

Biogeochemical Assessment of a Zn-Contaminated Site Using Scots Pine (*Pinus sylvestris* L.) Needles as Phytoindicators

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Received: 18 February 2016

Accepted: 27 May 2016

Abstract

The aim of our study was to evaluate the mobility of Zn in soils subjected to the activity of a zinc smelter and to assess the contamination of 1- and 2-year-old needles of Scots pine species sensitive to and tolerant of heavy metal contamination, particularly zinc. The trees were selected on the basis of their morphology, i.e., tolerant ones exhibited quite normal growth shape, but sensitive ones were more or less dwarves. Mineralogical composition revealed the prevalence of kaolinite in all soils except for two samples, where smectite dominated. Zinc contamination exceeded 30 times the geochemical background, and the reactive Zn forms represented 34.6% of total Zn content (Zn_{total}). Proton generation capacity (α) indices calculated for the bioavailable Zn fraction (Zn_{Bio}) were 59% higher compared to the reactive Zn pool (Zn_{Reac}). Two-year-old tolerant (T) pine needles accumulated 21.9, 38.2, and 13.6% more Zn, Fe, and Mg, respectively, as compared to 1-year-old ones. For sensitive (S) needles, the range followed: 12.4, 48.8, and 7.3%, respectively. Iron was considered a “strategic survival element” for both pines growing under high zinc pollution.

The transfer of Zn ions from the soil environment to plants still remains the basic source of maintaining its high concentrations in the needles, since air emissions ceased in 1996. The amounts of Zn accumulated by Scots pine at crucial years of metallurgical emissions may be considered as an additional time-constant source due to the evergreen vegetative lifecycle.

Keywords: soil, Zn, contamination, mineralogy, Scots pine, Mg, Fe, needles

Introduction

The natural zinc (Zn) concentration in the soil is generally not toxic for the flora and fauna. Several physical as well as chemical soil processes interact to maintain Zn in the soil solution at levels not impairing any biological functions [1]. Unfortunately, the anthropogenic pressure and the resulting environmental pollution *via* industrial emissions of chemicals, including Zn, are of great concern [2-3]. These actions tend to drastically raise the concentrations of Zn beyond safe thresholds in the soil environment, leading to the worsening of biological activities and simultaneously increasing Zn phytoaccumulation [4]. Zinc occur in soils under different compounds sorbed by mineral and organic colloids or as ions in the soil solution [5]. Its mobility in soil compartments and uptake by plants is strongly regulated and controlled by soil pH [6-7]. Several functions and physiological processes such as growth hormones and protein and carbohydrate synthesis are mediated by Zn in plants. High concentrations may induce negative plant responses, resulting in chlorosis and necrosis [8].

In general, organisms used for evaluating the state of the natural environment are defined as indicators (bioindicators), of which phytoindicators represent one of the core groups [9]. According to European Parliament data [10], more than 42% of EU land area is covered with forest and other wooded land. The main component of coniferous forests growing in Europe is *Pinus sylvestris* L., a species commonly used in passive biomonitoring of environmental pollution [11-12]. The advantage of conifers as evergreen species is that, in contrast to deciduous trees, they can accumulate atmospheric pollutants over several years. The intensity of these stressors are reflected by the chemical composition of the needles [13-15].

The aims of the current work were to investigate Zn content and its potential mobility in soils contaminated by a Zn smelter. In addition, the study deals with the assessment of Zn as well as Mg and Fe concentrations in one- and two-year-old Scots pine (*Pinus sylvestris*) needles of sensitive (S) and tolerant (T) species for evaluating the direct impact of Zn pollution on Mg and Fe phytoaccumulation. The link between Zn in soils and in needles with its possible phytotoxicity was also investigated.

Materials and Methods

Sampling Zone Characteristics

The study area is the impact zone of the Zn smelter located at Miasteczko Śląskie (N 51°41'03" and E 15°57'12", Poland). The investigated zone is dominated by northern and southwestern (21.4%) plus western (18.7%) winds. This creates much more pollution concerns for areas located to the east and southeast toward the smelter emitters. The course of biannual emissions of dusts and metallic Zn (Fig. 1) was irregular, but there was

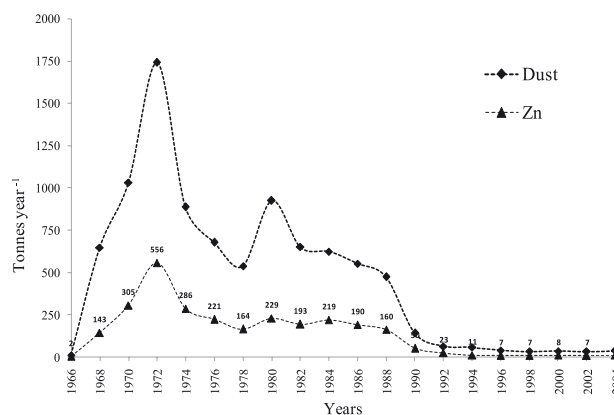


Fig. 1. Course of biannual emission of dusts and metallic Zn for the time-range 1966-2004 [12].

a decreasing trend from 1966 to 2004 [12]. For the time-range 1996-2004, emissions stabilized at mean levels of 34 and 7 tons per year for dusts and Zn, respectively.

