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**STUDY OF THE INFLUENCE OF FLOODING ON THE ABSOLUTE AND
RELATIVE CONTENT OF PLANT PIGMENTS IN URAL LICORISE
(*GLYCYRRHIZA URALENSIS*).**

Annotation. In this work, the influence of the 2024 spring flood on the content of plant pigments in Ural licorice (*Glycyrrhiza uralensis*) was examined using UV-Vis-spectroscopy. A comparative study of samples collected in 2023 and 2024 clearly shows that flooding of plant growth areas has a significant impact on the content of natural pigments in them. The change in different pigments content both towards an increase and a decrease is explained by the plant's adaptive response to the negative effects of water stress on growing conditions, leading to reduced light access and disturbances in gas changes. Results shows that these changes lead to both positive and negative imbalances in both the absolute and relative content of all plant pigments. Pearson's correlation analysis revealed important dependencies between the content of all studied pigments and established the nature of the influence of water stress on changes in their content and ratio. It has also been found that leaves of *G. uralensis* are the richest in chlorophylls and carotenoids, while the roots and flowers contain the highest levels of anthocyanins.

Keywords: *Glycyrrhiza uralensis*; plant pigments; anthocyanins; carotenoids; chlorophyll; flood; water stress; plant adaptation; medicinal plants; environmental factors; Pearson's correlation; UV-Vis-spectroscopy.

Introduction

Pigments play a crucial role in plants as they are important for plant survival and reproduction. Pigments are involved in the regulation of plant growth and development and respond to environmental stresses [1]. Chlorophylls are essential for absorbing light energy, which is converted into chemical energy during photosynthesis. This process is crucial for plant growth and energy production [2]. Pigments such as anthocyanins and carotenoids give flowers and fruits vibrant colors, attract pollinators, and aid in seed dispersal [3]. These pigments also protect plants from ultraviolet (UV) and visible light damage and act as antioxidants, reducing oxidative stress and protecting plant tissues [4].

Chlorophylls are the pigments that make plants green and are perhaps the most important compounds on Earth because they are needed to collect and convert light energy in photosynthesis. Most of the light energy absorbed and converted during photosynthesis



occurs through direct light absorption by chlorophylls. Therefore, any negative influence to chlorophylls content and structure will have a major impact on the ability of plants to perform photosynthesis.

Anthocyanins are phenolic water-soluble glycosides or acyl glycosides of anthocyanidins [5]. These compounds are secondary metabolites of plants that protect them from biotic and abiotic stresses. They are the most common derivatives of cyanidin, delphinidin and pelargonidin. Anthocyanins are responsible for the pink, red, blue and purple color of flowers, fruits and vegetables, the color being related to the substitution pattern (position and chemical groups) in the aromatic rings. They have been used as natural food colorants and are proving to be promising ingredients in the food and nutraceutical industries [6].

Carotenoids are another set of natural plant pigments that are primarily responsible for the red, yellow and orange colors of vegetables and autumn leaves (when all chlorophyll is broken down), but are also found in dark green vegetables [7]. Carotenoids are mainly synthesized by photosynthetic organisms such as plants and algae, as well as some microorganisms such as fungi and bacteria. They fulfill several important functions in the plant kingdom: they act as light collectors, growth regulators, inhibitors of photooxidation during photosynthesis and, due to their bright colors, as attractors for pollinators [8,9]. The most common carotenoids in plant leaves are lutein, β -carotene, violaxanthin and neoxanthin. Consumption of these compounds by people who are unable to synthesize them appears to play an important role in reducing a number of diseases such as eye cancer, immune disorders, cerebrovascular and cardiovascular diseases [10]. Carotenoids are also of great industrial importance and are used as food dyes, cosmetics and nutraceuticals [11].

The biosynthesis of pigments in plants is a complex process involving various pathways and regulatory mechanisms, which are crucial for maintaining pigment stability and function [12]. External factors like light, temperature, and nutrient availability can significantly affect pigments biosynthesis [13]. Light is a major regulator of pigment biosynthesis, particularly for anthocyanins and carotenoids. It affects the expression of genes involved in these pathways, enhancing pigment production under high light conditions [14]. Drought conditions can lead to increased pigment production as a protective response. Anthocyanins, for instance, help mitigate oxidative stress caused by water deficiency [15]. On the other hand, flooding can lead to a decrease in chlorophyll content, affecting photosynthesis efficiency. This reduction is often due to impaired gas exchange and photosynthetic activity under waterlogged conditions [16]. Flooding can induce oxidative stress, which can alter pigment composition. Plants may increase the production of protective compounds, but overall pigment content, including chlorophyll and carotenoids, often decreases [17].

