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GENETIC-ENVIRONMENTAL CONTROLS OF THE TOLERANCE OF FOREST TREES TO INDUSTRIAL POLLUTION

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GENETYCZNO-ŚRODOWISKOWE UWARUNKOWANIA TOLERANCJI DRZEW LEŚNYCH NA ZANIECZYSZCZENIA PRZEMYSŁOWE

Opracowanie porusza problematykę genetyczno-środowiskowych uwarunkowań tolerancji drzew leśnych (na przykładzie sosny zwyczajnej) na zanieczyszczenia przemysłowe. Na poziomie badanej populacji sosny zaobserwowano zróżnicowaną odpowiedź poszczególnych osobników na stres antropogenny, co stanowiło podstawę hipotezy o genetycznym podłożu zjawiska. Procedurę badawczą poprzedzono diagnozą warunków środowiskowych uwzględniającą szczególnie stan zanieczyszczeń atmosfery i pedosfery, jako tła dla oszacowania poziomu zmienności i zbadania struktury genetycznej grup osobników podatnych, bądź odpornych na zanieczyszczenia. Analizie poddano populację sosny pochodzącej z naturalnego odnowienia, pozostającą w strefie bezpośredniego oddziaływania zanieczyszczeń z Huty Cynku „Miasteczko Śląskie". W jej obrębie wyróżniono dwie grupy drzew: S (sensitive) i T (tolerant) charakteryzujące się odmiennymi parametrami genetycznymi. Zaobserwowane prawidłowości (zmniejszenie się tempa podziałów komórkowych, obniżenie wartości indeksu miotycznego w stosunku do drzew grupy kontrolnej, wysoki poziom aberracji chromosomowych) wskazują na bezpośredni wpływ zanieczyszczeń na materiał genetyczny drzew.

Summary

Genetic-environmental controls of the tolerance of forest trees to industrial pollution are discussed on the example of the Scots pine. Within the pine population under study, various responses to man-made stress were observed in individual specimens, which gave rise to the hypothesis about a genetic origin of the phenomenon. The research procedure was preceded by an assessment of the environmental conditions which focused especially on the pollution of the atmosphere and pedosphere as a background for estimating the level of variation and examining the genetic structure of specimens sensitive to, or tolerant of, the pollution. The analysis covered a pine population coming from natural forest regeneration growing in the zone of direct impact of pollution from the Miasteczko Śląskie Zinc Works. Two groups of trees were distinguished: S (sensitive) and T (tolerant), characterized by different genetic parameters. The observed tendencies (slower cell division rates, lower values of the mitotic index than in the control group, a high level of chromosomal aberrations) indicate a direct effect of the pollution on the genetic material of the trees.

INTRODUCTION

An analysis of the migration of man-made pollution in the natural environment system shows the state of an ecosystem to be connected with industrial emissions, and the genetic material of the model plant to be affected by the pollution. Therefore, in present article a research was conducted to provide a basis for:

- a diagnosis of the state of atmospheric air,
- a diagnosis of the state of the soil environment, and
- an assessment of the genetic-environmental controls of the tolerance of forest trees to industrial pollution.

For many years in Poland studies have been made on the effect on plants of emissions of such noxious gases as SO_2, NO_x, CO and HF, and the deposition of dust containing heavy metals [3, 7, 10, 19]. Stunted growth, disturbances in the root structure, leaf necrosis, etc., are only some of the observed external symptoms of genotoxicity resulting from the fact that air and soil pollutants directly damage vegetative tissues of plants and affect their physiological processes. In extreme cases, specimens with genotypes sensitive to pollution die. Adverse effects of heavy metals have been studied in a number of plants, like *Allium cepa* [7, 11, 15, 16], *Vicia faba* [10], *Lathyrus odoratus* [12], *Festuca rubra* [17], *Sorghum* sp. [17], *Pinus banksiana*, and *Picea glauca* [4]. It has been noted that at the population level particular specimens tend to respond to stress in an individual way, which raises the question of whether the response is genetically motivated and hence, whether it is reflected in a different genetic structure of each plant. In the present analysis, an examination was carried out of a Scots pine (*Pinus sylvestris* L.) population growing in an area with a high level of environmental pollution. The Scots pine is the dominant forest-forming species in Poland (about 70% of the forest stock [1]) and one that shows an especially low tolerance to industrial pollution, which makes it an ideal model for research on the effect of industrial pollution on plants and on micro-evolutionary processes taking place as a result of human impact [20].

The research was carried out in the impact zone of the Miasteczko Śląskie Zinc Works. The premises are surrounded in the north, west and east by a large Lubliniec Forest complex, and in the south-east by the localities of Żyglin and Żyglinek, quarters of Miasteczko Śląskie. In the south the works is separated from the town by a tall green belt, its buffer strip (Fig. 1).

