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Research Article

PINE NEEDLES (*PINUS SYLVESTRIS* L.) AS BIOINDICATORS IN THE ASSESSMENT OF URBAN ENVIRONMENTAL CONTAMINATION WITH HEAVY METALS

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ABSTRACT
The studies on Zn, Pb and Mn content in the needles of *Pinus silvestris* and the surface layers of soil (0.0–0.2 m and 0.4–0.6 m) were conducted in 2010. The research stations covered residential areas, municipal parks, neighborhoods of busy streets, industrial establishments, waste dump sites and sewage treatment plants. The tests showed variability of the analyzed elements in the needles of *P.silvestris* and in municipal soils, depending on concentration of urban and industrial activity, road traffic and the depth of the examined layer of soil. The content of heavy metals in soil varied. Zn was characterized by the highest variability, depending on the depth (50.3–52.4%), Mn slightly lower (38.5–42.2%), and Pb, the lowest (29.1–33.2%). It was evidenced that the values of the heavy metals enrichment factors of the needles are closely connected with concentration of the examined metals in the soil. Along with the rise of Zn, Mn and Pb content in the soil, the values of enrichment factors decreased. The strongest negative correlations were found in the case of Zn, both in 1-year old needles ($r = -0.82$, $p<0.05$), as well as in 2-years' old ones ($r = -0.83$, $p<0.05$), slightly weaker in the cases of lead and manganese.

Keywords: polluted area, soil, needles, pinus sylvestris, accumulation, enrichment factor.

INTRODUCTION

Municipal areas are ecosystems of the prevailing human impact, in which buildings and transport networks dominate. In waters, soils and plants of such ecosystems, increase of concentrations of various substances of anthropogenic origin, including heavy metals, is usually observed [Buszewski et al. 2000, Yang et al. 2001, Grzebisz et al. 2002, Dąbkowska-Naskręt and Różański 2009]. Emissions from industrial establishments and transportation means constitute main sources of heavy metal pollution of the environment [Lis and Pasieczna 1995, Krauss et al. 2000]. Heavy metals, as constituents of dust (PM10 i PM2.5) and aerosols, can be transported in the air at substantial distances, leading to pollution of ecosystems situated at longer distances from the source of emission [Brozek and Zarembski 2011]. Heavy metals deposited at the surface of the soil are fixed by the solid phase of the soil, absorbed by living organisms or they undergo migration along with the filtering water. Intensity of such processes depends on the complex of physical and chemical properties of the soils. Reaction plays a very important role and decides about mobility of heavy metals in the soil as well as on their bioavailability and toxicity in reference to living organisms [Tyler 1992, Kabata-Pendias and Pendias 1999, Karczewska 2002]. Toxic impact of heavy metals on living organisms appears after the threshold concentration of bioavailable forms characteristic for each of them has been exceeded [Lis and Pasieczna 1995, Gambuś 1997, Selim and Sparks 2001, Martinez and Motto 2000, McAlister et al. 2006]. Bioavailability of most metals increases along with the decrease of pH. In neutral and alkaline reaction conditions, labile forms are immobilized by creation of relatively durable complex connection with organic matter of the soils. In municipal ecosystems, alkalization of soils being the consequence of deposit of alkaline dust, has positive impact on this process. Absorption of metals in the soil, however, leads in many cases to their excessive pollution. There is also a risk that in case of change of conditions in the soils, the volume of metals absorbed by the soil becomes bioavailable and gets to the food chain undergoing bioaccumulation. At adequately high concentration, functions of ecosystems can be disturbed causing threat to living organisms [Kabata-Pendias and Pendias 1999, Gruca-Królikowska and Wacławek 2006]. Their contamination hazard increases with subsequent chains of the trophic chain, at the top of which, there is the man. Studies of pollution of municipal agglomerations found in the literature, in most instances refer to soils. Due to toxicity of heavy metals, systematic control of the status of pollution of soils [Czarnowska and Gworek 1991, Madrid et al. 2002, Imperatoa 2003, Pasieczna 2003] as well as needles of *Pinus silvestris*, especially at urban areas [Dmuchowski and Bytnerowicz 1995, Czarnowska 1997, Dmuchowski et al. 2011] is especially important.

The paper aimed at: (i) determination of their level Zn, Mn and Pb in the soil of the city of Słupsk in the layers: 0.0–0.2 m and 0.4–0.6 m,(ii) determination of the content of the tested metals in 1-year old and 2-year-old needles of Scots pine, (iii) specification of accumulation coefficients of the above mentioned metals in the needles of *Pinus silvestris* L. and (iv) evaluation of the level of heavy metals pollution of soils and in the needles of pine trees at the area of the city of Słupsk.

