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POTENTIAL FOR *Salix schwerinii* E. Wolf TO UPTAKE HEAVY METALS IN THE CONTAMINATED TERRITORIES OF MINING INDUSTRY IN THE NORHT-WEST RUSSIA

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The study was carried out on the industrial territory of Joint Stock Co. «Karelsky Okatysh» (Russia, Republic of Karelia, Kostomuksha). The species *Salix schwerinii* E. Wolf (Finland) was used as a phytoremediant and was cultivated on the contaminated territory of the mining enterprise – the tailing dump (the main pollutants being Ni and Fe). After one year, the willow plant samples were divided into two groups: healthy plants with higher biomass production (HBP) and suppressed plants with lower biomass production (LBP). The root system of HBP and LBP plants had no differences, but aboveground biomass was higher in HBP willow plants. The content of photosynthetic pigments was low in both groups of willows at (1.62 ± 0.10) mg/g wet weight. SLA index (specific leaf area) was 1.53 $mm^2\ mg^{-1}$ and 1.21 $mm^2\ mg^{-1}$ in HBP and LBP groups, correspondingly. About 50–90 % of Ni, Fe, Mn, Co, Cu, Cr were absorbed by the roots and leaves of willow. All of these elements accumulated in maximal amounts in the roots (70 %) of plants from the HBP group, while in the LBP group they accumulated mostly in leaves (70 %). Pb was deposited in the roots, leaves and bark (20–30 % for each structure), Cd, Zn – in the bark (50–60 %). According to the coefficient of biological absorption, heavy metals in the willow plants formed the following sequence: Zn (8) > Mn (6–8) > Cd (4–6) > Cu (4–5) > Pb (3) > Co (1) > Ni (0.6) = Cr (0.5–0.7) > Fe (0.2).

Keywords: *Salix schwerinii, phytoremediation, heavy metals, photosynthetic pigments, biometric parameters, specific leaf area, joint stock company* «*Karelsky Okatysh*», *Republic of Karelia.*

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INTRODUCTION

One of the most acute problems of technogenic landscape recultivation is choosing an effective decontamination method, which is restricted by the local peculiarities of the industrial territories. The development of modern mining and metallurgical industry with high level of emissions into the atmosphere of acidifying substances and heavy metals, leads to the degradation of terrestrial ecosystems of adjacent territories and formation of industrial barrens (Lukina, Nikonov, 1998; Remon et al., 2005; Kozlov, Zvereva, 2007). The predominant part of the industrial barrens is located in the northern hemisphere in the territory of the European Union, USA, Canada and Russian Federation. This connects with the distribution of industrial enterprises for processing of non-ferrous metals, as well as with higher vulnerability of northern ecosystems to the negative effects of emissions of these enterprises (Alexeyev, 1995; Androkhanov, 2012).

