonoids is very large: nowadays, several thousand of them are described and, according to general opinion, it is only a small part of these compounds.

Flavonoids are low-molecular compounds that can be attributed to the plant metabolome [4]. The metabolome is a set of all low-molecular metabolites of a cell, tissue or organism, which is determined by the genome, on the one hand, and, on the other hand, is regulated by adaptive processes under the action of various environmental factors [5-7]. In the practice of chromatographic studies, it looks like chromatograms with a changing number of peaks, the time of their release, as well as the area of particular peaks. To emphasize the individuality of such chromatograms, the term "fingerprint" was taken from criminalistics [4-7].

The total number of flavonoids and other low-molecular compounds in metabolomes is estimated in thousands, so in real conditions, all these compounds are either very difficult, or expensive, or simply impossible to identify [4-5]. Therefore, the dynamics of the general mechanisms of metabolomes formation, in particular, the role of stochastic processes, is under investigation currently. In the most common form, the stochasticity of biological objects is revealed with the help of the fractal analysis apparatus [1, 8].

If the properties of stochasticity are manifested for a wide range of chromatograms of extracts of different plants from different populations, then any chromatograms can be interpreted as stochastic fractals [8]. If the formation of chromatograms of extract in biosystems of different levels – from a single plant through coenopopulations – to the areal in some geographical boundaries forms self-similar structures throughout the selected range, the chromatograms are a fractal system. Fractal systems of any nature show "a unique property that allows on the basis of available (almost always incomplete) information about a part of the object make a statistically correct conclusion about the object as a whole..." [1]

The work objective of this paper is to assess whether it is possible to consider chromatograms of flavonoids and compounds with similar physical and chemical properties from *Oxycoccus palustris* Pers. as fractal objects with stochastic properties.

METHODS AND MATERIALS.

Ecological conditions. *Oxycoccus palustris* Pers. was investigated in natural conditions of oligotrophic swamps. The swamp contour with ecologically homogeneous conditions was chosen for the analyses [9-10]. Natural homogeneity of conditions was determined by a number of parameters: 1) the drainless swamp without water flow from other landscapes, and the movement of water flow within the borders of the swamp is chaotic, influenced by temperature, wind, melting of snow, etc. Water pH = 3-5; 2) raised swamps, by definition, have no supply of nutritional agents, with the exception of the removal of the underground substrate. Removal practically stops with the growth of peat, and the plants on the surface get resources from decaying peat masses. Ash content of soils is within 2-5% [11]; 3) the vegetation of the selected area is homogeneous. Syntaxonomically, the whole investigated area is covered by plants of the class *Oxycocco-Sphagnetea Br.- Bl. et R.Tx., 1946.* Dominant species belong to the *Ericaceae* family. *O. palustris* is about 20-33% of the plant composition [10].

Leaves for analysis were selected in the first ten-day period of July. All plant material was dried

to air-dried basis, crushed to a particle size of no more than 2 mm.

Extraction was carried out with a weighed quantity of 20 mg in stages: with hexane (in three doses with a total volume of the solvent of 100 ml), after evaporation of hexane, the vegetable fiber was extracted with 70% ethanol (in three steps, combining extracts filtered with glass filters). The use of hexane as a preliminary extractant was caused by the necessity to clean the samples from various non-polar organic substances not related to phenolic metabolites. Further on, during the analysis of samples with the HPLC method, these extracts were not analyzed.

HPLC – high performance liquid chromatography of cranberry extracts. High performance liquid chromatography HPLC.

Sample preparation. Preparation of vegetable raw materials was carried out in two stages: to clean the vegetable raw materials from the fat-soluble fraction, hexane was used. Vegetable raw materials were incubated three times with hexane for 15 minutes with continuous shaking, filter through glass filters. Then, the vegetable raw material was incubated for 45 minutes in 70% aqueous solution of ethanol three times, filtering and combining the obtained extracts. The extracts were evaporated in a water bath to obtain a concentrated solution; the solution was dried to obtain a constant dry weight.

Flavonoids analysis. For the analysis, the weighed quantity of the extracted sample was taken, dissolving it in eluent 50:50 water-acetonitrile. Chromatography run was carried out by the HPLC method on a chromatograph Waters "Breeze". The chromatography run of extracts of leaf samples was carried out in the inverse phase mode on a column Luna C18 250×4.6 mm, 5 μm. Standards and substances in the samples were detected at a wavelength of 360 nm (Fig. 1).

