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Effect of Smoke Caused by Fires on the Enzymatic Activity of Forest Soils in the North Caucasus (Russian Federation)

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Abstract: Forest fires can have a significant impact on soils, resulting in changes in biological indicators. Due to fire, high temperatures, and intensive generation of smoke from burning materials of different origin, the activity of soil enzymes is decreased. In this study are presented the results of modelling experiments on the impact of smoke on forest soils (Cambisols according to the World Reference Base for Soil Resources rating) of the Republic of Adygea, Nickel settlement (Russia). The findings demonstrated significant smoke exposure on the enzymatic activity of this type of soil. A decrease in the activity of such enzymes as catalase, peroxidase, polyphenol oxidase, and invertase within 60 min after soil treatment with smoke from burning materials of plant origin (pine sawdust) was established. A significant decrease in the activity of catalase relative to the control by 36%, phenoloxidases by 54–58%, and invertase from the hydrolase class by 31% was found. The integral index of soil enzymatic activity (IIEA) of the studied soils was also calculated. In addition, one of the informative diagnostic indicators is the pH of the soil suspension. The pH value for fumigated water was also determined to identify differences with the suspension. A reduction in the pH towards acidification was observed. The obtained findings may be used in a comprehensive assessment of pyrogenic effects on forest soils. Moreover, indicators are sensitive to this effect, which was confirmed by the results of the present research.

Keywords: indicators; cambisols; smoke; pH; soil enzymes

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1. Introduction

The rate at which the area of burned forest has increased, not only in Russia, but worldwide, is becoming devastating. According to the reports of the Federal Forestry Agency, fires are destroying huge areas, spreading over great distances. According to official data, about 18 million hectares of forest were destroyed in Russia alone in 2021 [1]. Not to be forgotten also is the dense smoke that reaches other regions, which can lead to smog. Hazardous gaseous substances can be deposited on the soils or fall as precipitation.

Forest fires cause a serious risk to land ecosystems. The consequences of pyrogenic factors deal with changing soil properties, the content of chemical elements essential for the functioning of living organisms. As a result of the impact of fire, vegetation coverage, soil, and supra-soil biota are destroyed, leading to a reduction in biodiversity. Fires directly affect soil properties: physico-chemical, and biological [2].

It has been observed that considerable changes in microbiological (microbial biomass) [3,4] and biochemical properties [5] of soils were seen as a result of the impact of main damaging factor, is flames. The effect of smoke from burning vegetation on soils is also noted, where the temperature factor plays a key role [3,6]. Fires have an important role in changing the thermal conductivity of soils [7]. As a result of high temperatures under pyrogenic effect, the diurnal amplitude increases, the temperatures of the upper layer's increase, creating extreme conditions for living organisms, changing soil properties, and affecting their fertility [8–11]. Nevertheless, these changes can be noticed only during the heat period of the year, while during rainy weather or at night the differences between the background and pyrogenic sites are less obvious [12]. It was noted previously [13] that smoke has an impact on soil, leading to a decrease in enzymatic activity. The duration of soil exposure to gaseous substances plays an important role and the extra-high temperature of the smoke [6,13]. The penetration of smoke into soil has also been studied formerly [14]. It was found that the activity of soil enzymes significantly changes only in the surface layer, and the effect becomes less pronounced with depth.

To detect changes occurring in soil, biodiagnostics is carried out using various indicators. One such parameter is the enzymatic activity of soils. The enzymatic activity of soils is characterised by the combined processes of input, immobilisation, and action of enzymes in the soil. The source of enzymes is the living matter of soils: plants, microorganisms, animals, fungi, soil algae, etc. [13]. The activity of soil enzymes concerns the most important periodic transformations in the biogeochemical cycle of carbon, nitrogen, phosphorus, sulphur, and other organogenic elements and redox processes [15]. Soil enzyme activity can be used as diagnostic indicators [16], as they are sensitive indicators [17,18]. Soil enzymes are considered suitable indicators of soil quality due to their association with nutrient cycling and biochemical [19] transformations and their influence in determining the extent of soil degradation in both natural and agro-ecosystems [20]. It is also necessary to take into account an integral index of soil enzymatic activity (IIEA) of the studied soils, which can show the degree of influence of the stressor.