The industrial territories of the mining plant Joint Stock Co. «Karelsky Okatysh» (the city of Kostomuksha, Republic of Karelia, Russia) is example of area, where industrial barren is the result of dumping of waste raw materials (sludge, pulp) called tailing dump (Markovskaya et al., 2015). Natural recovery successions are very difficult on such territories in the North. Constant emissions of pollutants (heavy metals and SO_2), their entry in to ground and accumulation in the surrounding terrestrial ecosystems, migration to the aquatic environment (Kashulina, Saltan, 2008) as well as lack of soil organic matter and nutrients are the reasons for the low rate of recovery. Cleaning and deforestation of industrial areas is possible with use of phytoremediation (Kumar et al., 1995; Salt et al., 1995; Pulford, Watson, 2003). The phytoremediation method is gaining widespread recognition, which provides for phytoextraction – the extraction of heavy metals by way of accumulating them in the plants. This method has a number of advantages over other methods of contaminated area restoration: low costs and, with rotation, a possibility to use ameliorant plants as biofuel (Licht, Isebrands, 2005; Sassner et al., 2008). In general, the plants of *Salix schwerinii* vary in their requirements to the different environmental factors. In relation to various environmental factors, plants, belonging to the species *Salix schverinii,* are semigeliophytes (on demand to lighting conditions), at the request of the humidity – mesophytes, on demand to nutrient status of the habitat – oligomesotrophs, on demand to temperature factor – mesothermophytes. *Salix* species are highly attractive as a phytoremediants due to their high level of genotypic polymorphism and genetic flexibility (Newsholme, 1992; Skvortsov, 1999; Valyagina-Malyutina, 2004; Rockwood et al., 2004; Tlustos et al., 2007; Efimova, 2012; Nesedko, 2012), which is determined by the phylogenetic youth of *Salix* gen. (Kulagin, 1998). Species of this genus also have particular biological and physiological features: high photosynthesis intensity (Liu et al., 2003; Kaipiainen, Pelkonen, 2007) associated with high stomatal conductance (Wikberg, 2006) and a well-organized water metabolism structure (Wikberg, 2006; Kuzovkina, Volk, 2009): resistance to xylem vessel cavitation (Wikberg, Ören, 2007), high hydraulic conductivity (Aasama et al., 2001), high water-retaining and water-absorbing capacity (Kulagin, 2003). All these properties ensure their flexibility and viability and facilitate their quick dispersal, successful introduction and acclimatization in a wide range of ecotopes. It is known that willows are tolerant to industrial contaminants (Smirnov, 1980; Minchenko, 1989; Sergeichik, 1994; Kulagin, 1998; Zakharova, 2005; Fischerova et al., 2006; Mleczek et al., 2009; Marmiroli et al., 2011; Pesonen et al., 2014) and capable of accumulating high concentrations of heavy metals in the tissues and organs and ensure the phytoremediation of contaminated landscapes.

Joint Stock Co. «Karelsky Okatysh» has territories contaminated with heavy metals and it is interested in remediation of industrial areas. Phytoremediation studies on industrial territories of this mining plant with using willows species were carried out for the first time in the project of ENPI CBC program «Development of Tree Plantations for Tailings Dumps Afforestation and Phytoremediation in Russia». These studies began in 2012 and it is important to evaluate the growth, development and accumulation levels of trace metals by plants at the early stages of their ontogeny at the beginning of phytoremediation willows plantation on the territory of this mining plant.

The purpose of the present study was to assess the potentiality of phytoremediation with *Salix schwerinii* E. Wolf on the territory of Joint Stock Co. «Karelsky Okatysh» based on accumulation and distribution of heavy metals within willow plants.

MATERIALS AND METHODS

Study area. The study was carried out on the industrial territories of Joint Stock Co. «Karelsky Okatysh» (Kostomuksha, Karelia, Russia) (64°34′15″N, 30°34′36″E). The main emission components of this mining and processing works are sulfur dioxide (the average annual concentration is 0.03 mg/m^3), dust emissions containing heavy metals, carbon oxide and nitrogen oxides. 18 elements were detected in the airborne dust. According to their concentration in the dust the microelements were divided into two groups: with concentration of 1–0.1 mg/g (Ni, Fe, Mn, Cr, Pb, Zn, Cu, Sr) and with concentration of < 0.1 mg/g (Zr, As, Br, Mo, Se). Iron is abundant in dust emissions, the nickel concentration being lower. In 2012, the pollutant emissions of Joint Stock Co. «Karelsky Okatysh» totalled: solid matter – 5 667 000 t, sulfur dioxide – 40 934 000, carbon oxide and nitrogen oxide – 1 687 000 and 1 872 000 t respectively (Gosudarstvennyi doklad…, 2010). SO_2 was the most common component of the emissions.

Field trail conditions. The experimental plants were planted on the industrial area – the sand medium of the tailing dump (pulp storage) on the territory of Joint Stock Co. «Karelsky Okatysh». There is continuous natural moistening of the growing medium with the waste waters of the works.

