БИОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ

BIOLOGICAL SCIENCES

DOI: 10.12731/2658-6649-2021-13-1-11-34
UDC 577.15

ACTIVITY AND ISOZYME COMPOSITION OF PEROXIDASE IN SCOTS PINE (*PINUS SYLVESTRIS L.*) NEEDLES EFFECTED BY TECHNOGENIC EMISSIONS FROM VARIOUS ENTERPRISES AND VEHICLES

O.V. Kalugina, T.A. Mikhailova, L.V. Afanasyeva, O.V. Shergina

Background. *The technogenic pollution leads to excessive production of reactive oxygen species (ROS) in plants which are highly reactive and toxic and cause damage to biomolecules. Plants have a complex antioxidant defense system that protects cells from the ROS and maintain homeostasis. The most important link this system is enzymes, in particular, peroxidase. It was of interest to determine the expression of the protective properties of one of the sensitive species of coniferous plants under the influence of technogenic emissions from various enterprises and vehicles.*

Purpose. *Investigation the activity and isoenzyme composition of peroxidase in the needles of Pinus sylvestris L. under the influence of technogenic emissions of different compositions in the Baikal region.*

Materials and methods. *The pine needles were collected on sample plots located near an aluminum plant, thermal power plant, chemical plant, coal mining enterprise, and the highway. The activity of soluble guaiacol-dependent peroxidases was defined by spectrophotometry in a reaction mixture with citrate-phosphate buffer, hydrogen peroxide, and guaiacol. Native polyacrylamide gel electrophoresis was used for determination of peroxidase isoforms.*

Results. *It was shown that an increase in the total guaiacol-dependent peroxidase activity ranged from 6 to 22 times in the pine needles in polluted areas. Maximum enzyme activity was found in needle samples collected near the aluminum smelter, whose emissions are characterized by large amounts of fluorides and polycyclic aromatic hydrocarbons. The high variability of peroxidase isoform* *composition in Scots pine needles under industrial pollution was revealed. It was expressed in the emergence of new isoforms in the zone of fast-moving (Rf from 61 to 100) and medium-moving (Rf from 31 to 60) items. The maximum number of isoforms (nine) was found in pine needles near the aluminum smelter with only two ones detected in the background area.*

Conclusion. *Peroxidase activity and the number of its newly formed isoforms can adequately reflect the degree of technogenic pollution and trees decline. The indicators can also be used in monitoring of coniferous forests condition.*

Keywords: *Pinus sylvestris L.; induced-guaiacol peroxidase; peroxidase isoforms; technogenic pollution*

For citation. *Kalugina O.V., Mikhailova T.A., Afanasyeva L.V., Shergina O.V. Activity and isozyme composition of peroxidase in scots pine* (Pinus sylvestris L.) *needles effected by technogenic emissions from various enterprises and vehicles.* Siberian Journal of Life Sciences and Agriculture*, 2021, vol. 13, no. 1, pp. 11-34. DOI: 10.12731/2658-6649-2021-13-1-11-34*

АКТИВНОСТЬ И ИЗОФЕРМЕНТНЫЙ СОСТАВ ПЕРОКСИДАЗЫ В ХВОЕ СОСНЫ (*PINUS SYLVESTRIS L.*) В УСЛОВИЯХ ЗАГРЯЗНЕНИЯ ТЕХНОГЕННЫМИ ЭМИССИЯМИ РАЗНЫХ ПРЕДПРИЯТИЙ И АВТОТРАНСПОРТА

О.В. Калугина, Т.А. Михайлова, Л.В. Афанасьева, О.В. Шергина

Состояние вопроса. *Воздействие техногенного загрязнения инициирует развитие в тканях растений окислительного стресса, связанного с избыточным образованием активных форм кислорода (АФК). Для предотвращения негативного влияния АФК у растений функционирует антиоксидантная система защиты, важнейшим звеном которой является фермент пероксидаза. Исследования активности этого фермента проводились многими авторами при загрязнении растений определенными поллютантами – озоном, диоксидом серы, тяжелыми металлами. Представляло интерес выяснить проявление защитных свойств одного из чувствительных видов хвойных растений при воздействии разных типов техногенного загрязнения.*

Цель работы *– исследовать активность и изоферментный состав пероксидазы в хвое Pinus sylvestris L. при воздействии техногенных эмиссий разного состава на территории Байкальского региона.*

Материал и методы. *Активность гваякол-зависимой пероксидазы определяли спектрофотометрически в реакционной смеси, содержащей цитратно-фосфатный буфер, перекись водорода и гваякол. Определение изоформ пероксидазы осуществляли с помощью нативного электрофореза в полиакриламидном геле.*

