Heavy Metals and Sulphur in Needles of *Pinus sylvestris* L. and Soil in the Forests of City Agglomeration

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Abstract: The content of sulphur and copper (Cu), iron (Fe), manganese (Mn), zinc (Zn) and of assimilation pigments in the needles of Scots pine (*Pinus sylvestris* L.) in the forests of Bydgoszcz, Poland was determined. The content of those metals and the activity of dehydrogenases (DHA) in the rhizosphere of the trees was assayed. The average total sulphur (TS) content in 2-year-old pine needles was 832.4 mg kg⁻¹ d.w. No significant correlation was found between TS and Cu, Fe, Mn and Zn in needles and the content of assimilation pigments indicating no phytotoxic effect of sulphur dioxide (SO₂) and metals on Scots pine. The content of metals in the needles pointed to an inconsiderable degree of human impact. The soils in the surface layer were not contaminated with heavy metals. With the principal component analysis (PCA) two principal components were identified which accounted for 68% of the total change in variation. The variables that determined the principal components were the soil content of organic carbon (TOC), total nitrogen (TN), TS and sulphates (SO₄²⁻), the soil content of Mn, Zn, available forms to plants of Cu, Mn, and the content of Cu, Fe in needles.

Keywords: environmental pollution; scots pine; trace elements; assimilation pigments; dehydrogenases

1. Introduction

Scots pine (*Pinus sylvestris* L.) is a species in the Pinaceae family and is the main forest-forming species in Eurasia which, due to a high ecological and economic importance, attracts much interest of foresters and naturalists [1,2]. Both the needles and the bark of Scots pine can be used medicinally. In the bark phenolic compounds like myricetin, eleutheroside, quercetin, vanillic acid, catechin, ferulic acid and taxifolin were identified as well as volatile compounds like α-pinene and β-pinene [3]. More than 70 constituents were identified in the essential oil from pine needles. The most important of them are α-Pinene, δ-3-carene, germacrene-4-ol, and bornyl acetate. The main sesquiterpenes are β-caryophyllene, germacrene D, bicyclogermacrene, δ-cadinene, γ-cadinine, germacrene D-4-ol, cubenol and α-cadinol [4,5]. Research has shown that essential oil exhibits anti-parasitic, anti-hyperglycemic, anti-allergenic, anti-viral, anti-spasmodic properties, as well as insecticidal and larvicidal properties [6,7].

One of the major problems of contemporary civilisation is a risk of pollution of the natural environment caused by human activity [8,9]. Scots pine, due to a high ecological and economic importance, attracts much interest of foresters and naturalists [10]. The assimilation apparatus is considered a sensitive indicator of environmental changes, and hence there is frequent use of pine needles for evaluating the air pollution [10–12]. Scots pine shows little sensitivity to the presence of heavy metals in soils. The content of metals in needles is mostly connected with their content in the air [13,14]. One of the indicators of the physiological changes in the plant caused by air pollution with sulphur can be the content of assimilation pigments [15]. One of the basic parameters determining the
pollution of the environment is the content of heavy metals in soils [8,16]. Their excessively high accumulation can be toxic to the plants, animals and people [17]. Those trace elements, triggering abiotic stress, disturb the metabolism of microorganisms and, frequently, limit the count of microorganisms. They also contribute to the deterioration of the chemical soil properties [18,19]. Elements such as Cu and Zn affect the enzymatic soil activity [20]. The indicator of the potential biological activity showing a high sensitivity to environmental changes are dehydrogenases (DHA) representing the class of redox enzymes [21].

The activity of DHA is considered an indicator of the oxidative metabolism in soils and thus of the microbiological activity [21], as, being exclusively intracellular, the activity is linked to viable cells. An excessive content of heavy metals in soil poses a threat to the adequate functioning of microorganisms as it can limit their number [19].

The aim of the study has been to evaluate the content of S and Cu, Fe Mn, Zn as well as the content of assimilation pigments (chlorophyll and carotenoids) in the needles of Scots pine growing in the forest of a city. We assayed the content of metals and the activity of DHA in the rhizosphere soil.

2. Materials and Methods

2.1. Study Area

The soil was sampled from selected locations in the region of Bydgoszcz (Poland). Sampling locations: (A)—Białe Błota (53°05’59.0” N 17°55’29.8” E), (B)—Belma Electomechanical Plant (53°07’45.6” N 17°53’59.1” E), (C)—Zachem Chemical Plant (53°05’55.0” N 18°04’18.7” E), (D)—the Zbigniew Załuski Park (53°08’25.2” N 18°02’06.9” E), (E)—Forest Park of Culture and Recreation (53°09’47.4” N 18°02’05.3” E). Białe Błota (A) is a suburban village, about 2 m south-west from the borders of the city of Bydgoszcz (population: 440,000). Location B is one of the oldest industrial plants in the region. The origins of its operation date back to 1868 (the manufacture of equipment for the rail, automotive, arms and mining sectors). Location C stands for to the Zachem Chemical Plant manufacturing, in 1948–2014, dyes, polyurethane foams and organic chemical semi-finished products. Location D—the Zbigniew Załuski Park, is a city park about 17 hectares in size. The Forest Park of Culture and Recreation (E) is found 5 km away from the centre of Bydgoszcz, in an immediate vicinity of a busy transport route. It is the biggest city park in Poland, 830 hectares in size.

