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USE OF CHEMICAL MARKERS IN ANTARTIC ECOSYSTEM STUDIES OF DEMARIA MOUNT

L.V. Chepeleva [1], Z.A. Sizova [1], G.D. Yukhno [1], S.Yu. Utevsky [2], Yu.G. Gamulya [3], A.Yu. Utevsky [2], A.D. Roshal [1]

[1] Institute of Chemistry
[2] Department of Zoology and Animal Ecology
[3] Department of Botany and Plant Ecology V.N. Karazin Kharkiv National University, 61022 Kharkiv, Ukraine, E-mail: autevsk@yandex.ua

Research is devoted to using chemical markers for studying and description of ecosystems. Photosynthetic pigments – carotenoids and chlorophylls, and soil polymers – humic, fulvic acids and their salts, were used as chemical markers. Correlations between concentrations of these markers in samples and parameters of "total nitrogen" and ash were studied. Complex chemical analysis for nine samples collected on a Demaria hill in the altitude range from 47 m to 408 m above see level was carried. It was concluded that in meager antarctic-like ecosystems the content of carotenoids and chlorophylls adequately reflects the quantity of a whole phytomass and of biomass. Total content humic and fulvic acids can be used to estimate quantity of organic substances in soils. Comparison of photosynthetic pigment concentrations with "total nitrogen" parameter allows to separate biogenic phytomass nitrogen and animal waste products.
Keywords: chemical markers, ecosystem, carotenoids, chlorophylls, humic acids

Использование химических маркеров в исследованиях антарктических экосистем горы Демария
Л.В. Чепелева, З.А. Сизова, Г.Д. Юхно, С.Ю. Утевский, Ю.Г. Гамуля, А.Ю. Утевский, А.Д. Рошаль
Реферат. Исследования посвящены проблеме использования химических маркеров для изучения и описания экосистем. В качестве химических маркеров использованы фотосинтетические пигменты – каротиноиды и хлорофиллы, а также почвенные полимеры – гуминовые, фульвиновые кислоты и их соли. Изучена корреляция между концентрациями данных маркеров в пробах и показателями «общего азота» и зольности. Комплексный химический анализ проведен для девяти проб, собранных на экологическом профиле, заложенном на склоне горы Демария Антарктического полуострова между высотами 47 и 408 м над уровнем моря. Показано, что в примитивных экосистемах, подобных антарктическим, содержание каротиноидов и хлорофиллов коррелирует с общим количеством фитомассы и биомассы. Суммарное количество гуминовых и фульвиновых кислот может быть использовано для оценки общего количества органического вещества в почвах. Сопоставление концентрации фотосинтетических пигментов и «общего азота» позволяет определить соотношение фракций биогенного азота фитомассы и продуктов жизнедеятельности фауны.

Використання хімічних маркерів у дослідженнях антарктичних екосистем гори Демарія.
Л.В. Чепелєва, З.О. Сізова, Г.Д. Юхно, С.Ю. Утєвський, Ю.Г. Гамуля, А.Ю. Утєвський, О.Д. Рошаль
Реферат. Дослідження присвячено проблемі використання хімічних маркерів для вивчення та опису екосистем. В якості хімічних маркерів використано фотосинтетичні пігменти – каротиноїди і хлорофіли, а також ґрунтові полімери – гумінові, фульвінові кислоти та їх солі. Вивчено кореляцію між концентраціями даних маркерів у пробах і показниками «загального азоту» та зольністю. Комплексний хімічний аналіз було проведено для дев'яти проб, зібраних на екологічному профілі, закладеному на схилі гори Демарія Антарктичного півострова між висотами 47 і 408 м над рівнем моря. Показано, що у примітивних екосистемах, подібних до антарктичних, вміст каротиноїдів і хлорофілів корелює із загальною кількістю фітомаси і біомаси в цілому. Сумарна кількість гумінових

і фульвінових кислот може бути використана для оцінки кількості органічної речовини у ґрунтах. Співставлення концентрації фотосинтетичних пігментів та «загального азоту» дозволяє встановити співвідношення біогенного азоту фітомаси та продуктів життєдіяльності фауни.

1. Introduction

Ecosystems structure and functioning have cyclic pattern occurred on different levels of vital activity in biological systems. First of all, it is a case of nutrition cycles based on various trophic chains [1, 2]. The lower level cycles are associated with similarity and repeatability of biochemical processes in ecosystems components. Such approach describes the ecosystems evolution as an evolution of different biochemical, particularly metabolic cycles [3]. Geochemical and biogeochemical cycles belong to lower level, which include micro- and macroelements migration [2, 4].

