Conference Paper

Leaf Mesophyll Structure and Photosynthetic Activity in *Calla palustris* L. from Natural Habitats with Different Level of Technogenic Pollution

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Abstract

A study of leaf structure and CO_2 assimilation rate was conducted on the emergent plant *Calla palustris* L. from natural habitats with different levels of technogenic pollution (the Sak-Elga River, upstream and downstream of the Karabash copper smelter, Chelyabinsk region, Russia). It was found that both chlorophyll *a* content and the rate of CO_2 assimilation decreased twofold in plants from the downstream site. No significant changes were observed in leaf mesophyll structure and the volume of aerenchyma in the leaf. It was shown that in plants from strongly contaminated site, the decrease in spongy mesophyll cell volume was compensated by the increase in their number, whereas the decrease in the number of chloroplasts per cell was accompanied by a growth in volume. It is concluded that the changes in the numerical and dimensional characteristics of mesophyll cells and chloroplasts provide for the viability of *C. palustris* under prolonged technogenic impact and demonstrate the plasticity of the photosynthetic apparatus.

Keywords: emergent plant, heavy metals, adaptation, leaf structure, CO_2 uptake

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1. Introduction

Photosynthesis is the most important function of plants, providing them with the energy and metabolites necessary for growth, development and performing all other functions, including maintaining homeostasis and adapting to environmental conditions. Under adverse conditions, the adaptive rearrangements of all life-supporting systems, including photosynthetic apparatus, can occur in plants that provides for optimal functioning [1, 2]. However, the changes in leaf mesophyll structure and functional activity under conditions of prolonged technogenic impact to which plants are exposed *in situ* near emitting pollutants enterprises have not been studied enough.

The aim of our study was to assess the changes in leaf structure and level of CO_2 uptake in the emergent plant *Calla palustris* L. from natural habitats with different levels of technogenic pollutions.

2. Methods

Calla palustris L. from the Araceae family is a holarctic boreal species widespread in Eurasia and North America. It is a rhizomatous herbaceous perennial plant belonging to the helophytes [3]. The plant material was collected in July 2016 and 2017 (in the period of blooming) on the Sak-Elga River, upstream and downstream of the Karabash copper smelter (KCS), Chelyabinsk region (Southern Urals, Russia). The territory adjacent to KCS has been declared a zone of ecological disaster. Copper smelting is the cause of pollution of the nearby area through dust emissions, acid gases and wastewater discharge. In addition to sulfur dioxide and its soluble form (sulfurous acid, which causes the strong acidification of surface water), the territory and nearby water bodies are contaminated by metals (copper, cadmium, iron, nickel, lead, zinc, etc.) [4, 5].

Heavy metal (HM) content in water, sediments and leaves of *C. palustris* was determined using a graphite furnace and an inductively coupled plasma atomic-emission spectrometer (ICP-AES, iCAP 6500 Duo, Thermo Fisher, USA) after wet digesting with 70% HNO_3(analytical grade). The pH and electrical conductivity of water were measured using a portable pH meter/conductometer ('Hanna Instruments', Germany).

Based on the physicochemical characteristics of the surface water and sediments, the two sites were distinguished according to the degree of toxic load: low contaminated (the Sak-Elga River upstream, 3 km above the KCS, 55.4466° N, 60.1685° E) and strongly contaminated (the Sak-Elga River downstream, 2.6 km below the KCS, 55.4456° N, 60.2256° E). The total toxic load index (*Si*) was calculated as in (1):

$$Si = (1/n)\Sigma(S_{sc}/S_{lc})_i \qquad (1)$$

where S_{sc} is the analyzed i^{th} metal concentration in the water or sediments of the strongly contaminated site, S_{lc} is the concentration of i^{th} metal in the low contaminated site, *n* is the number of studied metals [6].