Subjected to analysis was population of pine as a part of artificial restoration, mainly of mixed forest, sporadically of mixed wood. The trees in eastern part of woodlands (most contaminated, on the line of dominant winds) are 20 to 30 years old, when the age of woodlands located on the western side of Zinc Works is typical and cross-sectional, between 5 to 109. The analyzed population is a self-sowing; same as in S (sensitive) and T (tolerant) groups and is neither experimental nor economic area.

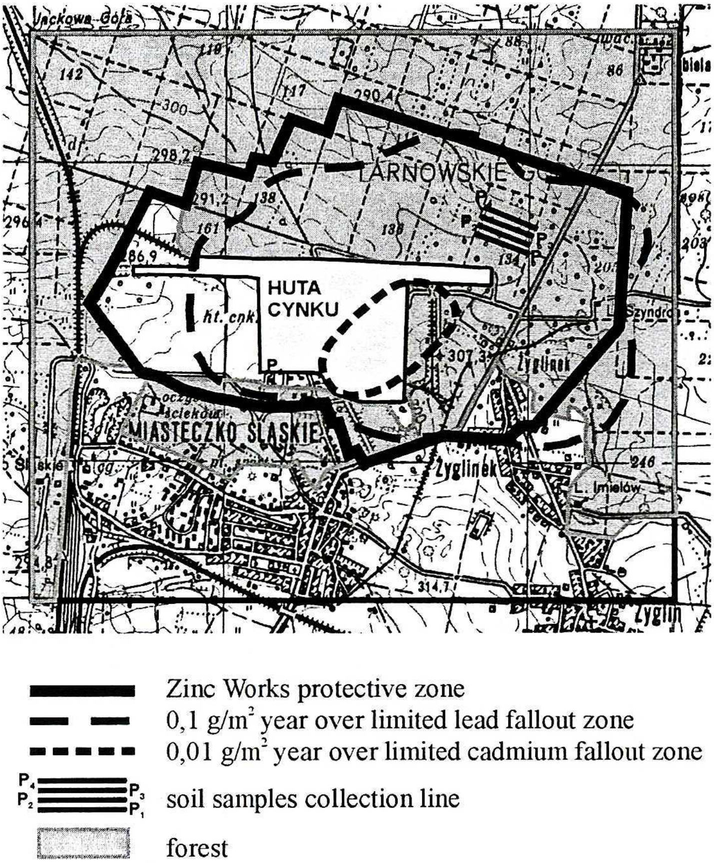

Fig. 1. Draft plan of Zinc Works

DIAGNOSIS OF THE STATE OF ATMOSPHERIC AIR

At a macroscale, the state of atmospheric air in the study area was, and is, determined by the Upper Silesian Industrial District (VSID) and transborder pollution flows. At a microscale, air pollution is the effect of the operation of the only source of industrial emission in the area, viz. the Zinc Works. At present there are 15 sources of organized pollution emission on the premises, the highest being four chimneystacks, at h = 120 m, discharging gases and particulates into the air; the height of the other emitters does not exceed 45 m. The gases and particulates produced as a result of the technological process employed include dust containing heavy metals – Zn, Pb, and Cd, as well as gases, mainly SO_2, NO_2 and CO from the burning of natural gas, and hydrogen. Some pollution is released into the atmosphere by various facilities of the works in the form of non-organized emission whose magnitude is impossible to measure or estimate. They are usually low emission sources crucially determining the pollution level of and threats to the areas in the immediate vicinity of the works.

Of crucial significance for the dispersal of harmful substances in the atmosphere are the speed and direction of wind. In the Miasteczko Śląskie region, the prevailing winds are from the south-westerly (21.4%) and westerly (18.7%) quarters, hence the pollution emitted by the Zinc Works poses the greatest threat to areas north-east and east of the plant. The mean wind velocity is 2.5 m/s, while calms, so unfavorable for the dispersal of pollutants, occur on 11.8% of days in the year. The study area discussed in the present analysis lies along the line of their dispersal. A detailed distribution of wind directions and speeds for Miasteczko Śląskie is listed in Table 1 and Figure 2.

