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The Impact of SO_2 and NO_2 Industrial Emissions on Anatomical Stem of *Salix alba*

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ABSTRACT

The paper examines the influence of industrial emissions SO_2 and NO_2 on the anatomical structure of the annual shoot stems of *Salix alba* L. in conditions of steppe zone of Ukraine. It reveals high stability of histological characteristics of the studied type to phytotoxic pollutants, which was proved by thickening of almost all anatomical stem parameters. It was ascertained the toxic gases chronic effect results in increasing the thickness of the stem cortex in the research object through the increase of the primary cortex where collenchyme and parenchyma grow thicker, and through thickening the secondary cortex as hard bast indices rise. The study proved that toxic gases do not affect the wood radius and pith diameter, though the biggest trachea diameter increases significantly. It has shown the thickening of both primary cortex and secondary bast which contribute to the change of that the diameter of annual shoot stems of *S. alba* in conditions of technogenesis. The high adaptive capacity of anatomical indicators of this his plant species stem to industrial pollution has been demonstrated, and therefore it was suggested that *S. alba* can be used for planting the areas subject to chronic actions SO_2 and NO_2.

1. INTRODUCTION

Technogenic pollution is a powerful factor that leads to the degradation and de forestation worldwide [1, 2]. Ukraine is one of the most ecologically disadvantaged countries in Europe [3]. The highest level of contamination is observed in the Prydniprov'ya region. The city of Dnipropetrovsk is its powerful industrial center. Plants and works of metallurgical, machine-building, chemical, power and other industries are concentrated in the city. Every year they emit millions of tons of toxic substances into the atmosphere, soil and water bodies [4] which have detrimental effect on the living organisms. Planting and establishing green spaces around the industrial areas and along the highways are the measures that are of primary importance in the system of environment protection [5].

Woody vegetation effectively acts as the "lungs" of the city, purifying air from harmful substances. However, the plants themselves undergo constant anthropogenic pressure, which affects their growth and development, aesthetics appearance and fertility [3, 6, 7]. The geographical location of Dnipropetrovsk effect in the steppe zone of Ukraine exacerbates the ongoing situation, as there is an enormous discrepancy between conditions needed to forest ecosystems and the real geographical and environmental conditions. Besides the anthropogenic loading, woody vegetation has to adapt to the complex unfavorable environmental conditions such as drought, strong dry winds, freezing winters and hot summers. Therefore, the creation of urban forest ecosystems for environmental protection requires the selection of tree species selection taking into account both their resistance to toxic gases, heavy metals, dust and other anthropogenic ingredients and their ability to adapt to adverse abiotic factors of the environment.

The use of ecological anatomical method is crucial to the study of nature of the plant adaptive responses to extreme environmental factors [8, 9]. It gives an idea of mutual subordination of plant organism parts required to establish the relationship between a structure and its function [9]. In the ecological plant physiology histological and histochemical changes make it possible to determine the location and pathways of toxic pollutants to plant tissues and organs [8]. Anatomical plant indices can also be used as technogenic pollution bioindicators and sensitive test parameters in studies monitoring the state of plants in contaminated areas [10, 11].

However, today the chronic impact of industrial emissions of sulfur oxides (IV) and nitrogen (IV) on the anatomical structure of the stem of woody species has not been thoroughly studied. There is little research devoted to the *Salix* L. genus ecological and anatomical studies under conditions of anthropogenic territories of the steppe zone of Ukraine. The present paper aims to analyze the impact of SO_2 and NO_2 pollution on histological parameters *Salix alba L.* stems in conditions of Prydniprov'ya region.

2. MATERIALS AND METHODS

Monitoring points

Storage plant material was collected in September 2013 in two monitoring points. The pilot (contaminated) area is located 2 km from the plant "Dnipropress", the city of Dnipropetrovsk (Ukraine), where the average concentration rates of SO_2 was 0,29 mg/m^3, and NO_2 - 0,24 mg/m^3. The reference area (relatively clean) is located in the Botanical garden of Dnipropetrovsk National University named after Oles' Honchar, where according to the City Sanitation Committee the concentrations of sulphur (IV) and nitrogen (IV) dioxides do not exceed the maximum permitted values.