Результаты. *В хвое деревьев сосны на техногенно загрязняемых территориях обнаружено увеличение активности пероксидазы от 6 до 22 раз. Максимальная активность фермента и наиболее высокая изменчивость спектра изопероксидаз отмечаются в хвое при загрязнении фторидами и полиароматическими соединениями. Новые изоформы выявлены в зоне среднеподвижных (Rf от 31 до 60) и в зоне быстрых (Rf от 61 до 100) компонентов. На фоновой территории найдено только две изоформы.*

Заключение. *Активность пероксидазы и число ее изоформ в хвое адекватно отражают степень техногенного загрязнения и угнетения древостоев и могут быть использованы при мониторинге состояния лесов.*

Ключевые слова: *Pinus sylvestris L.; гваякол-зависимая пероксидаза; изоформы пероксидазы; техногенное загрязнение*

Для цитирования. *Калугина О.В., Михайлова Т.А., Афанасьева Л.В., Шергина О.В. Активность и изоферментный состав пероксидазы в хвое сосны (Pinus sylvestris L.) в условиях загрязнения техногенными эмиссиями разных предприятий и автотранспорта // Siberian Journal of Life Sciences and Agriculture. 2021. Т. 13, № 1. С. 11-34. DOI: 10.12731/2658-6649-2021-13-1-11-34*

Introduction

Technogenic pollution is one of the significant anthropogenic factors causing pathological changes in plant organisms. The nutritional status of plants changes and many metabolic processes are disturbed due to the foliar and soil absorption of pollutant elements. At the biochemical level, the development of oxidative stress in plant tissues which is related to the increased production of reactive molecules – reactive oxygen species (ROS) – is the initial link of the disturbances [15]. The most important ROS for living cells are hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), superoxide radical (O_2^-), singlet oxygen (O_2^*). Oxygen radicals and their derivatives pose a serious threat to plant organisms since they can suppress the activity of enzymes, cause changes in nucleic acids, degradation of proteins, and change the membrane permeability [13; 22].

The multicomponent antioxidant defence system (ADS) functions effectively to eliminate ROS without the formation of any toxic compounds in plants. The system includes low and high molecular weight compounds [17]. By means of it, regulatory and detoxification mechanisms are launched, which act at the cellular, molecular, and tissue levels. The mechanisms act at the level of the whole plant, too [29]. Peroxidase is the most important highly molecular plant antioxidant, which directly detoxifies ROS [30; 37]. It is a two-component enzyme with iron-porphyrin in the prosthetic group, which has diverse substrate specificity [12]. The enzyme is one of the first to be activated and involved in the neutralization of ROS, ensuring thereby the regular functioning of oxidative processes. Moreover, peroxidase plays an important role in the respiration of plants, in nitrogen metabolism, in the regulation of growth processes, and in the formation of cell walls. In addition, it plays a key role in the process of lignification [1; 6; 32].

There is a lot of information about changes in the activity and isozyme composition of plant peroxidases under the conditions of technogenic pollution. Most researchers consider the quantitative and qualitative variability of the enzyme in herbaceous and woody plants under the influence of one type of pollution. These types are: heavy metals [40], ozone [26; 41], nitrogen dioxide [20], sulfur dioxide [21; 24], fluorides [23; 34], and polycyclic aromatic hydrocarbons [39]. Therewith, the authors note that peroxidase activity increases with an increase in the technogenic load. The increase in its activity indicates the occurrence of protective reactions of a plant organism in adverse conditions even long before the appearance of visible damage in plants. However, there is evidence of a decrease in the activity of this enzyme in plants in response to exposure to high concentrations of pollutants. Thus, with the increase in radionuclide contamination, the decrease in the peroxidase activity in Scots pine needles was noted [18], as well as in tobacco leaves in response to exposure to high nitrobenzene concentrations [38]. Moreover, a number of studies aim at studying the peroxidase activity of one or several tree species growing in urban environments [16]. According to the authors, it is possible to determine the degree of plant adaptive abilities and identify urban areas with different pollution levels by the enzyme activity changes. Not without interest is to assess the protective and adaptive abilities of one plant species growing in territories that differ in the type of technogenic pollution. We did not find corresponding studies.

The Baikal region (Eastern Siberia, Russia) is interesting in this aspect since forests, as the most important natural resource, are especially significant here. They are the main factor that ensures the sustainability of the ecosystem of the unique Lake Baikal and adjacent territories. Natural undisturbed stands still exist in most parts of the region. They represent the standard of species, population and ecosystem diversity. At the same time, in the southern part of the Baikal region, where more than ten large industrial enterprises with the annual volume of air emissions of more than 600 thousand tons of pollutants [2] are located, there is a tendency towards a decrease in the environmental protection and in the water-regulating potential of forest ecosystems [11].