Forty soil samples were collected on June 2012 at a depth 0-20 cm. Sampling proceeded along a transect, mostly in an easterly direction toward the smelter (like the number five on a dice). Next, eight composite soils were made (from thoroughly mixing five single samples), representing eight localities as reported in Table 1.

Soil Chemical Analyses

Prior to physical and chemical analyses, soil samples have been dried at ambient room temperature and passed through a 2 mm sieve mesh. Soil particles were determined using the methods described by soil survey staff [16], where three fractions (sand, silt, clay) were separated. Soil pH_{H_2O} as well as electrical conductivity (EC) were measured in an aqueous solution at a soil/distilled water ratio as 1:2.5, [17]. Moreover, pH was additionally

Table 1. Distribution of respective soil sampling sites.

Soil No	Location
1, 2	Experimental site with young pines, distant at 500 m from the emitter, ESE direction
3	Cynkowa street, distant at 100 m from the emitter, E direction (Miasteczko Śląskie)
4	Dworcowa street, distant at 500 m from the emitter, SE direction (Miasteczko Śląskie)
5	Brynicka street, distant at 500 m from the emitter, SE direction (Zyglin)
6	St Mark street, distant at 1500 m from the emitter, SE direction (Zyglin)
7	Zyglińska street, distant at 4500 m from the emitter, E direction (Brynica)
8	Staromiejska street, distant at 6000 m from the emitter, E direction (Bibiela)

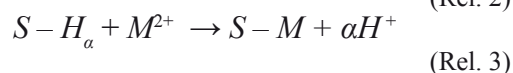
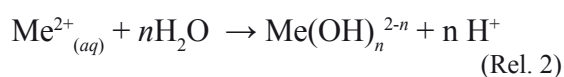
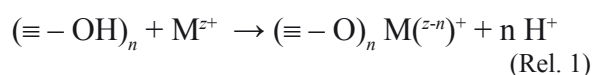
determined in a 0.01 mol CaCl₂ dm⁻³ solution at a ratio of 1:5 and designated as pH_{CaCl2} [18]. The content of oxidizable organic carbon was assayed according to Wang et al. [19], whereas the effective cation exchange capacity (CEC_{ef}) was evaluated first by extracting Ca²⁺, Mg²⁺, K⁺, and Na⁺ using 1 mol CH₃COONH₄ dm⁻³ (pH 7), and next summing these ions along with hydrolytic acidity (where necessary) [20-21].

Total Zn content was determined by hot extracting soil samples with *aqua regia*: (HCl_{conc.}:HNO_{3conc.} 1:3 ratio), [22]. Next the concentrations of bioavailable (Zn_{Bio}) as well as reactive (Zn_{Reac}) zinc forms were assessed by the chemical tests 0.10 mol NaNO₃ dm⁻³ [23] and 0.11 mol CH₃COOH dm⁻³ (first fraction), according to the BCR method [24], respectively. All elements (Ca, Mg, K, Na, and Zn) extracted by the reported tests were determined with the absorption spectrometry method (AAS) by using a Varian 250 Spectra Plus (Australia).

Bulk soil mineralogy of selected soil samples was assessed by random powder x-ray diffraction (Thermo ARL SCINTAG X'TRA X-ray diffractometer, USA, with Cu K α irradiation). Clay mineral fraction < 2 μ m was obtained using the sedimentation method. The samples were centrifuged twice for 15 min using a Sigma 4-15 centrifuge. The clay suspensions were pipetted onto a glass slide and left to dry at an ambient temperature. Clay mineral species were determined based on the oriented air-dried (untreated) samples, and ethylene glycol-solvated and heated (550°C) samples according to the methods described by Śródoń [25]. Because the clay mineral peaks were well resolved, no treatment was necessary for the removal of carbonates or organic matter. Measurements were done at 40 kV and 40 mA and scan rates from 3 to 30° 2 θ (or from 2 to 30° 2 θ) at 0.02° 2 θ min⁻¹ step size. Phase identification and peak area determinations were done using Win XRD software. XRD analyses were performed at the Institute of Geology, Adam Mickiewicz University, Poznan, Poland.

Evaluation of Zn Geochemical Processes

The reaction of Zn with soil colloids was evaluated by applying the index expressing the Proton Generation Capacity (PGC), (α) as described by the relations (Rel. 1, 2, 3) [26]. It is assumed that deprotonation of various functional groups takes place when Zn sorption and retention proceed. This leads to the release of H⁺, which consequently tends to enhance Zn mobility and activity in the soil solution, as reported below:



...where M⁺ and Me²⁺ express metallic ions (i.e., Zn²⁺), S-H α is active soil sorptive sites, α is proton index expressing the number of H⁺ moles generated when Zn reacts with soil, M²⁺ is soluble/active Zn ions, and S-M is sorbed Zn ions.

$$K = \frac{(S - M) * (H^+)^\alpha}{(M^{2+}) * (S - H_\alpha)} \quad (1)$$

$$\text{Log}(K_d) = \alpha \text{pH} + \text{log } K * (S - H) \quad (2)$$

$$K_d = \frac{\text{Amount of Zn sorbed in the soil (mg kg}^{-1}\text{)}}{\text{Concentration of Zn in the soil solution (mg dm}^{-3}\text{)}} \quad (3)$$

...where K_d expresses the distribution coefficient (dm³ kg⁻¹).