MATERIAL AND METHODS

Soil and plant sampling

The samples for laboratory tests were taken in September 2010 from 25 research stations all over the city of Słupsk (Central Pomerania, Northern Poland), Figure 1. Research stations were distributed around the city including the residential buildings areas, municipal parks as well as vicinity of busy streets, industrial establishments, surrounding area of waste dumps and sewage treatment plants. Soil samples were taken at two depths: 0–0.2 m and 0.4–0.6 m [Lis and Pasieczna1995]. Needles samples (1-year old and 2-year-old) were taken from the seventh whorl. Collective samples of 10–20 g were prepared on a basis of the collected plant matter, taking into consideration the age of the needles (separately 1-year old needles and

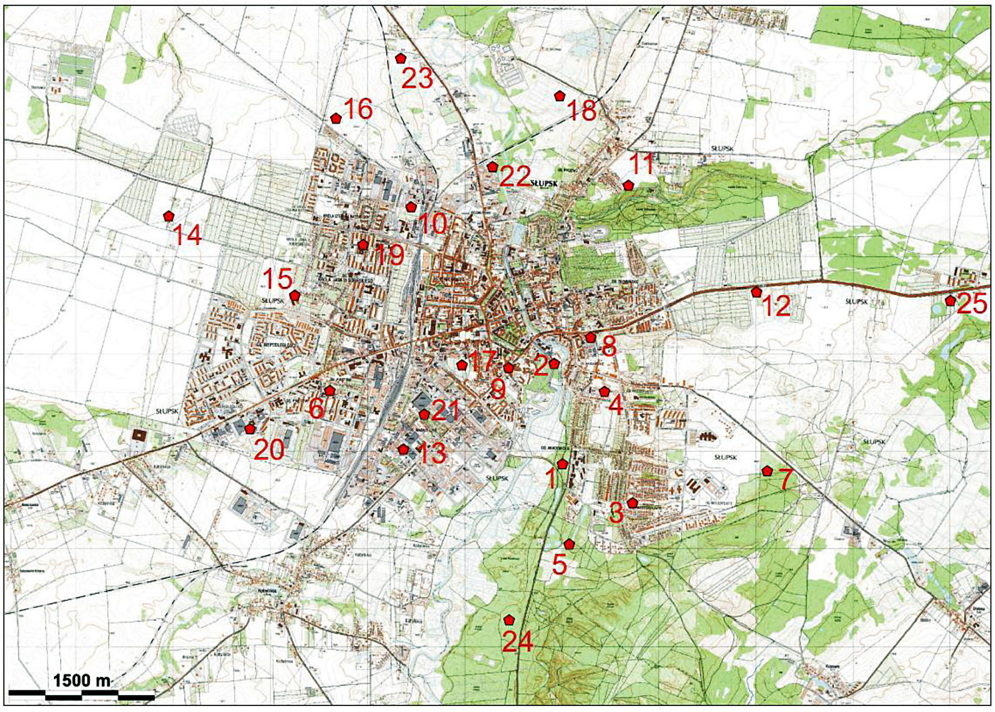

Figure 1. Location of sampling points on the city of Slupsk

2-years' old needles) according to the recommendations ICP Manual Forest [Rautio et al. 2010].

Soil and plant analysis

Soil samples were dried at the temperature of 65 °C and sieved through a sieve with the mesh diameter of 2 mm. Subsequently, the following were determined: active acidity (pH, H_2O) and exchangeable acidity (pH, KCl) by potentiometric method, organic matter content by the method of loss on ignition at the temperature of 550 °C, granulometric content by the sieve method, the content of Zn, Mn and Pb by AAS method (Perkin Elmer Aanalyst 300) in a solution after mineralization in a mixture of the concentrated HNO_3 and 30% H_2O_2. The analyses were performed in the oxy-acetylene flame. The tests were carried out following the original standards (Merck KGaA, 1 g/1000 ml). After bringing to the laboratory, the pine needles were washed in deionized water, dried at the temperature of 65 °C and homogenized in a laboratory grinder. The content of Zn, Mn and Pb was determined by the same method as in the case of soils.

Data processing and statistical analysis

In order to compare concentration of the examined heavy metals in the needles of Scots pine and in the soil, average, minimum and maximum values, standard deviations and coefficients of variation were calculated (CV). Coefficients of correlation of Spearman rank as well as coefficients of enrichment (*Enrichment factor, EF*) of the needles of Scots pine in heavy metals were calculated (Zn, Mn, Pb):

$$EF_{(Zn)} = C_{n(Zn)} : C_{s(Zn)},$$

where: $EF_{(Zn)}$ – enrichment factor of zinc,
$C_{n(Zn)}$ – zinc content in needles,
$C_{s(Zn)}$ – zinc content in soil.

RESULTS AND DISCUSSION

The content of Zn, Mn and Pb in soils

Anthropogenic soils of the city of Słupsk are made of sandy deposits in which coarse- grained (4.98–50.42%) and medium-grained sand (8.28–32.90%) dominate. Participation of framework fraction in the layer 0.0–0.2 m was 1.4–27.8%, and in layer 0.4–0.6 m 1.4–22.0%. The dust content did not exceed 6.9%.

The organic matter content in the tested soil was small and remained, depending on the station, at the level from 0.9 to 7.9% at the layer 0.0–0.2 m and from 0.8 to 4.6% at the layer 0.4–0.6 m, showing variation at the level of 44–46% within the area of 25 stations (Table 1). The soils were characterized by the reaction close to neutral, with values from 6.7 to 7.0 at the layer 0.0-0.2 m and from 6.7 to 7.2 (0.4–0.6 m). High pH values of the tested soils are likely to be a consequence of the deposition of alkalic dust of anthropogenic origin, mostly from coal combustion [Lis and Pasieczna 1995, Morel 1997]. According to Brożek and Zarembski [2011] average concentration of dust hanging over the area of the city of Słupsk in 2010 was: PM10 = 27–28 $\mu g \cdot m^{-3}$ and PM2.5 = 19 $\mu g \cdot m^{-3}$. Influence of urbanization on the reaction of soils was discovered both in the central part of the city and at its outskirts.