Plant material. Plants of *Salix schwerinii* E. Wolf were used as phytoremediative species. The cuttings were brought from Finland (University of Eastern Finland, Joensuu, 2000 pieces). Willows were planted on the territory of 0.3 ha with density 16 000/ha. The duration of the experiment was 1 year (May 2012 – August 2013). At the end of the experiment, biometric parameters of tested plants were measured. It is worth noting that the experimental site of the tailing dump is very heterogeneous when it comes to its granulometric and mineralogical composition, as well as the availability of waste waters, which resulted in the different growth response of the planted cuttings of *Salix schwerinii*. There was a visual distinction between two groups of experimental willow plants: the HBP group – taller plants, without defects, with high biological productivity and a high level of viability, and the LBP group – lower plants, with partial defects, low biological productivity and suppressed viability (Alexeyev, 1989). The ratio of LBP plants to HBP plants was 30 : 70 %.

10 typical plants from the HBP group – taller plants and 10 typical plants from the LBP group – lower plants were sampled. Each willow plant was splinted into organs – stem, roots and leaves. The bark was separated from wood on the stems.

The morphometric measurements included: the estimation of the leaf area and mass, the calculation of the specific leaf area (SLA). SLA is the one-sided area of the fresh leaf divided by its oven-dry mass, expressed in $mm^2\ mg^{-1}$ (Cornelissen et al., 2003).

The estimation of content of photosynthetic pigments in leaves was performed in ethanol extracts with a SF (selective filter) («UNICO 2800»), the peak absorption being 665 and 649 nm for chlorophyll (Chl) *a* and *b* respectively, and 470 nm for carotenoids (Car). The pigment concentration was calculated by using the formulas of I. F. Wintermans, De Mots (1965). All measurements were done in triplicate.

The coefficient of biological absorption (CBA) of metal by a whole plant was calculated as a ratio of the metal content in the plant (total metal content in the roots, leaves, bark and woody tissue) to the metal content in the soil.

Samples of soils were taken from the sites where tested plants grew. The soil medium testing included: the data on crop-producing power, pH of the water extract from the growing medium, total content of carbon, nitrogen, phosphorus, potassium, calcium, magnesium using standard procedures (Wolf, Beegle, 1995). The metal concentration in the soil, plant tissues (bark, wood) and organs (roots, leaves) was estimated using the atomic absorption method (the atomic absorption spectrophotometer AA-7000 with a flame atomizer, Shimadzu 7000 (Japan). The samples were first dissolved in a mixture of concentrated acids (HNO_3, HCl, in the ratio 3:1) in the microwave digestion system (speed wave four, Berghof, Germany). All the tests of heavy metal content were performed using the certified equipment of the Shared Use Centre «Analytical laboratory» of Forest Research Institute of the Karelian Research Centre of the RAS (ISO 11466 : 1995, NBN EN 13657 : 2002, ISO 11407 : 1998, ISO 20280 : 2007). All measurements were done in triplicate.

Statistical analysis. Statistical analysis was performed using SAS software (version 9.2, SAS Institute, Cary, NC). The MIXED procedure for analysis of variance was used to determine statistical differences ($P < 0.05$) from HBP and LBP field plants. Initially, the data were tested for homogeneity of variance and normality, however, violations were found. Average values are reported with transformed lettering according to Fisher's LSD at $P \leq 0.05$.

RESULTS AND DISCUSSION

Support medium chemical testing. According to obtained results, the content of macronutrient such as carbon in the soil of tailing dump was not high compared with the background values (Table 1).

The higher content of phosphorus was identified in the soil of experimental plantation on industrial area of Joint Stock Co. «Karelsky Okatysh». It was almost in seven times higher than in background values. Amount of potassium was at low level in soil of experimental plantation in comparison with background level. Acidity (pH) of soil from tailing dump has a value close to neutral.

