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DYNAMICS OF FOLIAR CONCENTRATIONS OF PHOTOSYNTHETIC PIGMENTS IN WOODY AND HERBACEOUS PLANT SPECIES IN THE TERRITORY OF AN INDUSTRIAL CITY

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Background. Anthropogenic activities associated with environmental pollution have a negative effect on plants growing in urban ecosystems. Plant photosynthesis is one of the processes that are particularly affected by environmental conditions, including the presence of pollutants in the atmosphere and soil. However, the dynamics of photosynthetic pigments, namely chlorophylls (Chl) and carotenoids, in plant species growing in urban ecosystems have not been sufficiently studied. The aim of this study was to analyze the effect of technogenic load on the concentrations of pigments of photosynthesis in the cells of woody and herbaceous plants common in industrial cities, using the example of the urban ecosystem of Lviv located in the western part of Ukraine.

Materials and Methods. The study was carried out at four experimental sites within the city of Lviv with different levels and types of technogenic load. Site S1 selected in the central part of the Stryiskyi park was considered as a control one. Site S2 was chosen in an area with a combined technogenic load, including road and rail traffic, and the operation of the combined heat and power plant-1 (CHPP-1). Sites S3 and S4 were subjected mainly to vehicular traffic. Leaves of six plant species were collected at sites S1–S4, including woody plants (*Acer platanoides* L., *Aesculus hippocastanum* L. and *Tilia cordata* Mill.) and herbaceous plants (*Plantago major* L., *Taraxacum officinale* F. H. Wigg. and *Urtica dioica* L). The concentration of Chl *a*, Chl *b*, the total Chl concentration and the concentration of carotenoids were determined spectrophotometrically.

Results. The analyzed plant species growing at site S1 had the highest levels of total Chl and carotenoids compared to other sampling sites. In plant leaves collected at sites S2–S4, the total Chl concentration was 1.5–3.2 times lower than in the leaves collected at site S1. The ratio of Chl *a* and Chl *b* concentrations was generally lower in plants growing at sites S2–S4 compared to plants from site S1. The concentration of carotenoids in the leaves of woody plants collected from sites S2 and S4 was 1.4 and 2.4 times lower, respectively, compared with site S1, while in the leaves of herbaceous plants sampled at sites S2–S4, the concentration of carotenoids was 1.5–2.6 times lower than at site S1.

Conclusions. The results of the study suggest that the concentrations of both chlorophyll and carotenoids in the leaves of the analyzed herbaceous and woody plant species growing in the territory of an industrial city are influenced by anthropogenic impact associated with atmospheric pollution in the areas of plant growth. These indices are lower in plants growing in areas with technogenic load compared with plants growing in the green zone of the city. At the same time, the concentration of chlorophylls in the leaves of the analyzed plant species growing under urban conditions was found to be more sensitive to anthropogenic load compared to the foliar concentration of carotenoids. Therefore, the concentration of chlorophylls in plant species growing in cities can be one of the biomarkers for assessing the level of pollution caused by anthropogenic load in urban ecosystems.

Keywords: pigments, photosynthesis, chlorophyll, carotenoids, urban ecosystems, technogenic load, anthropogenic pollution

INTRODUCTION

Anthropogenic impact on the environment is accompanied by changes in the species composition and cellular metabolism in organisms inhabiting natural and artificial ecosystems. This is especially true for plants that grow in the territory of industrial cities. As it is known, urban ecosystems combine areas with different levels of technogenic load. Along with industrial zones and areas with high traffic intensity, there are so-called green zones in cities (including forest patches, parks and squares) inhabited by herbaceous and woody plants, and other biota groups (Hornung *et al*., 2018; Sonti *et al*., 2021). Industrial areas also contain green islands with street trees and other groups of vegetation. Green spaces in urban ecosystems play a vital role in providing oxygen to the atmosphere, maintaining local climate balance and beautification of the city, as well as removing atmospheric pollutants (Li *et al*., 2022; Wang *et al*., 2022).

However, plants, similarly to other biotic components of urban ecosystems, are adversely affected by anthropogenic factors, especially those associated with atmospheric and soil pollution. Plants growing in industrial zones and areas with heavy automobile traffic suffer from air dustiness and the presence of harmful substances in the environment (Hariram *et al.*, 2018). In particular, environmental pollution with sulfur and nitrogen dioxides, ozone, and heavy metals is often associated with inhibition of plant growth and productivity due to metabolic and physiological disturbances in plant cells (Weber *et al*., 1994; Vacek *et al.*, 2020). Plant photosynthesis belongs to the processes that are particularly influenced by environmental conditions (Wang *et al.*, 2019; Teng *et al.*, 2022).