The heavy metals content in the soil of the city of Słupsk varied, depending on the depth and location of the point of test sampling. The highest quantity of Zn, Mn and Pb was found in the surface layer of the soil (0.0–0.2 m). According to Pasieczna [2003] heavy metal accumulation increases with the number of inhabitants of the cities and is more evident at the surface level. In case of zinc, the values from 12.3 to 93.6 $mg \cdot kg^{-1}$ were determined in the layer 0.0–0.2 m (average 45.3 $mg \cdot kg^{-1}$) and from 5.8 to 90.2 $mg \cdot kg^{-1}$ (average 35.7 $mg \cdot kg^{-1}$) at the depth of 0.4–0.6 m (Table 2).

Table 1. Selected properties of the soils

Parameter	Organic matter [%]		pH (H_2O)		pH (KCl)	
Depth [m]	0.0–0.2	0.4–0.6	0.0–0.2	0.4–0.6	0.0–0.2	0.4–0.6
Minimum	0.9	0.8	6.7	6.7	6.4	5.7
Maximum	7.9	4.6	7.0	7.2	7.4	6.1
Average ± SD	3.1 ± 1.4	2.5 ± 1.1	6.8 ± 0.2	6.9 ± 0.3	6.4 ± 0.5	5.9 ± 0.2
Median	2.9	2.4	6.8	6.7	6.6	5.8
CV, %	46	44	2.6	4.3	7.8	3.6

SD – standard deviation, CV – coefficient of variation.

Table 2. Heavy metals contents (mg·kg^{-1}) in the soils

Parameter	Zn		Mn		Pb	
	0–0.2 m	0.4–0.6 m	0–0.2 m	0.4–0.6 m	0–0.2 m	0.4–0.6 m
Minimum	12.3	5.8	68.3	47.0	10.8	15.9
Maximum	93.6	90.2	343.5	313.4	65.1	55.6
Average ± SD	45.3 ± 22.8	35.7 ± 18.7	138.8 ± 53.5	129.4 ± 54.6	35.8 ± 11.9	31.9 ± 9.3
Median	45.8	33.3	136.8	115.8	38.6	31.4
CV, %	50.3	52.4	38.5	42.2	33.2	29.1

In surface areas, at 9 stations, the highest value from 40 to 50 mg·kg^{-1} Zn was discovered (Figure 2). In the deeper layer, the dominating values were from 30 to 40 mg·kg^{-1} Zn. Concentrations of Zn in soil samples exceeded the geochemical background values for a tested area, but were within the acceptable limits for municipal areas, not exceeding toxic values [The Minister of environment 2002]. The largest quantities of Zn were discovered at the stations located in the neighborhood of busy streets. According to Ottesen et al. [1999] about 1.5–2.0% of zinc comes from abrasion of car tyres. In other cities of Poland the level of Zn in anthropogenic soils was: 44.8–893.7 mg·kg^{-1} in Opole [Kusza et al. 2009], 9.0–400.0 mg·kg^{-1} in Poznań [Grzebisz et al. 2002], about 19.9 mg·kg^{-1} in Toruń [Buszewski et al. 2000]. The average volume of zinc in sandy soils of Poland varies usually from 7 to 150 mg·kg^{-1} [Kabata-Pendias and Pendias 1999].

Concentration of manganese in the surface layer of the soil (0.0–0.2 m) was from 68.3 to 343.5 mg·kg^{-1} (average 138.8 mg·kg^{-1}), and in the layer 0.4–0.6 m from 47.0 to 313.4 mg·kg^{-1} (average 129.4 mg·kg^{-1}) (Table 2). At 18 from among 25 stations, concentration of Mn was found at the level of 100–150 mg·kg^{-1} (0.0–0.2 m) and at 12 stations in the level of 0.4–0.6 m (Figure 2). The obtained results for the soils of the city of Słupsk exceed the values of geochemical background of manganese, but do not constitute threat to living organisms. The highest concentration of that metal was found at the stations in the central part of the town. According to Kabata-Pendias and Pendias [1999], the occurrence of manganese in the soil depends on its content in the parent rock as well as on the character of soil-forming processes, and average content for various kinds and types of soils is usually from 100 to 1300 mg·kg^{-1}.

The content of Pb in the layer 0.0–0.2 m was between 10.8 and 65.1 mg·kg^{-1} (average 35.8%), and in the 0.4–0.6 m layer between 15.9 and 55.6 mg·kg^{-1} (average 31.5 mg·kg^{-1}) (Table 2). In 8 from among 25 test stations, the content of Pb was at the level of 40–50 mg·kg^{-1} in the surface layer (0.0–0.2 m), and in 10 – at the level of 30–40 mg·kg^{-1} in the layer (0.4–0.6 m). The largest concentration of lead was found in the vicinity of the streets and in the central part of the town. No excess of acceptable values of Pb was found. Much higher variation of Pb content was found in the soils of Opole from 14.6 to 1378 mg·kg^{-1} [Kusza et al. 2009], Białystok 50.6–145.6 mg·kg^{-1} [Czubaszek and Bartoszuk 2011] or Poznań 5.4-280 mg·kg^{-1} [Grzebisz et al. 2002].