The purpose of our research is to study the activity and isozyme composition of peroxidase in needles of Scots pine (*Pinus sylvestris* L.), an indicator species, under the influence of technogenic emissions from various enterprises and vehicles.

Material and methods

The studies were carried out in the southern part of the Baikal region (Baikal region, East Siberia, Russia) at sample plots located 2-3 km away from the aluminum smelter, the thermal power plant (TPP), the chemical enterprise, and the coal-mining enterprise. They were also carried out 150-250 m away from the major motor road. Emissions from aluminum production are characterized by the highest content of fluorides and polycyclic aromatic hydrocarbons (PAHs); the amount of sulfur dioxide and aerosols of heavy metals is also high. Sulfur dioxide and aerosols of heavy metals account for a large proportion of TPP emissions. The predominant phytotoxicants in the chemical enterprise emissions are oxides of carbon, sulfur and nitrogen, formaldehyde, furfural, and benzo(a)pyrene, as well as aerosols of mercury, zinc and nickel. The coal-mining enterprise emits a large amount of inorganic dust into the atmosphere. The dust contains heavy metals; oxides of sulfur, nitrogen, and carbon are recorded in the gas fraction of the emissions. A large number of lead compounds are noted in the emissions from vehicles along with carbon and sulfur oxides, aldehydes and PAHs [2].

Field surveys of Scots pine trees, the main forest-forming species of the region, were carried out in 2018-2020 using permanent sample plots (SP) technique in stands similar in age and bonitet [27]. Relief features, regional wind regime, and local air mass circulation were taken into account. There were established 11 sample plots in the tree-stands polluted by technogenic emissions from various industrial enterprises and vehicles. We studied Scots pine tree-stands of III bonitet class growing on gray forest soil. Background (unpolluted) tree-stands were 100 km away from the industrial territory and did not fall under emission transfer. We studied herb-rich III bonitet class Scots pine trees growing on gray forest soil.

In the middle of the vegetation period (from July 20 to July 30), second-year needles, as the most physiologically active, were selected from the middle part of the crowns of 5-6 40-year-old trees (II class according to the Kraft classification) from the south and southwest sides. The shoots were selected into Kraft paper bags using the Gardena pruner. In the laboratory conditions, needles of the second year of life were separated from the shoots with forceps. Needles taken on each SP were thoroughly mixed to form averaged samples. Next, one part of the needles was dried for 48 h at 60°C, ground to powder using the Bosch electric mill, and sieved through a 0.5 mm mesh. The dry material obtained was used to determine inorganic elements. The other part of the needles was left fresh and stored in a freezer. PAHs, activity and isozyme composition of peroxidase were determined in frozen needles.

To assess the level of industrial pollution, Scots pine needle content of the main inorganic pollutants was determined (fluorine, sulfur, lead, cadmium, mercury, zinc, iron, copper, nickel, lithium, molybdenum, vanadium, arsenic, tungsten, chromium, tellurium, silicon, aluminum) and the sum of 16 priority PAHs. Dry needles were mineralized in a muffle furnace at 450°C for three hours. The ash was then dissolved in 0.1 M nitric acid to determine the trace elements, and for the determination of sulfur – in 2 M hydrochloric acid. The elemental chemical composition in the obtained solutions was determined by the atomic absorption spectrophotometry and photocolorimetry [33; 35] using the instrumentation of the Bioanalitika Shared Instrumentation Center (Irkutsk): the AAS Vario 6 (Germany), the IR-spectrophotometer FT-IR Apectrum One, Perkin Elmer, AAA (Czech Republic). The fluorine content in Scots pine needles was measured spectrophotometrically at a wavelength of 540 nm with xylenol orange after dry ashing of the sample and distillation of the resulting ash with water vapor in perchloric acid using silver sulfate to remove the accompanying chlorine impurities. The mercury concentration in the needles was determined by the cold vapor atomic absorption technique [42]. PAHs were determined using the method of chromatography-mass spectrometry technique according to A.G. Gorshkov [19].

The content of hydrogen peroxide, an indicator of the oxidative stress in needles, was determined spectrophotometrically according the formation of a colored complex compound – titanium peroxide from titanium sulfate $Ti_2(SO_4)_3$[14]. Using the Specord spectrophotometer (Germany), the total activity of soluble guaiacol-dependent peroxidases was determined by changing the optical density (wavelength 580 nm) in the reaction mixture composition: 0.1 M citrate-phosphate buffer (pH 5.5), 0.3% hydrogen peroxide, 0.05% guaiacol, and a sample [3]. The determination was carried out at 25°C immediately after the enzyme extraction from needles samples. The determination of peroxidase isoforms in needles was carried out using native polyacrylamide gel electrophoresis (PAGE) [5]. The diaminobenzidine method modified by Loyda et al. [4] was used to detect the enzymatic activity in PAGE.