Table 1. Distribution of wind directions and velocities for Miasteczko Śląskie in the 1992–2001 period

	Calms	Wind quarters								Total
		N	NE	E	SE	S	SW	W	NW	
Percentage (%)	11.8	5.6	6.8	9.8	8.1	6.9	21.4	18.7	10.9	100.0
Mean velocity (m/s)	–	1.8	2.1	2.6	2.4	2.7	3.6	3.2	2.5	2.5

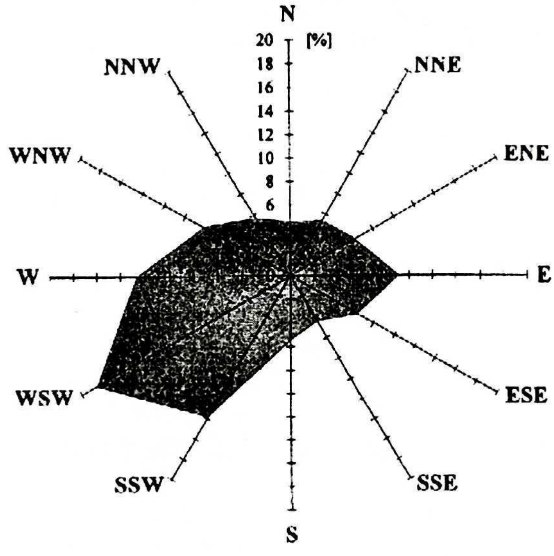

Fig. 2. Compass Rose for Miasteczko Śląskie

The zone of direct impact of the emitted industrial pollution is defined as an area with a radius of 50 times the height of the tallest emitters. In the case of the Zinc Works, the radius equals 6,000 m (50 x 120 m).

The current status of air pollution in the immediate impact zone of the Zinc Works is characterized by data prepared by the Silesian Voivodeship Sanitary-Epidemiological Station [25]. Table 2 shows that at none of the measuring sites do maximum dust deposition figures exceed the acceptable limits. The highest dust deposition levels were recorded at Radzionków–Śródmieście, at 83 g/m^2 year, Rojca, 75 g/m^2 year, as well as Tarnowskie Góry–

Śródmieście and Bobrowniki, 72 g/m^2 year each. In the immediate vicinity of the works, in Miasteczko Śląskie the deposition amounted to 52 g/m^2 year, while in the localities east of the plant, viz. Żyglin, Brynica and Bibiela, 43, 63 and 23 g/m^2 year, respectively.

Table 2. Deposition of dust and metals in dust at selected measuring localities (2000) (permissible amounts: deposition of dust 200 mg/m^2 year, deposition Pb – 100 mg/m^2 year, deposition Cd 10 mg/m^2 year)

Locality	Deposition of dust g/m^2 year	Deposition of metals in dust (mg/m^2 year)								
		Pb	Zn	Cd	Cu	Cr	Ni	Fe	Mn	Co
Kuczów (Kalety)	20	22	–	0.21	–	–	–	–	–	–
Miotek (Kalety)	34	53	–	0.64	–	–	–	–	–	–
Śródmieście (Kalety)	49	–	–	–	–	–	–	–	–	–
Zielona (Kalety)	26	68	–	0.7	–	–	–	–	–	–
Krupski Młyn	58	17	81	0.25	13	1.1	16.4	1.8	25	0.66
Bibiela (Miasteczko Śl.)	23	30	81	0.61	7	0.4	8.5	0.5	10	0.1
Brynica (Miasteczko Śl.)	63	44	149	1.16	10	1.0	9.1	1.1	30	0.42
Śródmieście (Miasteczko Śl.)	52	365	233	2.33	20	5.1	13.3	7.0	35	0.84
Żyglin (Miasteczko Śl.)	43	79	525	7.03	35	2.0	12.4	1.6	33	0.50
Pyrzowice (Ożarowice)	71	26	122	1.22	8	0.9	7.3	1.2	17	0.46
Tąpkowice (Ożarowice)	37	23	58	0.65	7	0.7	4.8	0.6	11	0.28
Rojca (Radzionków)	75	68	1414	1.07	19	3.5	15.1	3.7	59	0.84
Śródmieście (Radzionków)	83	29	223	0.93	13	3.4	10.0	3.9	69	0.84
Chechło Nowe (Świerklaniec)	51	29	112	1.01	9	0.9	7.9	1.03	19	0.36
Nakło (Świerklaniec)	23	20	74	0.47	6	0.8	4.1	0.5	14	0.23
Świerklaniec	48	33	112	0.70	12	1.5	10.0	2.2	28	0.56
Bobrowniki (Tarnowskie Góry)	72	32	149	1.35	9	1.2	4.4	1.3	36	0.37
Czarna Huta (Tarnowskie Góry)	62	42	1768	2.65	34	2.8	10.4	6.9	59	0.98
Pniowiec (Tarnowskie Góry)	25	24	149	0.51	7	0.7	6.2	1.3	23	0.33
Repty (Tarnowskie Góry)	71	24	158	0.79	9	1.9	13.0	4.1	37	0.65
Strzybnica (Tarnowskie Góry)	26	38	149	0.84	10	2.2	7.7	1.1	40	0.37
Śródmieście (Tarnowskie Góry)	72	52	335	0.96	13	2.1	13.2	2.9	40	0.80

An analysis of metal deposition in dust (Table 2) shows lead deposition to be the most serious threat; its highest values, 365 and 142 g/m^2 year, were recorded at two sites located in Miasteczko Śląskie, which means 3.5 times the permissible limit in the case of the former figure.