In the soil of tailing dump the content of such metals as Ni, Fe and Mn was 1.5–2 times higher compared to background values. Content of Cd was at level of 0.50 mg/kg in the soil which corresponds

Table 1. Characteristics of soil from tailing dump of Joint Stock Co. «Karelsky Okatysh»

Sample plot	pH (water)	C	N	P	K	Na	Ca	Mg
		%			mg/kg			
Tailing dump	7.60 ± 0.08	0.27 ± 0.02	0.01 ± 0.0	0.70 ± 0.04	6429 ± 915	124 ± 21	8576 ± 523	3796 ± 40
Background*	4.90	45.70	2.17	0.10	14191	19231	11460	4938

Sample plot	Ni	Fe	Mn	Cd	Pb	Cu	Co	Cr
	mg/kg							
Tailing dump	41.48 ± ± 1.56	39 505 ± ± 2058	366.54 ± ± 60.32	0.49 ± ± 0.15	4.97 ± ± 0.65	9.10 ± ± 0.65	4.16 ± ± 0.20	25.12 ± ± 1.42
Background*	27.5	17 505	282	0.5	15.5	18.5	11.6	37.2
MPC**	50	38 000	1500	3	32	100	50	100

Note. *Background – concentrations in mineral upper soil layers – average data for Karelia (Markovskaya et al., 2015); **MPC – maximum permissible concentrations (MPC), total content. Heavy metal concentrations were compared with the regional background values for mineral soil horizons and the current MPC for metals in soils (Predel`no Dopustimye Kontsentratsii …, 1994).

to background values. Average content of such metals as Pb, Cu, Co, Cr in the sampled soils of tailing dump was lower than background values.

Wastewater chemical testing. The wastewater from the works holding pond is discharged into the territory of the tailing dump. The characteristics of the elemental composition of this water are presented in Table 2.

Contaminated water contains concentration of ammonium ions that is in 24 times higher than MPC values for water bodies of Russian Federation. The content of potassium in the wastewater exceeded the MPC by 2 times in average. Heavy metals Ni, Cu, Zn as well as Mn in the wastewater exceeded amounts compared to MPC. The concentration of nitrate ions, total phosphorus, chlorine ions and sodium was lower than MPC values. These values do not exceed the maximum permissible concentration. The concentration of other elements in the wastewater was also registered at level which doesn't exceed MPC. The acidity of the wastewater was neutral.

Plant biometric parameters. The development of the root system of plants *Salix schwerinii* of HBP and LBP groups was at same level and the average length of roots was (13.7 ± 1.3) cm (Table 3).

The length of the shoots of willows was higher in HBP group in comparison with LBP plants. The number of leaves per stem of plants from HBP group was significantly higher than these values for plants of LBP group. They had average area of leaves at (300 ± 15) mm^2. Water content was highest in willows of HBP group compared with willows from LBP group.

Photosynthetic pigment content. The content of photosynthetic pigments was low in both groups of willows at (1.62 ± 0.10) mg/g wet weight. The content of chlorophyll *a* was at (0.92 ± 0.17) mg/g wet weight, chlorophyll *b* – at (0.54 ± 0.18) mg/g wet weight and carotenoids – (0.13 ± 0.04) mg/g wet weight. However, values of SLA were higher in HBP plants than in willows from LBP group (Table 4).

Table 2. Concentration of chemical elements (mg/l) and pH of waste water of Joint Stock Co. «Karelsky Okatysh»

Chemical elements	NH_4^+	NO_3^-	P	K	Cl	Ca	Na	pH
Average values (m = 0.01–1.00)	12.17	20.73	0.05	110.00	41.90	292.97	40.20	6.32
MPC**	0.5	40	1.14	50	300	180	120	6.5–8.5

Chemical elements	Cd	Pb	Cu	Co	Ni	Zn	Mn	Fe	Cr
Average values (m = 0.00001–0.01)	0.0001	0.001	0.006	0.001	0.017	0.051	0.432	0.043	0.0004
MPC**	0.0050	0.006	0.001	0.010	0.010	0.001	0.010	0.100	0.02–0.07

Note. MPC** MPC for surface water bodies of the Russian Federation. Order of Rosrybovodstvo (Russian Fish Husbandry) of 18.01.2010 № 20 and Sanitary Regulations and Norms 2.1.5.980.-00 (2010).

