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**Heavy metals accumulation in Scots pine stands of different densities
growing on not contaminated forest area (northwestern Poland)**

**Zur Anreicherung von Schwermetallen in Kiefernbeständen
unterschiedlicher Bestockungsdichte auf unbelasteten Böden
in Nordwest-Polen**

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Schlüsselbegriffe: *Cadmium, Chrom, Nickel, Blei, Pinus sylvestris, Forstwirtschaft*

Abstract

Plants are able to absorb polluting elements from the environment and store them in their tissues. Forest trees can thus play a considerable role in phytoextraction of heavy metals. Toxic effects of heavy metals on plants can be used for bioindication of environmental pollution. In order to use Scots pine as a phytoextractor or bioindicator a precise knowledge about the accumulation of trace elements in various tree compartments is necessary. In this study we assessed the influence of stand density (number of trees per area) on the accumulation level of trace elements such as cadmium (Cd), nickel (Ni), chromium (Cr) and lead (Pb) in specific tree compartments.

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Significant correlation was found for the accumulation of Cd and Cr in branch biomass and Scots pine stand density.

Zusammenfassung

Pflanzen sind in der Lage in ihrem Gewebe Umweltverschmutzungen zu speichern. Waldbäume können somit eine wichtige Rolle in der Phytoextraktion von Schwermetallen spielen. Die toxische Wirkung von Schwermetallen auf Pflanzen kann als Bioindikator der Umweltverschmutzung verwendet werden. Der Einsatz der Gemeinen Kiefer als Phytoextraktor oder Bioindikator benötigt umfassendes Wissen hinsichtlich der Akkumulation von Spurenelementen in verschiedenen Baumkompartimenten. Diese Studie untersucht den Einfluss der Bestockungsdichte (Anzahl Bäume pro Fläche) auf die Akkumulation von Spurenelementen, wie Cadmium (Cd), Nickel (Ni), Chrom (Cr) und Blei (Pb) in den einzelnen Baumkompartimenten. Ein deutlicher Zusammenhang zwischen der Akkumulation von Cd und Cr in der Astbiomasse und der Bestockungsdichte von Kiefernbeständen konnte festgestellt werden.

1. Introduction

Heavy metals contamination of soil is a very serious problem worldwide. Sources of such pollution in various ecosystems can be of natural origin (erosion, volcanic activity) but usually, large amounts of heavy metals are introduced to the environment due to human activities (mining, industrial waste, pesticides). The toxicity of heavy metals towards plants is generally known (Seregin & Ivanov, 2001, Nagajyoti et al., 2010, Küpper & Andresen, 2016) and they harm the forest environment in several ways. They impede the nutrient cycling in forests by reducing the availability of essential nutrients (Kabata-Pendias & Pendias, 2001) or by hindering plants ability to access and transport water (Barceló & Poschenrieder, 1990). They inhibit a litter decomposition (Derome & Nieminen, 1998) and have a toxic effects on roots and mycorrhizas (Jentschke & Godbold, 2000, Kahle, 1993). A high level of heavy metals in soils can be toxic or lethal for soil microfauna and plant communities (Kabata-Pendias & Pendias, 2001).

In this study, the amount of such heavy metals as cadmium (Cd), nickel (Ni), chromium (Cr) and lead (Pb) in the biomass of mature stands of Scots pine (*Pinus sylvestris* L.) was estimated. Zinc and copper, although toxic when in excess, were not included as they are also nutrients, important for plants growth. The accumulation of these elements were described in a separate study by authors (Węgiel et al., 2019).

Detailed knowledge about the influence of stand density on accumulation and allocation of heavy metals in Scots pine trees may prove useful in at least two cases: (1) by bioindication of environmental pollution and (2) by phytoremediation of degraded soils.

Next to mosses and lichens, Scots pine is the most important bioindicator of air and soil contaminations in Poland (Samecka-Cymerman et al., 2006, Marko-Worłowska et al., 2011, Chrzan & Marko-Worłowska, 2012, Gworek et al., 2011, Chrzan, 2013, Parzych & Jonczak, 2013, Kosiorek et al., 2016, Pająk et al., 2015, Chrzan, 2015) and other European countries: Estonia (Ots & Mandre, 2012), Finland (Lippo et al., 1995, Poikolainen, 1997, Rautio et al., 1998, Harju et al., 2002, Pöykiö et al., 2005), Germany (Huhn et al., 1995, Schulz et al., 1999), Slovakia (Mičieta & Murín, 1998), Spain (Santamaría & Martín, 1997), Turkey (Yilmaz & Zengin, 2004), United Kingdom (Lageard et al., 2008) and Lithuania (Čeburnis & Steinnes, 2000). Also, some other pine species were used for bioindication as well (Dogan et al., 2010, Kuang et al., 2007, Sawidis et al., 2011, Lehndorff & Schwark, 2008, Chiarantini et al., 2016, Al-Asheh & Duvnjak, 1997).