Quantitative traits of leaf mesophyll were measured according to Khramtsova et al., 2003 [1]. The average leaf sample was taken from 10–15 plants at each studied site. Transverse sections of the leaves were obtained using a freezing microtome MZ-2

(Russia). All measurements were carried out using the SIAMS MesoPlant program (Russia) and the light microscope Meiji MT 4300L ('Meiji Techno', Japan).

The content of chlorophylls (*a*, *b*) and carotenoids was determined using a UV-visible spectrophotometer (PD303-UV 'Apel', Japan) in 80% acetone extracts according to Lichtenthaler [7]. The rate of CO_2 uptake was measured with an infrared gas analyzer LI-6400XT ('LI-COR', USA) at a saturating light intensity of 1600 μM/(m^2 s), temperature of +23 °C and humidity of 50%.

The content of metals (in the water, sediments and leaves), pigments and the intensity of photosynthesis were determined in 4 replicates. Measurements of leaf structure traits were carried out in 30 replicates. Mean values and standard errors (SE) are presented in the figures and tables. The significant difference between the means was determined by the non-parametric Mann–Whitney *U-test* at $P < 0.05$ (Statistica 7.0) and marked with asterisks.

3. Results

The value of *Si*, calculated for Cd, Pb, Hg, Ni, Cu, Sr, M, Zn, Fe and Mn, was 41 relative units for water and 16 relative units for sediments at the strongly contaminated site (the Sak-Elga River downstream).

Prolonged technogenic pollution caused a 5-fold increase in electrical conductivity and a decrease in water pH in the Sak-Elga River from 6.8 (upstream of KCS) to 5.4 (downstream of KCS). The metal content in *C. palustris* leaves was significantly higher at the strongly contaminated site (Figure 1); for example, the content of Sr was 5.4 times higher, Cu and Co – 2.0 times, Pb – 1.8 times, Zn, Mg and Mn – 1.6 times and Cd – 1.2 times compared to the low contaminated site. The exception was for Ni and Fe, the content of which in the leaves of *C. palustris* did not change downstream of KCS.

The study of leaf anatomy showed the dorsoventral mesophyll type in *C. palustris*, which has also been confirmed by [8, 9]; so mesophyll was performed by palisade and spongy cells and airenchyma was well developed. Plants from the strongly contaminated site had a thinner leaf blade due to reductions in the epidermis thickness (by 25%), see table. Airenchyma volume in leaves did not differ in plants from both sites.

In *C. palustris* growing downstream of KCS, the number of mesophyll cells per unit leaf area was significantly higher both in palisade (30%) and spongy (18%) tissues. At the same time, the volume of palisade cells did not differ significantly (Table 1), while the volume of spongy cells decreased (by 35%) compared to the site upstream of KCS.

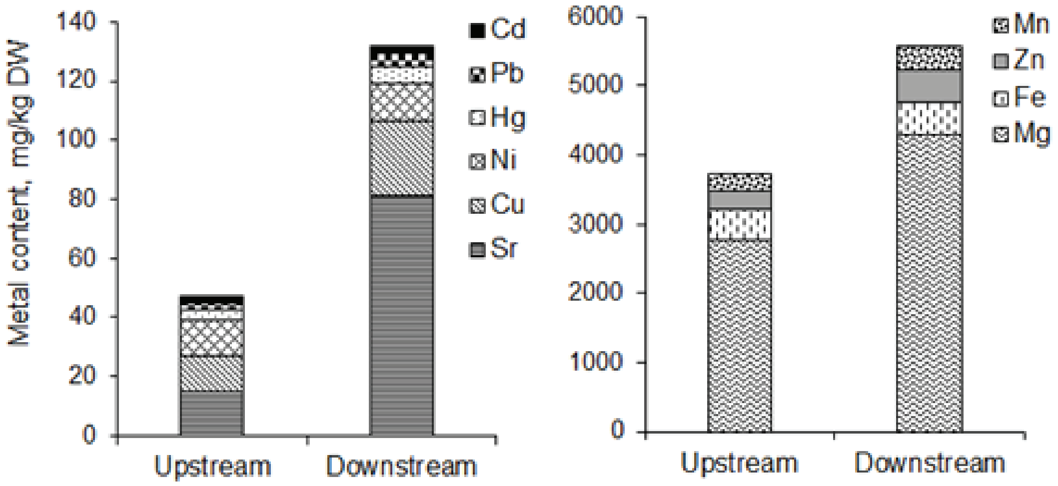

Figure 1: The content of metals in the leaves of *C. palustris* growing on the Sak-Elga River upstream and downstream of the Karabash copper smelter. Source: Authors' own work.