The content of the analyzed heavy metals in the soil varied to different extent. Zn (50.3–52.4 %) was the most changeable depending on the depth, Mn was slightly less so (38.5–42.2%), and Pb the least changeable (29.1–33.2%). Similar diversification of elements was observed in the soils of other agglomerations in Poland [Grzebisz et al. 2002]. Highly vital from statistical point of view, positive values of correlation coefficients between concentration of Zn, Mn and Pb in the layers of soil: 0.0–0.2 m and 0.4–0.6 m, reflect small diversification of pollutants of anthropogenic origin depositing independent of the depth of the samples of taken soil (Figure 3).

The content of Zn, Mn and Pb in the needles of *Pinus Sylvestris*

Heavy metal content in the needles of the *Pinus silvestris* within the area of Słupsk varied, depending on the metal, age of needles and the place of sampling (Table 3, Figure 4). From among the tested metals, manganese appeared in the highest quantity, zinc in slightly lower, and lead the lowest (Mn>Zn>Pb). At all examined stations, higher contents of the above mentioned metals were found in 2-years' old needles.

Concentration of zinc remained at the level from 45.8 to 85.2 mg·kg^{-1} in 1-year old needles and from 44.3 to 88.8 mg·kg^{-1} in 2-years; old needles. The highest Zn content in the needles

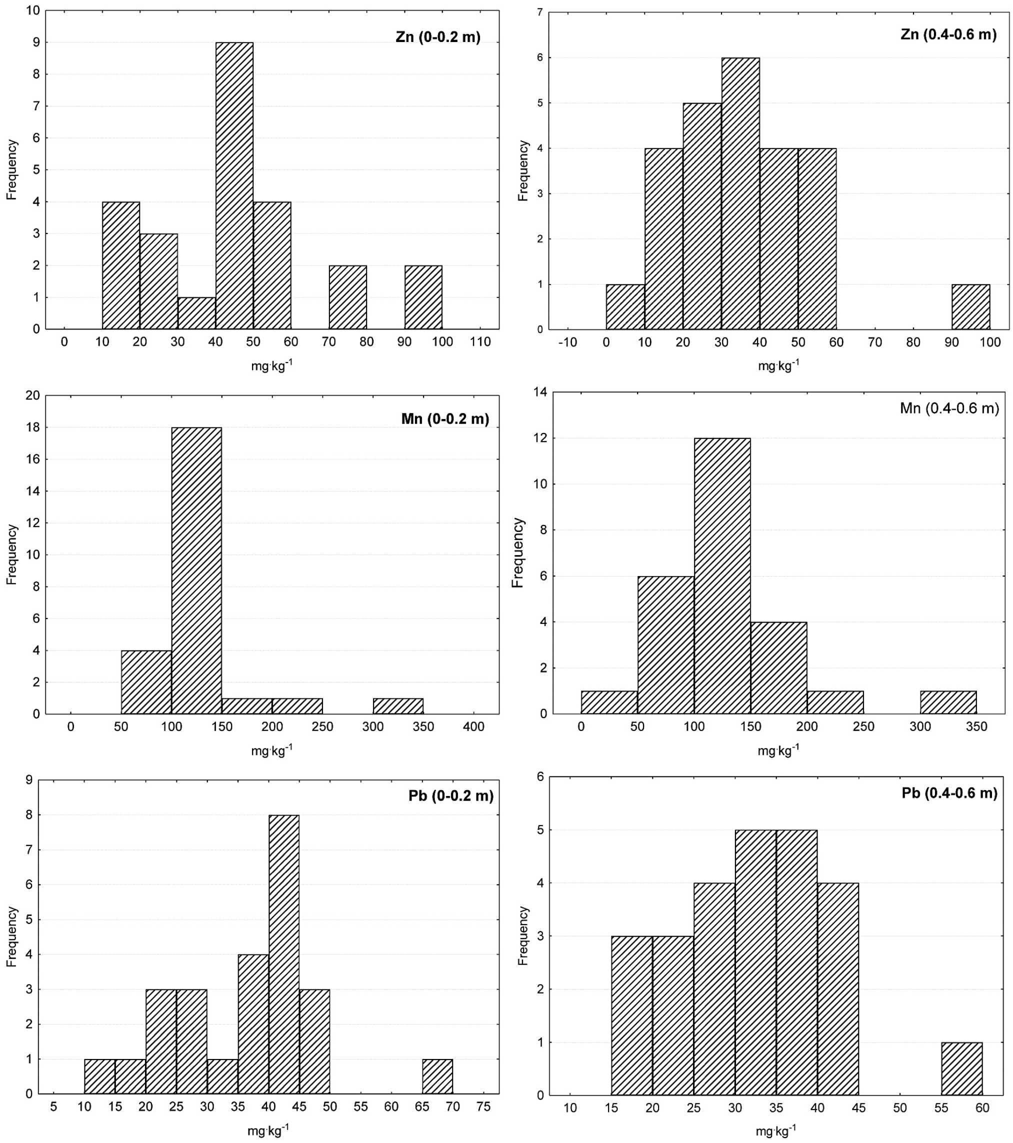

Figure 2. Histograms of heavy metals (Zn, Mn, Pb) concentrations in the soils of Słupsk