The results of such research gave the insight into the nature of the pollution (elements) and its intensity (concentrations). Using plants biomass for detecting the deposition, accumulation and distribution of trace elements in ecosystems is an efficient and valuable tool of monitoring the pollution. It is also one of the cheapest and simplest methods of monitoring atmosphere contamination (Rautio et al., 1998, Yilmaz & Zengin, 2004, Sawidis et al., 2011, Parzych & Jonczak, 2014). Different compartments of trees can be used for analyzes. Many studies focus on bark or needles (Gałuszka, 2005, Dmuchowski & Bytnerowicz, 1995, Huhn et al., 1995, Schulz et al., 1999, Parzych et al., 2017, Kandziora-Ciupa et al., 2016, Chrzan et al., 2016). It is, therefore, not sure which of these compartments is better suited for biomonitoring (Dogan et al., 2010, Alahabadi et al., 2017).

Very promising technique of biological soil remediation and purifying the environment is phytoextraction. This process is innovative and cost-effective and can be applied to extract heavy metals and other pollutants from soil (Saladin, 2015, Pajević et al., 2016). After growing trees on contaminated soil, aboveground biomass is harvested and incinerated to produce energy and concentrate the trace elements for reuse in various sectors (Pan & Eberhardt, 2011, Nzihou & Stanmore, 2013).

Plants that are the most effective in the process of phytoextraction can store the above-average level of heavy metals in their tissues without showing its toxic effects (Baker & Brooks, 1989, Verbruggen et al., 2009, van der Ent et al., 2013). These plants are called hyperaccumulators and are mostly herbaceous. This is some disadvantage, as their reduced root system does not reach deeper soil layers. Additionally, the biomass yield is relatively low and requires regular harvest and re-planting (Saladin, 2015).

Trees have a deep root system and do not require frequent management. This makes woody plants well suited for phytoextraction and reduces the cost of this process. Other factors supporting the use of trees are: fast and high biomass production that is economically valuable, genetic variability, established cultivation practices, high degree of public acceptance, contribution to site stability and prevention of further dis-

persion of heavy metals by leaching, wind or water erosion (Pulford & Watson, 2003, Pajević et al., 2016). Some studies have shown that a various tree species are capable of storing the same or greater amount of heavy metals than herbaceous plants. Especially fast-growing and intensive biomass producing trees as poplars and willows are recognized as a promising media for decontamination of polluted soils (Di Lonardo et al., 2011, Zacchini et al., 2011).

Coniferous tree species are not well studied in terms of phytoextraction capabilities, due to their limited capabilities in terms of heavy metal accumulation. However, these could be increased by metal-tolerant ectomycorrhizal fungi (Colpaert et al., 2011, Krznicar et al., 2010, Babu et al., 2014). Furthermore, comparing to willows and poplars, conifers are better suited to colder regions and drier and poorer soils (Saladin, 2015). Scots pine in particular has a low nutritional requirements and can thrive on acidic and dry soils. Some field experiments confirm that, due to its high adaptability, it can be used in the remediation of soil (Placek et al., 2016). Scots pine, while currently not being used for phytoremediation, is frequently used for reclamation of soils damaged by industry (Baumann et al., 2006, Karu et al., 2009, Kuznetsova et al., 2011, Pietrzykowski et al., 2014, Šebelíková et al., 2016). There is a considerable number of Scots pine stands growing on polluted soils, which potentially could be used for phytoremediation. Because this stands are mostly middle-aged, the question arises: how to manage them in order to increase their function (Figure 1). In particular, is it beneficial to thin these stands or better leave them unthinned until final cutting. The answer could be deduced by investigating the correlation between stands density and the amount of accumulated heavy metals.

We predict: in Scots pine stands of similar age there is a correlation between the stand density and the amount of accumulated heavy metals. In stands of lower densities lower amounts of various elements are stored in the biomass. This might be due to fact, that in stands of higher densities the total foliage (needles) area is greater, so trees can absorb more elements from the air.

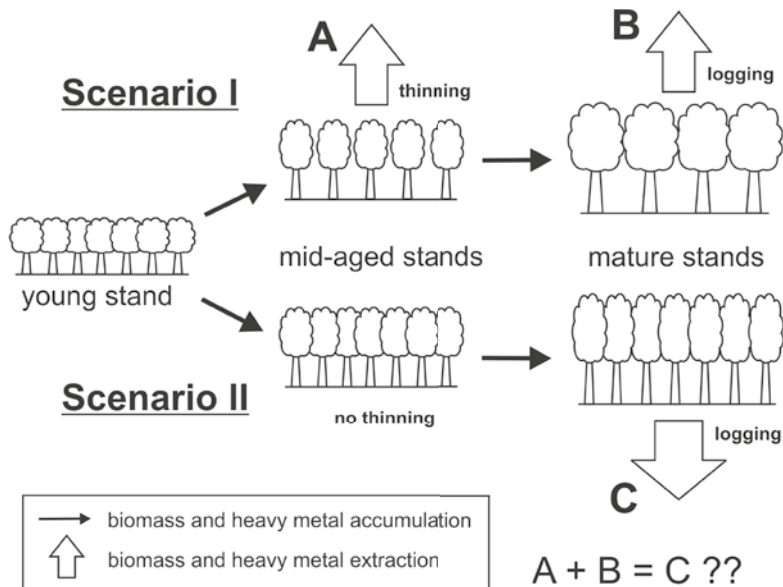


Figure 1: Two management scenarios of stands on degraded soils: Scenario I – thinnings resulting in a mature stand of low density, Scenario II – no thinning, resulting in a mature stand of high density. The question is: Is the total amount of exported heavy metals different between these scenarios?