The number of chloroplasts per cell was significantly lower (by 14%) in plants from the strongly contaminated site and did not change when calculated per unit leaf area. There was a tendency for the number of chloroplasts per cell in *C. palustris* leaves from the site downstream of KCS to increase, but the difference was insignificant.

Table 1: Structural traits of mesophyll and CO_2 assimilation rate in *C. palustris* from the Sak-Elga River upstream and downstream of the Karabash copper smelter.

Characteristics		**Upstream of KCS**	**Downstream of KCS**
Leaf thickness, μm		370.9 ± 7.2	311.3 ± 8.0*
Epidermis thickness, μm		70.2 ± 4.2	52.4 ± 3.5*
Airenchyma volume in the leaf, %		27.6 ± 1.6	27.6 ± 1.1
Number of mesophyll cells, $10^3/cm^2$	Palisade	262.4 ± 9.5	340.2 ± 13.7*
	Spongy	370.7 ± 15.3	435.8 ± 12.8*
Volume of mesophyll cells, 10^3 $μm^3$	Palisade	15.2 ± 1.4	14.2 ± 1.2
	Spongy	23.7 ± 2.5	15.3 ± 1.5*
Number of chloroplasts per cell	Palisade	22.0 ± 1.0	18.0 ± 1.0*
	Spongy	20.0 ± 1.0	18.0 ± 1.0*
Number of chloroplasts, $10^6/cm^2$	Palisade	5.7 ± 0.2	6.0 ± 0.2
	Spongy	7.5 ± 0.3	7.6 ± 0.2
Volume of chloroplast, $μm^3$	Palisade	63.5 ± 4.8	68.0 ± 4.4
	Spongy	74.4 ± 5.0	77.0 ± 7.5
Total chlorophyll content in chloroplast, mg/10^9		1.7 ± 0.1	1.4 ± 0.1
CO_2 uptake	μmol/(m^2 $_*$ s)	3.7 ± 0.5	2.0 ± 0.2*
	μmol/(g chlorophyll $_*$ s)	16.9 ± 2.0	10.8 ± 1.4*
	μmol/(10^{12} chloroplast $_*$ s)	28.3 ± 4.0	14.7 ± 1.9*
Source: Authors' own work.			

The chlorophyll *a* (Chl *a*) content in plants from the strongly contaminated site was significantly lower than those from the low contaminated habitat (by 18% per dry weight, Figure 2(A)). The content of chlorophyll *b* (Chl *b*) and carotenoids (CAR) did not change. A similar trend was observed for pigment content calculated per leaf area unit. The average amount of chlorophyll per chloroplast was lower in *C. palustris* from the strongly contaminated site (Table 1) compared from low contaminated site, but the difference was not reliable.

According to [9], the ratio Chl *a/b* can vary from 2.7 to 4.3 in plants with emergent leaves. According to our data, this ratio in *C. palustris* leaves was about 2.3, and there was no significant difference between the studied sites (Figure 2(B)). The ratio of the total chlorophyll content to carotenoids in plants downstream of KCS was significantly lower (by 17%) compared to the site upstream of KCS, which is explained by a substantial decrease in Chl *a* content and by the unchanged content of Chl *b* and carotenoids.