A number of visual and morphometric parameters were determined at all SPs for assessing the vital state of pine trees: crown defoliation, needle mass on second year shoots, second year shoot length, number of needles on second year shoots and needle age.

The content of inorganic elements was expressed in mg/kg dry weight, PAHs – in ng/g of dry needle mass, enzyme activity – in standard units per mg wet weight. All parameters were determined in five biological and nine analytical replicates; for morphometric parameters, 60-100 measurements were performed. Statistical data processing was carried out using the application package MS Excel 2010 and with the software «R», version 3.1.1. (2014). To assess the accumulation level of pollutant elements in Scots pine needles, a concentration coefficient was calculated, which is the ratio of the content of any element in the studied object to the background content [9]. The figures and tables show the average values of each parameter ± standard deviations (SD). The Shapiro-Wilk test was used as a normality test. Pearson's correlation coefficient was used [28]. Differences significant at $\alpha=0.05$ are discussed.

Results and discussion

According to the results of the studies, the content of elements in Scots pine needles changes at SP nearby the technogenic pollution sources; the increases in concentrations of those elements included in the emissions are observed. Their highest content in needles is noted near the aluminum smelter. The sum of inorganic pollutants here is 1682 mg/kg, organic – 1650 ng/g, which exceeds the background level by 4.0 and 30 times, respectively. Figure 1 shows the content of individual pollutants in needles expressed in terms of concentration coefficients (Cc). High sulfur concentrations in pine needles (up to 820 mg/kg with the background 310 mg/kg) are recorded near all the pollution sources. That is due to the wide distribution of sulfur dioxide, its presence in the atmospheric emissions from all the industrial enterprises and vehicles. The highest fluorine level (147 mg/kg against the background 10 mg/kg) in pine needles was found near the aluminum smelter. Moreover, fluorides were revealed to be emitted by a number of other enterprises (chemical plants, thermal power plants, etc.), though in much smaller quantities than those emitted by the aluminum smelter.

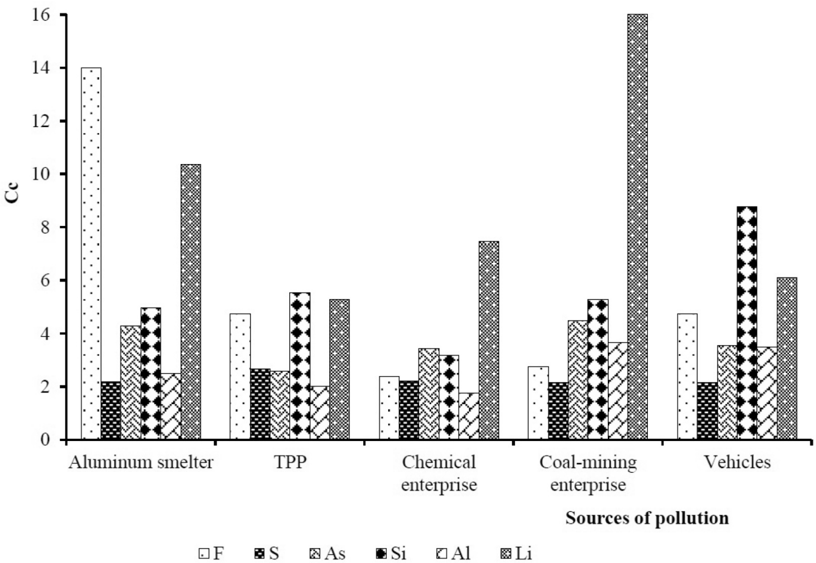

Fig. 1. Concentration coefficients (Cc) of pollutants in Scots pine needles polluted by technogenic emissions and vehicles.

In Scots pine needles nearby all the industrial enterprises, the concentrations of arsenic, silicon, aluminum, and lithium were increased (Cc was from 2 to 16.0) (see Fig. 1). When studying the distribution of aerosols of heavy metals, we identified SPs with high needle concentrations of heavy metals and SPs with low pollution levels (Table 1). The highest values of mercury and nickel were found near the chemical enterprise. High levels of tungsten, cobalt, molybdenum and cadmium were found near the TPP. High levels of zinc, lead, copper, iron, chromium, vanadium and tellurium were found near the motor roads.

PAHs are markers of atmospheric air pollution by persistent organic pollutants (POPs). When studying the accumulation of PAHs in Scots pine needles, their high level was recorded near all the industrial enterprises (Fig. 2). The highest level was marked near the aluminum smelter (one of the powerful sources of POPs emissions).