Table 3. Heavy metals contents ($mg{\cdot}kg^{-1}$) in the needles of Scots pine in Słupsk

Parameter	Zn		Mn		Pb	
	1-needles	2-needles	1-needles	2-needles	1-needles	2-needles
Minimum	45.8	44.3	239.5	351.0	6.7	7.8
Maximum	85.2	88.8	579.0	789.0	22.1	23.9
Average ± SD	59.4 ± 9.9	66.8 ±12.1	372.7 ± 113.1	532.0 ± 137.4	12.2 ± 3.9	13.3 ± 4.1
Median	56.5	64.4	322.0	512.7	12.2	13.0
CV, %	16.7	18.1	30.3	25.8	31.9	30.8

was collected at the stations in the central part of the town and in so called *Słupsk Economic Zone* in which production facilities are concentrated. None of the research stations showed toxic levels of Zn (>89 $mg{\cdot}kg^{-1}$) in needles. The average zinc content in overground parts of the plants, which are not under influence of pollutants, usually remains at the level of 10–70 $mg{\cdot}kg^{-1}$. Concentration in the leaves of most of plants between 15–30 $mg{\cdot}kg^{-1}$ [Pasieczna 2003] is enough to cover physiological needs of most plants. Similar zinc levels were found in the needles of *Pinus silvestris* over the area of Stalowa Wola: from 31 to 61 $mg{\cdot}kg^{-1}$ (1-years; old needles) and from 45–99 $mg{\cdot}kg^{-1}$ (2-year-old needles) [Samecka-Cymerman et al. 2006].

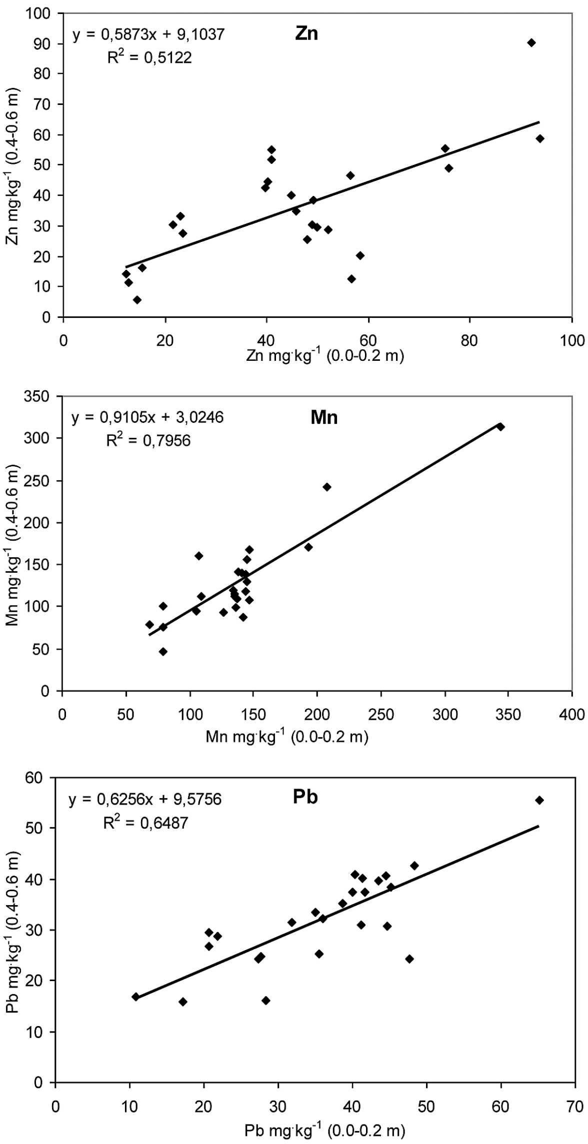

Figure 3. Relation between concentration of heavy metals in soil (0–0.2 m) and (0.4–0.6 m), $p<0.05$, n=50

Concentration of manganese in the needles of *Pinus silvestris* remained at the level from 239.5 to 579 mg·kg^{-1} in 1-year old needles and from 351.0 to 789.0 mg·kg^{-1} in 2-year old needles (Table 3, Figure 4). According to various authors, concentration of Mn in plants at the area outside the influence of pollutants is most often: 1540–3952 mg·kg^{-1} [Kozanecka et al. 2002], and in municipal agglomerations such values may be substantially higher. In case of concentration of Mn in needles, increased values of (> 400 mg·kg^{-1}) were found at 9 research stations in 1-year old needles and at 19 stations in 2-years' old needles. The lowest Mn content was found at the research stations found at the outskirts of cities.

The lead content in the needles of *Pinus silvestris* remained at the level from 6.7 to 22.1 mg·kg^{-1} in 1-year old needles and from 7.8 to 23.9 mg·kg^{-1} in 2-years' old needles (Table 3, Figure 4).

Increased content of Pb (>9 mg·kg^{-1}) was found in the needles originating from the research stations located in the central part of the city as well as in those collected in the vicinity of busy streets. In other cities of Poland, increased concentrations of that element in needles were found as well: 5.8–11.5 mg·kg^{-1} in Toruń [Buszewski et al. 2000] and 50.6–145.6 mg·kg^{-1} in Białystok [Czubaszek and Bartoszuk 2011].