Table 3. Biometric parameters of *Salix schwerinii* from tailing dump of Joint Stock Co. «Karelsky Okatysh»

Test scheme	Root length, cm	Shoots length, cm	Number of shoots	Number of leaves per shoot	Leaf area, mm^2	Wet weight of	Dry weight of	Water content in
						10 leaves, g		
HBP	13.5 ± 2.6[a]	11.4 ± 3.5[a]	3.7 ± 0.3[a]	13 ± 5[a]	326.5 ± 27.5[b]	0.63 ± 0.10[b]	0.24 ± 0.10[a]	0.39 ± 0.50[b]
LBP	14.0 ± 1.8[a]	8.9 ± 2.5[b]	5.5 ± 1.3[b]	9 ± 4[b]	273.1 ± 17.4[b]	0.46 ± 0.10[b]	0.19 ± 0.10[b]	0.27 ± 0.48[b]

Note. Values are mean ± S. D. (n = 10). Data presented with different letters in the same column indicate a significant difference at $P \leq 0.05$ from field (HBP, LBP) plants to Fisher's LSD test. HBP – high-productive plants; LBP – low-productive plants.

Table 4. Photosynthetic pigment content and SLA values of *Salix schwerinii* from tailing dump of Joint Stock Co. «Karelsky Okatysh»

Test scheme	SLA, mm^2/mg	Pigment content mg/g of wet weight				
		Total	Chl *a*	Chl *b*	Car	a/b
HBP	1.53 ± 0.07[a]	1.69 ± 0.15[a]	0.96 ± 0.14[a]	0.57 ± 0.19[a]	0.14 ± 0.07[a]	1.8
LBP	1.21 ± 0.05[a]	1.55 ± 0.20[a]	0.88 ± 0.20[a]	0.53 ± 0.21[a]	0.13 ± 0.01[a]	1.8

Note. Values are mean ± S. D. (n = 10). Data presented with different letters in the same column indicate a significant difference at $P \leq 0.05$ from field (HBP, LBP) plants to Fisher's LSD test. HBP – high-productive plants; LBP – low-productive plants.

Heavy metal accumulation and distribution in plant organs. Willow plants, which grew on contaminated territory, accumulated metals significantly in comparison with the initial level of metals in cuttings. Thus, content of metals by whole plant increased in 2.6 times for cadmium, in 6.1 times for lead, in 5.1 times for cobalt, in 6 times for copper, in 2.5 times for chromium, in 9.3 times for manganese. The maximum level of increasing was fixed for main pollutants – iron (in 68 times) and nickel (in 24 times).

Analysis of biological absorption coefficient of metals showed that the highest values observed for Mn and Zn (6–8 in average), Cd (5–7), Cu (2–5). The content of Co, Fe, Ni and Cr were about 1 or lower at (0.7–0.2) (Table 5). The raw of metal accumulation in willows on phytoremediation sample plots was as follows:

$$Zn > Mn > Cd > Cu > Pb > Co > Ni = = Cr > Fe.$$

The total concentration of Ni, Fe, Mn in willows from LBP group was higher than in HBP plants. The total concentration of Cr, Pb, Cu in the willows from HBP and LBP plants had no differences. The total concentration of Cd in the willows, from HBP and LBP was the same at the level of 2.29–2.99 mg/kg.

Ni, Fe, Mn and Co – trace metals that were accumulated mainly (80–90 %) in roots and leaves of willows from phytoremediation plot depending on their vitality. According to results, 77 % of Ni was contained in roots of HBP plants and 18 % – in the leaves; 11 % Ni had LBP plants in roots and 67 % – in the leaves. Accumulation of Fe in HBP willows was at 51 and 36 % in the roots and leaves, correspondingly. In LBP plants 34 % of Fe was registered in the roots and 45 % – in leaves. Concentration of Mn in HBP willow was at 63 % in the roots and 15 % – in leaves. In LBP willows 12 % of Mn was in the roots and 68 % – in leaves. The concentration of Co in HBP willows was 72 % in the roots and 13 % in the leaves, while in the LBP plants it was 16 and 73 %, respectively. The remaining 10–20 % of Ni, Fe, Mn and Co was deposited in bark and wood: in bark it was higher (approximately 15 %) than in the wood (approximately 5 %) (Figure).