The general quantitative ecosystems assessment is estimated using such characteristics as biodiversity [5] and biomass [6] which demonstrate average result of all cyclic processes activity within ecosystems. Biodiversity and biomass are approximate response which estimation is quite difficult as well as some various additional parameters representative of interspecies relations within some substructures in biological cycles.

The simplest way to characterize condition and quantitative parameters of ecosystems can be carried out using formal chemical markers which are present in the majority of ecosystem components. It could be primary and secondary metabolites or decomposition products. The examples of such important markers are chemical elements, which total concentrations allow to estimate the efficiency, rates and other biogeochemical cycle parameters underlying ecosystems existence. Thus, such marker as “total organic carbon” allows estimating the biomass of any whole ecosystems or of part thereof [6, 7].

Use of chemical markers can substantially simplify ecosystems studies, because analytical procedures are less time- and labour-consuming processes then routine biological fieldworks.

The important issue of marker usage is their adequacy, i.e. correspondence to some quantitative biological parameters in ecosystems under studying. The solution of a problem can be achieved by correlation analysis between marker concentration and some ecosystem parameters, as well as by inter-correlations studies between parameters of new and previously used markers. Objects the most suitable for such studies are simple biosystems, such as Antarctic ecosystems. Low average annual temperature, short vegetation period, low destruction rates of plant residues and fauna waste products result in lack of mature soils, and consequently relatively low biodiversity and biomass within Antarctic ecosystems.

The use of some chemical markers in Antarctic ecosystems studies was earlier discussed in [8], where the interplay between an important chemical marker “total nitrogen” and biomass parameter has been analyzed. As the storm activity is low in the Antarctic region, quantity of biotic nitrogen (due to nitrogen monooxide formation under atmospheric electrical discharge) seems to be insignificant. It was shown in [8], samples collected at altitudes higher 250 m above sea level contained low concentrations of “total nitrogen”, however at lower altitudes the “total nitrogen” concentration is substantially increased.

Ash parameter (hereinafter “ash”), relation between sample mass after and before calcination, is another marker which indirectly reflects total quantity of organic material. The smaller ash mass after sample calcination, the more organic material in this sample. Of note that this marker can give overestimated values, because the calcination of samples containing mineral components results also to destruction of mineral carbonates and crystalline hydrates. However the ash mass values demonstrated good correlation with “total nitrogen” marker [8].

In the present article, we have analyzed concentrations of potential markers of higher level such as methabolytes and organic destruction products.

Considering that solar energy is the main energetical sourse of any ecosystems, the ecosystems activity could be characterized by concentration of photosynthetic pigments (PP). The

more PP concentration observed in samples, the higher photosynthetic activity and the faster biomass accumulation. That is why, the most important PP (chlorophyls and carotenoids) were chosen. A total content of chlorophylls is directly associated with the light absorption and therefore evidences photosynthetic ecosystem activity. Carotenoids also demonstrate photosynthetic activity [9]. Unlike chlorophylls, they are more stable and accumulated not only into plant cells, but also into fatty tissue of herbivores [10, 11, 12]. Therefore carotenoids could be more general marker of ecosystems activity.

An important quantitative characteristic of intensity of processes taking place in ecosystems is total content of soils organic material. Humus is the most significant organic component of soils [13] directly related to the activity of ecosystems, decomposition efficiency of flora and fauna residuals, and, finally, formation of mature high-quality soils which are necessary for further biogeochemical cycles functioning. The main components of humus are biopolymers: humic acids and their salts (humates), as well as parent compounds of lower molecular mass – fulvic acids and fulvates [14]. The total concentration of humic and fulvic derivatives (HD) can be additional chemical marker describing ecosystem efficiency.

The present article is devoted to studying the possibilities to use total concentrations of chlorophylls, carotenoids and HD as potential chemical markers for ecosystems characterization. Studies were carried out within one of relatively simple Antarctic ecosystems in Antarctic Peninsula located on Demarria Mount. The concentrations of mentioned markers in collected samples were compared with amounts of "total nitrogen" and values of ash parameters. The inter-correlations between potential markers were analyzed, as well.

2. Experimental part

Collection and primary analysis of samples.

Studies were carried out using samples collected on the landscape test range – Demaria Mount ($65^0$17'S $64^0$06'W, Graham Land, Antarctic Peninsula). Sampling was made on a route which passes through relatively flat northwest hill (with a slope 45º, approximately) covered by moss fields, away from bird nesting areas (Figure 1).

