Photosynthetic pigments activity in plants leaves under the conditions of weather anomalies

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Abstract. The global climate change growing rate causes an increase in the frequency of weather anomalies, expressed in seasonally atypical temperature rises in various regions. Meteorological anomalies affect all components of living ecosystems, in particular – plant communities, and often have a negative impact, resulting in suppression of its components physiological functions, including photosynthetic activity of plants. In 2021, 2022 and 2024, summer weather anomalies were recorded in St. Petersburg at different pheno-phases of vegetation. In all three cases, the content of photosynthetic pigments (chlorophylls A and B, carotenoids) in leaves of higher plants was measured under conditions of exposure to the stress caused by seasonally atypical for the region weather conditions, as well as after the end of this factor impact in order to consider the plants capacity to restore photosynthetic pigments levels. It was shown that weather anomalies occurring in the earlier vegetation stages, when most plants of the studied region have not yet reached their maximum resistance to external stress, cause the most significant changes in their physiological state, which is slowly and not fully recovered after weather conditions normalization, while in the later pheno-phases chlorophylls levels were preserved to a greater extent.

1 Introduction

The last decades, weather anomalies have become more frequent due to the increased rates of global climate change [1]. The current tendency of climate of middle latitudes in the northern hemisphere softening, accompanied by warming, is characterized by unstable weather patterns with increasing regularity of meteorological parameters anomalous deviations in different seasons of a year, in particular it is manifested in record seasonally atypical temperature rises [2]. Such phenomena are a stress factor for components of living ecosystems, in particular for phytocenoses [3]. Urban ecosystems, which are exposed to the combined effect of weather and anthropogenic factors, are subjected to the most negative, oppressive impact [4].

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Weather anomalies occurring at different pheno-phases of vegetation cause physiological changes of varying degrees, often irreversible [5]. The degree of plants susceptibility to stress produced by such conditions depends on a number of factors, such as species and origin (relict or introduced), plant age, geochemical and physicomorphological parameters of the soil, its hydrological regime, edaphic conditions, light level of the growing site, location relative to industrial and economic objects: linear and point, stage of vegetation, and etc.

The result of exposure to oppressive impact is degradation of ecological functions, including barrier functions and accumulation of a number of external substances, among which numerous highly toxic pollutants, such as heavy metals, as well as a decrease in the efficiency of one of the most important processes in plants – photosynthesis [6], the consequence of which can be not only a violation of the balance in cenoses, but also a decrease in the carbon dioxide assimilation with its subsequent concentration in the atmospheric air of residential areas [7].

One of the integrated indicators of plants reaction to the environmental conditions transformation is changes of the phenological phases dates in their life cycle. Each plant species at the population level, attached to a certain range of geographical latitudes and climatic conditions determined by them, adapts to the appropriate range of climatic parameters of a particular region in their seasonal dynamics. Occurring at different pheno-phases of the growing season, weather anomalies can cause changes in its duration [8, 9].

The main photosynthetic pigments - chlorophylls A and B and carotenoids – levels and their ratios in relation to a specific time period and the pheno-phase corresponding to it normally can represent the degree of changes in the plants physiological state on the example of the photosynthetic apparatus [10, 11].

In 2021, 2022 and 2024, weather anomalies, manifested in prolonged periods of increased relative to the norm temperatures, accompanied by a precipitation deficit, were recorded in St. Petersburg during the summer season and the beginning of autumn. In 2021, the meteorological anomaly fell in the middle of June and early stages of vegetation, corresponding to blossom of most higher plant species planted in urban green spaces. The abnormal heat, which fell at a time when plants had not yet reached their maximum resistance to external stress factors, lasted for more than 2 weeks, during which average daily temperatures exceeded the norm by more than 5^{o}C and maximum daily temperatures were 34-36^{o}C. The summer of 2022 was also characterized by a weather anomaly that occurred in August, when plant resistance was higher compared to the 2021 case described above, but a prolonged period of similarly high temperatures combined with the previous one significantly impacted the inner-city plant communities. The weather anomaly of 2024 fell in September and lasted more than 3 weeks with temperature maxima at 26^{o}C on 16.09.2024 – under such conditions there was a shift of pheno-phases of vegetation to later dates.