Abbildung 1: Zwei Behandlungsszenarien für Bestände auf degradierten Böden: Szenario I – Durchforstungen, erntereife Bestände geringer Bestockungsdichte, Szenario II – ohne Durchforstungen, erntereife Bestände mit hoher Bestockungsdichte. Die Frage ist, ob die Summe der ausgetragenen Schwermetalle in beiden Varianten gleich ist.

The goal of the study was to determine the impact of stand density on the accumulation of four heavy metals (Cd, Cr, Ni and Pb) in the above-ground biomass of a mature Scots pine stands. The assessed values were the concentration and the amount of these trace elements in the aboveground biomass in 5 mature stands of Scots pine of different densities: 476 to 836 trees per hectare. These stands were commercial, managed forests, within a large forest complex, with no source of industrial pollution nearby. The calculated concentrations of heavy metals in different tree compartments and the amount per hectare could constitute a reference level for comparison with polluted areas.

2. Materials and methods

This study was carried out in 5 sample stands of Scots pine (*Pinus sylvestris* L.), located in the Drawno Forest District, northwestern Poland (E 15°50'-16°0', N 53°10'-53°13'). This area is characterized by nutrient-poor habitats on podzolic soils, where the dominant tree species is Scots pine. Scots pine mostly forms uniform stands with a small admixture of other tree species, usually birch. It can be assumed that the stands have been established in a similar way (the same initial spacing) and similarly managed (the same owner and manager). The sample plots, 0.5 ha each (Table 1), were selected so that density would be the distinguishing variable. Plots were established in even-aged, 82-year-old, single-species, single-layer stands growing on the same soil type classified as Carbic Podzol. The stands had not been thinned over the previous 5 years. The research area was located within a large forest area without any sources of air pollution nearby.

Table 1: Main characteristics of five 82-year-old Scots pine sample stands.

Tabelle 1: Kennwerte der fünf beprobten 82-jährigen Kiefernbestände.

Sample plot	Density, tree·ha ⁻¹	Relative spacing	Mean DBH (±SD), cm	Mean height (±SD), m	Top height (±SD), m	Basal area, m ² ·ha ⁻¹	Volume, m ³ ·ha ⁻¹
SP1	476	0.187	28.2±4.5	22.9±1.7	24.5±0.5	30.5	319
SP2	590	0.182	25.7±4.7	20.8±1.4	22.6±0.4	31.5	302
SP3	672	0.180	23.6±4.3	19.6±1.5	21.5±0.4	30.3	275
SP4	756	0.163	23.9±5.3	20.1±2.0	22.3±0.4	35.6	337
SP5	824	0.163	21.8±4.0	19.3±1.7	21.4±0.3	31.7	286

On the sample plots, diameter at breast height (DBH) was measured for each tree on each plot, and the height for 20% of the trees was measured. Based on this data, for each plot separately Näslund's height curves were developed to establish the height of each tree (Siipilehto, 2000). For each of the 5 sampled stands, the mean DBH, the mean height and the top height (mean height of 50 trees with the biggest dbh for each sample plot) were determined, as well as the relative spacing (the ratio of the average distance between the trees to the average dominant height of the stand) by Wilson (1946), the basal area (in m² per hectare) and the merchantable volume (in m³ per hectare).

The dry mass of all the trees on the five experimental plots was calculated with allometric equations (Picard et al., 2012). On each 0.5 ha plot, 10 model trees were selected, with DBH representing the range of the measured diameters, resulting in 50 model trees in total. Model trees were felled and divided into defined compart-

ments: stem, dead branches, thick branches (diameter of more than 5 mm), thin branches and needles. From the stem, samples were taken as 10 cm cross-sections that were cut every meter along the stem. The fresh mass of each compartment was measured. After weighing the fresh compartments, the samples were dried at 65° C to a constant mass to determine the dry mass. Using the sample proportion of dry and fresh mass, the dry mass of each compartment of each model tree was calculated.

Allometric equations were developed based on the data obtained for the 50 model trees across all plots. Their fit to data was assessed based on Akaike's Information Criterion (AIC), coefficient of determination (R^2) and residual standard error (RSE). For all the components the best-fitting model was the equation:

$$\text{Dry mass} = c_0 \cdot d^{c_1} \cdot h^{c_2}$$

d - diameter at breast height [cm],

h - tree height [m],

c_0 , c_1 , c_2 - equation parameters.

These allometric equations made it possible to calculate the dry mass of the aboveground component of every individual tree, using DBH and height as predictive variables. Mass was calculated for: stem ($R^2=0.936$), dead branches ($R^2=0.746$), thick branches ($R^2=0.728$), thin branches ($R^2=0.674$) and needles ($R^2=0.750$). The results were used to calculate the mass of each individual tree and the collective mass for the experimental plots.

Chemical analyses were conducted on a select number of trees used for biomass. For each sample area, 3 out of the 10 model trees were selected. From the list, ordered by increasing DBH, the second, fifth and ninth trees were chosen. Across the whole study area, a total of 15 trees were selected. Materials for the chemical analysis were taken beforehand from the samples used to determine dry mass. Samples from tree components, stem wood, dead branches, thick branches, thin branches and needles, were taken for chemical analysis. The material was ground, marked, and sent to the laboratory. Chemical analyses were performed in the Laboratory of Environmental and Soil Remediation Geochemistry at the University of Agriculture in Kraków. Laboratory samples were mineralized in HNO_3 and by using an Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES), the content of the elements were determined.