2.2. Soil Sampling and Laboratory Analyses

The soil was sampled 20 cm away from the trunk of Scots pine, from the depth of 0–25 cm. Each sample was a mixture of five subsamples taken in random places within each habitat examined. The samples were air-dried at room temperature and sieved using a 2 mm sieve. Soil texture was measured applying the Mastersizer 2000 analyser (Malvern Instrument, Malvern, UK). The total content of Cu, Fe, Mn, Zn in the soil samples was assayed. The soil for analysis were prepared in accordance with the standard method and pH was measured using the glass electrode in 1 M KCl solution (1:2.5 soil-solution ratio) [22]. The soil total organic carbon (TOC) was determined with the TOC analyser vario Max CN Elementar Analysensysteme GmbH (Hanau, Germany).

The total content of metals was assayed by digestion with HF and HClO₄ solutions, following the Crock and Severson method [23]. Certified reference materials (TILL-3, the Canadian Certified Reference Materials) were used to verify the accuracy of the results. The recovery rates for the elements analysed were as follows: 98%, 103%, 104%, 102%, for Cu, Fe, Mn, Zn, respectively. The contents of Cu, Mn (mg·kg⁻¹) and Fe (g·kg⁻¹), defined as the content of the local background, were: 40.6; 12.6; 309.9 and 16.5, respectively [24]. The content of available metal forms in soils was determined with 0.1 M HCl solution. The contents of metals in pine needles were assayed with the atomic absorption spectrophotometry (ASA), using a SOLAAR S4 spectrometer provided by ThermoElemental. The activity of DHA [E.C. 1.11.1.1.] was identified following the methodology by Thalmann [25] in three replications.
2.3. Plant Sampling and Laboratory Analyses

For the measurements of the total sulphur (TS) content, the total content of Cu, Fe, Mn, Zn and photosynthetic pigments, 2-year-old pine needles were collected from four randomly selected trees in five locations. The trees were of a similar age; about 40 years old. The average diameter and height of the analyzed trees were 0.30 m and 12 m respectively. About 30 needles were collected from each tree. The needles was tested separately from different trees for each location. Analyses were performed in triplicate for a total of 12 samples from each location. The needles were washed thoroughly with deionized water and dried at 50 \(^\circ\)C for 48 h. The amount of TS, the content of metals (Cu, Fe, Mn, Zn), TOC and total nitrogen (TN) were determined. The measurements of the physiological parameters covered the content of assimilation pigments (chlorophylls, carotenoids). The content of chlorophyll was assayed following the method by Arnon et al. \[26\], whereas the content of carotenoids with the method by Hager and Mayer-Berthenrath \[27\].

Plant samples were ground to powder for metal analysis. About 0.3 g of plant sample was digested with 5 mL 65% HNO\(_3\), 1 mL 30% H\(_2\)O\(_2\) mixture in the microwave digester, Speedwave Two (Berghof). The total content of Cu, Fe, Mn and Zn in pine needles was determined using the atomic absorption spectrophotometry (ASA), with a SOLAAR S4 spectrometer provided by ThermoElemental. In addition, in the dry weight of needles, the TOC and the NT were identified with the vario Max CN Elementar Analysensysteme GmbH (Hanau, Germany). The TS content was assayed according to Bardsley-Lancaster \[28\].

2.4. Statistical Analysis and Mathematical Calculations

Single-factor analysis of variance (ANOVA) was performed. The differentiation of means across the objects was determined by identifying homogeneous groups with the LSD test at a significance level of \(\alpha = 0.05\). The contents of all the components in soil and pine needles were evaluated using the principal component analysis (PCA) \[29\]. The content of TS and the content of sulphate sulphur (SO\(_4^{2-}\)) \[30\] were applied for statistical analysis. The coefficients of variation (CV) of the parameters was calculated \[31\].

The human activity impact on soils was estimated with the enrichment factor \((EF)\), based on the normalization of the metal measured against a reference metal. Fe was used as the reference element. The values of \(EF\) were calculated according to the formula \[32,33\]:

\[
EF = \frac{[C_n/Cn_{Fe}]}{[B_n/Bn_{Fe}]}.
\]

3. Results and Discussion

3.1. Physical and Chemical Properties

The soils were qualified as: sand for A, C and loamy sand for B, D, E \[34\]. They contained from 76.7% to 92.0% of sand fraction (2.0–0.05 mm), from 7.4% to 21.0% of silt fraction (0.05–0.002 mm) and from 0.63% to 2.33% of clay fraction (<0.002 mm).

The reaction of the soils was very acid, from pH 3.65 in A to pH 4.41 in E. The acidification of the soils was due to hydrogen ions released to the environment by pine tree roots while taking up mineral nutrients. Moreover, the acid reaction is affected by the activity of humus acids produced from the decomposition of pine needles and from the presence of mosses the product of decomposition of which are organic acids. Soil acidification can be also due to the chemical processes which take part in the weathering of minerals and from C and N cycling.

The soils demonstrated a similar content of organic carbon; the highest content was found in the soil sampled from location C (9.84 g kg\(^{-1}\)). The average content of TOC\(_s\) in soil was 7.63 g kg\(^{-1}\).