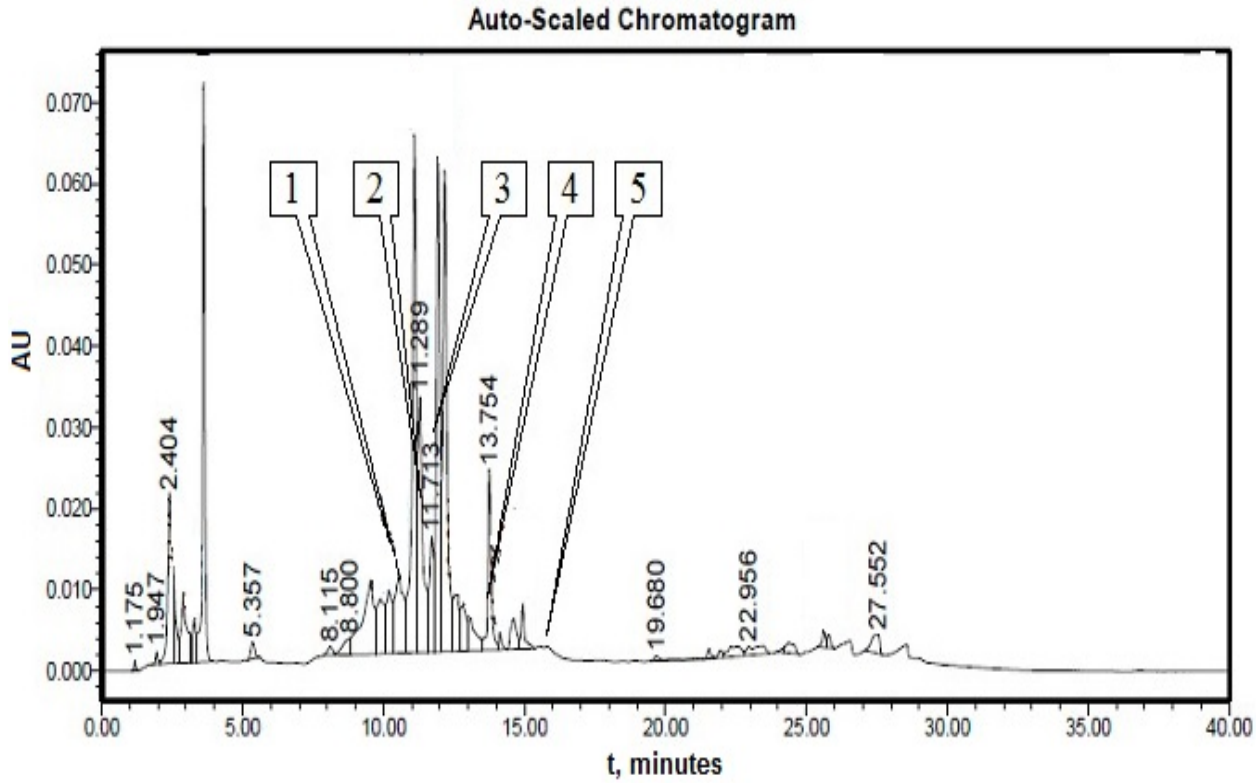

Fig. 1. Chromatogram of the *Oxycoccus palustris* leaves extract. On the "X" axis – time, on the "Y" axis signal intensity.1-5 – peaks identified according to the standards.1 – rutin, peak release time - 10,54 min; 2 – naringin, 10,30 min; 3 – dihydroquercitin, 11,94 min; fizetin – 13,54 min; 5 – quercitin – 15,33 min

Fractal analysis. At the first stage of testing for fractality, sampling and scaling are carried out – these are unformalized procedures, at the stage of which the researcher makes a hypothesis what is a self-similar structure, and what hierarchy these structures can form, preserving the property of self-similarity [1].

Further on, the fractal properties of the selected hierarchy are evaluated – calculation procedures, on the basis of which the hierarchy under investigation can or cannot be attributed to fractal ones (the calculation stages are given below).

The statistical processing was carried out with the help of Excel software package.

RESULTS AND DISCUSSIONS.

Fractal analysis of the chromatograms of *Oxycoccus palustris* extracts.