The highest zinc deposition was recorded at the Tarnowskie Góry–Czarna Huta site – 1,786 mg/m^2 year, Radzionków–Rojcy – 1,414 mg/m^2 year, as well as Miasteczko Śląskie–Żyglin – 525 mg/m^2 year, and Śródmieście – 233 mg/m^2 year.

The deposition of cadmium did not exceed the acceptable limit at any of the measuring sites. Its highest levels were recorded in Miasteczko Śląskie–Żyglin – 7.03 mg/m^2 year, and Śródmieście – 2.33 mg/m^2 year. Similar values were observed in Tarnowskie Góry-Czarna Huta – 2.65 mg/m^2 year.

Calculations of the immission of the particulate and gaseous pollutants in the impact zone of the Zinc Works performed with the help of a pollution dispersal model [5] show that substances in the form of suspended dust, carbon monoxide, sulphuric acid, and zinc and cadmium in suspended dust produce concentrations lower than or equal to 10% of the mean annual concentration limit. The highest concentrations were recorded in the case of sulphuric acid (48.43% of the mean annual limit D_a) and nitrogen dioxide (11.26% of D_a), but these figures do not exceed allowable levels.

What exceeds the limits is cadmium and lead deposition in the impact zone of the works. Cadmium deposition amounts to 0.000005–0.034 g/m^2 year, which is higher than the reference level of 0.01 g/m^2 year. The excessive levels are recorded on the premises and in the area situated about 500 m NE and E of its limits. Lead deposition varies between 0.00055 and 1.91 g/m^2 year, ranging between 0.01 and 0.135 g/m^2 year at sites located on the boundary of the works' buffer strip, which means exceeding the allowable level of 0.1 g/m^2 year in areas next to the buffer strip boundary as well as NE and E of the plant. The excessive-level zone includes the study area discussed here. It also lies within the range of maximum concentrations, which are recorded at a distance of some 550 m from the emitters [5]. Calculations of the maximum concentrations of pollutants released by the works in 2001 do not indicate the maximum values to be exceeded.

The above description of the state of atmospheric air for the years 2000–2001 shows most pollutants to have met the air quality standards. Excessive levels were only recorded in the case of two heavy metals: cadmium and lead. Among the gaseous pollutants, the highest concentrations, though still within the limit, were recorded for sulphur dioxide. This shows unambiguously an improvement in the pollution level of and threats to the atmosphere in the immediate impact zone of the Zinc Works, and a desirable tendency to restrict its detrimental effect. However, a full assessment of the status of the atmosphere as a factor affecting the other geocomponents, especially the soil environment, requires an approach to the atmospheric degradation in a wider time horizon. The particulate and gaseous pollution level is a result of emissions that have varied over the 38 years of operation of the plant. In 2001 the total dust emission equaled 39.208 Mg/year, as against 1.998 Mg/year in 1972, which means a 50-fold decline. Emissions of lead and cadmium in 2001 were 5.135 Mg/year and 0.0474 Mg/year, respectively, as against 315 Mg/year (Pb) and 4.5 Mg/year (Cd) in 1972. The distribution of emission levels of substances identified in this study as the greatest threats over the thirty-odd years is presented in Figure 3.

The permanent action of the air pollutants, though markedly limited recently, has contributed to the contamination of the soil environment in the vicinity of the works. This especially concerns areas situated NE and E of the plant.