The sampling was performed following a line between levels 47 m and 408 m above sea level along the direction of meltwater flow providing the migration of chemical components. The collection of samples was made in 9 points of the investigated area, which coordinates were referred by GPS. Coordinates of sampling points are listed in Table 1.

Samples were taken as a whole, including the biological material and the soil down to rock substrate. Samples were frozen at -18ºC, transported and stored at this temperature until beginning of their studies. After thawing of the samples, their description, catalogization, macro- and microphotography, chemical analysis, identification of plant and animal remains were carried out. Detailed description of vegetation cover, avifauna and primitive soil structure on the sample sites is given in [8].

Chemical analysis of the samples.

Representative sample points taken by quarter-point sampling method were vertical sections including both phytomass and soil. Results of chemical analyses of each marker were obtained as averaged values of three replicates. If the variation coefficient of average value exceeded 3%, the quantity of replicates was increased.

When analyzing PP, phytomass and soil were not separated, because soils also demonstrated photosynthetic activity due to containing photosynthetic diatomic algae (Figure 2). Thereby PP were formally used as markers characterizing general photosynthesis activity. The PP analysis was not carried out in samples rich in mineral components (pebbles). As living plants almost do not contain HD, the analyses of latter were performed only in the soils separated from the phytomass. The HD analyses in samples, where soil content was not enough, were not made either.

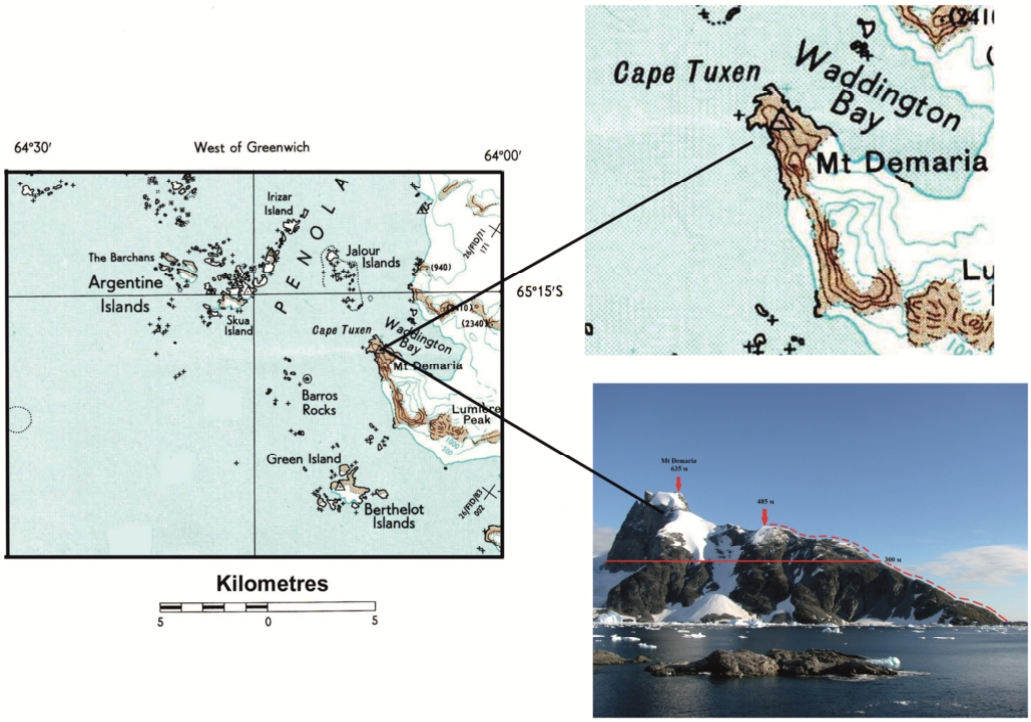

Figure 1. Location of sampling area on the hill of Demaria mount.

Table 1.

Coordinates of sampling points on Demaria Mount

Sample number	Altitude above sea level, m	Coordinates
1	47	S 65°16’07,5” W 64°07’17,7”
2	109	S 65°16’10,4” W 64°07’07,0”
3	134	S 65°16’13,1” W 64°07’01,2”
4	162	S 65°16’20,1” W 64°06’59,5”
5	166	S 65°16’22,8” W 64°06’59,0”
6	255	S 65°16’24,5” W 64°06’53,1”
7	304	S 65°16’31,0” W 64°06’42,3”
8	351	S 65°16’13,9” W 64°07’06,0”
9	408	S 65°16’20,1” W 64°06’59,5”

Quantitative PP analysis. The samples were dried to constant weight at temperature not higher than 60ºC. Water content in the samples was determined gravimetrically.