Table 1.

Concentration coefficients (Cc) of heavy metals in Scots pine needles polluted by technogenic emissions and vehicles

Heavy Metals	Sources of pollution				
	Aluminum smelter	TPP	Chemical enterprise	Coal-mining enterprise	Vehicles
W	3,03	25,46	4,79	2,69	8,62
Co	2,73	3,16	1,81	2,24	3,08
Mo	4,86	6,46	3,73	2,74	4,11
Cd	2,43	2,87	2,09	1,13	1,91
Hg	1,56	2,27	8,23	1,17	1,55
Zn	1,43	1,33	1,11	1,10	2,50
Ni	4,19	23,07	39,23	3,11	5,11
Pb	7,15	7,31	5,64	2,13	9,66
Fe	1,79	3,36	3,08	1,94	4,98
Cu	1,36	1,18	1,97	1,08	2,00
Cr	4,28	5,98	3,15	3,51	18,37
V	3,32	6,15	3,22	3,30	12,62
Te	2,74	4,17	4,51	2,58	7,95

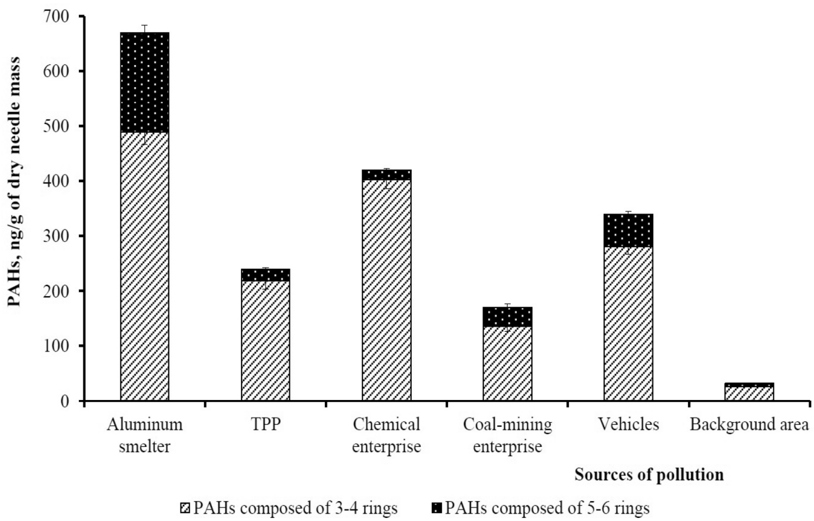

Fig. 2. The content of PAHs in Scots pine needles polluted by different sources of pollution and in the background area.

When analyzing needles, the prevalence of volatile PAHs composed of 3-4 aromatic rings (phenanthrene, fluoranthene, pyrene, chrysene) was shown. Their total amount can reach 70-96% of the total PAHs. Compounds with 5-6 aromatic rings (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, perylene, indeno[1,2,3-c,d]pyrene, benzo[g,h,i]perylene, and dibenz[a,h]anthracene) made up a smaller proportion (4-30%) in the total PAH content. The compounds are usually adsorbed on solid carriers (dust, soot) and are particularly toxic to biota [7].

The accumulation of pollutants is known to be associated with the development of oxidative stress, a prerequisite for which is the excessive accumulation of ROS. Hydrogen peroxide is a relatively stable ROS capable of diffusing from the place of formation [36]. Therefore, its generation can serve as one of the biochemical indicators of the state of plants during the alteration of intracellular processes. Our correlation analysis between the total content of pollutant elements in needles and the concentration of hydrogen peroxide there showed a significant effect of emissions from all the industrial enterprises on H_2O_2 production, correlation coefficient varied between 0.74-0.86 (α=0.05, n=18). Thus, in the vicinity of the coal-mining enterprise, the peroxide concentration was 1.5 times higher than the background values. It was 2.0 times higher near the chemical enterprise and the TPP; 2.5 times higher near the motor roads; 3.0 times higher near the aluminum smelter. With that in mind, we can talk about the high toxicity of emissions from the aluminum smelter, the TPP, and the chemical enterprise for plants. We can also notice somewhat lower toxicity of air emissions from the coal-mining enterprise.

The excess hydrogen peroxide that occurs in needles when exposed to industrial emissions is eliminated by specialized enzymes, including peroxidase. All groups of peroxidases participate in protective reactions: cationic (soluble) contained in vacuole, and anionic (weakly bound) localized in cellular components. Our study provides data on the total peroxidase activity. When determining the total activity of guaiacol-dependent peroxidase using omethoxyphenol as a reducing substrate, a significant increase was shown in Scots pine needles in technogenically polluted territories (Fig. 3).