To create a model for each tree in the test areas, one of 3 sample trees with the closest DBH, was assigned. Based on the dry mass and percentage content of the nutrients in the assigned sample tree, the nutrient mass in each tree component within the test area were calculated.

The next step was determining the nutrient content of each tree component for all experimental plots. For each tree, the nutrient content was estimated based on the dry mass of each given component and the content of 4 trace elements in the sample from this specific compartment. To calculate the element content for the remaining trees (other than model trees), specific coefficients were used based on proportions found in model trees that had the closest DBH to a certain tree. This way, the basis for calculations in smaller trees was the smallest model tree and for the bigger trees, the biggest model tree. The mass of any element contained in a tree compartment was calculated by multiplying the dry mass of this compartment by a specific coefficient (ratio) found in a model tree with the closest DBH.

Statistical analyses were performed using the "Multivariate Platform" tool in JMP 10.0 statistical software (SAS Institute Inc., Cary, NC, USA). Multiple linear regression analysis was carried out to identify the influence of multiple variables (stand density, mean DBH, mean height, top height and stand volume) on dependent variables (accumulation of heavy metals in each part of trees and total). The multicollinearity reduces the precision of the estimate coefficients, which weakens the statistical power of a regression model. Thus, to avoid running into estimation problem some variables were removed automatically; linearly dependent variables (variables with the largest partial correlation) were excluded from the analysis. A p-value less than 0.05 was accepted as statistically significant.

3. Results

The aboveground biomass was calculated for different compartments of the tree: stem wood, stem bark, thick branches, thin branches, dead branches and needles (Table 2). Basing on 15 model trees from 5 sample stands of different densities, the concentrations of 4 heavy metals (Cd, Ni, Cr and Pb) in the aboveground biomass of Scots pine trees were estimated (Table 3). Cadmium has reached the highest concentration in the stem bark, while Cr and Pb in the thin branches, and Ni in the needles. The lowest concentrations of Cd was found in the needles, Ni and Pb in the stem wood and Cr in dead branches (completely absent in the stem bark). A negative significant correlation was found for tree size and concentration of Ni in branches, concentration of Cr and Pb in stem wood and branches, and concentration of Cd in all compartments (Figure 2).

Table 2: Aboveground biomass ($Mg \cdot ha^{-1}$) of different compartments of five sampled Scots pine stands.

Tabelle 2: Oberirdische Biomasse ($Mg \cdot ha^{-1}$) von verschiedenen Baumkompartimenten in den fünf beprobten Kiefernbeständen.

Sample plot	Stem wood	Stem bark	Thick branches	Thin branches	Dead branches	Needles	Total
SP1	115.6	10.1	14.7	3.0	5.5	4.8	153.7
SP2	107.4	9.4	14.9	2.9	5.5	4.7	144.8
SP3	99.2	8.7	13.5	2.7	5.0	4.4	133.5
SP4	120.4	10.6	16.3	3.2	6.1	5.3	161.9
SP5	107.2	9.5	12.9	2.8	4.7	4.7	141.8

Table 3: Mean ($\pm SD$) concentrations of elements in the dry mass for all ($N=15$) sampled Scots pine model trees.

Tabelle 3: Mittelwerte (\pm Standardabweichung) der Elementkonzentrationen in der Trockenmasse aller beprobten Kiefern ($N=15$).

Element	Stem wood	Stem bark	Thick branches	Thin branches	Dead branches	Needles
Cd, $mg \cdot kg^{-1}$	0.24 \pm 0.07	0.97 \pm 0.29	0.47 \pm 0.11	0.41 \pm 0.08	0.36 \pm 0.11	0.10 \pm 0.03
Cr, $mg \cdot kg^{-1}$	0.99 \pm 0.95	0.00 \pm 0.00	1.06 \pm 0.75	1.27 \pm 0.80	0.74 \pm 0.89	1.10 \pm 1.84
Ni, $mg \cdot kg^{-1}$	0.25 \pm 0.09	0.76 \pm 0.43	0.46 \pm 0.12	1.21 \pm 0.33	0.52 \pm 0.27	2.89 \pm 2.41
Pb, $mg \cdot kg^{-1}$	0.79 \pm 0.74	1.41 \pm 0.90	2.59 \pm 0.59	2.80 \pm 0.88	1.70 \pm 0.67	2.10 \pm 1.59

The amount of four analysed heavy metals in the aboveground biomass of Scots pine stands of different densities is shown in Table 4. The greatest total content was found for Pb (0.087-0.242 $kg \cdot ha^{-1}$), followed by Cr (0.048-0.179 $kg \cdot ha^{-1}$), Ni (0.051-0.078 $kg \cdot ha^{-1}$) and Cd (0.033-0.053 $kg \cdot ha^{-1}$). All the analysed elements were most abundant in the stem wood: Cd (0.017-0.034 $kg \cdot ha^{-1}$), Cr (0.027-0.155 $kg \cdot ha^{-1}$), Ni (0.021-0.032 $kg \cdot ha^{-1}$) and Pb (0.019-0.150 $kg \cdot ha^{-1}$). In the needles, the scarcest elements were: Cd (0.000-0.001 $kg \cdot ha^{-1}$) and Pb (0.004-0.021 $kg \cdot ha^{-1}$). In the stem bark, the least accumulated was Ni (0.005-0.009 $kg \cdot ha^{-1}$) followed by Cr that was not detected at all.