It is also noteworthy that the pH of soils changes due to the pyrogenic factor. Especially the increase of salt content in the steppe soils Haplic Chernozem (Aric, Loamic, Pachic) after smoke treatment was found [14]. However, the response of forest soils may be different from steppe soils. Therefore, study of this aspect needs more research. Furthermore, the effect of fire also directly affects the organic (C) content of the soil [21–25]. Loss of organic (C) content and changes in soil structure are known to affect resource availability to plants by influencing the soil microbial community through changes in soil heterogeneity and substrate supply [26]. Despite the abundance of information on the effects of fire on soil properties, there is an understudied aspect. At present, the effect of smoke from fires on soil biological properties is poorly investigated compared with the organic carbon content. Extensive smoke from fires may also change soil properties.

The aim of the study was to investigate the effect of smoke from burning plant materials (pine sawdust) on forest soils located in the Republic of Adygea, Nickel settlement (Russia). The tasks of the study were to identify changes in the activity of soil enzymes of the oxidoreductase group (catalase, peroxidase, and polyphenol oxidase) and hydrolases (invertase). It was necessary to determine the most sensitive indicators to gaseous substances, to calculate the IIEA index for the investigated enzymes, and to investigate the hydrogen index (pH) of fumigated soils. The findings were compared with previously carried out studies on the effect of smoke on soils of the steppe zone—Haplic Chernozem (Aric, Loamic, and Pachic).

2. Materials and Methods

The Republic of Adygea extends from the main mountain range of Caucasus up to the Kuban river. The landscape of the region is variable, and this has contributed to the formation of the soil cover. The flat land of the Republic is located along Zakubanskaya lowland, in which the valley of the Kuban river and their tributaries are situated. The soils there are fragments on heavy to medium loams, in which drainage is difficult, and meadow-forest formations were created. In the floodplains, on the alluvial sediments of a lighter mechanical composition, were formed alluvial-meadow soils. Meadow-boggy soils appeared on soils of heavy mechanical composition in oxbow plains. The conditions outlined above applied to the piedmont area, which in turn forms part of the wider geomorphological zone of the Western Ciscaucasus [27].

In the piedmont areas, the landscape characterised by an erosional cuesto-sloping terrain with elevations of up to 1200 m. Forest soils are encountered in this area. The samples were selected for the research near the settlement of Nickel (Figure 1), where the elevation does not exceed 700 m.

Figure 1. Location of the Cambisols soil sampling plot for the research (Nickel settlement, Republic of Adygea, Russia).

The soil type selected for the experiments is Cambisols. At present, this type of soil is of scientific interest, as it is rarely found in Russia and is probably influenced by fires. Cambisols are represented by several subtypes: leached, carbonate (common), and representative [28]. The sampling was carried out near the settlement of Nickel. The site is located on the right bank of the Belaya River, where the right tributary of the Syuk River flows into it. The sampling site is located 9 km southwest of Dakhovskaya village and 55 km to the south of Maikop town. Geographical coordinates of the sampling location: 44°10′39″ N. 40°09′20″ E. The properties of Cambisols are presented in Table 1. Soil samples were taken in a three-fold replication from the 0–10 cm surface layer, as it is the most biologically active layer. The pyrogenic factor primarily transforms this very layer.

Table 1. Properties of Cambisols of the Republic of Adygea.

Indicator	Values
Humus layer thickness	10–20 cm
Humus, %	From 0.6 in the BC_g layer up to 8.1 in the A layer
pH	The pH of all subtypes is less than 6.5. At the sampling site 5.4–6.0 depending on soil layer
The level of the soil layers (%)	In the A_1 and AB layers it is 57–59%. In B_t and C 40.2–40.3%
Cation exchange capacity mg-eq./100 g	From 10.4 in the BC_g layer to 20.8 in A layer
Exchangeable acidity mg-eq./100 g	From 0.8 in BC_g layer to 2.5 in A layer
Ca mg-eq./100 g	From 13.1 in layer A to 5.5 in BCg layer
Mg mg-eq./100 g	From 4.1 in layer A to 3.3 in BCg layer
Al mg-eq./100 g	From 2.3 in layer A to 0.6 in BCg layer
H mg-eq./100 g	0.15–0.2