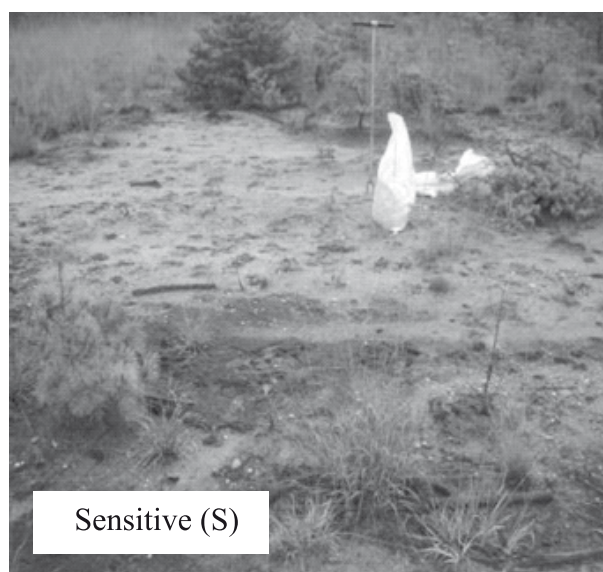
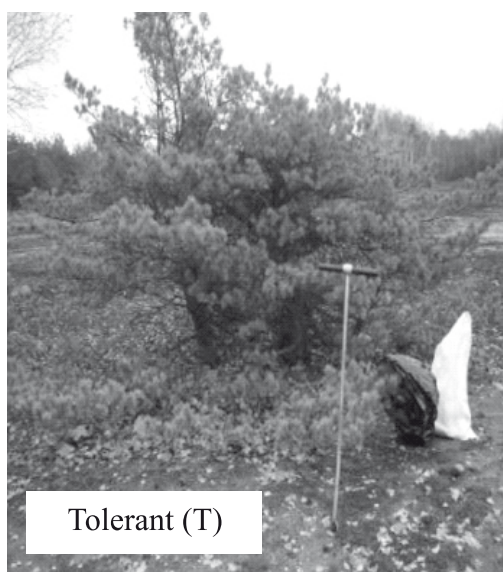


Photo 1: Tolerant (T) and sensitive (S) Scots pine species considered for the study (2012).

The expression from Eq. 2 outlines the linear form $y = ax + b$, where $y = \log K_d$, $a = \alpha$, $x = \text{pH}$, and $b = \beta$. This is a relationship established for the pairs: distribution coefficient (K_d) versus $\text{pH}_{\text{H}_2\text{O}}$ and/or $\text{pH}_{\text{CaCl}_2}$. The retention capacity (RC), (β), explains how strongly and intensively investigated soils are able to retain/immobilize Zn ions. The highest the β values, the much more Zn ions are retained by soils (and inversely).

Plant Materials (Pine Needles)

The plant materials consisted of 1- and 2-year-old needles sampled from two pine species subjected to Zn smelter activity. These pines exhibited tolerance and sensitivity to heavy metals, but particularly to Zn – the main polluting metal. The trees for needle sampling were selected on the basis of tress morphology [27], i.e., exhibiting normal growth shape as compared to sensitive ones, which were more or less dwarves (Photo 1). Needles were harvested from the middle third of the crown of randomly selected trees of both species: tolerant (T = 24 trees) and sensitive (S = 24 trees).

In total, 40 needles (10 needles per direction, i.e., east, west, north, south), 1- and 2-year-old were sampled from each tolerant and sensitive pine. Dusts and other impurities were washed from the needles using distilled water. Prior to grinding, needles were dried at 60°C for three days. Portions (0.250 ± 0.001 g) of ground needles were weighed into test tubes and mineralised in concentrated HNO_3 (MARS5, CEM Corporation, USA). The digests were filtered and Zn, Mg, and Fe were determined using AAS (Varian 250 Spectra plus). All analyzed (soils and needles) were run in duplicates. Simplified statistical summary and graphical sets were performed using Excel.

Results and Discussion

Soils within industrial areas are frequently subjected to contamination by several substances, of which heavy metals are of great concern due to the fact that they are not biodegradable [2]. These characteristics are responsible for their behavior in the biotic as well as abiotic media. In the case of Zn, it should be mentioned that this metal is important for the metabolism of the flora and fauna, but at relatively excessive concentrations both in the soils and plant shoots its effects are considered potentially harmful. The input of metalliferous compounds into soils additionally alters soil chemical properties such as pH and buffering properties, which in turn regulate and control metal geochemical processes. For such reasons, anthropogenic Zn occurs much more frequently in mobile and reactive forms [28], but the immobilization and retention rates depend mainly on soil properties [29-30].