The maximum values of enzyme activity exceeding the background ones by a factor of 22 were recorded in Scots pine needles in the vicinity of the aluminum smelter, where strong contamination with fluorides and PAHs was detected. Near the motor roads, the enzyme activity was 17 times higher than the background one. It was 15 times higher near the TPP; 12 times higher near the chemical enterprise; 6 times higher near the coal-mining enterprise.

Calculation of correlations between the level of hydrogen peroxide in needles and peroxidase activity revealed a close direct relationship between these parameters, correlation coefficient varied between 0.82-0.88 ($\alpha = 0.05$, $n = 18$). These results indicate a vivid display of the antioxidant properties of peroxidase, aimed at the removal of reactive oxygen species. Moreover, the higher the level of ROS in the needles is, the stronger the activity of peroxidase is. That indicates the great importance of this enzyme in maintaining the homeostatic state of needle cells under the influence of such a strong stress factor as technogenic emissions.

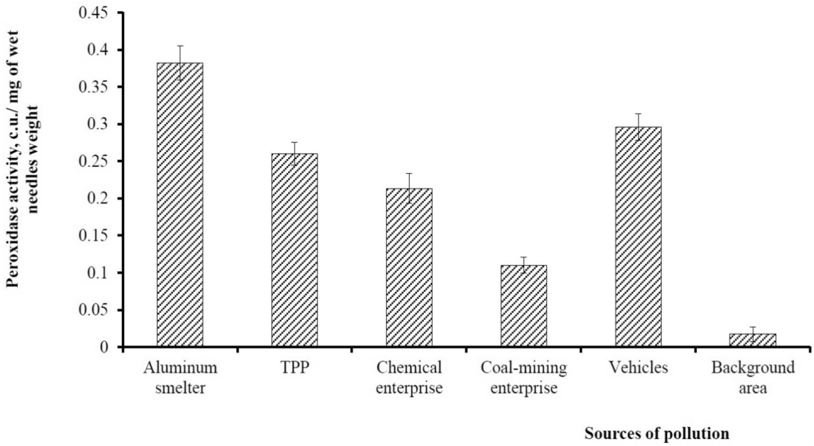

Fig. 3. Peroxidase activity in Scots pine needles polluted by technogenic emissions and vehicles.

The variety of functions performed and the active reaction of peroxidase in response to stressful effects are determined by the presence of a wide range of molecular forms of the enzyme – isoforms [1; 31]. Such heterogeneity of the spectrum of isoforms is the result of changes in the amino acid composition of the protein part of the enzyme molecule, the sugar composition of the carbohydrate part, or aggregation of low-molecular forms [8]. According to the relative electrophoretic mobility (Rf), isoperoxidases are divided into three zones: A-zone (slow isoforms), B-zone (moderate isoforms), C-zone (fast isoforms) [10; 25]. The set of the enzyme isoforms is characterized by high lability, which makes it possible to use it as an adequate indicator of the physiological state of plants [43]. These authors report an increase in the number of isoforms in case of deterioration in the condition of plants exposed to various stress factors.

The results obtained indicate the presence of two protein forms of the medium enzyme (Rf 0.56 and 0.68) in Scots pine needles in the background area. The Rf 0.56 isoform is stable and is observed in Scots pine needles in all the studied SPs (Table 2). A high variability of the peroxidase spectrum in needles is noted under the influence of technogenic pollution. The most profound transformation of the isoperoxidase spectrum was detected in Scots pine needles near the aluminum smelter: the number of newly formed components in the spectrum reached eight (plus the stable Rf 0.56 isoform). Four isoforms appeared in the moderate B-zone (Rf 0.42, 0.46, 0.52, 0.58); the rest (Rf 0.64, 0.73, 0.76, 0.81) appeared in the fast C-zone which consisted of components with the lowest molecular weight and fast electrophoretic mobility.

Table 2.

Determined peroxidase isoforms in Scots pine needles near the industrial enterprises and in the background area

Industrial Enterprises	Relative Electrophoretic Mobility (Rf)									
	0.42	0.46	0.52	0.56	0.58	0.64	0.68	0.73	0.76	0.81
Aluminum smelter	+	+	+	+	+	+	-	+	+	+
TPP	-	-	+	+	-	-	-	+	+	+
Chemical enterprise	-	-	+	+	-	+	-	+	-	-
Coal-mining enterprise	-	-	+	+	-	-	-	-	-	-
Vehicles	-	-	+	+	-	-	-	+	+	+
Background area	-	-	-	+	-	-	+	-	-	-

+ Isoform determined

- No isoform

In Scots pine needles growing near the TPP and motor roads, the isozyme spectrum includes (in addition to the stable Rf 0.56) 4 isoforms, one of which (Rf 0.52) appears in the B-zone and three (Rf 0.73, 0.76, 0.81) appear in the C-zone. Nearby the chemical enterprise, 4 isoforms (Rf 0.52, 0.56, 0.64, 0.73) were recorded in the isoform spectrum, including the stable Rf 0.56. Smaller spectrum changes expressed in the appearance of one new isoform (Rf 0.52) in the zone of moderately-moving components were found in needles of Scots pine growing near the coal-mining enterprise. Under technogenic pollution, we did not reveal isoforms related to the A-zone in Scots pine. However, there is evidence that new isoforms appear in Scots pine needles mainly in the A-zone under the conditions of chemical stress [10].