In this work, the content of chlorophylls, anthocyanins and carotenoids in different parts of *G. uralensis* was examined using UV-Vis spectroscopy and the effect of flooding on the content of these pigments was also estimated.

Materials and methods

Reagents and solvents.

All reagents are of analytical grade were purchased from commercial suppliers and used without any purification.

Collection and preparation of plant material.

The plants selected for our study are shown in Fig. 1.



Figure 1 – The appearance of *G. uralensis*: **A**-whole plant, **B**-inflorescences, **C**- stem, **D**- root, **E**-leaves

The plants were collected from their natural habitat. The samples were washed thoroughly with tap water to remove sand and dust, then with double-distilled water 2-3 times and kept in a shaded place in air until completely dry. The dried samples were then ground with a stainless-steel mill, sieved with a 1.0 mm sieve, and stored in dark glass vials at 4°C until further analysis.

Determination of total carotenoids content.

To 0.05 g of dry sample 5 ml of acetone were added and pulverized in a porcelain mortar in ice bath. Then, 1.0 g of anhydrous sodium sulfate was added and the solution mixed slowly followed by increasing the volume of acetone to 10 ml. The mixture was centrifuged at 26,000 rpm for 10 min. The supernatant was then removed and the absorbance measured at 662, 645 and 470 nm in 10 mm quartz cuvette. The total carotenoid content was calculated as follows [18]:

$$C_a (\mu\text{g/g}) = 11.24A_{662} - 2.04A_{645}$$

$$C_b (\mu\text{g/g}) = 20.13A_{645} - 4.19A_{662}$$

$$C_t (\mu\text{g/g}) = (1000A_{470} - 1.9C_a - 63.14C_b) / 214$$

where C_a stands for chlorophyll *a*, C_b for chlorophyll *b* and C_t is total carotenoid content. A_{470} is absorption at 470 nm (related to carotenoids), A_{645} - at 645 nm (related to chlorophyll *a*) and A_{662} - at 662 nm (related to chlorophyll *b*).

Determination of anthocyanins content.

To 0.02 g of dry sample 4 ml of 1% hydrochloric acid containing methanol were added and pulverized in a porcelain mortar. Solution was kept for 24 h in the refrigerator (4°C). Then, after centrifugation for 10 min at 13,000 rpm the absorbance of supernatant was measured at 530 and 657 nm in 10 mm quartz cuvette against blank. The blank solution was 4 ml of 1% hydrochloric acid solution containing methanol. The anthocyanins content (mg/g DW) was calculated by the following equation [18]:

$$\text{Anthocyanins (mg/g)} = A_{530} - (0.25 \cdot A_{657})$$

where “A” stands for absorbance at 530 nm and 657 nm respectively

Statistical analysis

Each experiment was carried out in triplicate ($n = 3$) and the data presented as an average of three independent determinations $\pm$ standard deviations (SD). Pearson's correlation analysis was performed using MS Excel 2019 software.

Research results

In our study, we performed a quantitative comparison of the content of plant pigments in *G. uralensis* over two years (2023-2024). The results of determination of the content of chlorophyll *a* and chlorophyll *b* for the specified time period are shown in Fig. 2.

As can be seen from Figure 2, the highest content of chlorophylls A and B is observed in the leaves of *G. uralensis*. Nevertheless, the amount of these pigments in all parts of the plant in 2024 exceeds their content in 2023. The high concentration of chlorophylls in the leaves is due to their crucial role in plant metabolism and adaptation. Chlorophylls are responsible for the green color and are essential for photosynthesis as they capture light energy to convert carbon dioxide and water into glucose and oxygen [19]. The increase in the content of photosynthetic pigments is most likely due to the consequences of the flooding in the region in spring 2024. The increase in chlorophyll content in plants after a flood can be attributed to physiological and biochemical reactions aimed at recovery and adaptation. Flooding initially causes stress, which leads to stomata closure and reduced photosynthesis [20].