The Merkel Standard smoke generator (Helikon, Russia) was selected for the model experiments, which was used to smoke the soil. Pine sawdust was placed in it, which burned and emitted gaseous substances. A container with a volume of 50 litres was chosen as the soil gas chamber. Soil in 0–1 cm layer was placed in 200 mL polypropylene containers. The weight of soil in each sample was 40 g. For analyses and experiments, only dried (at ambient temperature) forest soil was used. Methods of determining soil enzyme activity involve simultaneous collection of soil samples from control (non-fumigated soils) and experimental (fumigated soils) sites. The methods of enzyme activity determination involve drying of soil and this does not influence the determination of enzyme activity. A detailed schematic of the setup for the experiments is shown in Figure 2. Hailea ACO 208 air compressor (China) with adjustable air flow rate of 17.5 l/min was used for pumping smoke through the smoke generator and subsequent supply to the gas chamber. In the modelling experiment, high heat effects were excluded by the design of the equipment as this could have affected the results obtained. Temperature is one of the main factors of enzyme inhibition [29]. The air temperature during the experiments was 23 °C, relative humidity was 54–58%, and atmospheric pressure was 755–757 mmHg (100.67–100.93 kPa). The air environment parameters were determined using a meteometer MES-200A (Russia). The temperature of the gaseous combustion products was higher than that of atmospheric air and was 25.8 °C, which was determined using a laboratory thermometer TL-2 (Russia).

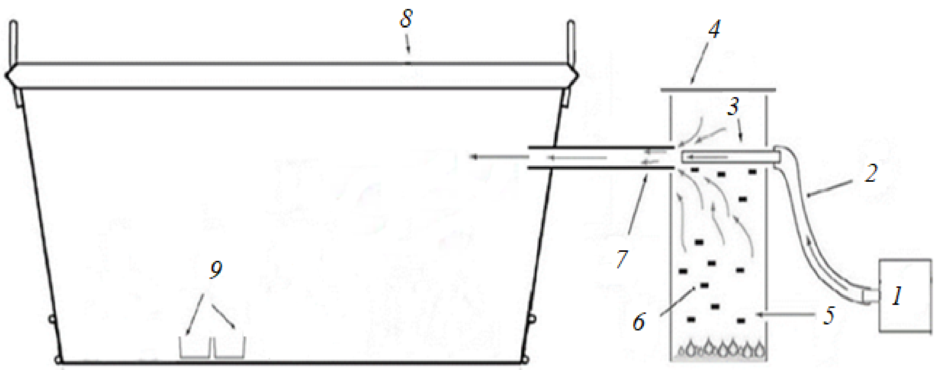

Figure 2. Scheme of the installation for soil fumigation: 1—Hailea ACO 208 air compressor; 2—hose from the air compressor for air supply to the smoke generator; 3—tube for air supply to the chimney; 4—Merkel Standart smoke generator; 5—holes for ignition of combustion materials; 6—smoke from thermal destruction of combustion materials; 7—chimney for smoke supply to the gas chamber; 8—gas chamber; 9—soil samples.

One of the enzymes with an important role in soil fertility is catalase, which can be related to the metabolic activity of aerobic organisms [30,31]. The activity of catalase was determined by the method of A.Sh. Galstyan, according to the decomposition of hydrogen peroxide (H_2O_2), formed during respiration of living organisms and other biochemical reactions into water and molecular oxygen within 1 min [32]. To determine the activity of catalase, a device consisting of two burettes connected with a rubber hose was used. The burettes were filled with water. The water level in the burettes was balanced and a certain level of water in them was maintained. This indicated that the temperature equilibrium was reached in the apparatus. A total of 1 g of soil was added to one of the compartments of the twin flask. A total of 5 mL of 3% hydrogen peroxide solution was added to the other compartment. The flask was tightly closed with a rubber stopper with a glass tube connected to a measuring burette with a rubber hose. After closing the stopper, the peroxide was mixed with soil by tipping the vessel while shaking the flask. The beginning of the experiment was marked on a stopwatch at the moment when the peroxide was mixed with soil and the contents of the vessel were shaken. Shaking of the mixture was carried out during the whole analysis, trying not to touch the flask with hands, holding it by the stopper. The released oxygen displaced water from the burette within 1 min. This enzyme is quite common as it is used in a variety of anthropogenic impacts on soil and it is a good diagnostic indicator [33,34]. The activity of the enzyme demonstrates reliable results, and it is sensitive to the slightest change in the soil.