Photosynthesis in plants is a globally important process that ensures carbon fixation, formation of organic matter and the release of molecular oxygen (Teng *et al.*, 2022). This process involves plastid pigments, primarily chlorophyll (Chl), which capture light energy and transfer it to other parts of the photosynthetic apparatus (Simkin *et al*., 2022). In higher plants, two main types of Chl are found, namely, Chl *a* and Chl *b* (Perez-Galvez *et al*., 2017; Zepka *et al*., 2019). Carotenoids are also involved in the process of photosynthesis, acting as accessory light-absorbing pigments (Hashimoto *et al*., 2016). Another important function of carotenoids in plant cells is their antioxidant function, which consists in the inactivation of reactive oxygen species (ROS) and protection of the components of photosynthetic apparatus against photodamage (Simkin *et al*., 2022).

Both chlorophylls and carotenoids are known to be highly sensitive to environmental factors, such as light intensity, temperature, humidity, as well as to anthropogenic pollution of atmospheric air (Giri *et al.*, 2013; Hazrati *et al.*, 2016; Teng *et al.*, 2022). The presence of toxic heavy metals and an excess of essential metals in the growth medium also negatively affects the content of photosynthetic pigments in plants (Li *et al.*, 2019; Zhang *et al*., 2020; Singh *et al*., 2021). However, the dynamics of photosynthetic pigments in plant species growing in urban ecosystems has not been sufficiently studied.

The aim of this study was to analyze the effect of technogenic load on the concentrations of pigments of photosynthesis in the cells of woody and herbaceous plants common in industrial cities, using the example of the urban ecosystem of Lviv located in the western part of Ukraine.

MATERIALS AND METHODS

Study area. The study was carried out in the city of Lviv using four experimental sites for sampling plants; the sites were characterized by different levels and types of technogenic load. Sampling site No. 1 (S1, 49°49′24.0″N 24°01′30.0″E) was selected in the central part of Stryiskyi Park with an area of 52.1 hectares located in the southern part of Lviv. There are no sources of technogenic pollution on the territory of the park; therefore, site S1 has been used as a control. Sampling site No. 2 (S2, 49°48′39.0″N 24°01′43.7″E) was chosen in the area of Luhanska Street loaded with automobile traffic. In addition, this area is subjected to the influence of the railway with rail traffic and the operation of the combined heat and power plant-1 (CHPP-1). Sampling site No. 3 (S3, 49°46′39.5″N 24°00′48.5″E) was selected at the end of Stryiska Street, one of the main thoroughfares of the city of Lviv, and sampling site No. 4 (S4, 49°50′03.0″N 24°04′02.5″E) was chosen in the vicinity of Pasichna Street with heavy traffic (daily load 1189±122 vehicles per 1 hour) (Polishchuk *et al.*, 2020).

Analysis of plant material. Six plant species belonging to flowering plants (Magnoliopsida) were used in the study. Among these, three species including *Acer platanoides* L. (Norway maple), *Aesculus hippocastanum* L. (horse chestnut) and *Tilia cordata* Mill. (small-leaved linden) belong to woody plants, and the other three belong to herbaceous plants, such as *Plantago major* L. (common plantain), *Taraxacum officinale* F. H. Wigg. (common dandelion) and *Urtica dioica* L. (stinging nettle). These plant species are widespread in the study area and are characteristic of the urbanized environment and anthropogenically modified landscapes (Otte & Wijte, 1993; Deljanin *et al.*, 2015; Nadgórska-Socha *et al.*, 2017; Lisiak-Zielińska *et al.*, 2021).

Plant material for experiments was sampled twice during the period from September 7 to October 7, 2021. Namely, the collecting of plant material from sites S1–S4 was carried out at a close time period during September, and then repeated at the beginning of October. From tree species, medium-sized leaves were collected from the accessible part of the crown, and from herbaceous plants, leaves of the middle tier of vegetative shoots were collected. The herbaceous plants sampled for the experiments had relatively similar growth conditions, including illuminance levels and temperatures. The average daytime temperature during sampling in September was 21°C, while during sampling in October it was 17°C. The Illuminance levels were in the range of 40,000–80,000 lx. The Illuminance levels were measured using the WT81 luxmeter (Wintact, China).