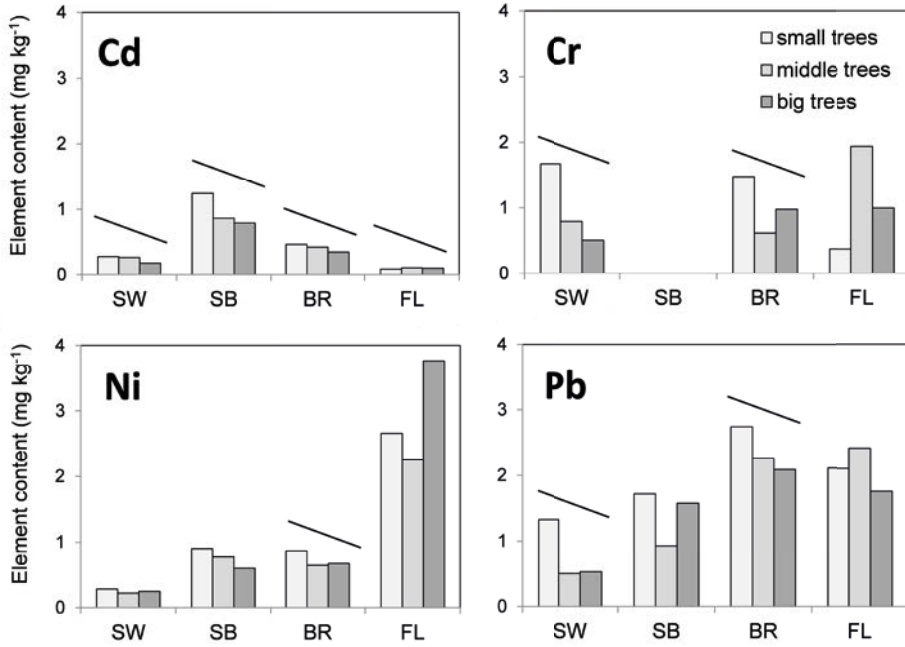


Figure 2: Average concentrations of heavy metals in tree compartments (SW - stem wood, SB - stem bark, BR - branches, FL - foliage) in model trees (N=15) divided into small, medium and big trees (according to DBH). Oblique lines show in which compartments concentration of heavy metals was significantly correlated ($p < 0.05$) with tree size. All correlations were negative.

Abbildung 2: Mittlere Schwermetallkonzentration in einzelnen Baumkompartimenten (SW – Stammholz, SB – Stammrinde, BR – Äste, FL – Nadelmasse) von 15 Probestämmen, die nach BHD in schwache, mittlere und starke Bäume unterteilt wurden. Die geneigten Linien zeigen an, für welche Kompartimente die Konzentration von Schwermetallen signifikant mit der Stärkeklasse korreliert war ($p < 0.05$). Alle Korrelationen waren negativ.

Table 4: Heavy metal accumulation ($\text{kg}\cdot\text{ha}^{-1}$) in stem wood, stem bark, branches, needles and total (all aboveground biomass) for Scots pine stands of different densities.

Tabelle 4: Schwermetallgehalte ($\text{kg}\cdot\text{ha}^{-1}$) in Stammholz, Stammrinde, Ästen, Nadeln sowie deren Summe (gesamte oberirdischen Biomasse) in Kiefernbeständen verschiedener Dichte.

Tree parts	Sample plots - Scots pine stands of different densities				
	SP1, 476 trees ha^{-1}	SP2, 590 trees ha^{-1}	SP3, 672 trees ha^{-1}	SP4, 756 trees ha^{-1}	SP5, 824 trees ha^{-1}
Cd (cadmium)					
Stem wood	0.017	0.023	0.025	0.029	0.034
Stem bark	0.007	0.008	0.009	0.010	0.008
Branches	0.008	0.010	0.008	0.013	0.008
Needles	0.001	0.001	0.000	0.001	0.000
Total	0.033	0.042	0.042	0.053	0.050
Cr (chromium)					
Stem wood	0.027	0.074	0.155	0.054	0.124
Stem bark	0.000	0.000	0.000	0.000	0.000
Branches	0.016	0.021	0.019	0.028	0.019
Needles	0.005	0.001	0.005	0.001	0.018
Total	0.048	0.096	0.179	0.083	0.161
Ni (nickel)					
Stem wood	0.032	0.021	0.032	0.026	0.024
Stem bark	0.007	0.009	0.005	0.009	0.006
Branches	0.011	0.012	0.011	0.013	0.014
Needles	0.028	0.009	0.007	0.011	0.014
Total	0.078	0.051	0.055	0.059	0.058
Pb (lead)					
Stem wood	0.019	0.065	0.098	0.091	0.150
Stem bark	0.013	0.010	0.014	0.011	0.014
Branches	0.047	0.056	0.050	0.056	0.057
Needles	0.008	0.009	0.007	0.004	0.021
Total	0.087	0.140	0.169	0.162	0.242