3.2. Content of TS\(_s\), Zn\(_s\), Cu\(_s\), Mn\(_s\), Fe\(_s\) and the Activity of DHA in Soils

The soils contained from 171 mg kg\(^{-1}\) to 343 mg kg\(^{-1}\) of TS\(_s\) and 14.76 mg kg\(^{-1}\) to 22.69 mg kg\(^{-1}\) of sulphate sulphur. Such a content of TS\(_s\) points to a low content of that
element in soil (class 1; content of TS from 160 to 500 mg S kg\(^{-1}\)), while the content of sulphate sulphur in the soil sampled in Białe Błota was classified as natural (class 0, content of SO\(_4^{2-}\) ≤ 15 mg kg\(^{-1}\)). In the other soil samples the content of sulphate sulphur was low (class 1, content of SO\(_4^{2-}\) ≤ 15 mg kg\(^{-1}\)) [35]. The contents of respective metals in soil samples can be ordered in a series as follows: Cu\(_s\) < Zn\(_s\) < Mn\(_s\) < Fe\(_s\) (Table 1). Evaluating the total content of the metals, one can find that they did not exceed the admissible contents provided for in the applicable law [36]. As compared with the contents of the geochemical background of the region’s soils [24], the mean accumulation of Cu\(_s\) in location C, Mn\(_s\) in A, and D, Zn\(_s\) in C as well as a considerable accumulation of Zn\(_s\) in sampling location D were found, which is evident from the values of enrichment factor (EF). EF is a comparatively simple and easy tool to assess the enrichment of elements in soils [37].

The contents of copper forms available to plants (Cu\(_{sa}\)) ranged from 1.02 to 6.70 mg ha\(^{-1}\); iron (Fe\(_{sa}\))—from 0.55 to 1.53 g ha\(^{-1}\); manganese (Mn\(_{sa}\))—from 26.5 to 161.0 mg ha\(^{-1}\); and zinc (Zn\(_{sa}\))—from 5.26 (E) to 33.9 mg ha\(^{-1}\) (Table 2). As reported by Ociepa [38], the availability of heavy metals to plants is affected by many factors, especially the type of the parent material, the soil reaction and the content of organic substance, clay minerals and the interaction with other elements.

As noted by Kabata-Pendias and Pendias [8] and Skwaryło-Bednarz et al. [39], Zn, even though one of the most mobile metals, is strongly bonded by organic matter, which was confirmed by the significant dependence recorded in this study. We identified significant positive dependencies between the content of TOC and the content of Cu and its available forms. Similar results were reported by Skwaryło-Bednarz et al. [39].

Determining the DHA activity in soil provides much information about the biological characteristics of soil that remains fertile and healthy [40]. The highest activity of DHA (2.054 mg TPF·g\(^{-1}\)·h\(^{-1}\)) was found in the soils sampled from location C, where the content of organic carbon was highest (Figure 1). We identified a positive correlation between the content of Zn\(_s\) and Cu\(_s\). The content of Cu\(_s\) is strongly related to organic matter [41]. Most of the soluble Zn usually occurs in a form of a free ion, while Cu occurs in organically-complexed forms [42]. The average accumulation of Cu\(_s\) and Zn\(_s\) in soil in location C had a stimulating effect on the activity of DHA in the soils. Therefore, it can be assumed that the content of metals and a relatively high content of organic carbon in those soil samples can have a stimulating effect on the microbiological activity of soils expressed as the activity of intracellular DHA.

![Figure 1](image_url). Activity of DHA in soil; A, B, C, D, E—explanations as in Table 1. The same lowercase letters above each column indicate that the average values of DHA activity (homogeneous group) are not significantly different at \( p < 0.001 \).
**Table 1.** Total content of sulphur (TS<sub>s</sub>), sulphate sulphur (SO<sub>4</sub><sup>2-</sup>), Cu<sub>s</sub>, Mn<sub>s</sub>, Zn<sub>s</sub> (mg kg<sup>-1</sup>), Fe<sub>s</sub> (%) in soil samples, and the values of enrichment factor (EF).