Physical and Chemical Properties of Soils

The mineral composition of the $< 2 \mu\text{m}$ fraction of the soils shows in most cases a predominance of kaolin-

ite, except for two samples (3 and 4), where smectite was the dominant mineral (Table 2). It should be mentioned that samples 1, 2, 5, 6, and 8 contained additionally illite (mostly in mixed layered smectite-illite), in contrast to 3, 4, and 7, where illite was not identified. Calcite (Cc), goethite (Gt), and quartz (G) have been also observed in samples 2, 7, and 8, respectively. The status of these soils implies that they were much more anthropogenic than geogenic, hence their mixed and multiphasic mineralogical character [31].

This mineralogy organizes the soils into two operational groups: kaolinite and smectite. The first one involves soils characterized by values of the cation exchange capacity (CEC) spreading within the range $1.4\text{-}9.9 \text{ cmol}_{(+) } \text{kg}^{-1}$, contrarily to the smectite group with the range $15.1\text{-}26.8 \text{ cmol}_{(+) } \text{kg}^{-1}$. This discrepancy is intrinsically related to the occurrence of layered minerals of the type 1:1 (specifically for kaolinite) and 2:1 (for the smectite). According to Hong et al. [32], the interlayer of these clays determines their reactivity, i.e., sorption/desorption power as well as the capacity for shrinking and swelling. These properties predestinate and are fundamental for forecasting the buffering capacities of soils.

It should be mentioned that the kaolinite group prevailed by representing 75% of all tested soils as compared to 25% for the smectite one. Therefore, it is expected that 3/4 of soils within the investigated area should potentially reflect the relatively weak Zn retention. Ratings for the cation exchange capacity (CEC) as suggested by Hazelton and Murphy [21] imply that the kaolinite group exhibited very low to low buffering properties, in contrast to the smectite (moderate to high). This stresses the fact that Zn is weakly to moderately retained by soils and should be more easily released (mobile). Next, in terms of retention mechanisms this process may be related to the decrease in the number of charges on soil colloids, a mechanism hampering the adsorption of Zn on exchange sites [33]. These geochemical conditions will favor zinc mobility and then raise its phytotoxicity.

It is generally accepted that among the various properties that regulate Zn availability, factors such as soil particles and organic matter as well as pH play a key role [34]. For soils investigated in the current study (Table 2), it was observed that sand prevailed with more than 60% of all soil fractions. Taking into consideration the fact that clay and silt fractions are much more responsible than sand for regulating Zn chemistry in the soils, summarizing their levels should give values indicative of weak or strong soil buffering capacities. This view is in line with the approach reported by Diatta et al. [35], which presented such practical and reasonable possibilities. In the case of data reported in Table 2, only three soils, representing the so-called smectite group, exhibited a [Silt + Clay] value $> 500 \text{ g kg}^{-1}$, as compared to the kaolinite group, where the contents of both fractions fluctuated within the range $180\text{-}400 \text{ g kg}^{-1}$. The mineralogical composition along with the contents of silt and clay were operating as the basic factors, but not the only ones, potentially shaping Zn behavior of the tested soils.

Table 2. Selected physical and chemical properties of investigated soils.

Sample No	Mineralogy ^a	Soil particles			C _{org} ^b	pH		EC ^c ($\mu\text{S cm}^{-1}$)	CEC _{ef} ^d ($\text{cmol}_{(+)} \text{kg}^{-1}$)
		Sand	Silt	Clay		H ₂ O	CaCl ₂		
		(g kg ⁻¹)							
1	Kaolinite > Illite	820	84	96	5.5	6.7	5.7	34.7	1.4
2	Kaolinite > Illite	760	148	92	9.1	6.2	4.9	41.6	1.8
3	Smectite > Kaolinite	248	488	264	15.5	7.6	7.2	189.1	26.8
4	Smectite > Kaolinite	500	240	260	20.0	7.5	7.1	128.5	15.1
5	Kaolinite > Illite	600	268	132	11.2	6.3	5.6	68.3	5.4
6	Kaolinite > Illite	784	132	84	7.5	6.8	5.9	72.3	5.9
7	Kaolinite > Smectite	462	410	128	31.0	6.0	4.6	65.7	9.9
8	Kaolinite > Illite	640	275	85	165.1	4.5	3.4	134.4	2.5

^a: Refer to Materials and methods; ^b: Organic carbon; ^c: Electrical conductivity; ^d: Effective cation exchange capacity