Peroxidase and polyphenol oxidase activities were determined by the method of L.A. Karyagina and N.A. Mikhaylova [35]. This is a colorimetric method based on the substrate hydroquinone, by photometric measurement of colour intensity of stained compounds (parabenzoquinone or purpurogallin) [29,36]. These enzymes in soils play an important role in humus formation processes. Polyphenol oxidase catalyses the oxidation of polyphenols into quinones in the presence of free air oxygen. Peroxidase catalyses the oxidation of polyphenols in the presence of hydrogen peroxide or organic peroxides. Peroxidases as oxidative enzymes, use H_2O_2 as an electron acceptor in order to generate radical species, which then can act as catalysts for biological reactions [37].

In the peroxidase assay, 1 g of soil was placed in 50 mL conical flasks. A total of 10 mL of freshly prepared 1% hydroquinone solution and 1 mL of 0.05% hydrogen peroxide solution were poured into each flask with soil. The contents of the flasks were placed in a thermostat for 30 min at 30 °C. The control variant was also placed in the thermostat. After incubation, 10 mL of ethyl alcohol each was added to the experimental and control flasks and mixed thoroughly. The resulting mixture was filtered and then colometrized on a PI 5300VI spectrophotometer (460 nm wavelength) in 1 cm wide laboratory cuvettes against control solutions for comparison. The amount of parabenzoquinone was calculated from the standard curve prepared using pure para-benzoquinone solution. Polyphenol oxidase assay is performed in a similar manner but without the use of hydrogen peroxide.

For the invertase analysis, a modified colorimetric method based on the determination of the optical density of Felling's reagent after reduction in $CuSO_4$ by glucose formed from inverted saccharose was used [14,36]. To analyse the invertase activity, 1 g of soil was placed in 50 mL flasks, 5 mL of 3% freshly prepared sucrose solution and one drop of toluene were added to each flask. The flasks were corked and placed in the thermostat at 30 °C for 24 h. Control samples were also placed in the thermostat. After incubation, 25 mL of distilled water was added to the control and experimental samples and filtered. After that, 6 mL of filtrate was taken into 20 mL tubes. A total of 6 mL of Fehling's reagent was added to the filtrate. The test tubes with solutions were heated on a boiling water bath for 10 min. In the process of heating a part of copper of Fehling's reagent was reduced and precipitated in red colour. The test tubes were then cooled and centrifuged in a LISTON C2201 centrifuge. The solutions were then colourimetrically detected on a PI 5300VI spectrophotometer at 630 nm in 1 cm wide laboratory cuvettes. The enzymatic activities of all enzymes were assayed in triplicate, for better precision and reproducibility.

To assess the impact of smoke on soil, an integral index of enzymatic activity was also calculated using the equation:

$$\mathrm{IIEA} = (\mathrm{E}/\mathrm{E}_{\mathrm{ref}}) \times 100\%$$

where E is the average estimated score of enzyme activity and E_{ref} is the control of enzyme activity.

In this research, the pH of soil suspension was also determined for control and fumigated soils. The results were obtained potentiometrically at a ratio of soil:water 1:2.5 by using a HANNA HI-98128-pHep-5 equipment (Germany). Additionally, a study on the effects of smoke on water was carried out to analyse the difference between the soil suspension results and the changes in water.

Statistical processing was by means of a one-way ANOVA test to verify the reliability of the results obtained in the laboratory analyses, with a statistical significance level of $p < 0.05$ in Statistica 12.0 software.

3. Results

In Figure 3a range diagram of the values of the investigated enzymes are presented, showing a significant difference between the control values and the smoke. Enzymes of the oxidoreductase class (catalase, peroxidase, and polyphenol oxidase) are particularly prominent. Invertase differences are noticeable as well, but the values between control and experimental samples are less pronounced compared with the rest of the enzymes.