Pb was accumulated in the leaves, roots and bark of willows of different groups as follows: in HBP – 22, 35 and 21 % and LBP – 44, 28 and 21 %, respectively.

Total content of Cu and Cr up to 40–70 % in the roots and leaves of willow, depended on their vitality. So, in the HBP willows of 58 % copper and 26 % of chromium were accumulated in the roots while 11 % of copper and 38 % of chromium – in the leaves. In the roots of LBP willows 13 % of Cu and 22 % of Cr were observed while in the leaves concentration of these elements was 57 and 20 %, respectively.

Part of the elements was accumulated in the bark: a high concentration of Cd in the HBP and LBP plant groups (67 and 61 %, respectively); Cr (26 and 45 %, respectively) and Zn (55 and 54 %, respectively) were reported. The others elements also found in the bark ranged from 5 to 20 %. The lowest values of element contents were found in the

Table 5. Concentrations of heavy metals (mg/kg) in tissue and organs and coefficient of biological absorption (CBA) by whole plant of *Salix schwerinii* from tailing dump of Joint Stock Co. «Karelsky Okatysh»

Metal	Plant condition	Roots	Leaves	Bark	Wood	Total concentration	Cuttnigs before plantings	Leaves of willows from contaminated soils	CBA
Cd	HBP	0.50 ± 0.15[a]	0.23 ± 0.07[a]	2.01 ± 0.05	0.25 ± 0.09	2.99 ± 0.01	1.00 ± ± 0.12	12.5*	6.1
	LBP	0.26 ± 0.15[b]	0.44 ± 0.09[b]	1.40 ± 0.13	0.19 ± 0.02	2.29 ± 0.12			4.5
Pb	HBP	4.88 ± 2.01[a]	3.08 ± 1.06[a]	2.86 ± 0.89	3.06 ± 1.28	13.88 ± 2.15	2.45 ± ± 0.10	23–180**	2.8
	LBP	4.17 ± 1.85[a]	6.50 ± 2.56[a]	3.05 ± 0.09	1.08 ± 0.05	14.80 ± 2.85			3.0
Cu	HBP	28.10 ± 5.86[a]	5.60 ± 1.25[a]	8.70 ± 2.54	6.20 ± 2.14	48.60 ± 4.25	7.00 ± ± 1.14	30–50*	5.3
	LBP	5.30 ± 2.13[b]	23.60 ± 4.85[b]	7.80 ± 1.07	4.10 ± 1.06	40.80 ± 3.25			4.5
Fe	HBP	4349 ± 65[a]	3061 ± 25[a]	1004 ± 102	93 ± 19	8507 ± 50[a]	134.0 ± ± 10.14	500****	0.2
	LBP	3342 ± 284[b]	4292 ± 183[b]	1924 ± 58	161 ± 23	9719 ± 20[b]			0.2
Co	HBP	3.26 ± 1.02[a]	0.61 ± 0.09[a]	0.58 ± 0.09	0.06 ± 0.01	4.51 ± 2.25	0.22 ± ± 0.12	–	1.0
	LBP	0.92 ± 0.08[b]	4.23 ± 1.04[b]	0.45 ± 0.10	0.11 ± 0.02	5.80 ± 1.25			1.4
Ni	HBP	17.67 ± 2.28[a]	4.20 ± 2.25[a]	1.04 ± 0.84	0.05 ± 0.01	22.96 ± 2.58[a]	1.00 ± ± 0.08	12***	0.6
	LBP	2.96 ± 0.25[b]	17.98 ± 1.01[b]	5.06 ± 2.13	0.64 ± 0.08	26.64 ± 1.25[b]			0.6
Cr	HBP	4.62 ± 0.23[a]	6.66 ± 2.87[a]	4.53 ± 1.78	1.82 ± 0.47	17.63 ± 1.85	6.00 ± ± 0.02	7.5****	0.7
	LBP	2.96 ± 0.87[b]	2.58 ± 0.54[b]	5.92 ± 0.85	1.66 ± 0.25	13.12 ± 2.59			0.5
Mn	HBP	1418 ± 80[a]	334 ± 54[a]	430 ± 25	45 ± 14	2227 ± 50[a]	277.00 ± ± 7.23	2012****	6.0
	LBP	363 ± 37[b]	2022 ± 57[b]	515 ± 15	55 ± 10	2955 ± 30[b]			8.0
Zn	HBP	46.60 ± 8.23[a]	25.00 ± 5.89[a]	109.7 ± 6.01	20.20 ± 2.45	201.2 ± 15.0	195.00 ± ± 5.25	1126*	8.2
	LBP	24.10 ± 2.18[b]	49.00 ± 3.25[b]	108.2 ± 5.87	18.90 ± 3.92	200.1 ± 12.6			8.1