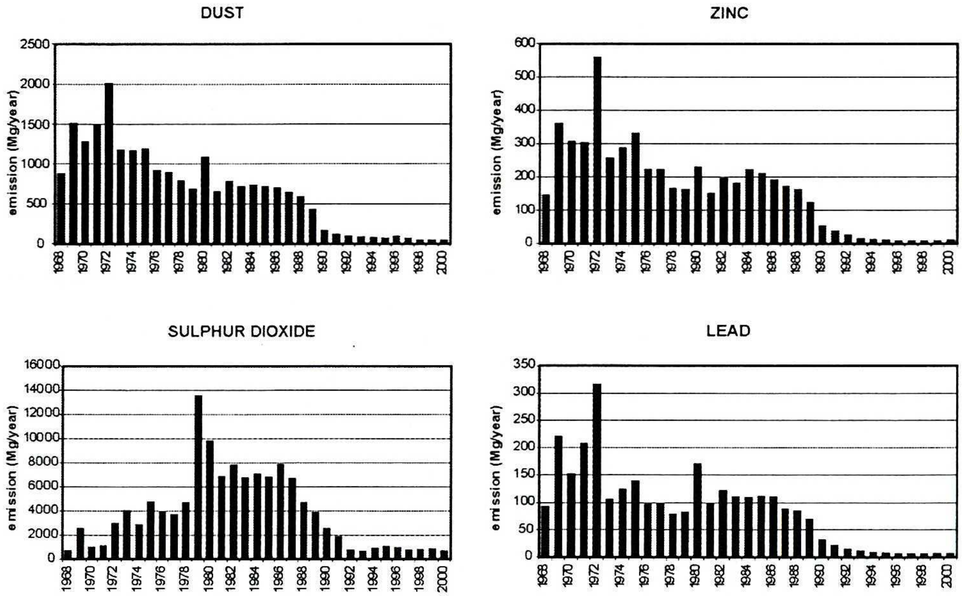

Fig. 3. Emission from the Miasteczko Śląskie Zinc Works in the years 1968–2001

DIAGNOSIS OF THE STATE OF SOIL ENVIRONMENT

Two processes responsible for the chemical degradation of soils were taken into consideration when making the diagnosis:

1. those leading to a change in the soil reaction, i.e. its acidification or alkalization, and
2. those leading to the accumulation of trace elements in the soil, especially heavy metals.

The evaluation of the level of soil contamination with heavy metals was performed on a research plot 400 by 200 m, supporting a 20–30-year-old pine stand and located 500 m NE of the Zinc Works within its buffer strip.

The analysis was carried out on samples taken from the top soil horizons (0–20 cm) along four 400-m profiles spaced every 50 m. The average sample (P1, P2, P3, P4) representing each profile line was a mixture of five single samples taken at 100-m intervals along a profile line. In each soil sample the following determinations were made:

– the soil reaction (pH), using the potentiometric method in a suspension of 1M KCL, and
– the content (mg/kg d.m.) of six trace elements (Cd, Cr, Cu, Ni, Pb, and Zn) using atomic absorption spectrometry (AAS).

Since the present research focused especially on the mobile parts of trace elements, the leaching of samples with hydrochloric acid (HCl 1:4) was chosen. The part of an element thus released and determined is one weakly bonded to the carrier (largely in sorption forms). The sorption form in which chemical elements occur determines their ease of migration, irrespective of whether they have been introduced into the environment by man or whether they come from natural sources. The weakly bonded, easily migrating part poses the greatest threat because it is absorbed by plants most readily [14].

In typological terms, the samples belonged to podzolic soils developed on weakly loamy and loamy sands. Ranked along the 10-degree resistance scale based on sorption capacity [23, 24], this type of soil displays poor sorption (a mean sorption capacity of 2–3 cmol/kg), which corresponds to degree 1–2 of the geochemical resistance to degradation. It should be emphasized, therefore, that the soils found in the vicinity of the works have a high level of susceptibility to pollution.

In geology, heavy metals are classified into the group of so-called trace metals which account for less than 1% of the Earth's crust. Because of wide differences in the metal content in rocks, soils often happen to be considered contaminated if they have developed on bedrock with an abnormally high content of some metals. Especially characteristic of some metal-bearing areas is the occurrence of wide, element-rich aureoles around polymetallic deposits, which is reflected in a generally higher level of metals in the soil there [2]. Hence, a very important step in establishing whether the concentration of heavy metals in the soils under study is greater than in the surrounding area is the determination of the local concentration background. In the present analysis, the reference level for the results obtained was provided by the data in the PIG Geochemical Atlas [14], which is justified by both studies employing the same methods of metal content evaluation (Table 3, column C). For a full picture of the degree of soil contamination with heavy metals, the table also lists such additional data as:

– boundary values (the background, zero level of pollution) of the natural heavy metal content measured in the top layers of various soils in Poland [8] – column A,
– mean heavy metal content in the soils in Poland [9] – column B,
– limits of heavy metal content in the soil according to the Minister of the Environment's Ordinance of 9 September 2002 concerning soil quality standards and land quality standards (Law Gazette no. 165, position 1359) for woodland, areas supporting trees and shrubs (column D), and industrial areas (column E).

Table 3. Heavy metal levels in the soil near the Miasteczko Śląskie Zinc Works

Metal	Research results				Reference level				
	mg/kg d.m.								
	P1	P2	P3	P4	A*	B	C	D	E
Cd	22.7	15.3	20.1	10.3	0.3–1.0	0.22	4–8	4.0	15
Cr	2.5	2.1	2.1	1.4	20–50	4.2	5–10	150	500
Cu	28.2	20.2	28.0	17.0	10–25	6.7	40–80	150	600
Ni	2.2	2.3	2.3	0.8	10–50	6.5	5–10	35	300
Pb	2972	2273	2228	1685	20–60	13.8	200–400	100	600
Zn	1480	2946	1337	1289	50–100	33.2	400–800	300	1000
(pH)	4.66	4.75	4.74	4.04	–	–	6.7–7.4	–	–

* explanation in the text

According to the Geochemical Atlas, the mean Zn content in the soils near Miasteczko Śląskie equals 400–800 mg/kg d.m.; Pb, 200–400 mg/kg d.m.; Cd, 4–8 mg/kg d.m.; and Ni, 5–10 mg/kg d.m. (cf. Table 3, column C).