The sampled plant material was prepared according to generally accepted methods. Pieces of leaves (1 cm^2) were cut from the central parts of fresh leaf blades, crushed, and extracted with 96% ethanol in the dark. After filtering the mixture, the concentration of chlorophylls *a* and *b*, the total chlorophyll content and the concentration of carotenoids were determined spectrophotometrically according to the method elaborated by H. K. Lichtenthaler and A. R. Wellburn (1983). The absorbance of the pigment solution was measured using the ULAB-102 spectrophotometer (China) at 470, 649 and 665 nm. Pigment concentrations were calculated using the formulas 1–3 (Lichtenthaler & Wellburn, 1983) as follows:

$$C_{Chl\ a} = 13.95A_{665} - 6.88A_{649} \quad (1)$$

$$C_{Chl\ b} = 24.96A_{649} - 7.32A_{665} \quad (2)$$

$$C_{car} = (1000A_{470} - 2.05\ C_{Chl\ a} - 114.8\ C_{Chl\ b})/245 \quad (3),$$

where $C_{Chl\ a}$ was the concentration of chlorophyll *a*, $C_{Chl\ b}$ was the concentration of chlorophyll *b*, C_{car} was the concentration of carotenoids in the pigment extract. The sum of the concentrations of Chl *a* and Chl *b* was considered as the total chlorophyll concentration. The final concentrations of pigments were expressed in μg per 1 g fresh weight (FW). Measurements were carried out in 3–5 replicates. The data obtained from the analysis of the plant material collected from the same sampling sites during September and October were combined and processed. The results were processed using the methods of variation statistics (Welham *et al.*, 2015).

RESULTS AND DISCUSSION

The data shown in **Fig. 1** demonstrate the dynamics of the total Chl concentrations in plant material collected at different sampling sites within the territory of the city of Lviv. It can be seen that the analyzed plant species growing at site S1 (Stryiskyi Park) possess the highest levels of total Chl compared to other sampling sites. This was especially true for tree species (*A. platanoides*, *A. hippocastanum* and *T. cordata*), which had higher foliar Chl concentrations in comparison with herbaceous plant species analyzed in this study. The overall values of total Chl concentration ranged from 1372±180 μg/g FW (in *A. platanoides* leaves) to 454±47 μg/g FW (in *P. major* leaves). However, the total foliar chlorophyll concentrations in the herbaceous plant species were somewhat lower compared to the values recorded in other studies (Znidarčič *et al.*, 2011; Loh *et al.*, 2012; Godlewska et al., 2020). This may be due to the fact that the studies were carried out at the beginning of the autumn period, when plants are generally characterized by a reduced level of chlorophyll in the leaves (Mattila *et al.*, 2018; Donnelly *et al.*, 2020).

In tree leaves collected in the areas of intense technogenic load (sites S2–S4), the total Chl concentrations were 1.7–2.8 times lower than in the leaves collected at site S1 ($p<0.01$–0.001). Similar dynamics of the total Chl concentration are observed in the leaves of herbaceous plant species sampled at sites S1–S2. Namely, in the leaves of *U. dioica* collected from sites S2–S4, the total concentrations of Chl were 1.5–1.7 times lower than the values recorded at site S1 ($p<0.05$–0.001); in the leaves of *T. officinale* sampled at sites S2–S4, the total Chl concentrations were 1.9–3.2 times lower than at site S1 ($p<0.01$–0.001), and in the leaves of *P. major*, the total Chl concentrations at sites S2–S4 were reduced 2.0–2.2-fold compared to the values recorded at site S1 ($p<0.01$).