The content of the analyzed heavy metals in the needles of *Ps* varied at the area of Słupsk to different extent. The most changeable, depending on the age of the needles was Pb (30.8–31.9%), slightly less changeable was Mn (30.3–25.8%), Zn was the least changeable (16.1–18.1%). Zn, Mn and Pb content in 1-year old needles and 2-years' old needles reflected statistically vital positive values of correlation coefficients (Figure 5).

The highest value of the enrichment factor was discovered in the case of manganese (EF = 1.4–9.5), slightly lower in the case of zinc (EF = 0.7–4.9), and the lowest in the case of Pb (EF = 0.2–0.6) (Figure 6). The high value of EF for manganese may result from the highest assimilability of that element by plants in soils of slightly alkaline reaction. It was also discovered that the value of heavy metals enrichment factors for the needles of *Pinus silvestris* are strictly connected with the concentration of the examined metals in the soil (Figure 6). Along with the increase of Zn, Mn and Pb content in the soil, the values of enrichment factor decreased. The strongest negative correlations were found in the case of Zn both in 1-year old needles ($r = -0.82$, $p<0.05$) as well as in 2-years'old needles ($r = -0.83$, $p<0.05$) and slightly lower in the case of lead ($r = -0.70$ and $r = -0,71$, $p<0.05$) and manganese ($r = -0.60$ i $r = -0.69$, $p<0.05$) (Figure 6, Table 4). Similar dependencies resulted from the research done by Gambuś [1997] and Królak [2003]. Substantially lower values of EF were found in the needles of

Table 4. The correlation coefficient (r) of heavy metals in relation: soil/1-needles, soil/2-needles, soil/EF-1-needles, soil/EF-2-needles. (n=50, $p<0.05$, $r_{crit.}= 0.30$)

r in relation:	Zn	Mn	Pb
soil/1-needles	0.47	0.11	0.12
soil/2-needles	0.28	-0.04	0.12
soil/EF-1-needles	-0.82	-0.60	-0.70
soil/EF-2-needles	-0.83	-0.69	-0.71