| Sampling Location * | TS<sub>s</sub> ** | SO<sub>4</sub><sup>2-</sup> ** | Cu<sub>s</sub> | Mn<sub>s</sub> | Zn<sub>s</sub> | Fe<sub>s</sub> | EF | EF | EF |
|---------------------|-----------------|-----------------|----------|----------|----------|--------|---|---|---|
|                     | mg kg<sup>-1</sup> |                  | %        |          |          |        |    |    |    |
| A                   | 340.0 ± 22.25   | 14.76 ± 2.87    | 2.82 ± 0.16 | 293.0 ± 9.87 | 19.5 ± 1.22 | 0.58 ± 0.03 | 0.64 | 2.69 | 1.22 |
|                     | CV = 6.54%  | CV = 19.44%     | CV = 5.67% | CV = 3.37% | CV = 6.26% | CV = 5.17% |
| B                   | 202.0 ± 1.70   | 22.69 ± 0.97    | 3.33 ± 0.21 | 109.0 ± 6.61 | 22.8 ± 1.36 | 0.53 ± 0.02 | 0.88 | 1.09 | 1.75 |
|                     | CV = 0.84%  | CV = 14.82%     | CV = 4.28% | CV = 6.06% | CV = 5.96% | CV = 1.06% |
| C                   | 343.0 ± 6.32   | 21.75 ± 1.45    | 15.70 ± 0.82 | 117.0 ± 6.01 | 84.2 ± 3.13 | 0.71 ± 0.03 | 2.91 | 0.88 | 4.82 |
|                     | CV = 1.84%  | CV = 6.67%      | CV = 5.22% | CV = 5.14% | CV = 3.72% | CV = 4.22% |
| D                   | 337.0 ± 9.55   | 21.94 ± 1.70    | 13.40 ± 0.77 | 88.1 ± 3.34 | 70.9 ± 3.41 | 0.42 ± 0.02 | 4.20 | 1.12 | 6.86 |
|                     | CV = 2.83%  | CV = 7.75%      | CV = 1.40% | CV = 3.86% | CV = 4.61% | CV = 4.76% |
| E                   | 337.0 ± 18.21  | 21.08 ± 2.23    | 5.37 ± 0.21 | 177.0 ± 7.11 | 27.7 ± 1.40 | 0.57 ± 0.03 | 1.24 | 1.65 | 1.97 |
|                     | CV = 10.65% | CV = 10.58%     | CV = 3.91% | CV = 4.02 | CV = 5.03% | CV = 5.26% |

* sampling location: A—Białe Błota, B—Belma Electomechanical Plant, C—Zachem Chemical Plant, D—The Zbigniew Załuski Park, E—The Forest Park of Culture and Recreation; average ± standard deviation, n = 12; ** Siwik-Ziomek et al. [30]; TS<sub>s</sub>—total content of sulphur in soil; SO<sub>4</sub><sup>2-</sup>—sulphate sulphur; Cu<sub>s</sub>—total Cu in soil, Mn<sub>s</sub>—total Mn in soil; Zn<sub>s</sub>—total Zn in soil; Fe<sub>s</sub>—total Fe in soil; EF—enrichment factor.

**Table 2.** Average content of Cu<sub>sa</sub>, Mn<sub>sa</sub>, Zn<sub>sa</sub> (mg kg<sup>-1</sup>) and Fe<sub>sa</sub> (g kg<sup>-1</sup>) available to plants.

| Sampling Location | Cu<sub>sa</sub> | Mn<sub>sa</sub> | Zn<sub>sa</sub> | Fe<sub>sa</sub> |
|-------------------|----------------|----------------|----------------|---------------|
|                   | mg kg<sup>-1</sup> | Content * Rating | mg kg<sup>-1</sup> | Content Rating | mg kg<sup>-1</sup> | Content Rating | g kg<sup>-1</sup> | Content Rating |
| A                 | 1.10 ± 0.03 | medium | 16.10 ± 9.11 | high | 13.10 ± 0.62 | high | 1.53 ± 0.05 | medium |
|                   | CV = 2.72%  |               | CV = 5.66%   |          | CV = 4.73%   |          | CV = 3.27%  |               |
| B                 | 1.02 ± 0.02 | medium | 28.9 ± 0.77 | medium | 5.96 ± 0.26 | high | 0.55 ± 0.03 | low |
|                   | CV = 1.96%  |               | CV = 2.66%   |          | CV = 4.36%   |          | CV = 5.45%  |               |
| C                 | 6.70 ± 0.39 | high | 28.2 ± 0.96 | medium | 27.50 ± 1.42 | high | 0.71 ± 0.04 | medium |
|                   | CV = 5.82%  |               | CV = 3.40%   |          | CV = 5.16%   |          | CV = 5.63%  |               |
| D                 | 5.95 ± 0.36 | high | 26.5 ± 0.93 | medium | 33.90 ± 1.50 | high | 0.74 ± 0.03 | medium |
|                   | CV = 6.05%  |               | CV = 3.51%   |          | CV = 4.42    |          | CV = 4.05%  |               |
| E                 | 1.41 ± 0.06 | medium | 85.0 ± 4.12 | medium | 5.26 ± 0.26 | medium | 0.97 ± 0.05 | medium |
|                   | CV = 4.26%  |               | CV = 4.85%   |          | CV = 4.94%   |          | CV = 5.15%  |               |

* A, B, C, D, E—explanations as in Table 1; average ± standard deviation, n = 12; Cu<sub>sa</sub>—available Cu in soil, Mn<sub>sa</sub>—available Mn in soil, Zn<sub>sa</sub>—available Zn in soil, Fe<sub>sa</sub>—available Fe in soil, *Content of metals provided for in the applicable Polish regulations.
3.3. Chemical and Physiological Pine Needle Analyses

The microelement increases the plant tolerance both to biotic and abiotic stress [43]. An unfavourable impact of SO$_2$, on the other hand, results in changes in cells, which is shown, e.g., in some damage to the root system, leaves and needles [44]. The phytotoxic effect of SO$_2$ on plants involves its penetration through stomata and, as a result, sulphate forming. It leads to a disappearance of chlorophyll and to disturbances in the process of photosynthesis as well as to a decreased assimilation of CO$_2$ [15].