Soils within the area impacted by the smelter were characterized by mostly slightly acidic to acidic reactions, except for site Nos. 3 and 4 representing the smectite group (mean $\text{pH}_{\text{CaCl}_2} = 7.15$). Such soil reaction may increase the mobility availability of Zn, particularly when 75% of investigated soils exhibited $\text{pH}_{\text{CaCl}_2}$ below 6.0. The extent of zinc speciation depends on stability constants of the species formed, ionic strength, pH, and the type and relative concentrations of cations and anions in the solution [36]. Data reported by Alloway [37] showed that zinc ions such as Zn^{2+} , ZnCl^+ , and ZnOH^+ (or also complexed with organic matter) occur in the soil solution where pH is below 6.5. The mobility and further availability of Zn compounds have undergone experimental measurements, which allowed the formulation of equilibrium constants [38-40]. The latter ones outlined the solubility of Zn as directly proportional to the square of proton activity as follows: $\text{soil-Zn} + 2 \text{H}^+ \leftrightarrow \text{Zn}^{2+}$, then their results generated a $\log K^0$ for this equation of 5.8. The transformation of this equilibrium reaction gives a $\log(\text{Zn}^{2+}) \leftrightarrow 5.8 - 2 \text{pH}$, implying that the solubility and therefore Zn availability increase with a decrease in soil pH [41]. This was the additional key factor decidedly controlling geochemical processes of Zn compounds and their further transfer to other environmental compartments [42-43]. These factors are the geo-environmental barriers efficiently reducing metals mobility and their further uptake by plants [40, 44-45].

Changes in Zn geochemistry are also regulated by the quantity and quality of organic matter [46]. Data listed in Table 2 show that the content of organic carbon varies from 5.5 to 165.1 g kg⁻¹, with a mean of 33.1 g kg⁻¹. When considering the value 165.1 g kg⁻¹ as an outlier, the mean site C_{org} content changes to 14.3 g kg⁻¹. The ranges suggested by Sequi de Nobili [47] i.e., C_{org} < 5.8 g kg⁻¹ (very low); 5.8-10.4 g kg⁻¹ (low), 11-14.5 g kg⁻¹ (moderate), and C_{org} > 14.5 g kg⁻¹ (high) indicate that the site investigated in the current study may be classified as moderately rich in organic matter. Therefore, the formation of metal-organic

complexes (for instance Zn-Organic matter), [45, 48-49] may proceed less efficiently. The moderate organic matter content along with soil mineralogy (dominated mostly by kaolinite and illite minerals) and particle size with the prevalence of sand confirm the concern of Zn mobility and hence the emergence of potential site-specific phytotoxicity.

Zinc Content and its Fractions in the Investigated Zone

Ecological risk assessment is a fundamental consideration for evaluating the effects of metal-polluted soils. The simulation of ecologically relevant conditions in the pedosphere requires the application of targeted soil tests discriminating mobile and mobilisable metal fractions [50]. The evaluation of zinc geo-bio-availability has been undertaken through three operational steps, where total (Zn_{Total}) as well as reactive (Zn_{Reac}) and bioavailable (Zn_{Bio}) Zn forms were determined (Table 3). The Zn_{Total} parameter should be considered basically as to be used for site state information about contamination and/or pollution as well as geochemical background purposes [51]. It does not provide details related to the geochemical behavior and further fate of Zn compounds [50].

The highest level of Zn_{Total} occurred at site No. 3 and amounted to 3,912.0 mg kg⁻¹, but the soil collected at site No. 4 exhibited a content quite half lower, i.e., 1,245.0 mg kg⁻¹. For the remaining sites, Zn_{Total} varied within the range 177.0-472.5 mg kg⁻¹. According to Kabata-Pendias and Pendias [52], the background level for Poland is fixed at 75.0 mg kg⁻¹, which means that this value was exceeded from 2.5 to 52 times. Next, in the case of natural Zn content (i.e., geochemical background), the suggested value amounts to 30.0 mg kg⁻¹ [53]. The state of soil contamination evaluated on the basis of this value revealed Zn concentration exceeding on average 30 times (from 6 to 130 times) for the whole area of study.

Table 3. Total Zn content (Zn_{Total}), reactive (Zn_{Reac}) and bioavailable (Zn_{Bio}) forms in investigated soils (mean, n = 5).

Sample No	Total (Zn_{Total})	0.11 mol CH_3COOH (Zn_{Reac})	0.10 mol $NaNO_3$ (Zn_{Bio})
	(mg kg^{-1})		
1	472.5±159.3*	302.4±153.7	28.50±2.47
2	418.5±296.0	240.9±248.1	27.67±7.59
3	3,912.0±1,224.3	1,135.0±454.7	21.30±4.29
4	1,245.0±793.2	493.9±297.8	14.66±1.52
5	246.0±31.1	80.2±18.8	5.19±0.55
6	369.0±324.3	66.8±35.4	2.30±0.39
7	177.0±62.2	52.1±18.5	0.43±0.06
8	204.0±60.6	68.2±21.8	0.15±0.02

*mean values ± standard deviation

These data clearly confirm the long-term cumulative Zn emissions as illustrated on Fig. 1 and the fact that the pollution occurred mostly at the upper soil layers. The latter ones are the pedo-rhizosphere zones for the dynamic growth of pine rootlets [54], which in turn take up and accumulate Zn compounds.