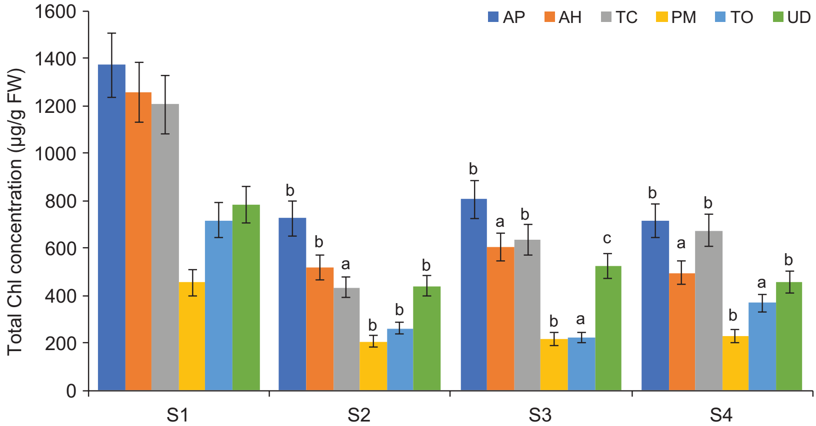

Fig. 1. Total chlorophyll concentration in the leaves of plant species sampled at sites S1–S4 with different levels of technogenic load in the city of Lviv during the period from September 7 to October 7, 2021
Comments: 1) in this and the following figures, the abbreviations are as follows: AP – *Acer platanoides*, AH – *Aesculus hippocastanum*, TC – *Tilia cordata*, PM – *Plantago major*, TO – *Taraxacum officinale*, UD – *Urtica dioica*; 2) letters *a*, *b* and *c* indicate the significance levels of differences between the values obtained in plants sampled at sites S2-S4, as compared to site S1 (*a* – $p<0.001$; *b* – $p<0.01$; *c* – $p<0.05$)

The data obtained indicate an inverse relationship between the total Chl concentration in the leaves of plant species and the level of technogenic pressure on the territory of plant growth. The most pronounced decrease in the total Chl concentration was observed in the plants collected from the site S2 compared to the plants sampled at site S1. Due to its location, site S2 experiences a high level of technogenic load, being subjected to combined pollution from CHPP-1, road and rail transport. It can be assumed that emissions from transport activities and the operating CHPP-1 have a detrimental effect on the photosynthetic apparatus of plants growing in the area, as a result of the induction of oxidative stress in plant cells. A number of studies have shown that air pollutants such as sulfur dioxide (SO_2), nitrogen dioxide (NO_2), ozone and heavy metals taken up by plant cells induce the formation of ROS and stimulate lipid peroxidation process (Li & Yi, 2012; Shahid *et al.*, 2014; Yue *et al.*, 2020; Sheng *et al.*, 2021). At the same time, ROS exposure causes chlorophyll degradation, inhibition of chlorophyll synthesis enzymes, and other deleterious effects in plants (Asada, 1994; Aarti *et al.*, 2006). In particular, a decrease in the concentration of Chl in plants has been reported under several conditions associated with atmospheric pollution (Thawale *et al.*, 2011; Hariram *et al.*, 2018; Mukherjee *et al.*, 2019; Banerjee *et al.*, 2021), as well as under influence of individual air polluting substances, including SO_2 (Mohasseli *et al.*, 2017; Duan *et al.*, 2019), NO_2 (Yue *et al.*, 2020) and ozone (Singh *et al.*, 2012; Xu *et al.*, 2022).

The results of calculating the ratio of Chl *a* and Chl *b* concentrations suggest that this index is generally lower in the analyzed plant species growing in the areas with anthropogenic load (sites S2–S4) compared to plants from site S1 (**Fig. 2**). In particular, Chl *a*/Chl *b* ratio in plant leaves sampled at site S1 was found to be in the range of 3.1–3.6, at site S2 this index was in the range of 2.3–2.8, and at sites S3–S4 it was in the range of 2.3–3.1. These data indicate a reduced concentration of both Chl *a* and Chl *b* in plants growing in the areas with technogenic load, with the decrease of Ch *a* concentration being more pronounced than concentration of Chl *b*.

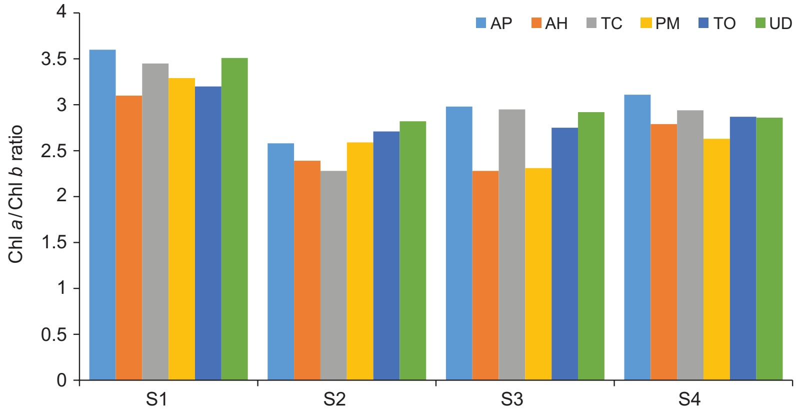

Fig. 2. Chl a/Chl b concentration ratio in the leaves of plant species sampled at sites S1–S4 with different levels of technogenic load