Isoforms differ in optimal conditions necessary for the launch of their catalytic activity [8]. The activity of certain peroxidase isoforms depends on many factors, such as natural characteristics of the habitat (biotope), climatic conditions, and the impact of negative factors. We assume that isoforms Rf 0.56 and 0.68, which were detected in background and polluted tree stands, are of greater significance in the physiological processes of Scots pine. Other forms of peroxidase present in needles only when exposed to technogenic industrial pollution seem to be involved in adaptive and protective reactions of a plant organism. Based on this, the data obtained on the increase in peroxidase activity and on the rearrangement of its isozyme system also speaks for the activation of the protective properties of Scots pine under the influence of a stress factor – technogenic pollution.

We compared the obtained data related to the biochemical protection of Scots pine, that is, we compared changes in the peroxidase activity in needles with data characterizing the state of Scots pine assimilation organs (namely, with the morphological parameters of needles and shoots) since their change can be used to estimate the inhibition degree of the tree growth processes. It was shown that a high activity of peroxidase was observed in needles of significantly inhibited trees with a high level of crown defoliation and a shorter length and mass of shoots and needles. Thus, the crown defoliation level near the aluminum smelter reached 65%, near the chemical enterprise and motor roads – 60%, near the TPP – 55%, near the coal-mining enterprise – 45%. It was on average 25% in the background area. The life span of Scots pine needles on the polluted SPs was reduced to 2-3 years, while it was 6 years on the background SP. The analysis of the morphological parameters of Scots pine shoots and needles under the influence of technogenic emissions also differs significantly from the background values, especially near the aluminum smelter and motor roads (Table 3). According to the table data, such parameters as shoot length, number of needles per shoot, and mass of needles on shoots are most significantly reduced. Near the sources of pollution, their values are 3.4-6.5 times lower than the background ones.

Judging by the results obtained, it can be assumed that under the influence of such a strong negative factor as long-lasting technogenic pollution, protective properties of trees are activated against the background of pronounced inhibition of the growth processes. The display of these processes depends on the degree of aggressiveness of pollutants. It was revealed that the degree of inhibition of growth processes, the level of peroxidase activity and its heterogeneity (the number of isoforms 9) are greatest near the aluminum smelter when exposed to highly toxic emissions containing fluorides and PAHs. Near the other enterprises (the chemical enterprise, the TPP, and the motor roads), the values of inhibition of growth processes, peroxidase activity, and number of isoforms (2 isoforms) are lower. Near the coal-mining enterprise, where the level of technogenic pollution of needles is the lowest, there is weak inhibition of growth processes, the lowest peroxidase activity and the smallest number of isoforms in comparison with other polluted SPs. Consequently, such indicators as peroxidase activity and number of its newly formed isoforms can adequately reflect the degree of technogenic pollution and inhibition of tree-stands. The indicators can be used in monitoring the state of coniferous forests.

Table 3.

Morphometric indicators (mean±SD) of trunks, shoots and needles of Scots pine trees polluted by emissions from industrial enterprises and vehicles

Indicators	Sources of pollution					Background area
	Aluminum smelter	TPP	Chemical enterprise	Coal-mining enterprise	Vehicles	
Length of shoots of the 2nd year, cm	7,5±3,3	7,8±2,9	11,2±2,8	15,7±2,1	6,7±1,5	24,3±2,5
Number of needles per shoot, pcs.	90,0±27,4	94,9±25,2	135,8±35,4	193,7±18,2	98,6±11,9	305,6±44,2
Mass of needles on the shoots, g	0,8±0,2	1,0±0,4	1,6±0,7	2,9±0,3	0,9±0,1	5,2±1,4
Mass of one needle, mg	8,9±0.3	10,5±0,3	11,8±0,3	14,5±0,5	9,1±0,3	17,0±0,6
Needle length, mm	47,8±7,9	50,3±9,2	51,3±5,8	53,6±4,9	48,6±5,2	55,7±7,2