Note. Values are mean ± S. D. ($n = 10$). The data on HBP plants presented with letters for all metals except Pb indicate a significant difference at $P \leq 0.05$ according to Fisher's LSD from the data for LBP plants, both in the roots and in the leaves. *Stoltz, Greger, 2002; **Zhivotovsky et al., 2011; ***Meers et al., 2007; ****Pulford et al., 2002.

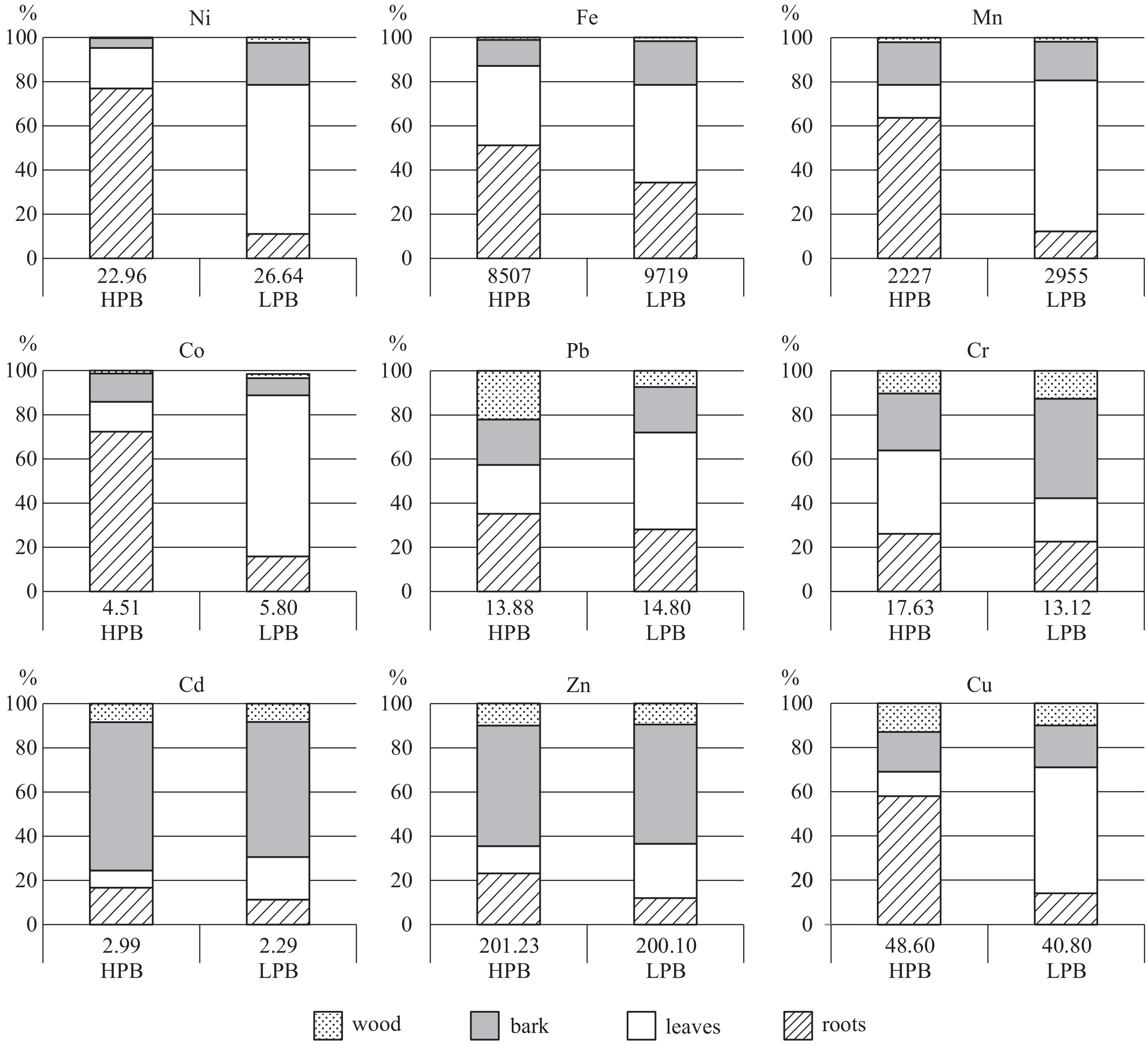
woody tissue: Pb < 22 %, Cd under 8 %, Cu, Cr, Zn under 13 %, and Fe, Co, Ni, Mn are present in minor, trace amounts.