The content of total sulphur in pine needles ($T_{S_n}$) was low and it ranged from 657.9 mg kg$^{-1}$ d.w. to 972.0 mg kg$^{-1}$ d.w. (Table 3). The results were similar to those recorded by Likus-Cieślak et al. [10], who report on the content of that element in pine needles in the region of Bydgoszcz ranging from 600–900 mg kg$^{-1}$ d.w. All across the country they assayed the content to be 537.5 to 2343 mg kg$^{-1}$ d.w. of pine needles, at the average content of 854.8 mg kg$^{-1}$ d.w. For a comparison, in Europe the content of sulphur ranges from 1050 mg kg$^{-1}$ to 1470 mg kg$^{-1}$ in 1-year-old needles [10]. In biomonitoring studies performed in Poland in the mid 1970s in the area of Bydgoszcz, Dmuchowski and Bytnerowicz [45] assayed higher contents of that element in pine needles, ranging from 910 to 1200 mg kg$^{-1}$ d.w. and reaching 2060 mg kg$^{-1}$ d.w. in south-eastern Poland. The results of the present study and those reported by other authors for the studies carried out over a few dozen years [38] suggest that since the 1980s the air quality in the Bydgoszcz region has improved.

As reported by Marska and Wróbel [46], the content of TS in plants can fall within a broad range from 700 to 14,000 mg kg$^{-1}$ of dry weight and the content in the plant depends on the plant species, the richness of soils in soluble sulphates, content of SO$_2$ in the air. For the right development, Scots pine needs 300 mg of sulphur per kg of dry weight [47]. As reported by Baciak et al. [48], in the strongly industrialised areas, the content of TS in Scots pine needles can be from 6000 to 10,000 mg kg$^{-1}$ d.w., whereas in the areas with no air pollution, the average content of TS$_n$ ranges from 300 to 1200.

We calculated the values of the ratio of carbon to nitrogen C:N in pine needles, which was similar in all the locations and ranged from 28.6 to 33.7 (Table 3). The values were lower than those reported by Griffin et al. [49], ranging from 39 to 64. Raven et al. [50] claim that the values of the C:N ratio in trees fall within a broad range up to more than 100.

The physiological response of Scots pine needles to the effect of SO$_2$ was assayed with the content of assimilation pigments: chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a+b (Chl a+b), and carotenoids (Carot). The most important assimilation pigments in plants affect the intensity of photosynthesis and biomass production [51]. Depending on the external factors, they also play a regulatory function in cellular differentiation, growth and metabolism [52].

The numerical data on the content of assimilation pigments in the two-year study of pine needles is provided in Table 4. The data reported by other authors indicate that the content of chlorophyll and carotenoids in the needles was increasing with the plant material age and in the 1-, 2-, and 3-year-old needles it was 1.42 mg g$^{-1}$ f.w. 2.08 mg g$^{-1}$ f.w., 3.79 mg g$^{-1}$ f.w. and 0.28 mg g$^{-1}$ f.w., 0.37 mg g$^{-1}$ f.w., 0.65 mg g$^{-1}$ f.w., respectively [53].

No significant correlations between the content of sulphur in 2-year-old pine needles and the content of assimilation pigments were found because of a lack of phytotoxic effect of sulphur on pine plants in the Bydgoszcz agglomeration (Table 5).
Table 3. Total nitrogen (TN$_n$), total organic carbon (TOC$_n$) (g kg$^{-1}$ d.w.), total sulphur (TS$_n$), total metals: Cu$_n$, Fe$_n$, Mn$_n$, Zn$_n$. (mg kg$^{-1}$ d.w.) and the ratio of carbon to nitrogen content (TOC$_n$/TN$_n$) in the dry weight of pine needles.

| Sampling Location | TOC$_n$ (g kg$^{-1}$ d.w.) | TN$_n$ | TOC$_n$/TN$_n$ | TS$_n$ (mg kg$^{-1}$ d.w.) | Cu$_n$ (mg kg$^{-1}$ d.w.) | Fe$_n$ (mg kg$^{-1}$ d.w.) | Mn$_n$ (mg kg$^{-1}$ d.w.) | Zn$_n$ (mg kg$^{-1}$ d.w.) |
|-------------------|-----------------------------|-------|----------------|------------------------------|----------------------------|---------------------------|---------------------------|---------------------------|
| A                 | 487.5                       | 17.03 | 28.6           | 972.0 ± 3.907$^a$            | 5.13 ± 0.092$^a$          | 248.6 ± 4.738$^a$        | 109.2 ± 0.778$^b$         | 58.75 ± 1.485$^a$         |
|                   |                             |       |                | CV = 0.40%                   | CV = 1.79%                | CV = 1.91%               | CV = 0.71%                | CV = 2.53%                |
| B                 | 485.3                       | 15.72 | 30.9           | 890.6 ± 6.664$^a$            | 4.56 ± 0.001$^b$          | 102.6 ± 12.586$^d$       | 65.00 ± 2.263$^c$         | 64.05 ± 0.919$^a$         |
|                   |                             |       |                | CV = 0.75%                   | CV = 0.02%                | CV = 12.27%              | CV = 3.48%                | CV = 1.43%                |
| C                 | 485.3                       | 15.60 | 31.1           | 825.4 ± 1.950$^a$            | 4.91 ± 0.163$^b$          | 203.3 ± 4.950$^b$        | 182.0 ± 1.909$^a$         | 54.30 ± 1.979$^b$         |
|                   |                             |       |                | CV = 0.24%                   | CV = 3.32%                | CV = 2.43%               | CV = 1.05%                | CV = 3.64%                |
| D                 | 478.1                       | 14.84 | 32.2           | 815.9 ± 2.36$^b$             | 5.51 ± 0.170$^a$          | 159.6 ± 12.021$^c$       | 104.5 ± 4.242$^b$         | 48.80 ± 2.404$^c$         |
|                   |                             |       |                | CV = 0.29%                   | CV = 3.09%                | CV = 7.53%               | CV = 4.06%                | CV = 4.93%                |
| E                 | 478.4                       | 14.21 | 33.7           | 657.9 ± 2.134$^c$            | 4.36 ± 0.064$^c$          | 154.6 ± 0.182$^c$        | 120.6 ± 13.152$^c$        | 56.10 ± 0.283$^b$         |