Air-dry samples, including mineral part, were grinded up to a powder. The exact portions (in range 150-250 mg) of the latter were extracted in Soxhlet extractor by 20 ml of chloroform during 6 hours. The obtained extracts were partially concentrated using a rotary evaporator, and then, transferred into 10 ml volumetric flask. The volumes of the extracts were brought up to 10 ml by chloroform. Absorption spectra of the extracts were recorded with a Hitach U3210. Depending on solution optical density, cuvettes of 1 mm or 10 mm across were used.

Content of chlorophylls was determined from values of optical density in absorption band maximum at 665 nm. Calibration curve was prepared using chlorophyll A standard solutions (Sigma-Aldrich).

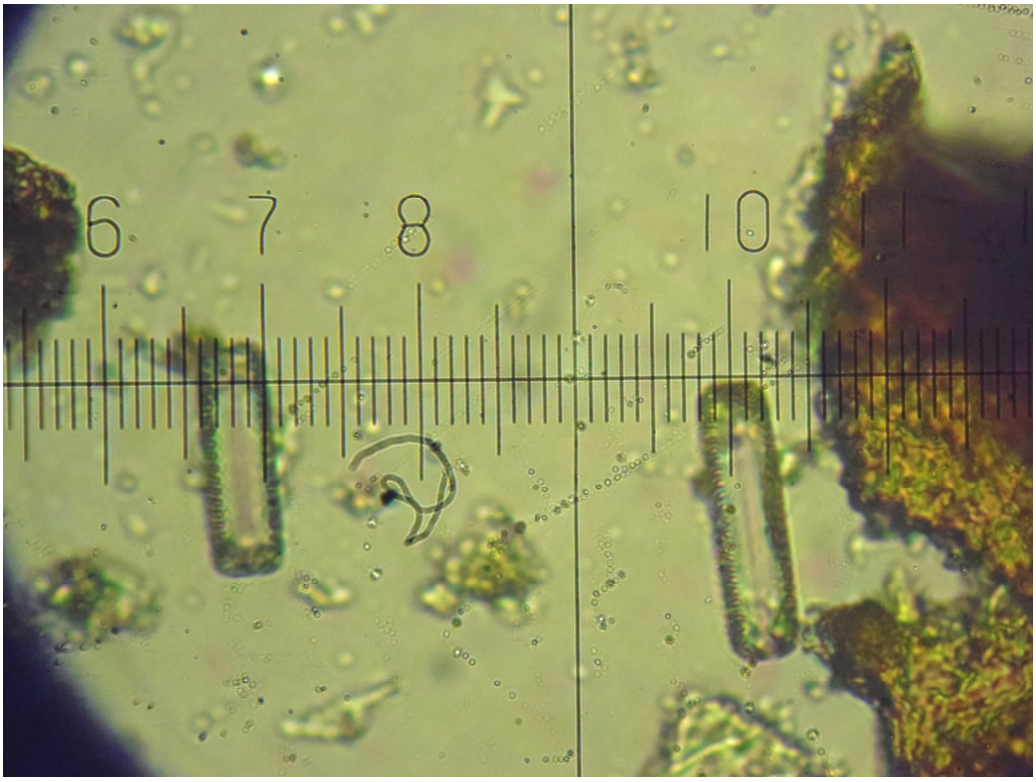

Figure 2. Diatom algae in soils of Demaria mount.

Absorption bands of carotenoids at 410-430 nm were extracted from absorption spectra of the extracts by deconvolution method using Spectra Data Lab soft package [15]. Content of carotenoids was calculated from optical density in maxima of carotenoids absorption bands. Solutions of β-carotene (Sigma-Aldrich) were used as standard ones.