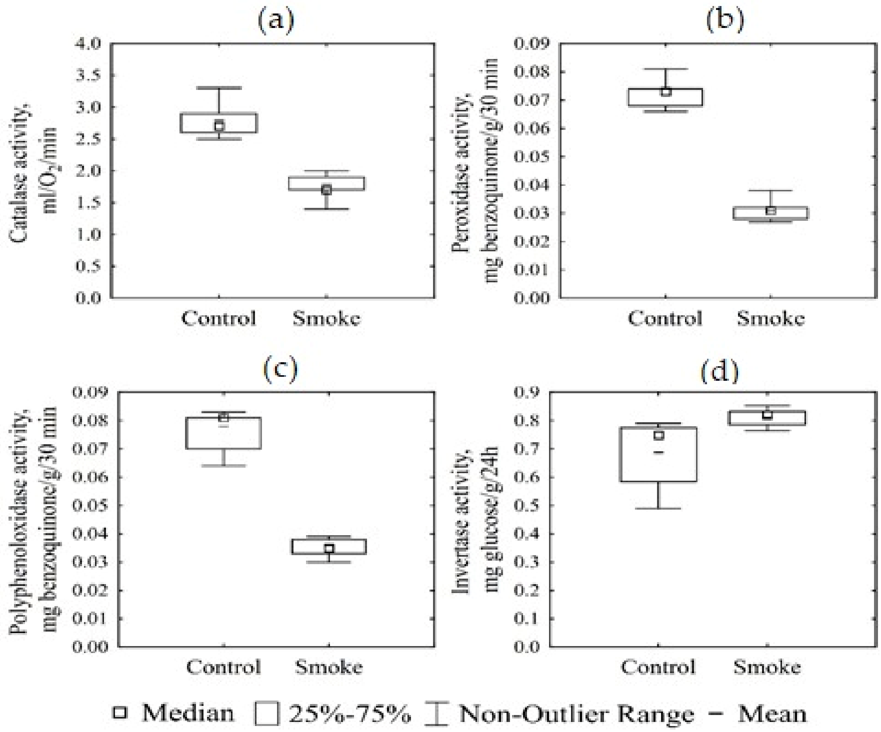

Figure 3. Range of values between control variants and smoke effects of the investigated soil enzymes in Cambisols: (**a**) catalase, (**b**) peroxidase, (**c**) polyphenol oxidase, (**d**) invertase.

Exposure to smoke from burning pine sawdust for 60 min revealed a significant decrease in the activity of the studied soil enzymes. According to the results obtained, the level of catalase activity decreased by 36% relative to control values. The greatest changes were observed in the activity of peroxidase and polyphenol oxidase, the values of which decreased by 58% and 54%, respectively (Figure 4A). The findings on the effect of smoke on forest soils of the Republic of Adygea were compared with steppe soils. The results are presented in Figure 4A,B. The figure shows that the changes are greater in steppe soils than in forest soils. The enzymes considered in the present study were significantly reduced. This confirmed the earlier suggestion of a significant effect of smoke from the fires on soils.

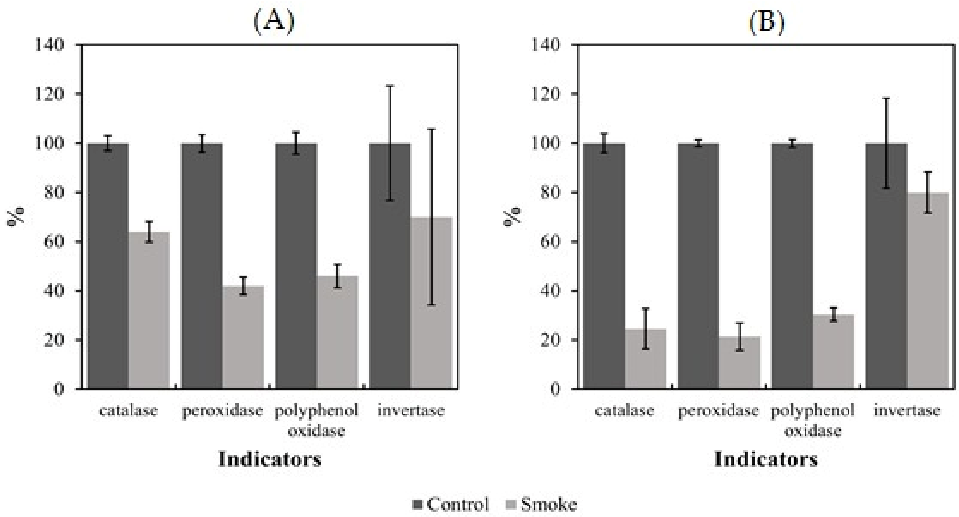

Figure 4. Changes in enzymatic activity of Cambisols (**A**) and Haplic Chernozem (**B**) after smoke exposure in model experiments (changes significant at $p < 0.05$ for catalase, peroxidase, and polyphenol oxidase).