Values followed by the same small letter within each column are not significantly different at $p < 0.001$; average ± standard deviation, $n = 12$; A, B, C, D, E—explanations as in Table 1; CV—coefficient of variation [%]; TN$_n$—total nitrogen in needles, TOC$_n$—total organic carbon in needles; TS$_n$—total sulphur in needles; Cu$_n$—total Cu in pine needles; Fe$_n$—total Fe in pine needles; Mn$_n$—total Mn in pine needles; Zn$_n$—total Zn in pine needles; d.w.—dry weight.

Table 4. Content of chlorophyll a (Chl a), chlorophyll b (Chl b), chlorophyll a+b (Chl a+b) and carotenoids in pine needles. (mg g$^{-1}$ f.w.) of the Bydgoszcz agglomeration area.

| Sampling Location | Assimilation Pigments Content mg g$^{-1}$ f.w. | Chl a/Chl b |
|-------------------|-----------------------------------------------|-------------|
|                   | Chl a                                         | Chl b       | Chl a+b | Carot | Chl a/Chl b |
| A                 | 1.860 ± 0.12$^a$                              | 0.736 ± 0.097$^a$ | 2.594 ± 0.222$^a$ | 0.539 ± 0.046$^a$ | 2.52          |
|                   | CV = 6.51%                                    | CV = 13.18%  | CV = 8.55% | CV = 8.53% |             |
| B                 | 1.753 ± 0.17$^a$                              | 0.621 ± 0.126$^a$ | 2.237 ± 0.254$^a$ | 0.377 ± 0.044$^b$ | 2.82          |
|                   | CV = 9.75%                                    | CV = 20.29%  | CV = 11.35% | CV = 11.67% |             |
| C                 | 1.958 ± 0.123$^a$                             | 0.660 ± 0.063$^a$ | 2.618 ± 0.387$^a$ | 0.573 ± 0.150$^a$ | 2.96          |
|                   | CV = 6.28%                                    | CV = 9.54%   | CV = 14.78% | CV = 26.18% |             |
| D                 | 1.280 ± 0.191$^b$                             | 0.620 ± 0.092$^a$ | 1.900 ± 0.183$^b$ | 0.619 ± 0.047$^a$ | 2.06          |
|                   | CV = 14.92%                                   | CV = 14.84%  | CV = 9.63%   | CV = 7.59%   |             |
| E                 | 1.880 ± 0.108$^a$                             | 0.821 ± 0.097$^a$ | 2.701 ± 0.305$^a$ | 0.526 ± 0.081$^a$ | 2.28          |
|                   | CV = 5.74%                                    | CV = 11.81%  | CV = 11.29%  | CV = 15.40%  |             |

Values followed by the same small letter within each column are not significantly different at $p < 0.001$; average ± standard deviation, $n = 12$; A, B, C, D, E—explanations as in Table 1; CV—coefficient of variation [%]; Chl a—chlorophyll a; Chl b—chlorophyll b; Chl a+b—chlorophyll a+b; Carot—carotenoid; f.w.—fresh weight.
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Figure 2. Configuration of variables in the system of the first two axes PC1 and PC2 of principal components. TOC—total organic carbon in soil, SO\textsubscript{4}\textsuperscript{2−}—sulphate sulphur in soil, DHA—dehydrogenase activity, TS—total S in soil, Cu—total Cu in soil, Fe—total Fe in soil, Mn—total Mn in soil, Zn—total Zn in soil, Cu\textsubscript{a}—available Cu in soil, Fe\textsubscript{a}—available Fe in soil, Mn\textsubscript{a}—available Mn in soil, Zn\textsubscript{a}—available Zn in soil, TN—total N in pine needles, TOC\textsubscript{n}—total organic carbon in pine needles, TS\textsubscript{n}—total S in pine needles, Cu\textsubscript{n}—total Cu in pine needles, Fe\textsubscript{n}—total Fe in pine needles, Mn\textsubscript{n}—total Mn in pine needles, Zn\textsubscript{n}—total Zn in pine needles, Chl a+b—total chlorophyll content in pine needles, Carot—carotenoids in pine needles; A—Białe Błota, B—Belma Electomechanical Plant, C—Zachem Chemical Plant, D—The Zbigniew Załuski Park, E—The Forest Park of Culture and Recreation.