The main stages of the fractality evaluation are [1]: 1) object selection that is considered as "self-similar"; 2) definition of a hierarchical system within which objects retain self-similarity; 3) the calculation stage at which it is determined whether the hierarchical system by self-similarity has this property or not.

The first stage: Sampling – the initial selective process, during which a kind of unified structure, checked for the properties of self-similarity, is chosen. The chromatogram of the "single" extract of a standard sample of plant material averaged over three analytical runs was taken as an elementary unit of the hierarchy (Fig. 1). A separate chromatogram was characterized in three parameters: 1) the release time of a single peak in a standard solvent system; 2) the number of peaks released in a particular chromatogram; 3) the size of peaks as a reflection of the substance concentration.

The second stage: Scaling – determination of the scale measure and range of levels of the organization (hierarchy), in which all incoming and hierarchy-forming structures have the properties of self-similarity. Scaling allows determining under what conditions and on what scale the studied chromatogram complexes are considered fractal objects.

For this purpose, a hierarchy is created where the separate elements (in this case, chromatograms) are combined. In this investigation, a hierarchy by creating "combined" chromatograms with an increasing number of combined chromatograms was created. Almost all peaks on the chromatograms were released within 40 minutes; therefore, a single interval from the beginning of the peaks to the 40th minute was chosen for the combined chromatograms. In case of increasing the scale (combination), all the peaks of the summed chromatograms were placed on a single *time* axis. In the case when the same (in time of release) peaks were detected on both chromatograms, they were summed at the time, characterized for these peaks. If a peak appeared, which is on one, but not on the other chromatogram, the "new" peak was placed on the corresponding point of the time scale of the peaks. Thus, the total number of separate peaks in the combined chromatogram can increase significantly. The entire population, in this case, is the combination of all peaks detected in all chromatograms. It can be seen from Table 1 that as the chromatograms are combined, the number of peaks increases.

Table 1: Chromatograms hierarchy for the 5 levels of combination

	Plants / grounds								
Levels of combining chromatograms	1	2	3	4	5	6	7	8	9
1. The number of peaks in individual chromatograms	29	27	26	30	25	23	24	26	21
2. Combining chromatograms in pairs	44		39		35			31	
3. Combining chromatograms of three	54			49			33		
4. Combining chromatograms in 2 groups	65					59			
5. Total number of peaks for all plants	76								