The aim of the work was to study the behavior of photosynthetic pigments in leaves of plants of inner-city areas under weather anomalies of 2021-2024 at different pheno-phases and to consider this issue in the context of the general physiological state of the higher plants photosynthetic apparatus representation under such conditions. For the cases of 2021 and 2022, when weather anomalies fell on earlier vegetation stages, the periods of physiological functions recovery after this factor had passed were considered in temporal dynamics, which made it possible to trace the character of reversibility of the occurred changes, and the degree of destructiveness of weather anomalies coming to different pheno-phases.

2 Materials and methods

The research was conducted in the inner-city areas of the largest Baltic and the world's northernmost metropolis – St. Petersburg. The impact of the 2021 and 2022 weather anomalies was recorded for plants of Admiralteysky district greenery in single and row

plantings in residential areas close to busy roads, as well as in the interior spaces of enclosed courtyards. The material accepted for the analysis was represented by five species of arboreal plants (ash-leaved maple (*Acer negundo* L.), horse chestnut (*Aesculus hippocastanum* L.), European white elm (*Ulmus laevis* Pall.), white poplar (*Populus alba* L.), common ash (*Fraxinus excelsior* L.)), four species of herbaceous plants, including one species of leafy moss (broadleaf plantain (*Plantago major* L.), common dandelion (*Taraxacum officinale* (L.) Webb ex F.H.Wigg.), meadow bluegrass (*Poa pratensis* L.), *Barbilophozia barbata*). Typical species growing in city green spaces represented by both relict for these latitudes and introduced plants, as well as one stranger species – *Barbilophozia barbata*, were chosen for the study.

The 2024 weather anomaly effect was recorded in contrast areas for their characteristic vegetation: in the Admiralteysky district and within the boundaries of the buffer zone of inner-city protected area – Yuntolovsky Sanctuary. Both on the territory of the Yuntolovsky Park, directly adjacent to the sanctuary, and on the protected area, anthropogenic transformation of landscapes of different types is pronounced, such as deforestation of some sites, soil morphological organization disturbance, pyrogenic and recreational pressure, permanent air and waterborne pollutants transfer, and changes in the hydrological regime of certain areas. Four sites, which ecosystems are subjected to anthropogenic load of different character and degree, with their characteristic vegetation were selected for sampling in this work (Table 1).

Table 1. Characterization of the sample sites established in the Yuntolovsky Park and the list of species accepted for the study.

Sample site №	General characteristics of the site	Anthropogenic transformation degree	Character of anthropogenic transformation	Sampled species
1	Soil is represented by a soil-like formation: clamps which upper organogenic fertile layer of soil was collected in. The site is deforested for construction works, but there are pronounced signs of secondary succession – there is an intensive overgrowth on clamps, including plants of arboreal life form, but there is no closed canopy	High	Deforestation, transformation of the soil cover into urbanozem with collection of the upper fertile layer in clamps	Grey alder (*Alnus incana* (L.) Moench), Berlin poplar (*Populus ×berolinensis* K.Koch), common aspen (*Populus tremula* L.), almond willow (*Salix triandra* L.), red fescue (*Festuca rubra* L.), wood horsetail (*Equisetum sylvaticum* L.)
2	Post-pyrogenic mixed forest site, canopy is closed. The herbaceous layer is poor. Weakly peaty soil with well-defined soil horizons	Medium	Post-pyrogenic forest site with abundant traces of recreational impact and signs of past creeping fire	Common aspen (*Populus tremula* L.), rowan (*Sorbus aucuparia* L.), common lady-fern (*Athyrium filix-femina* (L.) Roth ex Mert.)
3	Transitional mire with features of raised bog.	Low	Practically undisturbed wetland. The oppressive factor is the close	Bog cranberry (*Vaccinium oxycoccos* L.), bogbean