Plant material

The object of study was the autochthonous species of white willow *S. alba* (*Salicaceae*). In each of the multiple monitoring points 30 annual shoots from several model trees of same age group were selected. Samples were fixed in 70% ethanol. Stem cross sections of were made at a distance of 2 cm from the base of the shoot. To identify the lignified cells the cross sections were stained with 1% solution of phloroglucinol [12]. BRESSER Biolux LCD 40x-1600x a light microscope was used to measure the tissue thickness with an amplification of 100 times and the thickness of the trachea diameter with an amplification of 400 times. The replication was 30 stem cross sections for each monitoring point.

Statistical Analysis

The results of the study were handled using a multifunctional application software package «STATGRAFICS». Mean absolute error was calculated. To compare the anatomical parameters in *S. alba* stems of reference and test samples Student's t-test ($p < 0,05$). Normality allocation of the sample had been preliminary assessed.

3. RESULTS

The influence of industrial emissions of toxic gases on the anatomical structure of *S. alba* stem annual shoots revealed that the majority of histological parameters is increasing under the anthropogenic conditions of growth compared with these characteristics indices of plants in a relatively clean zone.

The impact of industrial emissions on *S. alba* stem primary cortex structure

As can be seen from the table 1, the thickness of *S. alba* annual shoot stem primary cortex under influence of phytotoxic pollutants is increasing significantly and makes 156.7% of the control index. This is due to the growth of the width of two histological elements of the stem primary cortex, mechanical tissue and parenchyma. The thickness of the cork in plants in the contaminated and the reference areas is almost similar.

The impact of industrial emissions on *S. alba* stem secondary cortex structure

As a result of chronic exposure to toxic gases stem secondary cortex thickness of annual *S. alba* shoots increased dramatically (by 22.7% compared to this indicator in plants in the reference area). The impact of pollutants is causing major thickening of phloem fibre (hard bast), which twice as big as the reference index. As for the area sieve tubes with companion cells and parenchyma (soft bast), their thickness does not change compared to the reference indices (the differences between the control and experimental indices are insignificant at $p \leq 0,05$).

Table 1. Effect SO_2 and NO_2 have on the size of histological elements of the annual *Salix alba* shoot stem primary cortex

Indicator	Control	Industrial area
Cork Thickness [μm]	16,13 ± 0,01	16,15 ± 0,02*
Collenchyme Thickness [μm]	49,17 ± 4,57	79,03 ± 2,89
Pimary Cortex Parenchyme Thickness [μm]	54,04 ± 5,65	91,95 ± 4,84
Primary Cortex Thickness [μm]	119,36 ± 9,69	187,11 ± 4,93

* – differ significantly from control ($p < 0,05$)

The impact of industrial emissions on the structure of the stem wood *S. alba*

We found that sulphur (IV) and nitrogen (IV) dioxides do not influence the radius of the wood in the research object (Table. 2). However, although the width of this histological parameter of the stele in technogenesis remains stable on the whole, some components of this tissue are still changing. Thus, the diameter of the largest timber vessels in plants in the industrial area rose to an extreme top of 125.0% as compared to the reference value.

Table 2. Effect of SO_2 and NO_2 on the size of histological elements of the central cylinder of annual *Salix alba* shoot stem

Indicator	Control	Industrial area
Hard Bast Thickness [μm]	85,49 ± 9,01	124,20 ± 7,25
Soft Bast Thickness [μm]	100,01 ± 5,27	104,84 ± 8,42*
Secondary Cortex Thickness [μm]	185,42 ± 12,00	227,43 ± 15,11
Wood Radius [μm]	493,58 ± 17,88	500,03 ± 9,03*
Largest Vessel Diameter [μm]	43,20 ± 4,41	54,00 ± 2,32
Pith Diameter [μm]	462,93 ± 23,09	435,51 ± 18,16*
Stem Diameter [μm]	2056,49 ± 30,71	2146,90 ± 32,32

* – differ significantly from control ($p < 0,05$)

The impact of industrial emissions on the pith structure and the stem diameter of *S. alba*

Industrial emissions of SO_2 and NO_2 do not cause changes in pith diameter stem of annual *S. alba* shoots (Table 2). As a result of the above mentioned changes (Table. 1-3) in indices of histological parameters of the central cylinder and primary cortex of the stem, its diameter in the research objects increases substantially under the influence of phytotoxic pollutants.