Among the heavy metals under analysis, Cd, Zn and Pb displayed the highest levels relative to the background values. A special threat is posed by lead, whose content was almost five times greater (P1) than the limit for industrial areas (Table 3, column E). Its concentration was ten times higher than the mean value of the local geochemical background (Table 3, column C), and exceeded the mean Pb content in the soils of Poland more than 200-fold. Despite the small size of the research plot (8 ha) and spacing of the profile lines along which the sampling was done (50 m), the lead concentration in the soil tended to decline as the distance from the works premises grew, which may suggest a decisive role of non-organized emission of particulates from industrial emitters. A similar tendency was displayed by the distributions of Cd and Zn within the research plot.

A wider spatial approach confirms the proportional dependence between the distance from the works and the heavy metal content in the soil within its impact zone. In research carried out by Rochel et al. [22] (Table 4), the highest zinc content in the soil was recorded at sites located in the immediate vicinity of the works: A – Miasteczko Śląskie with 1,560.43 mg/kg, C – Żyglin with 1,260.28 mg/kg, and D – Żyglin with 1,309.95 mg/kg. They were situated at a distance of 100, 500 and 1,000 m, respectively, east and south-east of the works. At sites E – Brynica and F – Bibiela located along the same direction of pollution dispersal but at a distance of 4,500 m and 6,000 m, respectively, the concentrations dropped to 270 mg/kg (E) and 160 mg/kg (F).

Table 4. Zn levels in the soil in the impact zone of the Miasteczko Śląskie Zinc Works

Site	Site description	Zn content in soil sample (mg/kg d.m.)
A	Miasteczko Śl. ul. Cynkowa, 100 m from Zinc Works, direction SE	1560.43
B	Miasteczko Śl. ul. Dworcowa, 500 m from Zinc Works, direction W	469.66
C	Żyglin ul. Brynicka, 500 m from Zinc Works, direction E	1260.28
D	Żyglin ul. Św. Marka, 1500 m from Zinc Works, direction SE	1309.95
E	Brynica ul. Żyglińska, 4500 m from Zinc Works, direction E	270
F	Bibiela ul. Starowiejska, 6000 m from Zinc Works, direction E	160

Site B – Miasteczko Śląskie, located at a distance of 500 m on the western side of the plant, had one-third of the Zn content (469.66 mg/kg), which corroborates the enormous role of climatic conditions in the spread and immission of contaminants. The Zn content in the soils surrounding the works exceeded the local background 5 times (P2), the limit for industrial areas nearly 3 times, and the mean Zn content in Polish soils, 90 times.

On a 5-degree scale of soil pollution [8], those surrounding the works are classified as a very highly polluted group (5th degree) on account of their above-standard lead and cadmium concentrations, and a highly polluted group (4th degree) on account of their zinc content. When assessing the persistence of soil contamination with heavy metals, one should keep in mind that a half-life of Cd ranges from 15 to 1,100 years and of Pb from 740 to 5,900 years, depending on the kind of soil and its physico-chemical properties. Because of this very slow process of soil self-purification and the tendency of heavy metals to accumulate, in making an assessment of threats to the soil environment one should take into consideration the entire duration of their detrimental influence.

However, the correlation between the level of elements in the soil and their availability to plants is not always simple. The ability of plants to take up and accumulate individual heavy metals is controlled, among other things, by the set of edaphic conditions among which an important role is played by the soil reaction (pH). A lowering of the soil pH makes the trace elements introduced pass into readily soluble forms, which increases their concentration in the liquid phase of the soil and in consequence makes their bioaccumulation and migration to ground- and surface water easier. The reaction of the soil samples under study ranged between 4.04 and 4.75, which puts them in the class of very acid soils. The high acid reaction of the soils and their poor buffer ability (low sorption capacity) decidedly facilitate the uptake and accumulation of heavy metals in plants tissues.

GENETIC-ENVIRONMENTAL CONTROLS OF THE TOLERANCE OF FOREST TREES TO INDUSTRIAL POLLUTION

The performed analysis of the state of the natural environment showed a high level of contamination of the soil in the vicinity of the Zinc Works with heavy metals, which according to Siuta [24] may bring about a higher uptake of those elements by plants growing in a habitat with their very high concentrations. What additionally facilitates this process in the study area is the genetic type of the soil and its reaction.