	Among the dominant environment-forming species is bog-myrtle (*Myrica gale* L.), which is characterized by high vulnerability and here indicates a favourable ecotoxicological situation at the site. The tree layer is poor, there is no closed canopy. There is a periodic disturbance of the sphagnum cover of the mire, with rapid recovery		proximity of city residential areas, busy motorways and a number of road junctions. Periodically, mainly seasonally (second half of summer - beginning of autumn) due to the popularity of forest food resources collecting by citizens from the neighbouring districts, there is partial disturbance of sphagnum cover, which is also can be explained by the position of this site close to the entrance to the Yuntolovsky Park	(*Menyanthes trifoliata* L.), woollyfruit sedge (*Carex lasiocarpa* Ehrh.), bottle sedge (*Carex rostrata* Stokes), flat-topped bogmoss (*Sphagnum fallax* Hugo Erich Meyer von Klinggräff)
4	The Glukharka river bank. Soils are transformed into urbanozems. The site is mostly exposed to pollution from motor transport emissions. There is increased eutrophication of the Glukharka river, which channel was partially transformed into buffer ponds with a closed system, practically devoid of water exchange. The herbaceous layer is extensive, represented by a large number of typical coastal wetland and meadow plants, among which a number of indicators of anthropogenically disturbed areas	Medium to strong	Coastal-aquatic site that underwent soil transformation to urbanozems and is affected by pollution due to the proximity of busy motorways and road junctions. The Glukharka riverbed was artificially divided into two channels, one of which was transformed into closed stagnant buffer ponds	Grey alder (*Alnus incana* (L.) Moench), Siberian larch (*Larix sibirica* Ledeb.), almond willow (*Salix triandra* L.), common bulrush (*Typha latifolia* L.), meadow vetchling (*Lathyrus pratensis* L.), meadow bluegrass (*Poa media* Schur)

The laboratory analysis was carried out in accordance with the Lichtentaller method in the variant of extraction with 96% ethanol with the further calculation of photosynthetic pigments concentrations in the raw mass of the analyzed material [12, 13].

In the first day after sampling (methodically – up to three days from the sampling date) chlorophyll fixation was carried out by partial extraction in 96% ethanol: for deciduous plants with a large lamina and pinnate, palmate or arc venation parts of the leaf of a fixed size extruded using a circle mould with a diameter of 8 mm in the number of several units (3-4), for plants with a small leaf size or parallel venation – parts of a fixed size cut from the leaf, for coniferous plants – cut needles parts of a certain length, analogically parts of twigs – for wood horsetail (*Equisetum sylvaticum* L.), in the case of mosses – the green part of one shoot of a fixed length taken for analysis, were transferred to a 1.5 ml eppendorf, which then was filled with ethanol with the addition of calcium carbonate to prevent overoxidation. The extracts prepared for photosynthetic pigment analysis were stored closed in a freezer until

further analysis (the shelf life of such a sample is up to 1 year). Using the above-described methods, for each of the plants taken for the study lamina units were stored until weighing in conditions that exclude loss of moisture and mass of the raw leaf.

The previously partially extracted samples were transferred from the eppendorfs to a ceramic mortar so that all the green material and ethanol involved in the sample preparation was carried away. Soda was also added to the mortar with the analyzed material at the rate of 2 g per 300 mg raw weight of plant material. The material was grinded with a pestle until homogeneous with the condition of no homogenate loss at all stages. Then the homogenate was transferred from the mortar into a 50 ml centrifuge tube. The indicator of complete overflow was the absence of traces of green colour in the mortar. The tubes with the diagonal balancing were placed in a centrifuge set at 3000 rpm and centrifuged for 5 minutes. After the first centrifugation, the bulk of the clear green supernatant was transferred to a clean tube, after which ethanol was added to the sediment, which had retained its green colour due to the chlorophyll not completely extracted, the closed tube was shaken and centrifugation was repeated – if it was necessary, an additional grinding of the sediment was carried out. This algorithm was repeated on average 3-5 times – the indicator of complete chlorophyll extraction was the absence of green colouring of the sediment or supernatant after centrifugation.