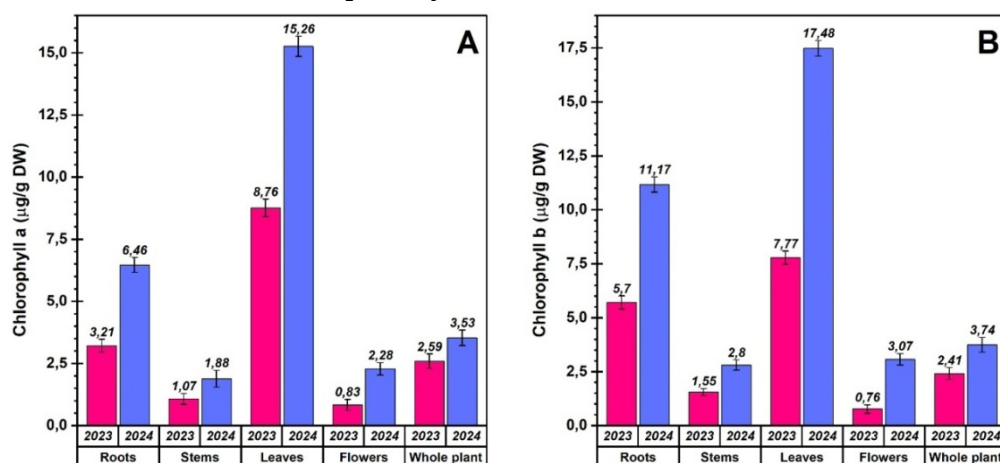


Figure 2 – The content of chlorophyll *a* (A) and *b* (B) in *G. uralensis*.

However, during the recovery period, plants improve oxygenation and water uptake, which facilitates the reopening of stomata and the resumption of photosynthesis to compensate for the initial loss of this function [21]. This is supported by increased amounts of photosynthetic pigments and improved photosynthetic rates observed in some species in the post-flood period [22].

The ratio of chlorophylls *a* and *b* for *G. uralensis* over the same period is shown in Fig. 3.

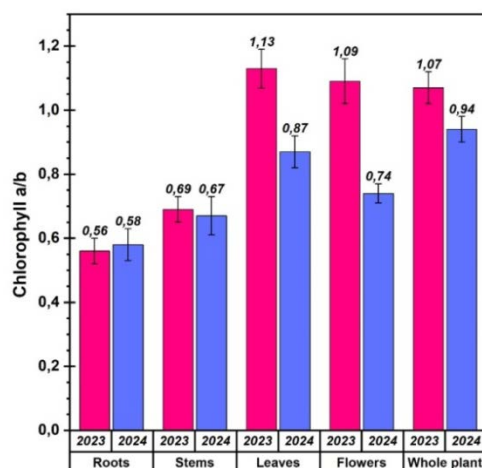


Figure 3 – The chlorophyll *a/b* ratio in *G. uralensis*.

Figure 3 shows that the Chlorophylls *a/b* ratio in the studied plant parts collected in 2023 was between 0.56 (root) and 1.13 (leaves), while in the plants collected in 2024 it was between 0.58 (root) and 0.94 (whole plant). Nevertheless, this ratio remained constant in roots and stems. At the same time, this ratio decreases by around 20% for leaves and flowers.

These results can also be explained by the influence of flooding, since it is known that flooding often leads to hypoxic conditions that can cause stress and alter the plant's metabolic processes. A key response is the increase in chlorophyllase activity, an enzyme that degrades chlorophyll, particularly chlorophyll *a*, leading to a relative increase in chlorophyll *b* [23]. In addition, the stress of flooding can lead to an imbalance in the synthesis and breakdown of chlorophylls. Chlorophyll *b* is more stable under stressful conditions than chlorophyll *a*, which can be broken down more easily [24].

The total anthocyanin content of the plants examined is shown in Fig. 4.

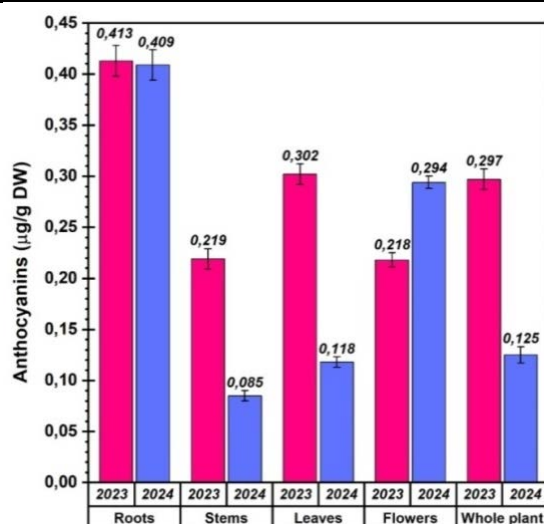


Figure 4 – Total anthocyanins content in *G. uralensis* (A - 2023; B - 2024).