The impact of industrial emissions on the ratio of histological parameters of *S. alba* stems

The research data presented in Tables 1, 2 are given in micrometers. Though to fully analyze the impact of industrial emissions on the development of stem histological elements we need to know the indices of anatomical parameters both in absolute and relative values, i.e. the percentage of each tissue compared to the total thickness of the primary cortex and central cylinder of the shoot. As it is seen in Fig. 1, cork fraction decreased by almost 5% to the total primary cortex thickness value in the stems of *S. alba* in terms of technogenic growth compared to the reference plants. It is due to the increase in the share of the primary cortex parenchyma, and partially collenchyme.

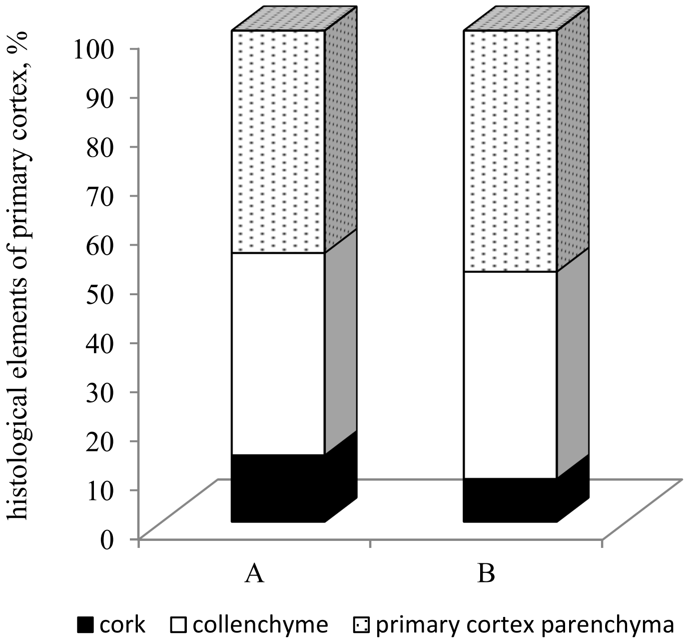

Fig. 1. The influence of SO_2 and NO_2 on the ratio of histological elements of primary cortex of annual shoot stems of *Salix alba* L., %: *A* – reference area; *B* – test area

As for the changes in the ratio of histological elements of the central cylinder of *S. alba* stem, the share of wood and pith in the experimental plants show a slight decrease which can be explained by the significant thickening of the hard bast tissue and a moderate thickening of soft blast tissue in the stems of the research objects (Fig. 2).

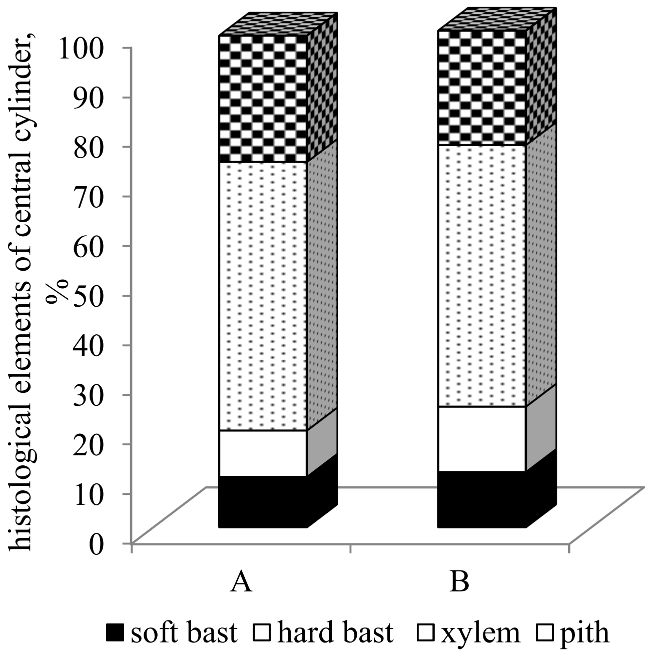

Fig. 2. The influence of SO_2 and NO_2 on the ratio of histological elements of the central cylinder of annual shoot stems of *Salix alba* L., %: *A* – reference area; *B* – test area