Quantitative HD analysis. Humic acids and their derivatives were analyzed by a method described in [16] with minor modifications. HD extraction was carried out from crushed portions of soil; the weight (exact) of these latter was in range 200-250 mg. Each portion was extracted by 25 ml 0.01M NaOH solution in an ultrasonic bath (40 kHz) during 30 min at 30ºC. The extracts containing soluble salts of humic and fulvic acids were filtered. Extract aliquotes were subjected to a reverse differential potentiometric titration by 0.01M HCl solution. Titration plots dpH/dV =*f*(V) (Figure 3) demonstrated the presence of two peaks, the higher one, at pH ~ 7, corresponding to neutralization point of NaOH, and the flatter one of lower intensity at pH~ 10 –11 corresponding to humic acid/humates equilibrium. As humic acids are less soluble in water than humates, the achievement of equivalent titration point was accompanied by precipitation of humic acids. The content of humic acids in soils was calculated using formula:

$$\omega = \frac{(C_{NaOH} \times V_{alq} - C_{HCl} \times V_{HCl}) \times M_{hum} \times V_{NaOH}}{V_{alq} \times m \times 1000},$$

where ω is the content of humic acids and their derivatives in a sample, mg/g; C_{NaOH} – NaOH concentration in the extragent, mol/l; V_{extr} – the volume of the extragent (0,01 mol/l NaOH), ml; V_{alq} – volume of an aliquote of extract taken for the titration procedure, ml; C_{HCl} – the titrant concentration, mol/l; V_{HCl} – volume of the titrant, ml; M_{hum} – average molecular weight of humic acids, 1500 g/mol [17]; m – the mass of portion, g.

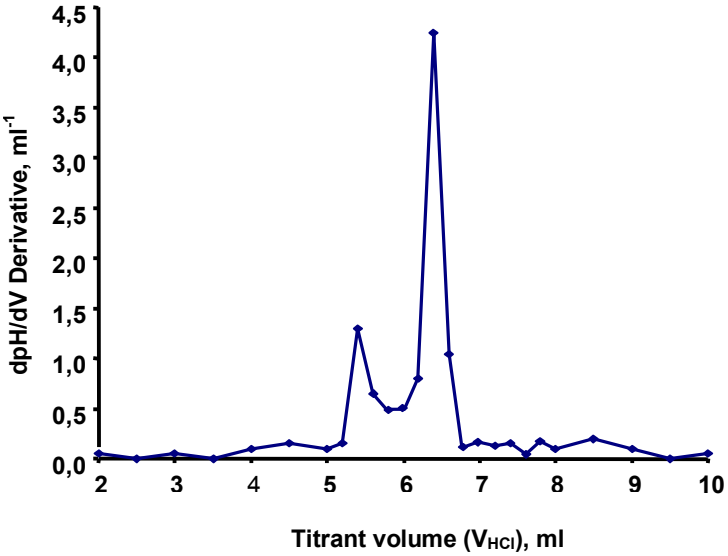

Figure 3. A typical curve of reverse differential potentiometric titration of humic acids in soil samples.

3. Results and discussion

Total concentrations of chlorophylls, carotenoids and HD are listed in Table 2. Dependences of concentrations of total chlorophylls and carotenoids on altitudes of sampling are depicted on Figure 4.

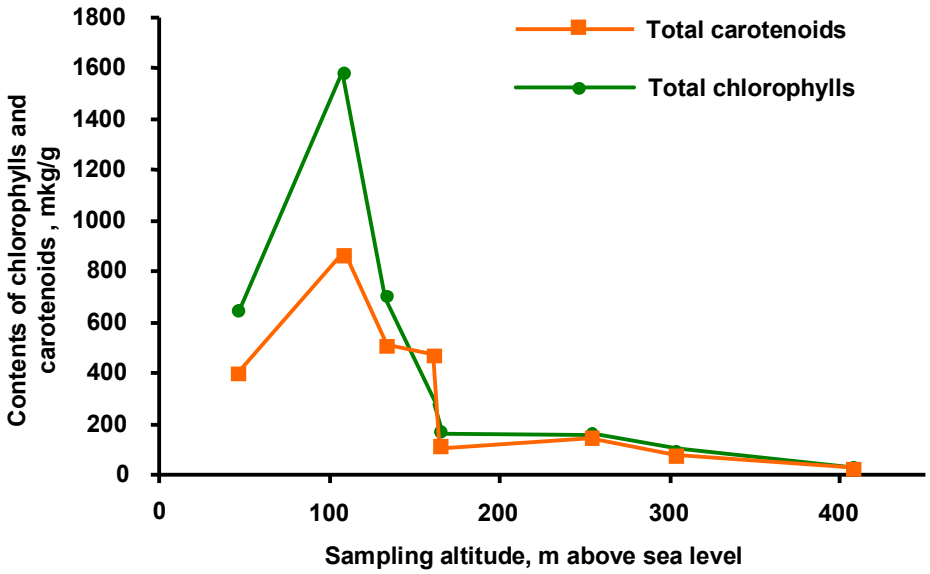

Figure 4. The dependencies of total chlorophyll and total carotenoid contents on sampling altitudes.