As can be seen from Fig. 4, the anthocyanin content also shows changes over the specified period. Anthocyanin levels in 2023 ranged from 0.218 µg/g in flowers to a high of 0.413 µg/g in plant roots. Furthermore, the anthocyanin content in the plant parts collected in 2024 ranged from 0.085 mg/g in the stem to a maximum of 0.409 µg/g in the plant roots. It was found that the content of anthocyanins in roots remains at the same level in 2024 as in 2023. In stems, leaves and whole plants there is a significant decrease in anthocyanin content by more than twice. In flowers, the content of these pigments was about 30% higher in 2024 than in 2023.

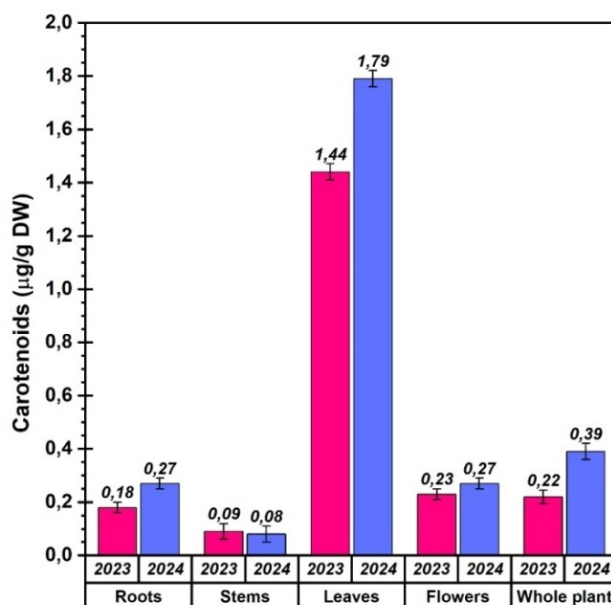


Figure 5 – Total carotenoids content in *G. uralensis* (A - 2023; B - 2024).

These facts can also be explained as stress response and hormonal changes in plant consequences of flooding. Flooding causes stress in plants and leads to changes in hormone levels, particularly abscisic acid (ABA). ABA is known to regulate anthocyanin biosynthesis under stressful conditions such as drought, but its role in flooding stress is less clear. Reduced ABA levels during floods could lead to decreased anthocyanin synthesis [25]. Furthermore, flooding results in hypoxic conditions and the lack of oxygen can disrupt the enzymatic activities required for anthocyanin production [26]. A reduction in light availability due to water cover and sediment deposition may also lead to a decrease in anthocyanin levels, as light is a crucial factor in their synthesis [27].

The total carotenoid content determined in *G. uralensis* is shown in Fig.6.

The results of the two-year study showed that the highest content of carotenoids is found in the leaves of the plant (1.38 and 0.67 $\mu\text{g/g}$). The lowest values of total carotenoids are found in the stem of the plant (0.19 and 0.18 $\mu\text{g/g}$). As can be seen, the carotenoid content varies slightly. The high concentration of carotenoids in leaves compared to other parts of plants is due to their essential role in photosynthesis and light protection. Carotenoids such as lutein and β -carotene are crucial for the absorption of light energy and protect the photosynthetic apparatus from damage caused by excess light and reactive oxygen species [28, 29]. In addition, carotenoids play a protective role by dissipating excess light energy as heat (non-photochemical quenching) and scavenging reactive oxygen species, thereby preventing oxidative damage to the photosynthetic machinery [30]. This is also due to the differential expression of carotenoid biosynthesis genes. These genes are highly expressed in leaves, leading to the accumulation of carotenoids necessary for photosynthesis and protective functions [31].

In this work, the Pearson's correlation analysis was also carried out for *G. uralensis* to compare the relationship between the content of anthocyanins, carotenoids, chlorophylls *a* and *b* and their ratio. The results are shown in Fig.7.