After fixing an extracts volume in clean tubes, spectrophotometric measurements at wavelengths corresponding to the light absorption maxima of chlorophyll A, chlorophyll B and carotenoids were carried out. The measurements were performed on a spectrophotometer in cuvettes with an absorbing layer thickness of 1.00 cm. The apparatus was calibrated with ethanol at each wavelength: the wavelength of 664 nm corresponded to the absorption maximum of chlorophyll A, 649 nm - to the absorption maximum of chlorophyll B, 470 nm - to the absorption maximum of carotenoids.

The photosynthetic pigments levels were calculated using formulas 1-3:

- formula 1 was used to determine chlorophyll A concentration:

$$C_{cl(A)} = (13{,}36 * A(664) - 5{,}19 * A(649)) * V/m \quad (1),$$

where $C_{cl(A)}$ – chlorophyll A concentration in raw leaf mass, mg/g, *A(664)* and *A(649)* – optical density of the extract obtained at wavelengths of 664 nm and 649 nm, respectively, *V* – extract volume, ml, *m* – sample weight, mg.

- the level of chlorophyll B was determined using formula 2:

$$C_{cl(B)} = (27{,}36 * A(649) - 8{,}12 * A(664)) * V/m \quad (2),$$

where $C_{cl(B)}$ – chlorophyll B concentration in raw leaf mass, mg/g, *A(664)* and *A(649)* – optical density of the extract obtained at wavelengths of 664 nm and 649 nm, respectively, *V* – extract volume, ml, *m* – sample weight, mg.

- carotenoids concentrations were determined according to the following formula:

$$C_{car} = \left(1000 * A(470) - 2{,}13 * C_{cl(A)} - 97{,}63 * C_{cl(B)}\right)/290 * \frac{V}{m} \quad (3),$$

where C_{car} – carotenoids concentration in raw leaf mass, mg/g, *A(470)* – optical density of the extract obtained at a wavelength of 470 nm, $C_{cl(A)}$ – chlorophyll A concentration in raw leaf mass, mg/g, $C_{cl(B)}$ – chlorophyll B concentration in raw leaf mass, mg/g, *V* – extract volume, ml, *m* – sample weight, mg.

In addition, ratios of photosynthetic pigments concentrations were calculated at different pheno-phases of vegetation in seasons accompanied by meteorological anomalies, which serve as plants physiological state indicators and which dynamics can be used to assess the recovery or suppression of photosynthetic activity after the end of the oppressive external factor beyond the norm.

3 Results

Data on the content of measured pigments in the leaves of the Admiralteysky district plants at the peaks of the recorded weather anomalies are shown in Figure 1.