Table 2.

Results of chemical analysis of the samples*

Sample number	Sampling altitude	Total humic acids	Total chlorophylls	Total carotenoids
	m above s.l.	mg/g	mkg/g	mkg/g
1	47	–	640,53	393,29
2	109	–	1580,55	861,72
3	134	60,52	697,15	505,04
4	162	56,21	280,38	466,49
5	166	50,26	162,34	105,64
6	255	–	159,31	142,75
7	304	27,54	89,57	69,61
8	351	40,13	–	–
9	408	24,9	26,50	30,55

* – all the results are average values of three and more replicates. Variation coefficients of the average values do not exceeds 3%.

In altitude range from 410 to 160 m above s.l., the chlorophyll concentration slowly increases from 26.5 to 162.3 mkg/g. Then, when further decreasing of the altitude, the chlorophyll content dramatically increases almost by 10 times, and, finally, at the altitude ~50 m above s.l., the chlorophyll concentration decreases twice times. Dependency of carotenes on the altitude demonstrates a similar tendency.

Figure 5 shows that changing content of chlorophylls and carotenoids demonstrate linear dependency with high determination coefficient – $R^2 = 0.981$ ($R^2 = 0.871$ taking into account a sample 5 outlier from general tendency). Correlation depicted in Figure 5 show that the chlorophyll content in the samples is 1.9 times higher than that of carotenoids. The intercept of dependency, different from zero, indicates accumulation of carotenoids: even when chlorophyll is absent, the residual quantity of carotenoids is 41 mkg/g, approximately. It evidences that carotenoids remain in the samples after flora dying. However, the correlation between contents of chlorophylls and carotenoids allows concluding that, independently on the accumulation or destruction rates, the PP give similar information about photosynthetic activity of ecosystems. It is worth to note that such a conclusion can be true only for Antarctic ecosystems, where is a lack of fauna feeding actively lichens and mosses. Perhaps, in ecosystems with high level of biodiversity, the linear relationship between concentrations of chlorophylls and carotenoids may be violated.

The dependence between PP content and ash parameter (Figure 6) has a power character with high values of determination coefficient (R^2 = 0.85-0.88). The higher concentrations of chlorophylls and carotenoids and, consequently, greater phytomass quantity, the lesser weight of the ash after sample calcination Such dependence allows making an important conclusion: ash parameter is directly associated with phytomass quantity, and the loss of the ash weight due to thermodegradation of mineral components of samples is minimal. This allows using the ash parameter for “total biomass” characterisation. In the case of meager Antarctic ecosystems the ash and PP content can be used as reasonably accurate markers for “total phytomass” estimation.

Figure 7 demonstrates plots of the PP concentrations against “total nitrogen” content. When decreasing sampling altitude from ~410 down to ~150 m above see level, PP concentrations and “total nitrogen” increase parallelly. Analysis of the plot allowed estimating an average value of the “total nitrogen” in phytomass of Demaria mountain ecosystems, which is in range 0.2–0.3 mg/g. The further growth of “total nitrogen” at altitudes lower 150 m above s.l. results in the decrease of PP content that corresponds to phytomass quantity decrease. This effect can be explained by increasing amount of the fauna biomass and by accumulation of vital activity products.

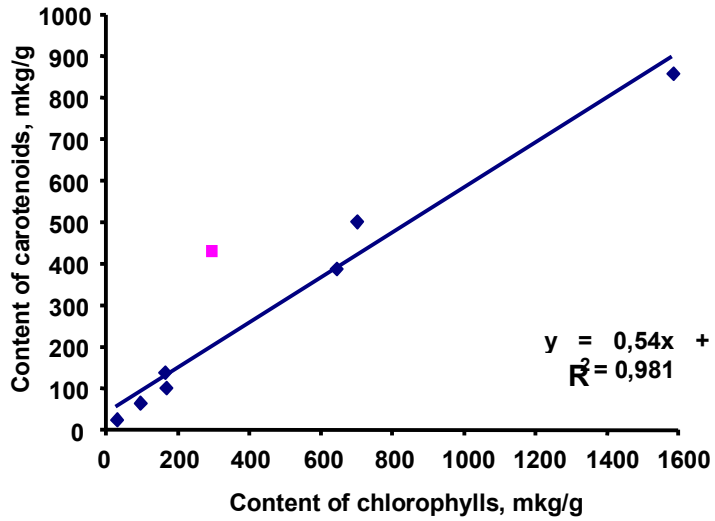

Figure 5. The relationship between contents of chlorophylls and carotenoids in studied samples. A point falling outside the linear dependence and marked by red color was not taken into account under correlation analysis. In correlation equation: x - chlorophyll content, y - carotenoid content.