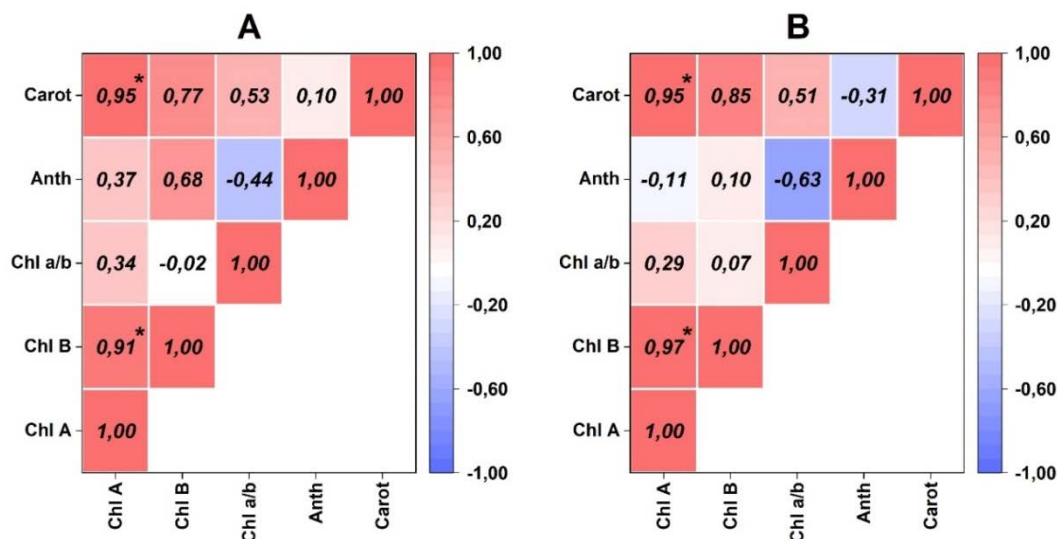


Figure 6 – Pearson's correlation coefficients (*r*) for the relationship between plant pigments of *G. uralensis*: A - 2023, B – 2024 (* - significant at $p < 0.05$).



The correlation analysis showed relatively stable relationships between the pigment contents determined in both years. Significant correlations ($p < 0.05$) were found between chlorophylls *a* and *b* ($r = 0.91$ (2023) and $r = 0.97$ (2024)) and chlorophyll *a* with total carotenoid content ($r = 0.95$ (2023) and $r = 0.95$ (2024)). The correlation between anthocyanins and the ratio of chlorophylls *a* and *b* becomes more negative in 2024 compared to 2023 ($r = -0.63$ and $r = -0.44$, respectively). This suggests that anthocyanin content tends to increase as the ratio of chlorophyll *a* to *b* decreases. This may be attributed to poor light conditions during the flooding period, where the chlorophyll *a/b* ratio decreases and anthocyanin synthesis is upregulated as part of the corresponding adaptive response of the plant [32]. A highly significant correlation between carotenoids and chlorophyll *b* was also observed ($r = 0.77$ (2023) and $r = 0.85$ (2024)). A significant positive correlation between chlorophyll *b* and total anthocyanin content was found with $r = 0.68$ in 2023 and only $r = 0.10$ in 2024. This decline can also be described as an adaptive response of plants to poor lighting conditions.

In addition, it was also found that the ratio of chlorophyll *a/b* does not correlate with the content of chlorophyll *b* ($r = -0.02$ (2023) and $r = 0.07$ (2024)) and only an insignificant negative correlation between total carotenoids and anthocyanins in 2024 ($r = -0.31$). In 2023, this relationship was only $r = 0.10$. This is because under stressful conditions such as flooding, plants may prioritize the synthesis of certain pigments over others. For example, anthocyanins are often produced in response to oxidative stress and may increase under such conditions, while carotenoid levels may not increase to the same extent or may even decrease due to resource allocation to stress mitigation [33]. Furthermore, the biosynthetic pathways of anthocyanins and carotenoids have common precursors. Under stress, the plant could divert more precursors to anthocyanin production at the expense of carotenoids, leading to a negative correlation between their levels [34]. Here, as in previous cases, reduced light conditions during flooding may reduce carotenoid synthesis while potentially increasing anthocyanin production as a protective response [35].

Conclusion

The main aim of this study was to determine the content of anthocyanins, carotenoids and chlorophylls in different parts of *G.uralensis* growing in the West Kazakhstan region. According to the results, there are significant differences in the total content of carotenoids and anthocyanins between the plant parts examined, as well as large differences in the content of all pigments. Our research has shown that flooding areas where plants grow significantly affects the concentration of all plant pigments. Such changes are caused by the adaptive response of plants to water stress and the inability to synthesize most pigments under insufficient lighting and limited air access. The results obtained may be useful for further studies aimed at investigating the relationships between the phytochemical composition and growth conditions of plants, including the influence of natural emergencies.