Table 5. Correlation coefficient (R), determination (R\textsuperscript{2}) and linear regression models for selected properties of soils and pine needles (n = 12; p < 0.05).

| Variables        | Regression Equation     | R   | R\textsuperscript{2} |
|------------------|-------------------------|-----|---------------------|
| Zn\textsubscript{n} | Zn\textsubscript{s}     | y = −0.6987 + 0.19686x | 0.998 | 0.996 |
| Cu\textsubscript{s} | Zn\textsubscript{s}     | y = −0.6987 + 0.19686x | 0.997 | 0.994 |
| Zn\textsubscript{s} | DHA                    | y = 4.0819 + 40.959x | 0.935 | 0.874 |
| Zn\textsubscript{a} | DHA                    | y = 0.06016 + 8.1080x | 0.939 | 0.882 |
| DHA              | Cu\textsubscript{s}    | y = 0.11242 + 0.10866x | 0.939 | 0.882 |
| TN\textsubscript{n} | TOC\textsubscript{n}   | y = 8.3151 + 0.00861x | 0.939 | 0.882 |
| TOC\textsubscript{n} | TN\textsubscript{n}    | y = 425.15 + 3.7316x | 0.908 | 0.824 |
| Cu\textsubscript{a} | TOC\textsubscript{s}   | y = −10.95 + 1.6991x | 0.906 | 0.821 |
| Cu\textsubscript{s} | TOC\textsubscript{s}   | y = −22.48 + 3.6716x | 0.931 | 0.867 |
| DHA              | TOC\textsubscript{s}  | y = −2.750 + 0.44927x | 0.985 | 0.970 |
| TOC\textsubscript{s} | Zn\textsubscript{s}    | y = 6.2752 + 0.04600x | 0.919 | 0.045 |
| TOC\textsubscript{s} | Cu\textsubscript{s}    | y = 6.4170 + 0.23628x | 0.931 | 0.867 |
| TOC\textsubscript{s} | Cu\textsubscript{a}    | y = 6.7837 + 0.48277x | 0.905 | 0.819 |
| TOC\textsubscript{s} | DHA                    | y = 6.1897 + 2.1574x | 0.985 | 0.970 |

Explanations as in Figure 2.
The value of the Chl a/Chl b ratio ranged from 2.06 to 2.96. Taller plants contain 2–4-fold more chlorophyll a than chlorophyll b [54].

The content of selected metals in needles was found in a decreasing series: \( \text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} \) (Table 3). Of all the metals studied, the lowest content in pine needles was recorded for Cu (Table 3). Drawing on the earlier research [45], the area of Poland under study, which covered the Bydgoszcz agglomeration, was classified in terms of pollution with Cu, as in zone II, with 5.1 to 10.0 mg Cu kg\(^{-1}\). Most plants need a negligible amount of Cu (2 mg kg\(^{-1}\)) to satisfy their physiological needs. The content of Cu in plants is usually below 4–5 mg kg\(^{-1}\) and its average content in the aboveground plant parts ranges from 5 to 20 mg kg\(^{-1}\) [8]. For the purpose of comparison, in Stalowa Wola the content of Cu in two-year-old needles ranged from 4.7 to 7.8 mg kg\(^{-1}\) [55], in the Białowieża Wild Forest—from 2.92 to 3.85 mg kg\(^{-1}\) [45], while in the Słowiński National Park [49] it was 5.9 mg kg\(^{-1}\). The statistical analysis of the results of this experiment did not identify, as for sulphur, any significant correlations between the content of the metals analysed in pine needles and the content of assimilation pigments, which shows a lack of phytotoxic effect of the metals on the plants (Table 5).

The content of Fe in the plants varied and it ranged from 102.60 mg kg\(^{-1}\) d.w. to 248.6 mg kg\(^{-1}\) d.w. The contents were similar to those reported by Samecka-Cymermanet et al. [55] in two-year-old needles of pines growing in Stalowa Wola, ranging from 77 to 270 mg kg\(^{-1}\). Slightly lower contents in pine needles, ranging from 107.1 mg kg\(^{-1}\) to 132.1 mg kg\(^{-1}\), were reported by Parzych and Jonczak [56] in the Słowiński National Park, one of the purest national parks in Poland.

The content of Mn in the two-year-old needles of pine ranged from 65.00 mg kg\(^{-1}\) to 182.0 mg kg\(^{-1}\) (Table 3). The content was lower than that recorded by Parzych and Jonczak [56] in the area without a human impact (the Słowiński National Park); from 185.1 to 368.7 mg kg\(^{-1}\) in 1-year-old needles and from 211.1 to 449.8 mg kg\(^{-1}\) in 2-year-old needles. According to other authors, the content of Mn in other plant species in the area outside the contamination impact was from 27.2 to 247.3 mg kg\(^{-1}\) [57], 122 to 837 mg kg\(^{-1}\) [58]. The demand for Mn in plants varies a lot depending on the species. In most cases the content from 10 to 25 mg kg\(^{-1}\) is enough [8]. As reported by Malzahn [59], the content of Mn in the plants growing outside an immediate pollution impact is, most frequently, 340-1339 mg kg\(^{-1}\).