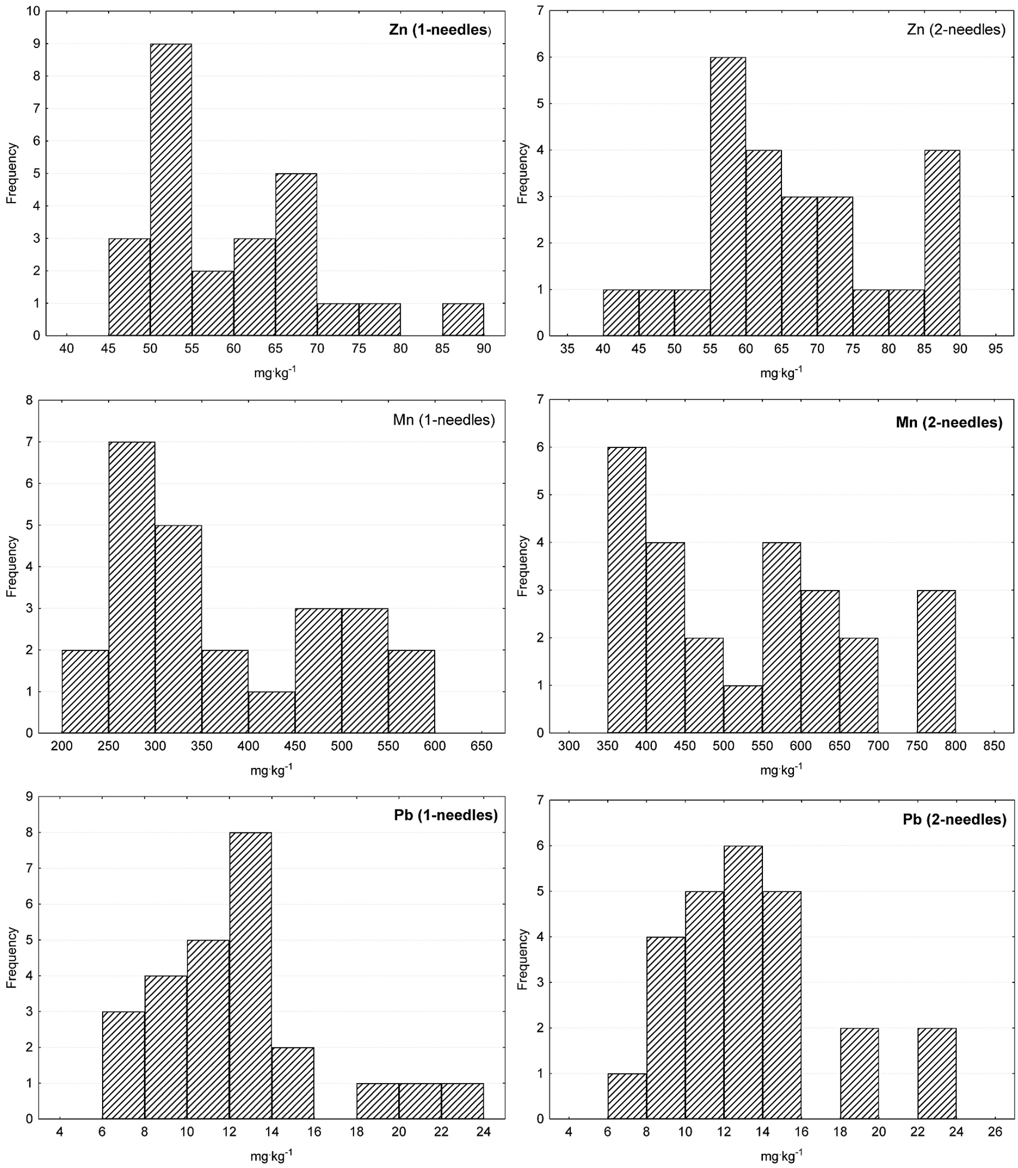

Figure 4. Histograms of heavy metals concentrations in needles of Scots pine in Słupsk

Scots pine at the territories at farther distance from anthropogenic sources of pollution [Parzych and Jonczak 2013]. Obtained *EF* values show that Mn showed highest cumulative properties of the tested metals. According to Kłos [2009] the values of enrichment factors *EF*<10 indicate the alluvial soil, as source of origin of metals. The obtained values of enrichment factors *EF* should be treated only for orientation purposes since they were determined on a basis of total concentrations of metals in the soil, where the plants accumulate only bio - available form of such contamination.

From among the examined heavy metals, only in the case of zinc, a vital statistically, positive correlation was found between the content of Zn in the soil and its concentration in 1-years old needles (Table 4). No vital statistical correlations between the content of Mn and Pb in the soil and the concentration of these metals in the needles of *Pinus silvestris* were found, which can suggest that these elements have been absorbed from the atmosphere. According to Kabata-Pendias [2001] from 73 to 95% Pb in plants is of atmospheric origin, and has not been absorbed from the soil. There are many factors which influence the availability of heavy metals for plants. One of them is soil reaction [Takáć et al. 2009]. In alkaline environment, most heavy metals are not available for plants. Activation of zinc takes place at pH<6.2, lead with pH <5.2 [Martinez and Motto 2000], and manganese in acid environment with pH<5.5 or alkaline with (pH~8), [Pasieczna 2003]. The lack of the above mentioned relationship may be also an effect of examination of surface layers of

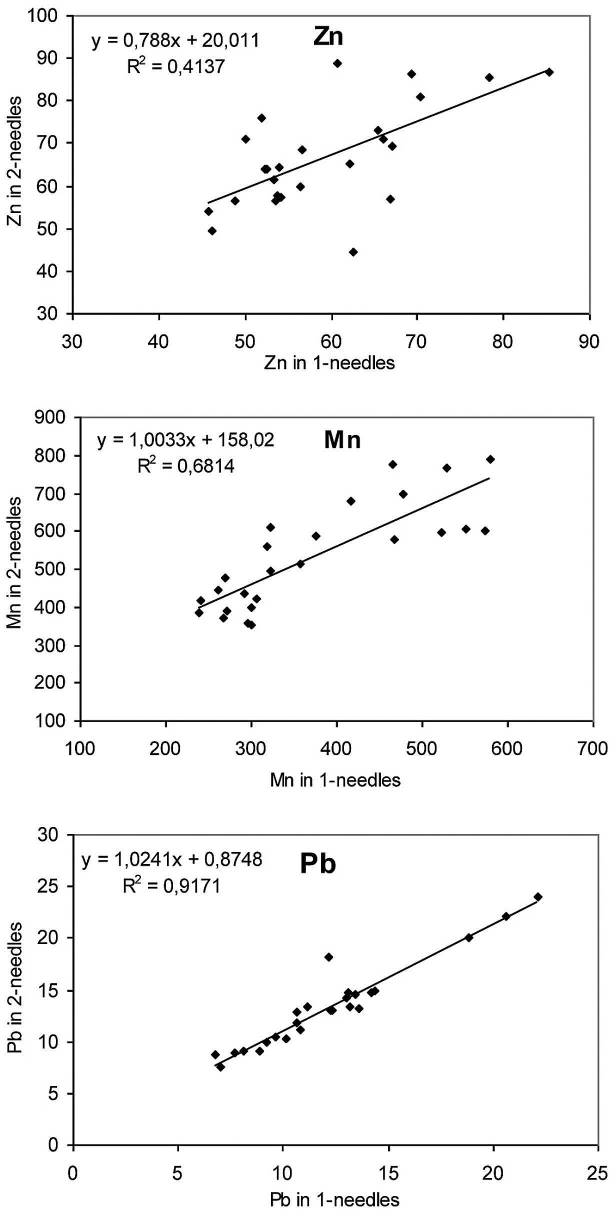

Figure 5. Relation between concentration of heavy metals in 1-needles and 2-needles of Scots pine, $p<0.05$, $n=50$

soil, which can in turn have impact on concentrations of heavy metals in plants, especially perennial ones.