4. DISCUSSIONS

It is known [8, 9], anatomical changes are often quantitative in environmental physiology and other areas of plant biology. This study also found significant changes in the size of a range of histological parameters of annual shoot stems of *S. alba* influenced by industrial emissions of SO_2 and NO_2.

The impact of industrial emissions on the structure of primary stem cortex of *S. alba*

The primary cortex is the part of stems of young shoots of trees and shrubs known to be sensitive to many stress factors of the environment. Thus, Jusypiva and Griztay (2014) discovered reducing the thickness of the primary cortex both in *Caragana arborescens Lam.* shoot base and shoot apex when the plants were affected with SO_2 and NO_2 emissions [13]. It was noted that there was a decrease in the width of primary cortex parenchyma, while at the shoot apex all the primary cortex components indices decreased. Griztay and Shupranova (2015) established the increase in the width of the primary cortex of the *Tilia L.* genus, namely *T. europaea L., T. platyphyllos Scop.* and *T. cordata Mill.* under the influence of power plant emissions (SO_2, NO_x, solid additions). However, the authors note that the first two types of proliferation of this part of the stem was due to protective tissues (cork and collenchyme) and primary cortex parenchyma, whereas in *T. cordata* the phellem and primary cortex parenchyma increased, while the mechanical tissue primary cortex reduced [10].

The present research findings (Table. 1) do not demonstrate qualitative changes in the structure of the stem primary cortex under conditions of anthropogenic stress. The outer layer of the stem of the studied species is periderm, which is a multiple layer tissue, the main component of which is cork. Its cells are suberized, do not pass gas and water [14], prevent the entry of pathogens and depredators, providing comprehensive stem protection from adverse environmental factors. Collenchyme placed right under the cork is an essential part of the primary cortex in young stems. It is a multifunctional tissue [9] as its thick walls not only protect deep rows of parenchyma living cells against compression (reinforcing function), but also contain a significant amounts of pectic substances and hemicellulose, which can be used by the plant as spare polysaccharides (storing function). Since collenchyme cells contain chloroplasts it provides photoassimilation. Also, it has the ability to acquire meristematic activity, i.e. the ability to form meristem. Cortex parenchyma performs mainly the storing function. It is evident that the development of these components of the primary stem cortex is essential for a condition of the normal functioning of the shoots of woody plants.

According to the research, *S. alba* primary cortex thickness increased significantly under exposure to toxic gases when compared with control plants stems indices. Since the plant gas resistance depends on how the stem protective tissues develop, we view it as an adaptive reaction of plants under the action of sulfur (IV) and nitrogen (IV) oxides. The primary cortex of the research objects, the *S. alba* plants, increased due to the cortex parenchyma thickening rate increased by 70.2% and collenchyme by 60.7% respectively compared to the reference indices. There was no record of phellem thickness index discrepancies between the stems of plants in both reference and contaminated areas.

The impact of industrial emissions on the secondary cortex of *S. alba* stem structure

Secondary cortex (phloem) is a peripheral part of the central cylinder of the stems of woody plants. Phloem is the leading tissue that is a set of hard bast or bast fibers and soft bast consisting of sieve tubes with companion cells and bast parenchyma. Phloem transports photosynthesis products from assimilation organs to places of consumption or storage areas [14]. Being the part of the plan transportation system, the plant stem secondary cortex is exposed to environmental factors impact, including the anthropogenic ones, which affects its functioning. So, Dmuchowski, Kurczynska and Wloch (1998) registered inhibition of cambium in the stems of plants *Pinus sylvestris L.* that grow in the area characterized by high rate of steelmaking plant emissions [15]. The authors note that inhibiting the mitotic activity of the cambial zone leads to reducing the number of phloem cells (at different stem height levels), and it is most significant in the second half of the vegetative season. Abdussalam et al. (2015) recorded the complete damage of the secondary phloem in the stems of

Boerhavia diffusa L. under the influence of heavy metals such as cadmium, chromium, mercury and lead [16].