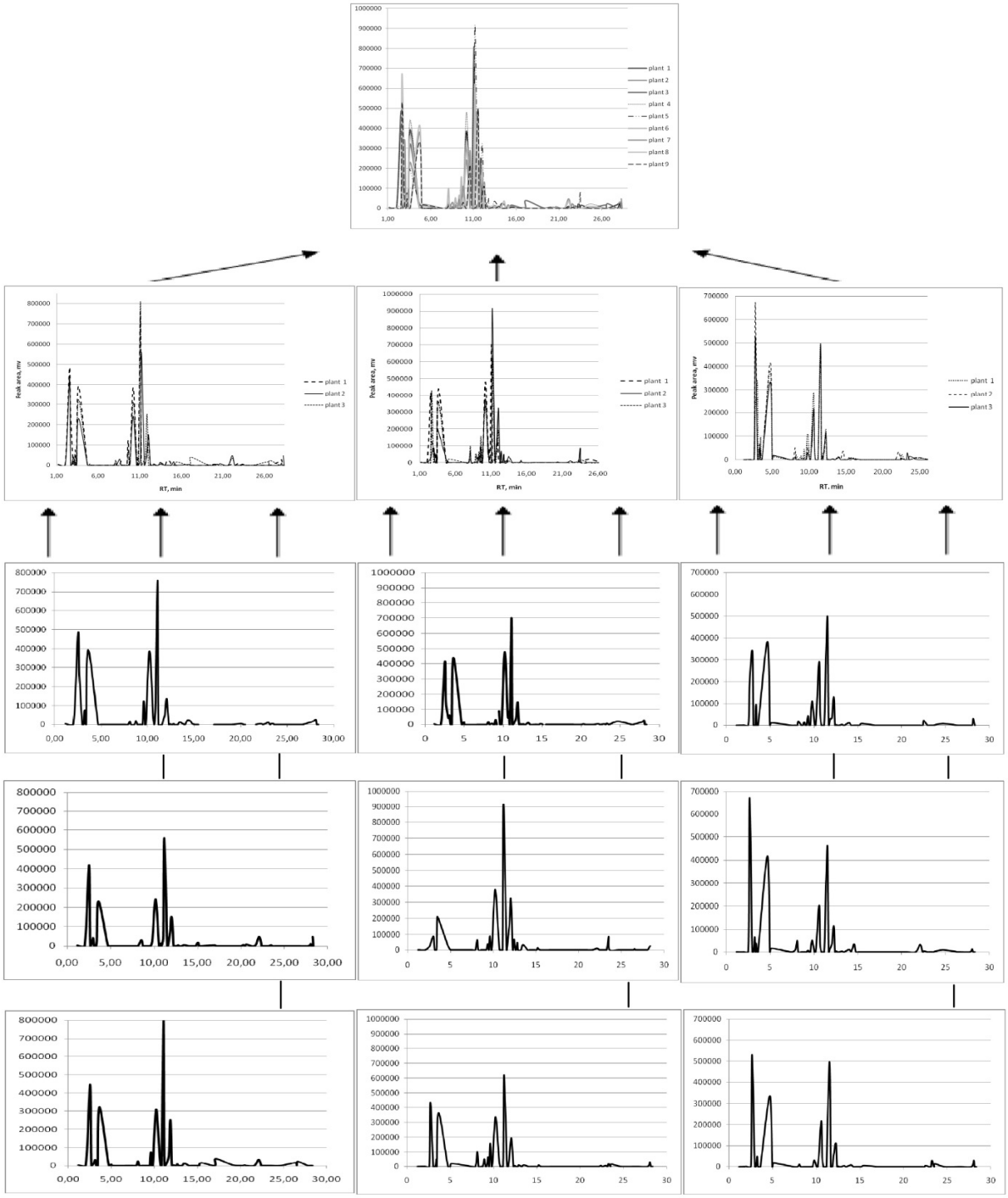

Fig. 2. The procedure of chromatograms combining by hierarchical levels.

The bottom three rows are the chromatograms of the particular extracts; the fourth row is the chromatograms combined in three; the top one is the combination of all chromatograms into the entire population of peaks found in all chromatograms. For perception convenience, the combined chromatograms of 2 and 4 levels are not given. The number of peaks is given in Table 1.

The investigation was carried out on the analogy of the study of the spatial structures: smaller sites are combined into larger ones, i.e., a hierarchy of scales is created [8]. The same method is used in phytosociology: the lists of specific plant species for separate sites are compiled, then these lists are combined on an *increasing* scale for areas covering biogeocenoses and landscapes, and flora of the

corresponding rank [1, 12]. Instead of individual plants, it seems possible to use individual peaks of chromatograms and their release time, and a separate whole chromatogram – as an analog of the phytosociological description on the site. This approach allows forming populations of realized peaks for a single plant if plant samples were taken from different areas on a single plant. Then, "entire" populations are formed for separate coenopopulations as the sum of selected plants, further on, for arbitrary groups of coenopopulations and, finally, for all studied plants of the region.

In this investigation, all chromatograms of plants with the ecologically homogeneous site of the oligotrophic swamp were combined (Fig. 2, Table 1).

The assessment of the fractal nature of the analyzed chromatogram selection. On the basis of the constructed hierarchy, a generalizing matrix was created (Table 1, Fig. 2), on which the following indicators were calculated:

Mq (N) is the generalized statistical sum of the substance diversity indices (p_i) calculated for data with this or that order of distribution (q). The expression recommended by the authors [1] was used to calculate this indicator:

$$M_q(N) = \sum_{i=1}^{(N)} p_i^q, \text{ where}$$

N – total sample size. This indicator ranged from 1 (single sample) to 9 (all investigated sites)

p_i – the index of diversity; that is, the part of each compound in the overall picture.

q – the order of distribution moment. This indicator is in the range recommended by the authors from -3 to 3 and actually sets the range of degrees to which p_i of each element should be raised, which, in its essence, the scaling is. This indicator is necessary to represent the data of the generalizing matrix in a wide range of power levels. It is necessary to confirm the self-similarity of the observed pattern in the given range of these levels [8].

At the first stage, a set of moments Mq was calculated for each value of q. The property of self-similarity is considered to be proved if in the whole range q a significant correlation between logMq from logN is seen [1]. Thus, the property of self-similarity is the first condition for the compliance of the object with the principles of fractal formalism.