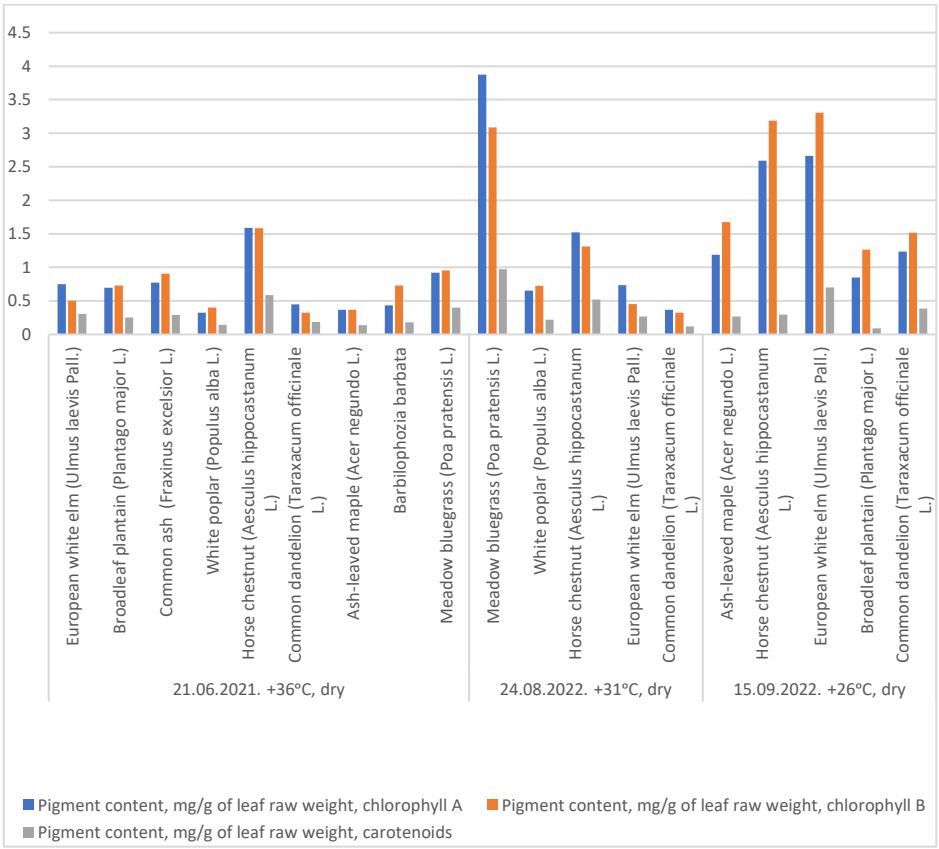

Fig. 1. Photosynthetic pigments content in the leaves of the Admiralteysky district plants under the weather anomalies of 2021-2024.

As it can be seen from the figure above, weather anomalies occurring at earlier vegetation stages have the most oppressive effect on the plants physiological state. For a number of plants even at earlier pheno-phases the predominance of chlorophyll B in the ratio of chlorophyll A to B, characteristic for later growing season stages, was observed [14]. Abnormal conditions falling at later stages have a negative impact of a lesser degree. However, the highest chlorophyll levels were shown under meteoanomalous conditions fell at the autumn season beginning (when normally defoliation occurs in most therophyllous species), indicating a shift of pheno-phases to later dates, which has its own risk at the onset of frosts with below zero temperatures and precipitation (snow).

The greatest stability of physiological functions was traced in horse chestnut (*Aesculus hippocastanum* L.), a species introduced from more southern latitudes, for which relict areal such seasonally atypical for St. Petersburg temperatures is a norm, and, accordingly, stable and resistant in such conditions [15].

Photosynthetic pigments levels under the 2024 weather anomaly conditions in leaves of plants of the Admiralteysky district green spaces and the protected buffer zone of the Yuntolovsky Sanctuary are compared in Figure 2 below.