This study demonstrates that the constant flow of SO_2 and NO_2 industrial emissions into the atmosphere results in an increase in the secondary cortex thickness of *S. alba* stems. The reason for this phenomenon is thickening of the hard bast by 45.3% compared with the same indicator in control plants. However, the soft bast zone remaines unchanged. It allows to state that the rise of the phloem mechanical elements thickness can enhance supporting function of the plant stem of the species under research when it undergoes human pressure on the environment.

The impact of industrial emissions on the structure of wood stems *S. alba*

It is known [9] that the wood has the biggest share of the woody plant stem. The xylem includes leading elements (tracheids and vessels), wood fiber (libriform) and wood parenchyma, and therefore this part of the central cylinder of the stem performs multiple functions: mechanical, transport, storage of nutrients and others. The changes taking place in the xylem serve as tree species response to anthropogenic pollution for many researchers. Csókáné (1990) recorded a decrease in the average width of annual rings in plants *Quercus cerris L.*, which have been exposed to chemical plant emissions for 20 years [17]. Hmelev and Hvatova (2003) observed the increase in the number of vessels by $1mm^2$ in the stems of annual stems of *Populus L.* under the influence of the steel mill emission, accompanied by their diameter and reduction and vessels segments shortening [18]. Jarmishko (2012) recorded the cases of partial and full loss of annual wood layers in P. *sylvestris* and received a significant negative indices correlation between the growth of stem diameter and the volume of sulfur dioxide and hard particules in the emissions of copper-nickel plant [19]. Segala (1995) noted the process of wood thinning, as well as increasing the number of vessels, reducing the size of wood fibers in *Cerropia glazioou L.* stems under the influence of metallurgical, chemical and petrochemical industries emissions [20].

Our research findings indicate that the wood radius in the experimental plants of *S. alba* under chronic SO_2 and NO_2 influence showed no significant change which is a clear evidence of this species resistance to aerogenic pollutants. In addition, there is statistically significant growth of the largest xylem vessel diameter in the plant in the contaminated area. Therefore the experimental data confirm an adaptive response to the action of phytotoxic pollutants because it creates favorable conditions to ascending transport of water and mineral compounds in the plant.

The impact of industrial emissions on *S. alba* pith structure and stem diameter

The central part of the stems of woody plants is the pith, which has almost the same volume as the wood in annual shoots of *S. alba*. It is the size of the two components of the central cylinder that largely defines the stem diameter. Several authors marked a decrease of stem diameter growth under the influence of sulfur (IV) and nitrogen (IV) oxides in a number of tree species: *Betula pendula Roth., Populus nigra L., B. pubescens Ehrh., Robinia pseudoacacia L.* [21, 22] and *T. cordata* [10, 22]. Kaakinen et al. (2004) found a statistically significant reduction of the distance between the core and the cortex in *Populus tremuloides Michx., Betula papyrifera Marsh.* and *Acer saccharum Marsh*., when they were exposed to elevated O_3concentration. In our work, industrial emissions of SO_2 and NO_2 do not change the pith diameter of the stem [23]. However, due to the increase of primary cortex and secondary cortex indices the stem diameter in the studied *S. alba* plant affected by phytotoxic pollutant increases substantially.

5. CONCLUSION

The results of this study suggest that high concentrations of toxic gases SO_2 and NO_2 do not cause qualitative changes in the anatomic structure of annual *S. alba* shoot stems. However, atmospheric pollutants significantly affect the size of the individual histological elements both in the cortex and the central cylinder. Because of long-term influence of sulfur (IV) and nitrogen (IV) oxides the thickness indices of primary and secondary cortex in the research object stems increased, leading to an increase in the stem diameter.

Due to high flexibility of its anatomical indices *S. alba* has proved to have high adaptive capacity to industrial emissions containing SO_2 and NO_2, and therefore this wood species can be recommended for planting in anthropogenic areas contaminated with toxic gases.

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