However, the same property of self-similarity (according to the authors of the technique) may be characteristic of objects of regular nature, described by relatively simple laws, and in which between the demonstrated indicators, a rigid functional dependence is always seen.

The authors propose to estimate the irreducibility of the observed pattern to simple mathematical models according to the Akaike information criterion (AIC). To assess the nature of the observed pattern of dependence of log Mq indicators from log N, the authors propose to use two models – "linear" and "quadratic."

This criterion for small samples was calculated by the formula:

$$AIC = ln\frac{RSS}{n} + \frac{n+k}{n-k-2},$$

where n – the volume of analyzed data, k – the number of model parameters (for a linear model, it is 3, for a quadratic model – 4), RSS – the sum of squared deviations from the values predicted by the model [1].

The Akaike information criterion (AIC) is calculated for both models over the entire range of values used in the calculations of the order of q distribution moments. In the investigated case, the values of the Akaike criterion were closer to the quadratic models.

The more accurate approximation of the value of the Akaike criterion to the quadratic model than to the linear model in the entire range of orders of distribution moments (q) from -3 to +3 was obtained as a result. This indicates the possibility of the object under investigation to have fractal (multifractal) properties. In other words, the Akaike information criterion indicates that the population investigated in the whole range (q) cannot be described by standard curves, but can be described as a stochastic fractal [8].

The presence of self-similarity in investigated samples was estimated according to the

reliability of the linear dependence of logarithms of the indices N and Mq, and applicability to the observed pattern of the fractal hypothesis – the results of the comparison of the Akaike criterion for the linear and quadratic models. Thus, all chromatograms obtained from *Oxycoccus palustris* plants are stochastic fractals, where the independent variables are the number, the size (area) and release time of each particular peak.

CONCLUSIONS.

Previously, the data on permanent microfluctuations of the chemical composition of soils and dirts in very different environments: from swamps to steppes and steppe solonchaks were obtained [2, 13-16]. Changes in the element concentration of soil solution and swamp waters act as regulatory factors for certain stages of plant metabolism. It is known that the system of flavonoid biosynthesis allows synthesizing a substance passing through metabolic branches and shunts in some cases [17-20]. The synthesis of particular compounds in plants is controlled by a large number of endogenous and exogenous factors, and the combination of stimulating and inhibitory regulators is constantly changing. Due to this fact, the response of plants to the constant fluctuations of the medium, the composition of the flavonoid metabolome is represented as the formation of a stochastic set, the composition of which continuously fluctuates. It is the evidence in favor of the fact that the chromatograms of the *Oxycoccus palustris* Pers. flavonoids are fractal objects with stochastic properties. Taking into account the fluctuating nature of the environmental parameters and the high mobility of the element synthesis of flavonoid metabolome, it is possible to expect two variants of case scenarios: a) the real response of the system of flavonoids synthesis has a neutral nature, i.e., it has mechanisms to choose among several equally likely events, which only partly depend on the specific factors of the external environment; b) the synthesis of this or that flavonoid is strictly determined by the regulators of metabolism operating here and now. In the presence of fractal properties of the flavonoid system, it is possible to realize the implementation of both neutral and deterministic variants of the synthesis of this or that flavonoid. The analysis of this alternative will be the topic of the future investigation.

ACKNOWLEDGEMENTS

The chromatographic analysis was carried out on the equipment of the common use center "Chemistry" of UIC of the RAS.

The investigation was sponsored by the RFBR grants 18-44-860006.

REFERENCES

1. Gelashvili D.B., Iudin D.I., Rosenberg G.S., Yakimov V.N., Solntsev L.A. Fractals and multifractals in bioecology. - Ed. Nizhny Novgorod University, 2013, 370 p. (In Russian).
2. Usmanov I.Yu., Yumagulova E.R., Ovechkina E.S., Ivanov V.B., Shcherbakov A.B., Aleksandrova V.V., Ivanov N.A. Fractal analysis of morpho-physiological parameters of Oxycoccus palustris Pers. in oligotrophic swamps of Western Siberia // Vegetos: An International Journal of Plant Research. 2016. Vol. 29. № 1. pp. 1-3.
3. Serafin A., Pogorzelec M., Bronowicka-Mielniczuk U. Habitat preferences of Oxycoccus palustris Pers. on peatlands in east Poland in the perspective of shaping the conditions of Ecological cultivation of the species // Applied Ecology and Environmental Research. 2018. Vol. 16. Issue 4. pp. 4015-4028.
4. Bundy J.G., Davey M.P., Viant M.R. Environmental metabolomics: a critical review and future perspectives // Metabolomics. 2008. Vol. 5. № 3.
5. Goncharov N.V., Ukolov A.I., Orlova T.I., Migalovskaya E.D., Voitenko N.G. Metabolomics: on the way to the integration of biochemistry, analytical chemistry, computer science // Successes of Modern Biology. 2015. Vol. 135. № 1. pp. 3-17. (In Russian).
6. Smolikova G.N., Shavarda A.L., Alekseichuk I.V., Chantseva V.V., Medvedev S.S. The metabolomic approach to the assessment of