CONCLUSIONS

The tests reflected variability of the analyzed elements in municipal soils and in the needles of *Pinus sylvestris* L., depending on concentrated urban – industrial activity and transport, as well as from the depth of the tested layer of the soil. Contamination of the soils of Słupsk with Zn, Mn and Pb is small. Their content exceeds the values of geochemical background of these elements but is within the limits of the concentrations acceptable for municipal areas. Manganese showed the highest concentration in the surface layers of the soil, while lead showed the lowest. Heavy metals content in the needles of *Pinus silvestris* also indicates small pollution of the city of Słupsk with the tested elements under consideration. The highest content of Zn, Mn and Pb in the soil and the needles of *Pinus silvestris* was found in the central part of the city, in the *Słupsk Economic Zone* as well as in vicinity of busy streets. The lowest concentrations in the soil and in the needles were found in the outskirts of the city. No vital statistical relations were found between the concentration of Mn and Pb in the soil, and the concentration of these metals in the needles of *Pinus silvestris*, which can reflect substantial assimilation of these elements from atmosphere. The largest values of the enrichment factors were found in the case of Mn, slightly lower in the case of zinc and the lowest in the case of Pb. The values of enrichment coefficients of the needles with heavy metals were strictly connected with concentration of examined metals in the soil. Along with the increase of Zn, Mn and Pb in the soil the values of enrichment factors decreased.

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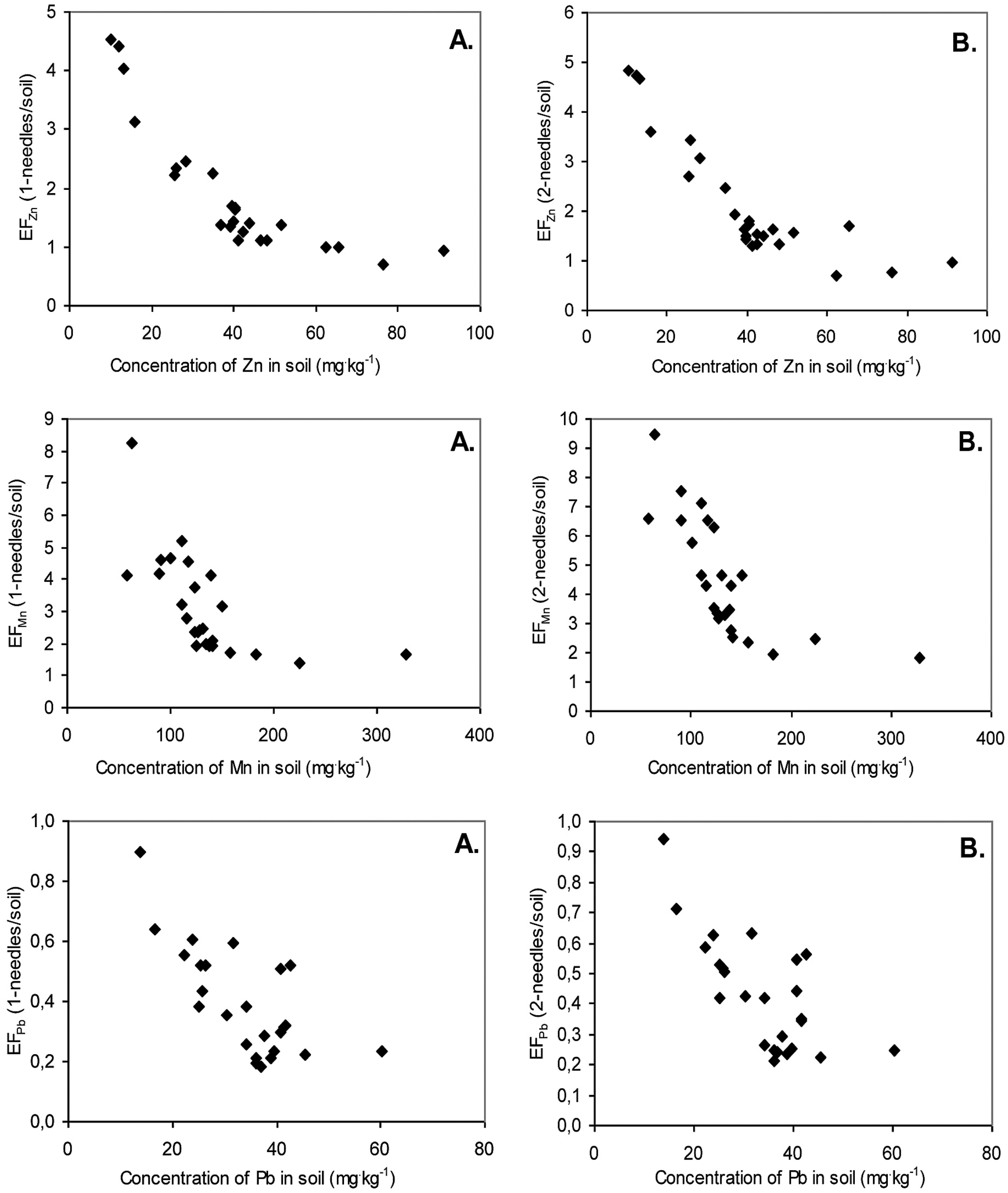

Figure 6. Relation between concentration of heavy metals in soil and enrichment factor (*EF*) in relation: A – concentration heavy metals in 1-needles, B – concentration of heavy metals in 2-needles

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