Among multiple procedures reported for evaluating and forecasting the impact of trace elements, including Zn (among others) on the biota, the BCR method [24] is frequently applied. For the current study, only the first fraction, i.e., Zn_{Reac} was considered (Table 3). The amounts of Zn extracted by this test represented from 18 to 64% of Zn_{Total} (mean 34.6%), which implies that zinc was relatively weakly bound and retained by investigated soils. The behavior of Zn compounds under such conditions is intrinsically related to the mineralogical composition, where kaolinite occurred in all tested soil and next as the dominating mineral, except for soils 3 and 4 (Table 2). The buffering properties of the latter ones are very extended due to the joint effects of smectite as well as pH and organic matter. These parameters decidedly control the mobility of metallic elements and simultaneously are responsible for their relatively low solution activity [55]. As a matter of fact, it should be mentioned that Zn is much more mobile than frequently reported heavy metals, for instance Cu, Pb, Ni, and Cd [56]. But the respectively high concentrations of Zn in the reactive fractions (Zn_{Reac}) as listed in Table 3 did not confirm this rule, indicating an enhanced mobility and potential phytotoxicity.

The tangible harmful effects that could be expected from Zn were those evaluated on the basis of the bioavailable fraction (Zn_{Bio}). According to Gupta and Häni [23] and Gupta and Aten [57], the threshold value for Zn toxicity to microorganisms [6] and plants is 0.50 mg kg^{-1} . This level was exceeded in almost all investigated soils, except for Nos. 7 and 8. The mean Zn_{Bio} content for the whole area amounted to 12.5 mg kg^{-1} , representing ca. 1.42% of Zn_{Total} . On the other hand, the risk of Zn-induced biological toxicity was raised 25 times (12.5 mg kg^{-1} : 0.5 mg kg^{-1}), but spread within the range from 0.3 to even

57 times. Such an impact of Zn-contaminated soil along with additional air pollution pertinently participates in the deterioration of forest stands [4].

Geochemical Evaluation of Zn Reactivity

The reaction of metallic ions with soil colloids is intermediated by functional groups, which generate charges enabling sorption and retention mechanisms to proceed properly. The resulting deprotonation process activated by metal binding should be a measure of potential geochemical reactivity and mobility of metals in the soil environment [26, 58]. This approach was outlined throughout the proton generation capacity (α) index (Table 4) with the values calculated for pH_{H_2O} and pH_{CaCl_2} and both Zn forms, i.e., Zn_{Bio} and Zn_{Reac} . The results revealed, that the Zn_{Bio} fraction was characterized by the highest values of α as compared to Zn_{Reac} , irrespective of soil pH. This finding is in line with data reported by Diatta and Chudzińska [43], implying that the reactions of zinc ions with active sites on colloid surfaces as well as with functional groups leads to the deprotonation mechanism. Consequently, this process increases the activity of Zn^{2+} in the soil solution. The initiated geochemical mechanism "secures" zinc ions and other metals against sorption by keeping them in relative solution activity. The α indices for the reactive Zn form (Zn_{Reac}) were markedly low as compared to Zn_{Bio} . This is particularly worth reporting due to the chemical characteristics of tests applied for extracting both forms of Zn. For Zn_{Reac} , the concentrations of H^+ incorporated with the use of the 0.11 mol CH_3COOH dm^{-3} test were high enough to hamper the geochemical process of proton generation, resulting in Zn sorption and retention.

This is clearly reflected by the relationships established for the pairs K_d (distribution coefficient) versus pH of investigated soils. The mean values of α (for the whole zone impacted by the smelter) revealed that this proton generation activity for Zn_{Bio} was 60 times higher than that observed in the case of Zn_{Reac} – proof of a controlling effect of pH on Zn soil solution chemistry. Investigations

Table 4. Evaluation of proton generation (α) and retention (β) capacities for bioavailable (Zn_{Bio}) and reactive (Zn_{Reac}) Zn forms.

Test	pH	α	β	r^*
		(mol H ⁺ mol ⁻¹ Zn ²⁺)	(mol _(c) kg ⁻¹ soil)	
0.10 mol NaNO ₃ dm ⁻³ (Zn_{Bio})	H ₂ O	0.481	0.85	0.513
	CaCl ₂	0.401	0.020	0.532
0.11 mol CH ₃ COOH dm ⁻³ (Zn_{Reac})	H ₂ O	0.004	2.09	0.026
	CaCl ₂	0.011	2.00	0.092

*Coefficient of correlation

carried out by Benjanin and Leckie [58] pointed out the process of Zn sorption/immobilization resulting in about 75% of more H⁺ generation as compared to metals such as Cd, Cu, and Pb. Others, for instance Christensen et al. [59], have indicated at 40%, respectively, but with reference to Cd, Co, and Ni.

The retention capacity (RC) index (β) expresses the degree at which the soil environment is capable of retaining or immobilizing metallic elements [43, 58]. Among investigated Zn forms, only in the case of Zn_{Reac} were the highest concentrations of soil charges (on average 2.05 mol kg⁻¹) responsible for sorption as well as retention observed. This is an important characteristic of the test 0.11 mol CH₃COOH dm⁻³, since the extracted Zn amounts were characterized simultaneously by relatively high activity (Zn_{Bio}) as well as stability ($\Delta = Zn_{Reac} - Zn_{Bio}$). These results confirm earlier reports about increased mobility of Zn due to its slightly weak retention by soils [60], thus creating a threat for the biota – particularly the investigated Scots pine trees.