At the same time, no significant values of invertase were found for Cambisols and Haplic Chernozem. Reliable values were only for enzymes of oxidoreductase class. This indicates a high sensitivity of these enzymes to the stressor.

An integral index of enzymatic activity was calculated for enzymes of the class of oxidoreductases of forest soils. IIEA was also obtained for soils of steppe areas and comparison of the results obtained with Cambisols is given (Figure 5). For steppe soils the figure was 25.3% and for Cambisols it was 50.6%.

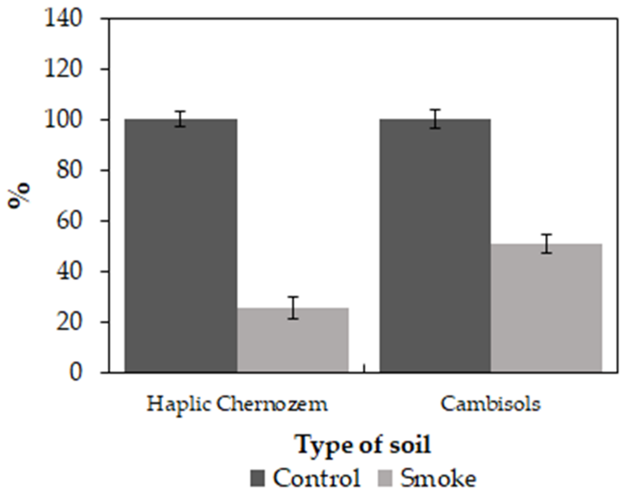

Figure 5. Changes in the integral index of enzymatic activity (IIEA) of Cambisols and Haplic Chernozem oxidoreductase enzymes (changes significant at $p < 0.05$).

The study also revealed significant differences in the soil suspension reaction (pH) of the experimental samples compared with the control samples that were not exposed to smoke in the model experiment. The results of laboratory analyses showed that 60 min after soil treatment with the gaseous substances, the value of hydrogen index in soil suspension decreased by 0.85 (Table 2) relative to the control. The pH of tap water, which was also exposed to smoke after burning pine sawdust, was measured using a similar method to compare the data. The decrease in pH in tap water was more significant and was 3.63. In this study, the pH of soil suspension was also compared for two types of soils (Cambisols and Haplic Chernozem). For chernozem, the value of the control sample was 8.16, whereas after smoke treatment this value decreased by 1.29 and was 6.87. This value is higher than for forest soils (4.55 after smoke treatment).

Table 2. Changes in the pH of Cambisols soil suspension and water after exposure to smoke.

Variant Exposure to Smoke	pH Cambisols	pH H_2O
Control	5.40	5.82
60 min of smoke effect	4.55	3.63

However, these were studies on modelling fires with smoke emission under laboratory conditions. Therefore, an additional experiment under realistic conditions was conducted, which revealed a decrease in the activity of Cambisols soil enzymes after exposure to smoke. The activity of catalase decreased by 24%, peroxidase by 26%, polyphenol oxidase by 29%, and invertase by 26%. The findings are approximated to the real conditions. Meanwhile, in this experiment, the experimental conditions (gas chamber, smoke generator, combustion materials, and fumigation time) were similar to the previous experiment. Also, as in the previous study, reliable values ($p < 0.05$) were registered only for oxidoreductases.

4. Discussion

It is assumed that the decrease in enzyme activity was caused by the slow sedimentation of smoke on the Cambisols and the resulting accumulation of toxicant substances such as CO_2, CO, and various hydrocarbons due to the combustion of pine shavings over 60 min. The combustion of the woody material resulted in the emission of soot. The oxidoreductase class enzymes have been revealed to be the most informative indicators. The data obtained indicate a significant impact of gaseous combustion products on both types of soils and a similar trend in the reduction in biochemical indicators can be observed.

The pH along with enzymatic activity is an important indicator of soil condition, it is a sign of soil resistance to various natural and anthropogenic influences. In the current study, there was a decrease in the pH of the soil suspension. As the hydrogen index decreases after smokiness, the vitality of numerous animals and plants becomes limited under such circumstances. However, a decrease in values to 4.55 is not sufficient to significantly reduce the activity of living organisms.