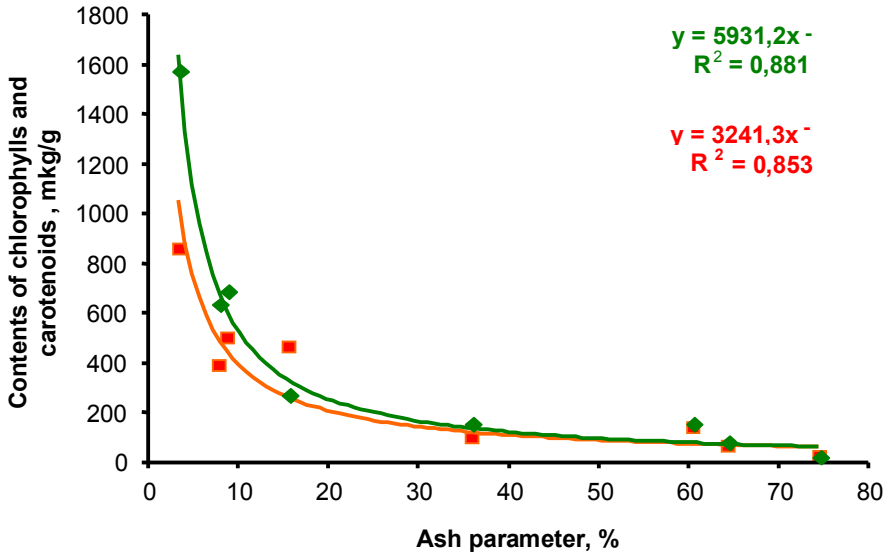

Figure 6. The relationship between total chlorophyll and total carotenoid contents and ash parameter of studied samples.

As noted above, HD analysis was made in six samples containing sufficient quantity of soil component. Figure 8 shows dependence of HD content and their derivatives in soils on the sampling altitudes. It can be seen, when altitude decreasing, the concentration of HD increases. This latter marker is not directly connected with phytomass quantity and PP content in whole (phytomass+soil) samples; in our opinion it shows biosystem ability to the crop residues decomposition after vegetation die away.

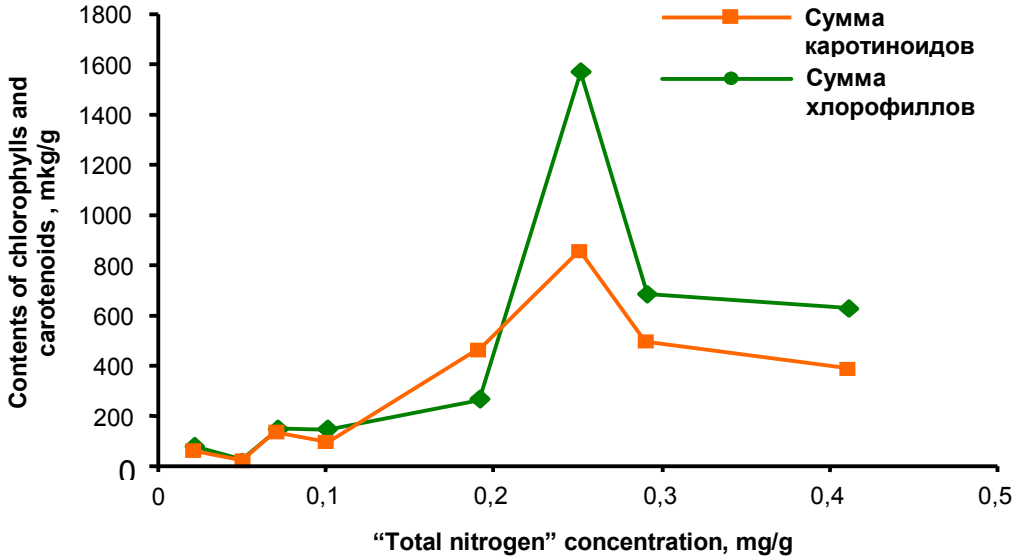

Figure 7. The relationship between PP contents and "total nitrogen" content in studied samples.