A multiple regression was run to predict one of four heavy metals accumulation in one of each tree part from stand density, mean DBH, mean height, top height and stand volume (Table 5). Mean DBH and top height were excluded from the model in each case. The other three variables significantly predicted Cd accumulation in branches ($F=807.20$, $p=0.026$, $R^2_{\text{adjusted}}=0.998$) and very highly significantly predicted Cr accumulation in branches ($F=3995503,85$, $p=0.001$, $R^2_{\text{adjusted}}=1.000$), statistically significant model ($p<0.05$) was developed, basing on these three variables. The model with three variables also significantly predicted Cd accumulation in total biomass ($F=1188.66$, $p=0.021$, $R^2_{\text{adjusted}}=0.999$), statistically significant model ($p<0.05$) was developed, basing on one variable (stand volume) (Table 5).

In each case, a graph of relationship between standardized residuals and predicted

values was done, and did not show any trend in any instance. This proves that the relationship between heavy metals accumulation and each analyzed parameter is linear.

The proportions between the contents of four heavy metals in various tree compartments are shown in Figure 3. In the stem wood the highest share was reached by Cr, in the stem bark – Cd, in the branches – Pb and in the needles – Ni.

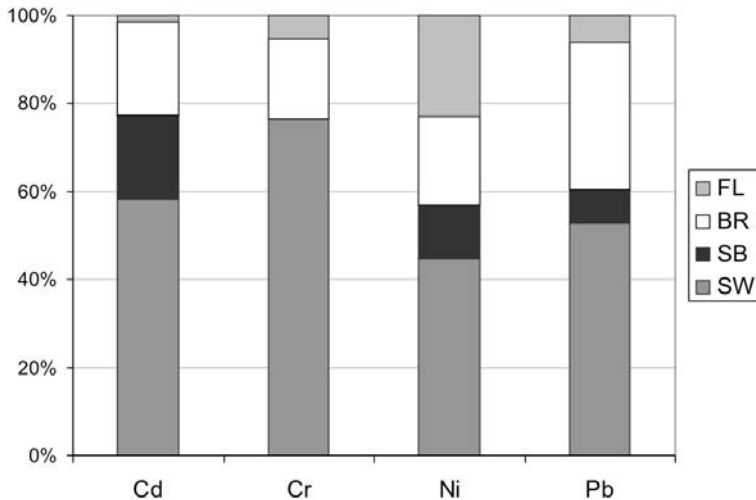


Figure 3: Proportions between the content of four heavy metals (Cd, Cr, Ni and Pb) in the aboveground biomass (FL - foliage, BR - branches, SB - stem bark and SW - stem wood), based on the average accumulation (kg ha^{-1}) for five sampled stands.

Abbildung 3: Beziehungen zwischen dem Gehalt (kg ha^{-1}) von vier Schwermetallen (Cd, Cr, Ni und Pb) in der oberirdischen Biomasse (FL – Nadeln, BR – Äste, SB – Stammrinde und SW – Stammholz) als Mittelwerte aus fünf beprobten Beständen.

Table 5: Multiple regression analyses for four heavy metal accumulations in different part of sampled Scots pine stands and different independent variables: stand density [trees·ha⁻¹], mean height [m] and volume [m³·ha⁻¹]. Two independent variables were excluded from the model in each cases: mean DBH [cm] and top height [m].

Tabelle 5: Multiple Regressionsanalyse der Schwermetallkonzentrationen in den Baumkompartimenten der beprobten Kiefernbeständen. Die unabhängigen Variablen sind Bestandesdichte [Stämme ha⁻¹], mittlere Baumhöhe [m] und Stammvolumen [m³ ha⁻¹]. Zwei unabhängige Variablen wurden entfernt, mittlere Durchmesser in Brusthöhe [cm] und Oberhöhe [m].