The researches conducted in previous years repeatedly proved that the needles of pines growing in the surroundings of Zinc Works Miasteczko Śląskie contain significantly largest quantities of zinc, lead and cadmium than the needles of pines growing distant from the emission centre. There was affirmed almost linear relationship between the content of zinc and lead in plants and their distance from the emission centre. Even if the population of plants was grown on contaminated soil, but in the clean atmosphere and there was no simple correlation between soil contamination and accumulation of heavy metals in leaves and needles, a correlation of concentration of e.g. zinc in roots and soil could be observed [17].

The pine population under analysis included specimens clearly resistant or susceptible to contamination, which suggested a genetic origin of the phenomenon, the more so as a high heritability of resistance of individual trees has been observed earlier [18, 20].

In view of the above, the aim of the research conducted was to assess the level of variation and examine the genetic structure of specimens sensitive or resistant to pollution, as well as the effect of the pollution on the process of mitosis and the occurrence of chromosomal aberrations. The object of study was the Scots pine (*Pinus sylvestris* L.) growing in the immediate impact zone of the Miasteczko Śląskie Zinc Works.

Methods

The material under study included winter buds, two-year-old needles, and cones from two groups of trees growing on the contaminated ground around the Miasteczko Śląskie Zinc Works. Group S (sensitive), numbering 39 specimens, displayed clear symptoms of injury, such as needle necrosis and chlorosis, an uncharacteristic growth habit, and poor growth. In group T (tolerant), also embracing 39 trees, no such damage was observed. Measurements were also taken of tree height (Table 2). The control in the cytogenetic study was a group of 15 trees growing in the Wielkopolski National Park (WNP) in soils whose heavy metal content was within the allowed limit. The cytogenetic study, which is one of the methods of examining genotoxicity, was carried out by crushing root apices of seedlings germinating from seeds collected from all three groups of trees, S, T and WNP. In groups S and T not all trees had cones (Table 5). Wherever possible, calculations were performed of the seed germination ability. Chromosomal aberrations were analyzed at various stages of the mitotic division of cells. To determine the proportion of dividing cells in the root meristem, a mitotic index (MI) was calculated. A study of isoenzyme variation served to determine the genetic structure of trees tolerant of and sensitive to pollution. Within each group of trees, for 18 gene loci in 11 enzymatic systems, determinations were made of such parameters as: frequencies of alleles and genotypes, mean number of alleles (A/L) and genotypes (G/L) per locus, observed (Ho) and expected heterozygosity (He), genotype polymorphism index (Pg), and Wright coefficient (F).

Table 5. Numbering of trees from which seedling was obtained and cytological analyses made

WNP*	T	T	S	S
Germinating ability	Number of tree analyzed cytologically	Germinating ability	Number of tree analyzed cytologically	Germinating ability
81%	42, 46, 48, 49, 50, 51, 52, 53, 54, 58, 60, 62, 64, 66, 69	69.2%	3, 4, 6, 8, 11, 14, 19, 23, 24, 29, 30, 31, 34, 38, 39	54.5%

* In the control group each tree had cones and analyses were made for all the 15 specimens.

Results

The trees from the two groups growing around the Zinc Works differed markedly in height while being of roughly the same age (15–20 years). The mean height in group T was 6.39 m as against mere 1.46 m in group S (Table 6). The height is treated as a manifestation of the tree being resistant to pollution. Seeds were obtained from 23 trees (out of 34 with cones) with no signs of injury (T), 17 trees (out of 27 with cones) showing damage (S), and all the control trees from the WNP. There were distinct differences in the ability of the particular groups to reproduce, which corroborates earlier observations of the effect of pollution on seed bearing [1]. The proportion of seed germination in group T was 69.2%, in group S 54.5%, and in the WNP group, 81% (Table 5).

The mitotic index of meristematic cells of root apices in the group of damaged trees was found to be lowered, while the figures for the healthy and control WNP trees were similar (Table 3). In group ***T***, the mean MI ranged from 2.3% to 4.1% (3.1% on average), as against 2% to 3.3% in group ***S*** (2.5% on average). The differences in MI values were not significant statistically.

Among the trees growing in Miasteczko Śląskie, chromosomal mutations observed most frequently included chromosome stickiness, acentric fragments, and chromosome bridges. In anaphase, multipolar divisions, lagging chromosomes and c-mitosis were observed with various frequencies. In group S the mean proportion of the aberrations was 17.8%, as against a mere 12.3% in group T (Table 7).