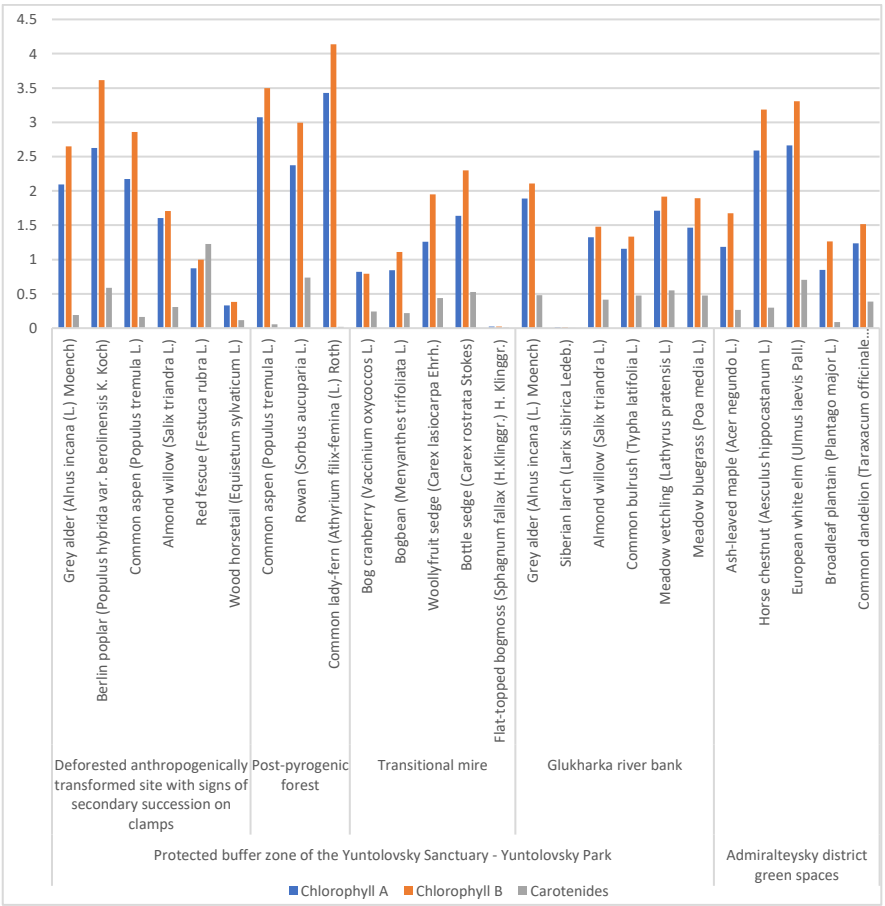

Fig. 2. Photosynthetic pigments levels in the leaves of the urban green spaces and the protected buffer zone of the Yuntolovsky Sanctuary plants under the weather anomaly of 2024.

There was no clear correlation between the growth sites of the studied plants and the degree of changes in the photosynthetic pigments content, which indicates the similar effect of the weather anomaly both on the green spaces of residential areas in the metropolis center in conditions of greater exposure to anthropogenic load, and on the plant communities of the protected nature area. However, the lowest values of photosynthetic pigments content were found in the vegetation of the transitional mire and the sample site established on the Glukharka river bank, characterized by the absence of tree layer and closed canopy.

The dynamics of changes in the photosynthetic pigments (total chlorophyll and carotenoids) content in 2021 and 2022 are shown in Figures 3-6.

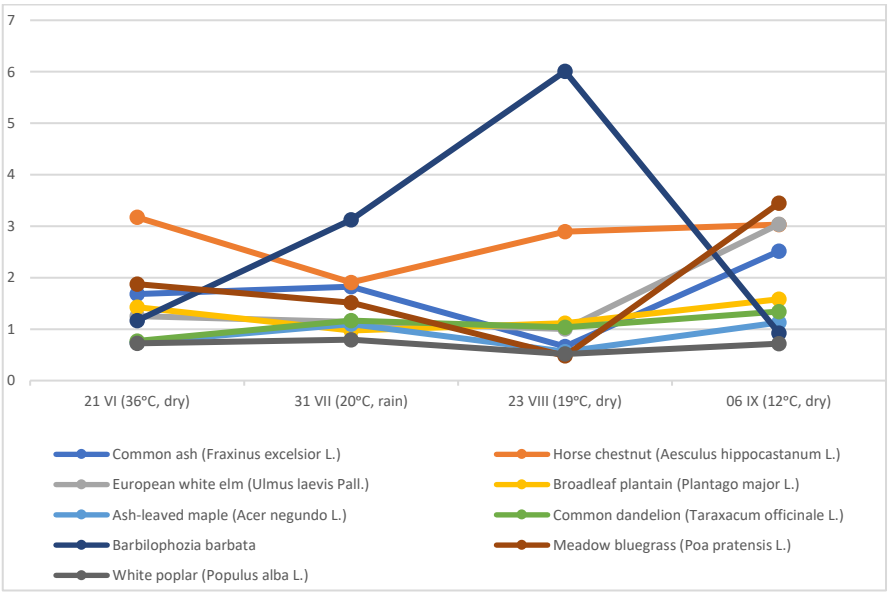

Fig. 3. Total chlorophyll in the weather dynamics of 2021.