cultivar specificity of Brassica napus L. seeds. // Vavilovskii Zhurnal Genetiki i Selektsii. Vavilov Journal of Genetics and Breeding. 2015. 19 (1). pp. 121-127. (In Russian).

7. Rees Ch.A., Nordick K.V., Franchina F.A., Lewis A.E., Hirsch E.B., Hill J.E. Volatile metabolic diversity of Klebsiella pneumoniae in nutrient-replete conditions // Metabolomics. 2017, Vol. 13. № 18.
8. Mandelbrot B.B. The fractal geometry of nature. - New York: Freeman, 1982. 468 p.
9. Lapshina E.D. Rastitel'nost' bolot yugo-vostoka Zapadnoj Sibiri [Vegetation in the South-East of West Siberian wetlands]. Khanty-Mansiisk, 2010. (In Russian).
10. Ovechkina E.S. Preliminary prodromus allocated for territories Samotlor field of hierarchical units of vegetation // Bulletin of Science and Practice. 2017. № 2. (In Russian).
11. Ivanov V.B., Kalinovskaya E.A., Ivanov N.A., Aleksandrova V.V., Usmanov I.Yu. Geochemical assessment of the impact of sludge pits on high bog soils // In the World of Scientific Discoveries. 2017. Vol. 9. № 2-2. pp. 23-28. (In Russian).
12. Mirkin B, Naumova L. Current state of the main concepts of the science of vegetation. - Gilem-press, 2012, 488 p. (In Russian).
13. Usmanov IYu, Scherbakov AV, Mavletova MV, Yumagulova ER, Ivanov VB, et al. (2016) Pulsing multidimensional environmental niche of plants: expansion In: News bulletin of Samara research centre of Russian Academy of Science 18: 525-529. (In Russian).
14. Usmanov I.Y., Yumagulova E.R., Ivanov V.B., Aleksandrova V.V., Ivanov N.A., Schaichmetova R.I., Scherbakov A.V., Mavletova-Chistuakova M.V. Physiological barriers for adventitious species invasion in oligotroph ecosystems of the Middle Ob area // Vegetos: An International Journal of Plant Research. 2017. Vol. 30. № 4. pp. 81-85.
15. Usmanov I.Yu., Semenova I.N., Shcherbakov A.V., Suyundukov Ya.T. Endemic ecological niches of the South (Bashkir) trans-Urals: multidimensionality and fluctuating modes // Bulletin of the Bashkir State Agrarian University. 2014. № 1 (29). pp. 16-22. (In Russian).
16. Usmanov I.Yu., Scherbakov A.V., Ayupov D.S., Ivanova L.G., Ivanov V.B. Fractal Dynamics in Growth of Seedlints of Zea mays // Vegetos: An International Journal of Plant Research. 2018. Vol. 31. № 2.
17. Ivanov V.B., Usmanov I.Yu., Aleksandrova V.V., Ivanov N.A., Ivanova L.G., Kalinovskaya E.A. Assessment of the impact of sludge pits on high bog soils // In the World of Scientific Discoveries. 2017. Vol. 9. № 1-2. pp. 66-71. (In Russian).
18. Usmanov I.Yu. Ecological and physiological characteristics of some plant species with different types of strategies from communities subjected to anthropogenic influence // Scientific reports of higher education. Biological sciences. 1986. № 10. pp. 66-70. (In Russian).
19. Bashirova RM, Usmanov I.Yu., Lomachenko N.V. Substances of special plant metabolism (Classification. Functions). The manual. - Ufa, 1998. (in Russian).
20. Caston K.J., Choun S.L. Neutrality and niche // Functional ecology. 2005. Vol.19, pp.1-6.