The highest Zn content in soil was 48.80 mg kg\(^{-1}\) to 64.05 mg kg\(^{-1}\) (Table 3). To satisfy the physiological needs of most plants, the content of zinc in shoots ranging from 15 to 30 mg kg\(^{-1}\) is sufficient and the shortage of that metal is most frequent for the content lower than 20 mg kg\(^{-1}\) [60]. Dmuchowski and Bytnerowicz [45] reported by on the environmental pollution of the territory of Poland, the area was not considered polluted if the content of Zn\(_n\) did not exceed 70 mg kg\(^{-1}\). The content in 2-year-old pine needles in Poland ranged from 10 to 70 mg kg\(^{-1}\) in the Słowiński National Park [56], from 45 to 99 mg kg\(^{-1}\) in Stalowa Wola [55], and from 40.9 to 173.7 mg kg\(^{-1}\) in the Białowieża Wild Forest [45]. The content of Zn\(_n\) recorded in this study in the needles of *P. sylvestris* in the Bydgoszcz agglomeration area was comparable to the level of the content in pine needles sampled from the regions considered the purest in Poland points to an inconsiderable content in the soils under study (Table 1).

The PCA facilitated determining the general dependencies between variables and describing and classifying the parameters defined by other variables, which helped define two principal components (PC1 and PC2), which accounted for more than 68% of the variation observed in soil (Table 6).
Table 6. Values of the two extracted factor loadings for 26 elements ($n = 12$).

| Elements      | Components Matrix |
|---------------|-------------------|
|               | PCA 1  | PCA 2  |
| $SO_4^{2-}$   | $-0.077$  | $-0.982$  |
| Chl a         | $0.529$  | $0.043$  |
| Chl b         | $0.324$  | $0.307$  |
| Chl a+b       | $0.473$  | $0.017$  |
| Carot         | $-0.471$  | $-0.638$  |
| TN s          | $0.766$  | $-0.550$  |
| TOC s         | $0.725$  | $-0.396$  |
| TS n          | $0.599$  | $-0.523$  |
| TS s          | $-0.781$  | $0.439$  |
| Cu s          | $-0.817$  | $-0.534$  |
| Fe s          | $0.232$  | $-0.292$  |
| Mn s          | $0.848$  | $-0.205$  |
| Zn s          | $-0.787$  | $-0.570$  |
| Cu sa         | $-0.774$  | $-0.612$  |
| Fe sa         | $0.703$  | $-0.343$  |
| Mn sa         | $0.810$  | $-0.168$  |
| Zn sa         | $-0.649$  | $-0.732$  |
| Cu n          | $-0.232$  | $-0.763$  |
| Fe n          | $0.393$  | $-0.794$  |
| Mn n          | $-0.314$  | $-0.578$  |
| Zn n          | $0.731$  | $0.458$  |
| sand          | $0.662$  | $-0.658$  |
| clay          | $-0.672$  | $0.658$  |
| silt          | $-0.558$  | $0.625$  |
| TOC s         | $-0.700$  | $-0.545$  |
| $pH_{KCl}$    | $-0.363$  | $0.747$  |

Values in bold are marked as significant; explanations as in Figure 2.

From Figure 2 it can be concluded that the vectors of variables representing the soil content of Zn, Cu and TOC as well as the activity of DHA showed the greatest negative linear dependencies, which groups them with the second principal component. Analysing the soil properties, one can found that the content of Cu and Zn in soil and their forms available to plants and the activity of DHA correlated most with the content of organic matter. DHA plays a crucial role in the biological oxidation of soil organic matter by transferring hydrogen from organic substrates to inorganic acceptors [61,62].

A comparison of stands A to E demonstrates that the soils sampled from locations C and D contained similar contents of Cu, Zn and TOC and a similar activity of DHA in soil.

4. Conclusions

The TS content in 2-year-old needles of Scots pine growing in a few locations of the Bydgoszcz agglomeration was low, which points to a lack of air pollution with SO$_2$. A low level of human impact is also seen from the total content of Cu, Fe, Mn and Zn. No significant correlations were found between the total content of S, Cu, Fe, Mn and Zn in Scots pine needles and the content of assimilation pigments (chlorophyll a, chlorophyll b, carotenoids), which points to a lack of their phytotoxic effect. The contents of elements satisfied the basic physiological needs of the trees. Medicinal raw material coming from the described locations can be safely used in phytotherapy. Pine needles can be successfully used as a bioindicator of the air quality.

In the soil surface layer no pollution with the heavy metals studied was found. A considerable Zn accumulation, as compared with the content of the geochemical background, was identified only in one of the locations, the Zbigniew Załuski Park. There, a significantly positive effect of organic matter on Zn accumulation in soil was noted. The presence of that metal and Cu stimulated the activity of DHA.
With the principal component analysis (PCA) we identified two principal components which accounted for 68% of the total change in variation. The variables which determined the principal components most were the soil content of TOC, TN as well as TS and sulphates (VI), the soil content of Mn, Zn and available forms of Mn and Cu and the content of Cu and Fe in pine needles.

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