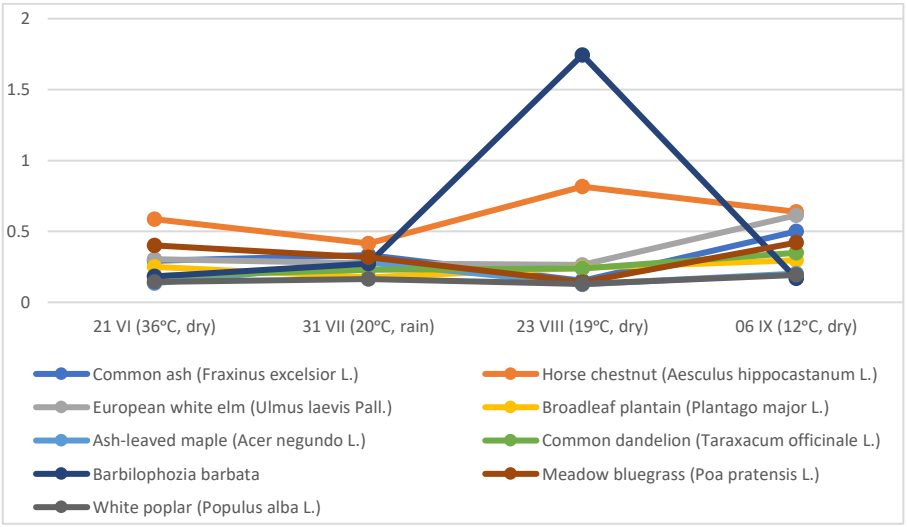

Fig. 4. Carotenoids in the weather dynamics of 2021.

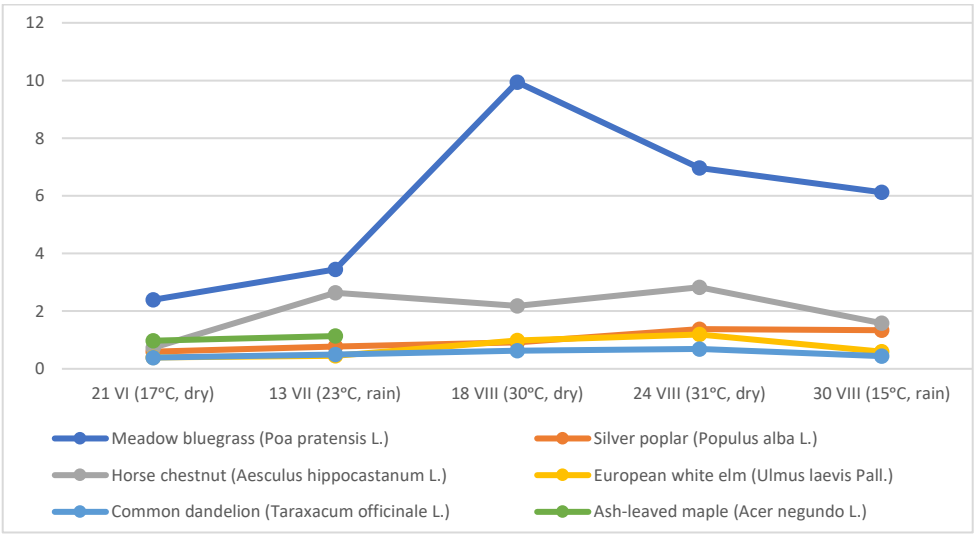

Fig. 5. Total chlorophyll in the weather dynamics of 2022.