Conclusion

Differences in the activity of guaiacol-dependent peroxidase and its isozyme spectrum in Scots pine needles polluted by technogenic emissions from the aluminum plant, the chemical enterprise, the TPP, the coal-mining enterprise, and vehicles are shown. The activity of this enzyme in polluted needles 6-22 times exceeded the background values, the number of isoforms increased to 9, and 2 isoforms were recorded in the background area (Rf 0.56 and 0.68). The strongest changes in peroxidase activity and number of isoforms were observed in needles near the aluminum smelter, and a high accumulation of fluorides and PAHs was detected in the needles. Somewhat smaller changes in peroxidase activity and composition of its isoforms were detected in Scots pine needles polluted by vehicles and emissions from the chemical enterprise and the TPP. The smallest changes in peroxidase activity and number of isoforms were found near the coal-mining enterprise. The results suggest that peroxidase activity and number of its newly formed isoforms can adequately reflect not only the specificity of needle protective properties under differing technogenic pollution but also indicate the inhibition degree of polluted trees. This is evidenced by the parallel study of morphostructural parameters characterizing growth processes of trees. The greatest inhibition of these processes was revealed in case of pollution by emissions from the aluminum smelter. The lowest inhibition was found near the coal-mining enterprise.

Conflict of interest information. The authors declare that they have no conflict of interest.

Funding information. The reported study was funded by RFBR and the Government of the Irkutsk Region, project number 20-44-380009.

Acknowledgements. This work was performed using equipment belonging to the Bioanalitika Shared Instrumentation Center of the Siberian Institute of Plant Physiology and Biochemistry, Sibrian Branch, Russian Academy of Sciences, Irkutsk. The authors are grateful to Maxim Zhivetyev for his help in determining the activity of peroxidase.

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DATA ABOUT THE AUTHORS

Kalugina Olga V., Cand. Sc. (Biology), Senior Researcher of the Laboratory of Natural and Anthropogenic Ecosystems Laboratory

Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of Russian Academy of Sciences

132, Lermontov Str., Irkutsk, 664033, Russian Federation

olignat32@inbox.ru

SPIN-code: 5805-2159

ORCID: 0000-0001-6500-748X

ResearcherID: I-9669-2018

Scopus Author ID: 36190781000

Mikhailova Tatiana A., Dr. Sc. (Biology), head of laboratory of the Laboratory of Natural and Anthropogenic Ecosystems
Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of Russian Academy of Sciences
132, Lermontov Str., Irkutsk, 664033, Russian Federation
mikh@sifibr.irk.ru
ORCID: 0000-0003-2214-8690
ResearcherID: I-9715-2018
Scopus Author ID: 15725975200

Afanasyeva Larisa V., Cand. Sc. (Biology), Researcher of the Floristry and Geobotany Laboratory
Institute of General and Experimental Biology Biochemistry Siberian Branch of the Russian Academy of Science
6, Sakhyanova Str., Ulan-Ude, 670047, Russian Federation
afanl@mail.ru
SPIN-code: 1244-1046
ORCID: 0000-0003-1339-7302
ResearcherID: J-6805-2018
Scopus Author ID: 57209328261

Shergina Olga V., Cand. Sc. (Biology), Senior Researcher of the Laboratory of Natural and Anthropogenic Ecosystems Laboratory
Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of Russian Academy of Sciences
132, Lermontov Str., Irkutsk, 664033, Russian Federation
sherolga80@mail.ru
SPIN-code: 8646-5549
ORCID: 0000-0002-6333-8821
ResearcherID: I-9646-2018
Scopus Author ID: 55902360500

ДАННЫЕ ОБ АВТОРАХ

Калугина Ольга Владимировна, канд. биол. наук, старший научный сотрудник лаборатории природных и антропогенных экосистем
Сибирский институт физиологии и биохимии растений СО РАН
ул. Лермонтова, 132, г. Иркутск, 664033, Российская Федерация
olignat32@inbox.ru

Михайлова Татьяна Алексеевна, д-р биол. наук, заведующий лаборатории природных и антропогенных экосистем
Сибирский институт физиологии и биохимии растений СО РАН
ул. Лермонтова, 132, г. Иркутск, 664033, Российская Федерация
mikh@sifibr.irk.ru

Афанасьева Лариса Владимировна, канд. биол. наук, научный сотрудник лаборатории флористики и геоботаники
Институт общей и экспериментальной биологии СО РАН
ул. Сахьяновой, 6, г. Улан-Удэ, 670047, Российская Федерация
afanl@mail.ru

Шергина Ольга Владимировна, канд. биол. наук, старший научный сотрудник лаборатории природных и антропогенных экосистем
Сибирский институт физиологии и биохимии растений СО РАН
ул. Лермонтова, 132, г. Иркутск, 664033, Российская Федерация
sherolga80@mail.ru