Element	Independent variable	Tree parts												Total		
		Stem wood			Stem bark			Branches			Needles					
		β	t	p	β	t	p	β	t	p	β	t	p	β	t	p
Cd	Stand density	1.137	4.350	0.144	-0.99	-0.838	0.556	-0.879	-14.901	0.043*	-1.076	-0.787	0.576	0.533	10.971	0.058
	Mean height	0.176	0.593	0.659	-2.019	-1.503	0.374	-1.723	-25.691	0.025*	-0.894	-0.575	0.688	-0.582	-10.523	0.06
	Volume	-0.068	-0.507	0.701	1.002	1.649	0.347	1.363	44.904	0.014*	0.986	1.4	0.395	0.438	17.497	0.036*
		R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p
		0.968	40.76	0.115	0.337	1.68	0.504	0.998	807.20	0.026*	0.111	1.17	0.577	0.999	1188.66	0.021*
Cr	Stand density	-0.094	-0.178	0.888	-	-	-	-0.762	-908.982	0.001**	2.488	10.624	0.06	0.147	0.31	0.809
	Mean height	-0.559	-0.927	0.524	-	-	-	-1.801	-1889.308	<0.001***	2.504	9.401	0.067	-0.378	-0.702	0.61
	Volume	-0.634	-2.324	0.259	-	-	-	1.218	2823.097	<0.001***	-1.217	-10.099	0.063	-0.644	-2.646	0.23
		R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p
		0.867	9.67	0.231	-	-	-	1.000	3995503.85	<0.001***	0.974	50.86	0.103	0.894	12.22	0.207
Ni	Stand density	0.165	0.064	0.959	-1.032	-0.834	0.557	1.614	1.144	0.457	1.591	3.832	0.163	1.411	1.241	0.432
	Mean height	0.698	0.238	0.851	-1.387	-0.986	0.505	0.936	0.584	0.664	2.539	5.378	0.117	2.28	1.763	0.328
	Volume	-0.421	-0.318	0.804	1.274	2.002	0.295	-0.02	-0.028	0.982	-0.556	-2.604	0.233	-0.449	-0.768	0.583
		R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p
		-2.151	0.09	0.955	0.273	1.50	0.526	0.056	1.08	0.593	0.918	15.95	0.182	0.386	1.847	0.486
Pb	Stand density	1.068	5.724	0.11	1.864	1.269	0.425	0.208	0.121	0.924	2.215	1.893	0.309	1.24	3.121	0.197
	Mean height	0.227	1.071	0.478	2.088	1.256	0.428	-0.715	-0.365	0.777	2.285	1.717	0.336	0.46	1.018	0.494
	Volume	-0.378	-3.935	0.158	-1.224	-1.619	0.352	0.392	0.443	0.735	-1.175	-1.951	0.302	-0.461	-2.257	0.266
		R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p	R ² adjusted	F	p
		0.983	80.32	0.082	-0.024	0.97	0.616	-0.41	0.61	0.709	0.35	1.72	0.499	0.925	17.45	0.174

* Significant at p < 0.05, ** significant at p < 0.01, *** significant at p < 0.001

4. Discussion

Cadmium is not necessary for plants growth, on the contrary – is highly toxic and mobile (Küpper & Andresen, 2016). In the studied Scots pine trees the average concentration of Cd ranged from 0,10 mg kg⁻¹ in the needles to 0,97 mg kg⁻¹ in the stem bark. These values were similar to the ones obtained by other authors for various compartments of Scots pine trees (Bramryd, 2001, Kandziara-Ciupa et al., 2016, Parzych et al., 2017, Huhn et al., 1995).

Chromium is extremely toxic for plants and hinders their growth and development (Nagajyoti et al., 2010). Although, some stimulating influence on plants growth was observed (Kabata-Pendias & Pendias, 2001), there is no evidence of its significant role in plants metabolism. In this study, the concentrations of Cr ranged from 0,74 mg kg⁻¹ in dead branches to 1,27 mg kg⁻¹ in thick branches. We did not find any Cr in the bark. Similar concentrations were found by other researchers for the needles (Rautio et al., 1998, Bramryd, 2001) and for the stem wood (Butkus & Baltrėnaitė, 2007), but Huhn et al. (1995) found substantially higher concentration of Cr in the bark (0,8-8,7 mg kg⁻¹).

Nickel is an element easily and quickly absorbed by plants, with a relatively low toxicity. In plants physiology, nickel is known to be a part of only one enzyme: urease (Küpper & Andresen, 2016). In our study, the average concentrations were between 0,25 mg kg⁻¹ in the stem wood to 2,89 mg kg⁻¹ in the needles, which is along the values reported by other authors (Butkus & Baltrėnaitė, 2007, Saarela et al., 2005, Parzych et al., 2017).

Lead is one of the most abundant and common toxic element in the soil. It is not required for plants growth and highly toxic (Krutul et al., 2017). In the analyzed Scots pine trees the concentrations of lead were on average from 0,79 mg kg⁻¹ in the stem wood to 2,80 mg kg⁻¹ in the thick branches, which corresponds with the results in the literature (Butkus & Baltrėnaitė, 2007, Dmuchowski & Bytnerowicz, 1995, Kandziora-Ciupa et al., 2016).

The obtained concentrations were lower than toxic levels given by Kabata-Pendias and Pendias (2001) at a range of 5-30 mg kg⁻¹ for Cd and Cr, around 10-100 mg kg⁻¹ for Ni and 30-300 mg kg⁻¹ for Pb. The obtained concentrations were, however, similar to the ones reported for Scots pine on some protected areas in Poland (Dmuchowski & Bytnerowicz, 1995, Gałuszka, 2005, Parzych & Jonczak, 2013). Because this research was carried out in area of low atmospheric emissions, far from industrial pollutants, the results can be treated as a reference level for concentrations obtained in cities or industrial areas.

When using Scots pine as a bioindicator of environment pollution, it is important to select a correct tree compartment because the concentrations of elements vary significantly between tree parts. In bioindication usually bark and needles are used for chemical analyses, however there is some discussion on which part is better suited for this role (Dogan et al., 2010, Kosiorek et al., 2016). For example, Dogan et al. (2010) studied the concentrations of metals in pines growing in urban environment, and concluded that the needles are better bioindicator than the bark. On the other hand, Samecka-Cymerman et al. (2009) and Alahabadi et al. (2017) reported that bark is better to indicate a long-term cumulative traffic pollutions, rather than needles.