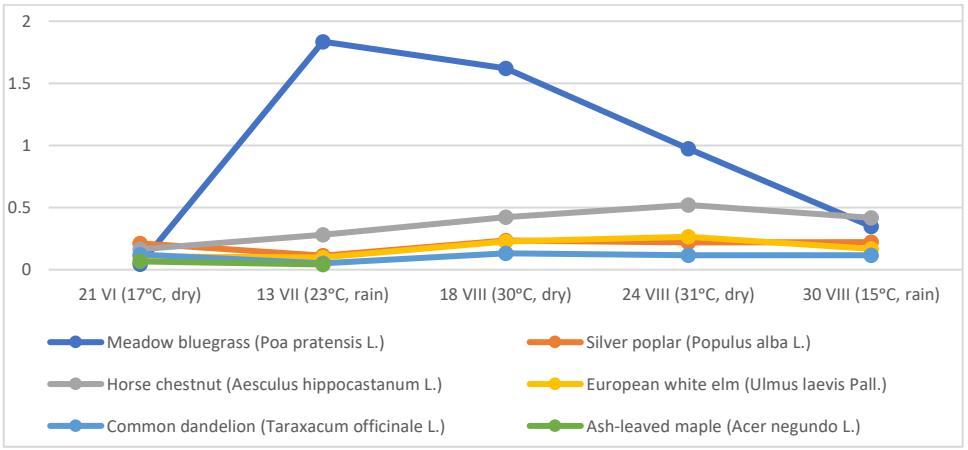

Fig. 6. Carotenoids in the weather dynamics of 2022.

4 Discussion

As consideration and comparison of photosynthetic pigments levels at the weather anomalies peaks and the dynamics of their changes after the end of the stress factor influence shows, meteorological anomalies occurring at earlier stages of vegetation most significantly affect the physiological functions of plants. A decrease in chlorophyll levels indicates a decline of plants photosynthetic activity, a parameter that is directly proportional to it, representing one of the most important ecosystem functions of plants, related to the carbon dioxide assimilation. Photosynthetic activity suppression, among other things, has the potential risk of CO_2 concentrations increasing in residential areas.

For a number of species under the stress conditions, an increase in the proportion of chlorophyll B to that of chlorophyll A not typical for early pheno-phases was observed, which is explained by a more intensive destruction of chlorophyll A under the elevated temperatures

[16]. Moreover, the percentage of carotenoids increased even in the first third of vegetation, indicating the deterioration of the plants physiological state.

In 2022, at the beginning of the growing season characterized by low and normal temperatures, the ratios of photosynthetic pigments did not correspond to the normal state, which may be related to the residual effects of experienced in 2021 stress; furthermore, some plants showed an early end of the vegetation with signs of desiccation, but the percentage of chlorophylls in the other plants increased towards the later pheno-phases, when the weather anomaly occurred, and their highest concentrations were recorded at its beginning, however, the period of abnormally high temperatures coupled with the precipitation absence was prolonged and chlorophyll A destruction was observed already in a week, but not as significant as in 2021, and taking into account the fact that this phenomenon fell on the second half of August – the end of vegetation, when chlorophyll A percentage normally the first of other chlorophylls begins to decrease, it can be seen that plants demonstrated greater resistance to the meteorological anomaly that occurred at the end of vegetation.

Under the conditions of the prolonged to later dates due to increased autumn temperatures growing season of 2024, high levels of chlorophyll B were observed in all studied plants, which is characteristic of late pheno-phases. The carotenoids present was abnormally low for the dates when defoliation normally occurs.

The highest chlorophyll concentrations were recorded under the conditions of weather anomaly, occurred at the end of vegetation and increased its duration practically without disturbance of physiological functions, traceable by the levels of photosynthetic pigments, despite the preceding of this stage to defoliation, which is always associated with the highest level of pollutants, inhibiting physiological processes, accumulated during the growing season in leaves.

5 Conclusion

Thus, based on the results of the study, it can be concluded that weather anomalies occurring at different stages of vegetation cause physiological changes of varying degrees, traceable by the levels of photosynthetic pigments in the higher plants leaves. The study of the behavior of chlorophylls and carotenoids in the leaves of St. Petersburg plants represented in two contrasting areas: the residential area loaded with motor vehicles and industry and the protected natural area, showed that weather anomalies have the similar effect on this parameter for different phytocenoses.

Weather anomalies occurring in the early stages of vegetation, when plants have not yet reached maximum stability, cause more significant changes in the content and ratios of photosynthetic pigments in leaves.

It was found that introduced species, due to the typicality of anomalous for St. Petersburg weather conditions (increased temperatures and precipitation deficit) for their relict areas, react to such meteoanomalies to a lesser extent.

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