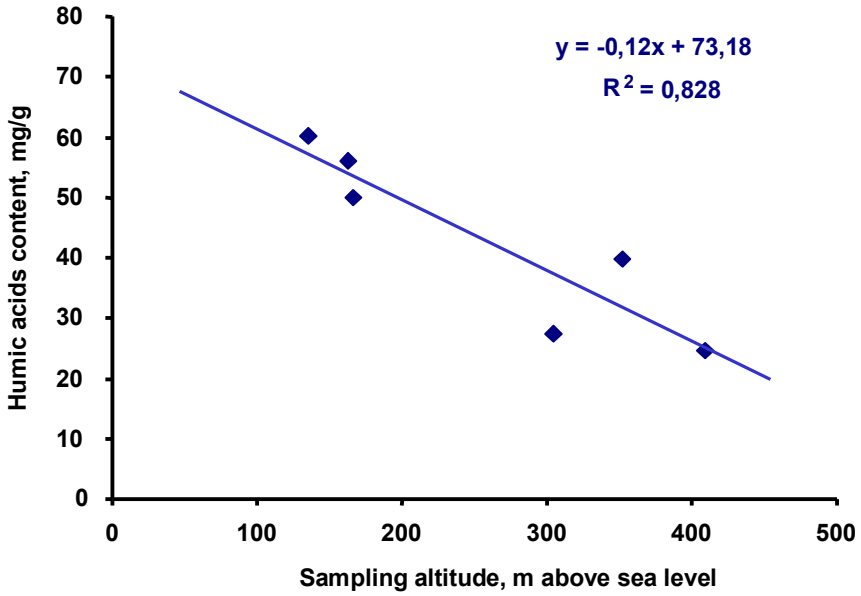

Figure 8. The dependency of total humic acid content on sampling altitudes.

According to the equation shown in Figure 8, when decreasing altitudes from 400 m down to 100 m above s.l., the HD concentration of humic acid derivatives increases by 2.4 times. Probably, this marker could correlate with average temperature at sampling points, because the growth of temperature leads to the acceleration of chemical and biological phytomass destruction.

Unlike PP concentrations characterizing living phytomass, HD content correlates linearly with ash parameter of soils (Figure 9). Different types of ash parameter dependences on PP content and HD content evidence different mechanisms of phytomass accumulation in vegetation process and phytomass humification.

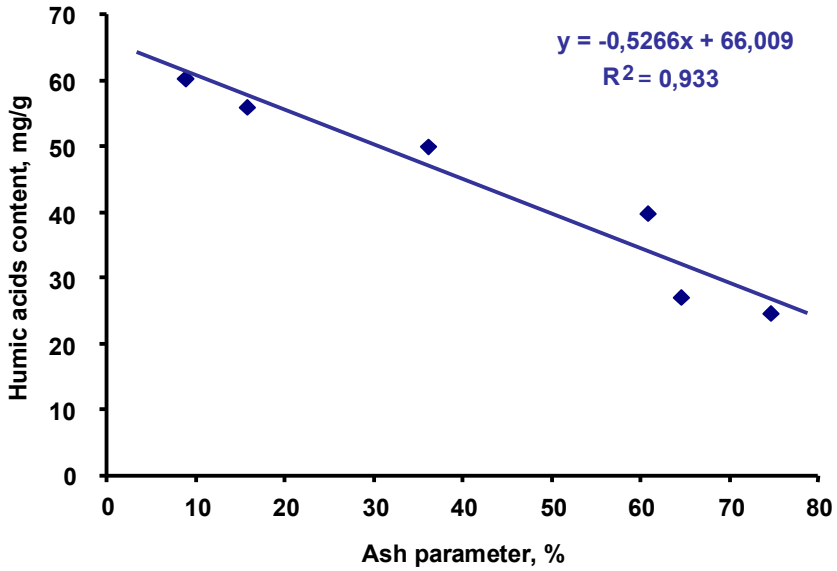

Figure 9. The relationship between the content of humic acids and ash parameter (In correlation equation: x - ash parameter, y - content of humic acids)

4. Conclusions

Results described above allow estimating quantitatively the phytomass accumulation, vegetation die-off and humification. These estimations are not based on physical measurements of phytomass weight or on analyses of soil morphology, but on the use of formal chemical markers such as concentrations of photosynthetic pigments (chlorophylls and carotenoids), total content of humic acids and their derivatives, "total nitrogen" content, ash. Use of chemical markers can accelerate and simplify studies in ecosystems.

The obtained chemical parameters give additional information about unique Antarctic ecosystem of Demaria Mount. They show phytomass distribution and humification dynamics depending on altitude above sea level.

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