Table 6. Numerical data on mean height (in meters) of damaged (S) and undamaged trees (T)

Trees	n	$\bar{x}$	S^2	S	R
S	39	1.46	2.98	1.72	0.35 – 2.70
T	39	6.39	4.00	6.32	2.70 – 10.0

$\bar{x}$ – mean values (cm), S^2 – variance, S – standard deviation, R – spacing

Table 7. Cytological parameters of the trees examined (mean values for each group)

Tree group	Division stage (mitosis)	Interphase	Mitotic index (%)	Proportion of chromosomal aberrations
WPN	16.8	282.4	5.52	3.7%
T	8.6	265.4	3.14	12.3%
S	6.8	261.8	2.52	17.8%

The examination of variations in enzymatic loci showed the genetic structure of the two tree groups to differ significantly (Table 8). In group T a lower number of alleles (40) and genotypes (52) per locus was recorded, as well as a predominance of rare alleles. The mean number of alleles per locus was 2.2 for population T and 2.4 for S. The mean value of observed heterozygosity for 16 loci was –0.286 for T and –0.255 for S.

Table 8. Genetic parameters in the tree groups S and T: number of alleles found, number of rare alleles (with a frequency of under 0.05), mean number of alleles per locus (A/L), number of genotypes found, and mean number of genotypes per locus (G/L)

Tree groups	Number of alleles found	Number of rare alleles	A/L	Number of genotypes found	G/L
S	43	10	2.4	56	3.1
T	40	8	2.2	52	2.9

For 9 loci displaying significant differences in the level of heterozygosity between S and T, it was observed that in group T the Wright coefficient indicated the population to be in a state of Hardy-Weinberg equilibrium (a balance between the number of homo- and heterozygotes), while in group S homozygotes were found to predominate (Table 9).

Table 9. Mean genetic parameters in the tree groups S and T calculated for 9 gene loci

Tree groups	He	Ho	F	Pg
S	0.316	0.263	0.168	0.456
T	0.330	0.327	0.010	0.456

Discussion

As a result of a substantial reduction in the emission of pollutants (from 82% to 98% over the years 1994–1996), one can positively report an improvement in the state of the atmospheric air in the direct impact zone of the Miasteczko Śląskie Zinc Works and a desirable tendency to restrict its detrimental effect. However, this has not been reflected in the state of the soil, in which the heavy metal content still exceeds the allowed limits, and in the response of plants to contact with heavy metals.

The *Pinus sylvestris* population under study responds to the environmental stress in which it grows in various ways. The two clearly distinguishable groups of trees, S and T, display different genetic parameters, like the level of heterozygosity, the number of cells dividing in root meristems, and the frequency of chromosomal aberrations. The observed slower rates of cell division and lower mitotic index values in comparison with the control are probably due to a higher concentration of zinc ions (Zn^{2+}) in the soil [7]. The high level of chromosomal aberrations (chromosome bridges, multipolar divisions, lagging chromosomes) is evidence of a clastogenic action of the pollutants under study. The observed regularities indicate a direct effect of the pollutants on the genetic material of the trees. Due to selection, some genotypes are favored (a higher seed production, a higher germination percentage) while others are not. The comparative research conducted in an area considered ecologically clean in which the heavy metal content in the soil stays within the limit has shown the condition of trees growing there to be much better, and their genotype variation to be considerably higher than that recorded in the Miasteczko Śląskie population. The obtained results may find use in the reclamation (afforestation) of contaminated land when selecting material with a suitable genetic composition.

SUMMING UP

The article is concerned with the effect that human impact has on individual plant species. An analysis of the migration of anthropogenic pollution in the natural environment system shows that a special role is played by interactions between the atmosphere, the pedosphere, and the biosphere. Contamination of the air and soil results in stunted growth, irregularities in the root structure, and leaf necrosis observed as external symptoms of genotoxicity. It was found that at the population level, various responses to man-made stress could be observed in individual specimens, which provided a basis for the hypothesis about a genetic origin of the phenomenon.

The aim of the research conducted was to estimate the level of variation and examine the genetic structure of specimens sensitive or resistant to pollution on the example of the Scots pine (*Pinus sylvestris* L.) growing in the direct impact zone of the Miasteczko Śląskie

Zinc Works. The research background was provided by a diagnosis of the state of atmospheric air and the soil environment. The analysis of the air on the basis of 2000–2001 data showed most pollutants to have met the air quality standards. Excessive levels were only recorded in the case of deposition of two heavy metals in dust: cadmium and lead. The permanent presence of air pollutants released during the 40-year-long activity of the works, though markedly limited recently, has contributed to the contamination of the soil environment in its vicinity. The study of the heavy metal content showed higher levels of lead, zinc and cadmium compared to the background values. Two groups of trees were distinguished within the *Pinus sylvestris* population under study: S (sensitive) and T (tolerant), characterized by different genetic parameters. The observed slower cell division rates and lower values of the mitotic index than in the control group is probably due to a higher concentration of zinc ions (Zn^{2+}) in the soil. The high level of chromosomal aberrations (chromosome bridges, multipolar divisions, lagging chromosomes) is evidence of a clastogenic action of the pollutants under study. The observed regularities indicate a direct effect of the pollutants on the genetic material of the trees.

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