Figure 3 shows the dynamics of the concentration of carotenoids in the leaves of the analyzed plant species. The results of the study indicate that, similarly to the content of Chl, the concentration of carotenoids in the plant material collected from sites S2–S4 tends to decrease as compared to site S1. At the same time, the decrease in foliar carotenoids concentration in plants collected from the areas with anthropogenic load was less pronounced than the decrease in the total concentration of Chl. In particular, the concentration of carotenoids in the leaves of all three woody plants collected at site S3 did not change compared to site S1; however, in the leaves of trees collected from site S2, this indicator was 1.8–2.4 times lower compared to site S1 ($p<0.05$–0.001). In the leaves of *A. platanoides* and *A. hippocastanum* sampled at site S4, the concentration of carotenoids was 1.4 and 1.8 times lower, respectively, compared with site S1 ($p<0.05$).

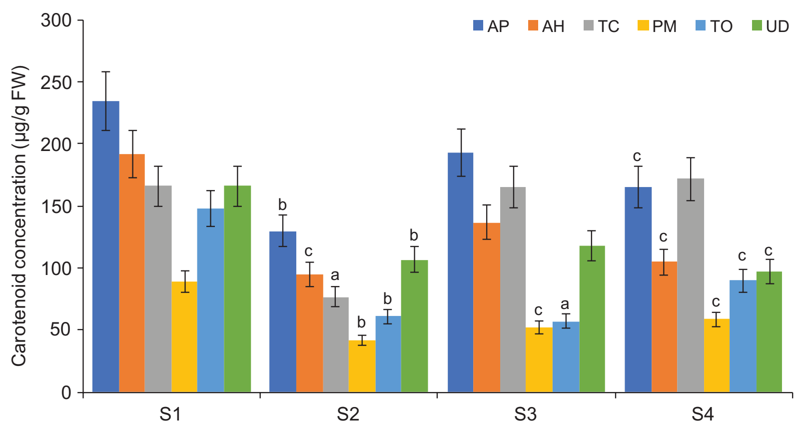

Fig. 3. Concentration of carotenoids in the leaves of plant species sampled at sites S1–S4 with different levels of technogenic load
Comment: letters *a*, *b* and *c* indicate the significance levels of differences between the values obtained in plants sampled at sites S2–S4, as compared to the site S1 (*a* – $p<0.001$; *b* – $p<0.01$; *c* – $p<0.05$)

Among the analyzed species of herbaceous plants, *U. dioica* had the most stable concentration of carotenoids in leaves. Namely, the concentration of carotenoids did not change significantly in the leaves of *U. dioica* collected from site S3, but it was 1.6 and 1.7 times lower in the leaves sampled at sites S2 and S4, respectively, compared with site S1. In the leaves of *T. officinale* and *P. major* sampled at sites S2–S4, the concentration of carotenoids was 1.5–2.6 times lower than at site S1 ($p<0.05$–0.001).

The decrease in the concentration of foliar carotenoids in plants growing in areas with technogenic load within the city can be explained by an increased degradation of pigments under the influence of air pollutants (Hariram *et al.*, 2018). In general, data on the concentration of carotenoids in the leaves of plant species growing within the city of Lviv suggest that this parameter is less sensitive to anthropogenic load compared to the concentration of leaf chlorophylls.

CONCLUSIONS

The concentration of photosynthetic pigments in plant cells is a sensitive indicator of the impact of environmental conditions on plant metabolism. The results of this study suggest that the concentrations of both chlorophyll and carotenoids in the leaves of the analyzed herbaceous and woody plant species growing in the territory of an industrial city are influenced by anthropogenic impact associated with atmospheric pollution in the areas of plant growth. These indices are lower in plants growing in the areas with technogenic load compared with plants growing in the green zone of the city. At the same time, the concentration of chlorophylls in the leaves of the analyzed plant species growing under urban conditions was found to be more sensitive to anthropogenic load compared to the foliar concentration of carotenoids. Therefore, the concentration of chlorophylls in plant species growing in cities can be one of the biomarkers for assessing the level of pollution caused by anthropogenic load in urban areas.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest: The authors declare that the study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

Animal rights: This article does not include animal studies.