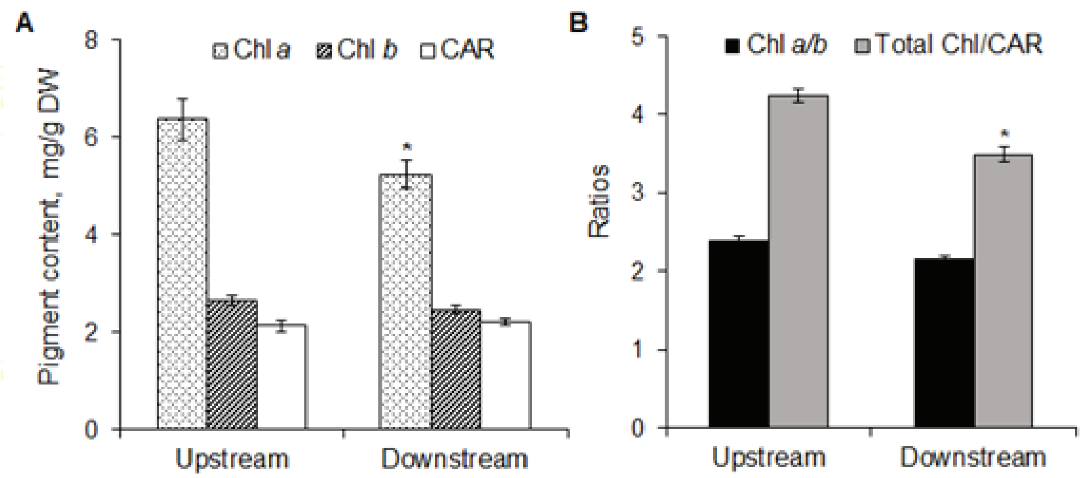

Figure 2: The content of photosynthetic pigments (A) and their ratio (B) in leaves of *C. palustris,* growing in the Sak-Elga River upstream and downstream of the Karabash copper smelter. Source: Authors' own work.

It is known that an excess of HM can cause structural and ultrastructural changes in chloroplasts through the peroxidation of membrane lipids [2]. Excess metals in cells can not only inhibit the key enzymes of chlorophyll synthesis, but also directly cause destruction of the pigment molecules [2, 10]. In addition, some metals have an inhibitory effect on both light and dark photosynthetic reactions. Among HM, mercury, cadmium, lead and copper are the most phytotoxic in high doses. They disrupt the transport of electrons in different parts of the electron transport chain of chloroplasts [10]. Excess HM can suppress RUBISCO activity, reducing carboxylase and increasing the oxygenase function of this enzyme [2].

It was found that the rate of carbon dioxide assimilation in the *C. palustris* leaves was significantly lower at the impact site, both per unit area and per chloroplast (almost 2 times), as well as per g of chlorophyll (1.6 times), see Table 1. Perhaps the reason for this fall in photosynthesis is not only the decrease in the amount of Chl *a*, but also the inhibition of RUBISCO. Since there were no significant changes in mesophyll leaf structure, the volume of the airenchyma was revealed. Consequently, the mesophyll conductivity for CO_2 should be the same in plants from both sites. The limitation of carbon dioxide diffusion through stomata is also unlikely, since this species belongs to helophytes and always has access to water.

4. Conclusion

During prolonged technogenic impact, plants are forced to adapt to a new environment. Acquired changes could affect the anatomical, morphological, and the functional characteristics of plants. One of the typical reactions of the photosynthetic apparatus to environmental contamination is the decrease in chlorophyll content and the rate of CO_2uptake, which was also confirmed by our studies. Besides the decrease in the spongy mesophyll cell volume and the increase in their number, a decrease in the number of chloroplasts per cell and an increase of their volume have been shown in plants from the strongly contaminated site when compared to the low contaminated site. The observed changes in mesophyll tissues could be considered as compensatory and indicate adaptive transformations of *C. palustris* leaves upon contamination of the aquatic environment by heavy metals. Thus, the changes in the numerical and dimensional characteristics of mesophyll cells and chloroplasts provide for the viability of *C. palustris* under a high level of technogenic pollution and demonstrate the plasticity of the photosynthetic apparatus.

Conflict of Interest

The authors declare no conflict of interest.

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