Impact of Zn on Mg and Fe Concentrations in Scots Pine Needles

Zinc, magnesium, and iron play key roles in the efficient course of photosynthesis, therefore these elements can constitute phytochemical indicators of plant resistance or sensitivity to industrial pollution – particularly Scots pine [61-63]. Next, the excess of Zn and most frequently the deficiency of Fe in plants hamper the photosynthesis process, which strongly disturbs plant growth [13-14]. The

assessment of the degree of Scots pine (*Pinus sylvestris*) needles contamination by Zn was carried out on two species, i.e., tolerant (T) and sensitive (S). Data listed in Table 5 point to a high degree of needle contamination as impacted by the Zn-Smelter.

Moreover, it should be mentioned that the concentrations of Zn, Fe, and Mg in the tolerant (T) pine are higher as compared to the sensitive (S) species, irrespective of the age of the needles. Data averaged for the whole pine populations showed that in the case of the T species, the 2-year-old needles accumulated 21.9, 38.2, and 13.6% more Zn, Fe, and Mg, respectively, with reference to the 1-year-olds. In the case of the S species, the trend follows similarly the proportions 12.4, 48.8, and 7.3%.

These data decidedly show that Fe was most accumulated by 2-year-old needles, irrespective of investigated species. Therefore, Fe should be considered a “strategic survival element” for both Scots species under high Zn pollution conditions. In their study, Başlar et al. [63] reported a similar trend, but induced by Cu concentrations in the case of *Pinus brutia*. The highest accumulation of Zn and Mg, particularly in the case of the tolerant (T) pine, may be related to physiological functions, where enzymatic activities are basically controlled by both elements.

The response of both pine species to high Zn contents in needles was illustrated by a similar course in the increase of Mg and Fe concentrations (Figs. 2a-b, 3a-b). It should be noted that the effect of Zn in plants is commonly agreed to be positive (as a micronutrient), but it is adverse in the case of pollution, as for the currently investigated

Table 5. Optimal ranges of Zn, Fe and Mg concentrations in dry matter of Scots pine needles (64)^a and data of the current study^b (n = 24 for each species and age of needles).

Element	Ranges ^a	Tolerant (T) ^b		Sensitive (S) ^b	
		Pine needles			
		1-year old	2-year old	1-year old	2-year old
mg kg ⁻¹					
Zn	17 – 88	347 ± 85 ^c	423 ± 101	322 ± 87	362 ± 82
Fe	100 – 850	55 ± 13	76 ± 29	41 ± 16	61 ± 24
Mg	300 – 1400	323 ± 108	367 ± 104	259 ± 108	278 ± 72

^c mean values ± standard deviation

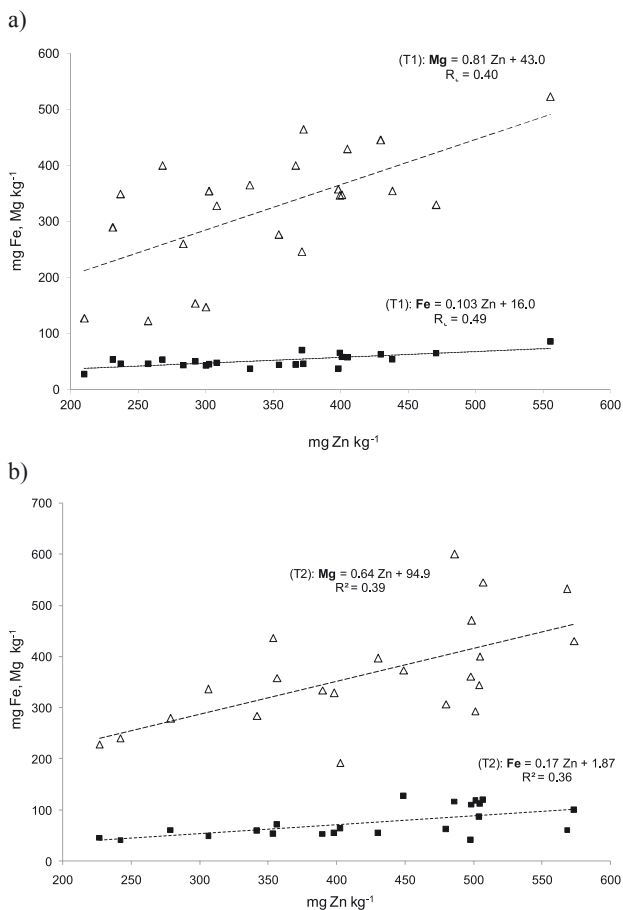


Fig. 2. a) relationship between Zn versus Mg and Fe concentrations in 1-year-old (T1) Scots pine needles of the tolerant (T) species, b) relationship between Zn versus Mg and Fe concentrations in 2-year-old (T2) Scots pine needles of the tolerant (T) species.