AUTHOR CONTRIBUTIONS

Conceived and planned the experiments [A. I. P. & H. L. A.]; conducted experiments and data collection [A. I. P.]; both authors analyzed and discussed the results and contributed to the final manuscript.

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ДИНАМІКА КОНЦЕНТРАЦІЇ ПІГМЕНТІВ ФОТОСИНТЕЗУ В ЛИСТКАХ ДЕРЕВНИХ І ТРАВ'ЯНИХ РОСЛИН НА ТЕРИТОРІЇ ПРОМИСЛОВОГО МІСТА

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Обґрунтування. На рослини, що ростуть у міських екосистемах, негативно впливає антропогенна діяльність, пов'язана із забрудненням навколишнього середовища. Фотосинтез є одним із процесів у рослин, на який особливо впливають умови навколишнього середовища, зокрема, наявність забруднювальних речовин в атмосфері та ґрунті. Проте динаміка пігментів фотосинтезу, хлорофілів і каротиноїдів у рослин, що ростуть у міських екосистемах, з'ясована недостатньою мірою. Метою цього дослідження було проаналізувати вплив техногенного навантаження на концентрацію пігментів фотосинтезу в листках деревних і трав'яних рослин, розповсюджених у промислових містах, на прикладі урбоекосистеми Львова, розташованої в західній частині України.

Матеріали і методи. Дослідження проводили на чотирьох дослідних ділянках у межах м. Львова з різними рівнями та видами техногенного навантаження. Контрольною вважали ділянку S1, вибрану в центральній частині Стрийського парку. Ділянку S2 обрали в районі з комбінованим техногенним навантаженням, зумовленим рухом автомобільного і залізничного транспорту та роботою ТЕЦ-1. Ділянки S3 і S4 зазнавали техногенного навантаження, переважно зумовленого автомобільним рухом. На ділянках S1–S4 проводили збір листя шести видів рослин, включаючи деревні рослини (*Acer platanoides* L., *Aesculus hippocastanum* L., *Tilia cordata* Mill.) і трав'яні рослини (*Plantago major* L., *Taraxacum officinale* F. H Wigg., *Urtica dioica* L). Концентрацію хлорофілів *a* і *b*, загальну концентрацію хлорофілу та концентрацію каротиноїдів визначали спектрофотометричним методом.

Результати. Найбільшу концентрацію хлорофілів і каротиноїдів виявлено у листках досліджуваних видів рослин, зібраних на ділянці S1, порівняно з іншими місцями відбору проб. У листках рослин, що росли на ділянках S2–S4, загальна концентрація хлорофілів була у 1,5–3,2 разу нижчою порівняно з листками, зібраними на ділянці S1. Співвідношення між концентраціями хлорофілу *a* і хлорофілу *b* було загалом меншим у листках рослин, що росли на ділянках S2–S4, порівняно з рослинами ділянки S1. Концентрація каротиноїдів у листках деревних рослин, зібраних із ділянок S2 та S4, була, відповідно, в 1,4 і 2,4 разу меншою порівняно з ділянкою S1, а в листках трав'яних рослин із ділянок S2–S4 концентрація каротиноїдів була меншою в 1,5–2,6 разу порівняно з аналогічними показниками ділянки S1.

Висновки. Результати дослідження свідчать про те, що на концентрацію хлорофілу і каротиноїдів у листках проаналізованих видів трав'яних і деревних рослин, поширених на території промислового міста, впливають антропогенні чинники, пов'язані з забрудненням атмосферного повітря. Ці показники нижчі у рослин, що ростуть на територіях з техногенним навантаженням, порівняно з рослинами, що ростуть у зеленій зоні міста. Водночас концентрація хлорофілів у листках досліджуваних видів рослин у міських умовах була більш чутливою до антропогенного навантаження порівняно з концентрацією каротиноїдів. Відповідно, концентрація хлорофілів у листках рослин на території міст може бути одним із біомаркерів для оцінки рівня забруднення, зумовленого антропогенним навантаженням у міських екосистемах.

Ключові слова: пігменти, фотосинтез, хлорофіл, каротиноїди, урбоекосистеми, техногенне навантаження, антропогенне забруднення

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