In our study, the concentrations of heavy metals were in the following order: in the

needles Ni>Pb>Cr>Cd, and in the bark Pb>Cd>Ni>Cr. For Turkish red pine the accumulation values of heavy metals in the bark and needles were: Pb>Ni>Cr>Cd and Cr>Pb>Ni>Cd (Dogan et al., 2010). Considering the Scots pine as a bioindicator, it needs to be taken into account that the concentrations of elements in various tree compartments depends not only on their amount in the environment, but also on tree size (Figure 2) and stand density (Table 5). Furthermore, our study show that a better indicator for Cr are the needles, as we did not found any Cr in the bark.

Concentrations of heavy metals differs significantly between various tree compartments. This can be due to their toxicity and the ability of plants to translocate the toxic elements to a specific compartment to avoid its harmful effect (Gielen et al., 2016). Therefore, a selection of bark or needles for analysis can give a different results. For this reason, the biomonitoring values obtained in different ways should not be directly compared. In case of main nutrients concentrations in trees, variations between tree compartments are also pronounced, usually however, they form the same pattern: the lowest concentration in the stem wood and the highest in the needles (Augusto et al., 2008, Barron-Gafford et al., 2003, Skonieczna et al., 2014).

The ability of Scots pine to accumulate the heavy metals is much lower compared to herbaceous hyperaccumulators. Furthermore, pine is not very resistant to their toxicity. Despite this, it can contribute to phytoextraction on the areas where it has been already planted (Saladin, 2015). Thanks to Scots pine low nutritional requirements, it can successfully grow on dry, acidic and poor soils, and is frequently planted on the reclaimed soil (Thiry et al., 2005, Prach & Pyšek, 2001, Pietrzykowski & Socha, 2011, Ortigosa et al., 1990, Margus et al., 2004, Karu et al., 2009, Kuznetsova et al., 2010).

Many stands on industrialized areas are middle-aged and a decision about management strategy needs to be taken. Is thinning necessary? How often and how intense should the stand be thinned? Or even – is it better to leave them without thinning up to the final cutting? How this affects their performance as a phytoextraction medium? Our results do not allow to unambiguously answer the question of which scenario is the best for phytoextraction (Figure 1). It is mostly due to the fact that we do not know what amount of heavy metals was exported from the stands during thinning.

It is, therefore, worth considering how to increase the accumulation of harmful elements by forest trees. One of the stand characteristic, that can be regulated by initial spacing and later by thinning intensity, is stand density. Among the analyzed heavy metals Cd and Cr showed a positive correlation with stand density (as well as with tree height and volume). This means that the more trees there are on the given area, the faster the accumulation occurs. This is not a result of higher biomass of stands with higher number of trees (no correlation), but of higher concentration of heavy metals in smaller trees (Figure 2). In stands of lower densities trees had smaller DBH (statistically significant correlation). The lack of statistically significant correlation between stand density and accumulation of Pb and Ni could be due to small sample size

(5 stands). The clue could be the fact that in some compartments their concentration was positively correlated with tree size (Figure 2).

Management strategies in the stands intended to be utilized for heavy metals accumulation, are opposite than in case of nutrient accumulation balancing. In case of phytoextraction of harmful elements, the suggested harvesting scenario would be the whole-tree harvesting (WTH). In regular managed forests this method can cause excessive nutrient export (Węgiel et al., 2018b, Palviainen & Finér, 2012, Wall, 2012) and is not recommended. However, thinnings seem to be unnecessary, because they increase the costs and produce stands of low densities, which inhibits phytoremediation.

We do not know any other studies concerning the influence of stand density on accumulation of heavy metals. For nutrients, a positive correlation with stand density was found for nitrogen, phosphorus and potassium (Węgiel et al., 2018a). Similar relationship was found in plantations of *Pinus taeda* and *Pinus elliottii* in USA – the stock of N, P and K was significantly higher in stands of greater density (Barron-Gafford et al., 2003). Different results were obtained in Korea for *Pinus densiflora* (Noh et al., 2013) and in Canada for various tree species (Paré et al., 2002), where no influence of density on the nutrient stock was found.

Conclusions

The demonstrated differences in concentrations of heavy metals in different tree parts of Scots pine can be used in bioindication of environment pollution. Information that the concentration of some elements is related to stands density and tree size, is useful and needs to be considered, when selecting the trees for biomonitoring. Furthermore, selection of needles or bark as an indicator would yield a different result. It is therefore necessary to continue research on how the concentration of elements in these compartments is correlated with their concentration in the soil and the atmosphere.

Scots pine is used for revegetation of degraded soils for a long time. These stands are reaching the age that the management strategy needs to be decided. Scots pine stands could be used for phytoextraction of heavy metals. In this case, the correlation between the accumulation of heavy metals and stand density can have a practical use. Increased accumulation of these elements would be obtained in stands of higher densities, so thinning may not be recommended. However, to unambiguously state that conclusion some further research is necessary, that will accommodate the amount of heavy metals exported whilst thinning of various intensities.

The knowledge about different levels of accumulation of heavy metals in various tree components, could also be used by phytoextraction. For example, for elements that

mostly accumulate in needles, like Ni, removal of fallen foliage might prove more efficient. For elements that mostly accumulate in branches, like Pb, pruning and removal can also be advisable. Further studies would also be recommended, to assess if these results would be similar in polluted areas.

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