needles. The evaluation made on the basis of the coefficients of determination (R^2) showed that the increase in Zn concentrations induced the strongest accumulation of Mg in the tolerant (T) species, both for 1- and 2-year-old needles as compared to the sensitive (S) species. A reverse trend was observed in the case of Fe. Therefore, it can be formulated that Mg constitutes a basic element alleviating the negative effects of Zn in the tolerant Scots pine, but the survival of the sensitive pine depended much more on adequate Fe concentrations in the needles. This is proven by a very high increase (48.8%) of Fe concentrations in the S species compared to T. The needle contents of Mg and Fe for both pines confirmed their biological strategy for survival adaptation. This process is strictly related to the whole Scots pine fresh biomass [65] as the basic receptor of environmental stresses. The transfer of Zn ions from the soil environment to plants still remains the basic source of maintaining its high concentrations in the needles, since air emissions have quite ceased from 1996 (Fig.1). Next, the plant accumulated Zn at crucial years of emissions may be considered as an additional *time-constant source* due to the evergreen vegetative cycle of Scots pine.

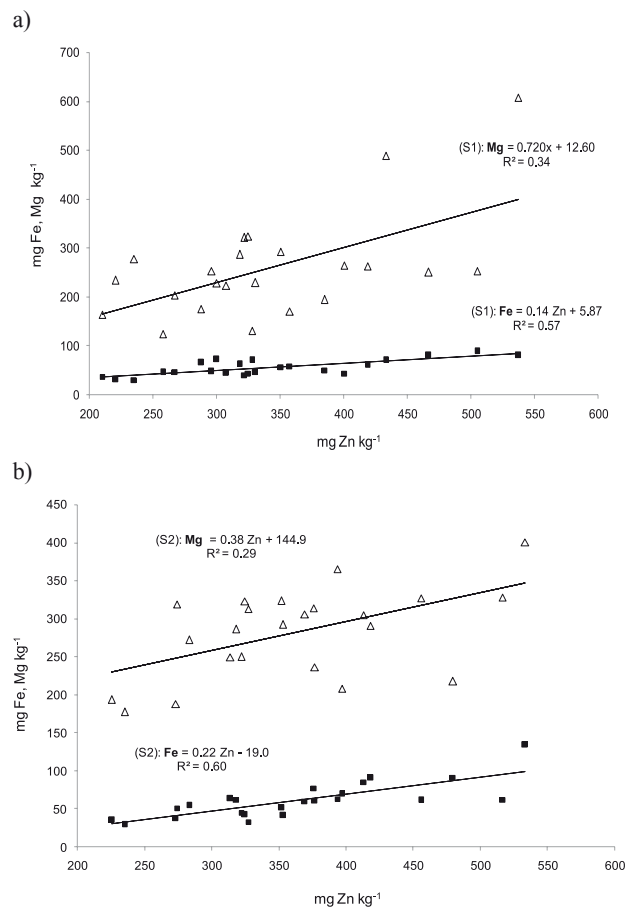


Fig. 3. a) relationship between Zn versus Mg and Fe concentrations in 1-year-old (S1) Scots pine needles of the sensitive (S) species, b) relationship between Zn versus Mg and Fe concentrations in 2-year-old (S2) Scots pine needles of the sensitive (S) species.

Conclusions

1. The mineralogical composition of the $< 2 \mu\text{m}$ revealed the occurrence and prevalence of kaolinite in all soils, except for two samples (i.e., 3 and 4), where smectite was the dominant mineral. This mineral structure was decisive in Zn geochemistry, but assisted by pH and organic matter.
2. The contamination state evaluated on the basis of the geochemical background value ($30.0 \text{ mg Zn kg}^{-1}$) revealed that this value was exceeded *ca.* 30 times within the investigated zone.
3. Amounts of reactive (Zn_{Reac}) Zn forms represented from 18 to 64% of Zn_{Total} (on average 34.6%), thus indicating relatively weak Zn retention by soils.
4. The values of proton generation capacity (α) for the whole zone show that α for the bioavailable Zn fraction (Zn_{Bio}) was 59 times higher as compared to the reactive fraction (Zn_{Reac}). This implies a strong influence of soil pH on Zn ion activity.
5. For the tolerant (T) Scots pine, the 2-year-old needles accumulated 21.9, 38.2, and 13.6% much more Zn, Fe, and Mg as compared to 1-year-old needles.

The sensitive (S) species exhibited the following trend, i.e., 12.4, 48.8, and 7.3%, respectively.

6. Iron concentrations in needles may be regarded as a “strategic survival element” for both pine species growing under conditions of high Zn pollution.
7. Amounts of Zn accumulated by Scots pines in crucial years of metallurgical emissions may be considered as an additional time-constant source due to the ever-green vegetative life cycle.

Acknowledgements

This study was financially supported by the Polish National Science Centre (grant No. NN 305058440), titled “Comparison of micro-evolutional processes taking place in the two bioindicative species; *Pinus nigra* Arn. and *Pinus sylvestris* L. growing in a heavy polluted environment.”

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