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Узақбай Г.Б., Исагат Р.И., Мендигалиев Е.Е., Максотова А.Е., Акатьев Н. В.*
ИССЛЕДОВАНИЕ ВЛИЯНИЯ ПАВОДКА НА АБСОЛЮТНОЕ И
ОТНОСИТЕЛЬНОЕ СОДЕРЖАНИЕ РАСТИТЕЛЬНЫХ ПИГМЕНТОВ В
СОЛОДКЕ УРАЛЬСКОЙ (*GLYCYRRHIZA URALENSIS*)

Аннотация. В настоящей работе методом УФ-Вид-спектроскопии исследовано влияние весеннего паводка 2024 года на содержание растительных пигментов в солодке уральской (*Glycyrrhiza uralensis*). Сравнительное исследование образцов, собранных в 2023 и 2024 годах однозначно указывает на значительное влияние затопления территорий произрастания растений на содержание в них природных пигментов. Изменение содержания пигментов в сторону как увеличения, так и уменьшения объясняется адаптивной реакцией растения на негативное влияние водного стресса на условия произрастания, приводящее к снижению освещенности и нарушению газообмена. Результаты показывают, что данные изменения приводят как к положительному, так и отрицательному дисбалансу как в абсолютном, так и в относительном содержании всех растительных пигментов. Корреляционный анализ Пирсона позволил выявить важные зависимости между содержанием всех исследованных пигментов и установить характер влияния водного стресса на изменение их содержания и соотношения. Также установлено, что листья *G. uralensis* наиболее богаты хлорофиллами и каротиноидами, в то время как в корнях и цветах обнаружено наибольшее содержание антоцианов.

Ключевые слова: *G.uralensis*; растительные пигменты; антоцианы; каротиноиды; хлорофилл; паводок; водный стресс; адаптация растений; лекарственные растения; экологические факторы, корреляция Пирсона; УФ-Вид-спектроскопия.

Ұзақбай Г.Б., Исағат Р.И., Мендіғалиев Е.Е., Максотова А.Е., Акатьев Н. В.*
ОРАЛ МИЯСЫ (*GLYCYRRHIZA URALENSIS*) ӨСІМДІК
ПИГМЕНТТЕРІНІҢ АБСОЛЮТТЫ ЖӘНЕ САЛЫСТЫРМАЛЫ
ҚҰРАМЫНА СУ ТАСҚЫНЫНЫҢ ӘСЕРІН ЗЕРТТЕУ

Аңдатпа. Бұл жұмыста 2024 жылғы көктемгі су тасқынының Орал миясының (*Glycyrrhiza uralensis*) өсімдік пигменттерінің құрамына УК-көрінетін-спектроскопиясы арқылы әсері зерттелді. 2023 және 2024 жылдары жиналған үлгілерді салыстырмалы зерттеу өсімдік өсіретін аумақтарды су басудың олардағы табиғи пигменттердің мөлшеріне айтарлықтай әсер ететінін анық көрсетеді. Пигмент құрамының көбеюі де, азаюы да өсімдіктің өсу жағдайларына су стрессінің теріс әсеріне бейімделу реакциясымен түсіндіріледі, бұл жарықтандырудың төмендеуіне және газ алмасуының бұзылуына әкеледі. Нәтижелер көрсеткендей, бұл өзгерістер барлық өсімдік пигменттерінің абсолютті және салыстырмалы құрамындағы оң және теріс тепе-теңдіктің бұзылуына әкеледі. Пирсонның корреляциялық талдауы барлық зерттелетін пигменттердің



мазмұны арасындағы маңызды қатынастарды анықтауға және олардың мазмұны мен арақатынасының өзгеруіне су кернеуінің әсер ету сипатын анықтауға мүмкіндік берді. Сондай-ақ, *G. uralensis* жапырақтары хлорофиллге және каротиноидтарға ең бай, ал антоциандардың ең көп мөлшері тамыры мен гүлдерінен табылғаны анықталды.

Кілт сөздер: *G.uralensis*; өсімдік бөліктері; антоцианиндер; каротиноидтар; хлорофилл; су тасқыны; су стрессі; өсімдіктердің бейімделуі; дәрілік өсімдіктер; қоршаған орта факторлары; Пирсон корреляциясы; УК-көрінетін-спектроскопия.