As part of this research, a large change in the pH of the water was seen, which has probably been caused by the accumulation of CO_2. Carbon dioxide dissolves in water to form carbonic acid. After an hour of smoke exposure, the pH value in the control sample decreased from 5.82 to 3.63. We can assume that the soil suspension pH decreases to a lesser rate than the water pH in the analysis as a result of the high buffering of the soil. Thus, the soil is a filter which can absorb toxic gases and prevent negative impacts from smoke on the soil organisms. Similar studies were conducted by us earlier on another soil type (Haplic Chernozem) [14].

The results can be compared with the works of other studies, where the authors noted a change in pH [3,38]. This is due to the fact that burning of plant material and organics on the soil surface releases alkaline cations such as Ca^{2+} and Mg^{2+}, and pH values decrease with increasing temperature. In the present work, the effect of high temperatures on smoke was not detected. The smoke temperature was no more than 25.8 °C while the pH varied significantly.

The research on the effect of smoke on the enzymatic activity of soils found that one of the crucial factors influencing the condition of soils, both forest and steppe, is the duration of exposure to the stressor. Moreover, the materials thermally destructed in the model experiments produced hazardous toxicants that had a negative impact on the biochemical indicators.

Hazardous toxicants in the form of gaseous matters may be emitted in a great range of ways. For example, if unfinished burning of materials is seen, CO, HNC, hydrocarbons, etc., are produced. In forests, a process that is accompanied by carbon monoxide emission can often be observed [39–42]. It is also known that the main particle size generated by forest fires (over 90%) is 10 micrometres or less. They can be both primary, being emitted into the atmosphere and deposited later on the ground owing to incomplete combustion, and secondary, formed by physical or chemical transformations. Primary particles can be elemental carbon, soot, or organic carbon particles. They can be produced secondary by hot vapour condensation (tar) [43].

When a fire occurs, soot particles are introduced into the air. They are well-known to consist of carbon and products of incomplete burning (wood, tree bark, forest floor, etc.). In turn, these organic substances, including phenolic compounds, are harmful pollutants [40]. Therefore, smokiness can be considered as one of the factors that have a significant effect on soil by fires. Despite this, there is no doubt that fire is a major damaging factor for ecosystems. And the effects of smoke contribute even further to the impact of pyrogenic effects on the environment overall. The model experiment conducted in the laboratory approximates burning in natural conditions with the formation of dense smoke and smog. In addition, we conducted an experiment on the effect of smoke on the enzymatic activity of soil in natural conditions. The activity of the studied enzymes also underwent significant changes.

When analysing the literature, information is often found that fire and extremely high temperature affect changes in microbiological (microbial biomass and nitrogen-fixing bacteria) and biochemical properties of soils [3]. It was found that it is hot smoke that has the greatest inhibitory effect on enzymes. The decrease in biochemical parameters depended on the soil depth. A change in microbial biomass and Azotobacter bacteria abundance as a result of hot smoke exposure was observed [3]. Both enzyme activity and abundance of living organisms depended on the time of smoke treatment. The depth of smoke penetration into the soil was studied [14]. However, such studies were conducted for steppe soils, but not for forest soils. This work is devoted to changes in enzymatic activity of forest soils after smoke exposure under fire modelling and comparison of the results obtained with data on steppe soils. The study of the effect of smoke from fires on Cambisols was carried out for the first time.

5. Conclusions

The greatest sensitivity was recorded for oxidoreductases such as catalase, peroxidase, and polyphenol oxidase. A significant decrease in IIEA was also found in soils. Thus, soil enzymes may be informative indicators of exposure to one type of pyrogenic exposure (smoke). Similar results were observed in earlier studies on the effect of combustion products on steppe soils.

The decrease in the pH of the soil suspension (4.55) as well as of tap water (3.63) indicates the deposition of toxic substances from the smoke onto the soil and water. Probably the crucial elements affecting soil properties can be considered CO2, CO, as well as various hydrocarbons and many other substances that are contained in smoke. As can be seen from the results, however, the pH value of water is significantly lower than that of the soil suspension. In this case, the soil appears to function as a barrier, preventing the penetration of toxicants and thus protecting the soil organisms from the negative effects of smoke. All the results obtained from smoke exposure can be used in a comprehensive assessment of the ecological condition of soils, along with the high temperature effects following forest fires. Any type of pyrogenic effect is detrimental to the environment, and the results presented in this study on the effects of smoke in fire modelling on soil confirmed this.

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