The data analysis concerning the distribution of mineral elements showed that the main deposition organs for Ni, Fe, Mn, Co, Cu, Cr are roots and leaves; for Pb – roots, leaves and bark and for Cd and Zn – bark.

The chemical composition of the soil. The analysis of substrates (soil) of tailing dump revealed very low content of carbon, nitrogen, potassium and a high content of phosphorus. The acidity (pH) of the pulp has a near-neutral value, optimizing the plant mineral nutrition. The analysis of heavy metals showed that in soil of tailing dump of Joint Stock Co. «Karelsky Okatysh» Mn concentration is the same as natural content. Fe and Ni concentrations are higher than the values of their natural level and are nearing the MPC, as for the rest of the studied elements their concentrations are lower than their natural concentration level and the MPC. It can be concluded that the soil of tailing dump contaminated with nickel and iron, has low content of biophilic macronutrients and quantitative indicators remaining microelements corresponds to the average value of their content in Karelia.

The use of wastewater from Joint Stock Co. «Karelsky Okatysh» which is rich in nitrogen, potassium and other macro- and micronutrients, for watering, leads to enrichment of substrates of technogenic territory and improving mineral nutrition of plants.

Plant biometric parameters. An analysis of willow plantation near tailing dump of Joint Stock Co. «Karelsky Okatysh» showed that there was a differentiation of plant *Salix schwerinii* on healthy with HBP (high biological productivity) and weakened plants with LBP (low biological productivity). This might be connect with the variability of the habitat conditions near the tailing dump as well as with realization of these conditions at high level of genotypic polymorphism and plasticity of the genus *Salix* (Newsholme, 1992; Skvortsov, 1999; Valyagina-Malyutina, 2004; Efimova, 2012; Nesedko, 2012). This genotypic polymorphism is base for hypothesis of different ways to adaptation of willows at level of physiological processes in plants under stress conditions. According to the



Total concentration (mg/kg) and metal content (%) in different parts of *Salix schwerinii* from tailing dump of Joint Stock Co. «Karelsky Okatysh».

some studies (Evlard et al., 2014) it was shown that different clones of *Salix fragilis* x *alba* growing in conditions of heavy metals contamination were also divided into HBP and LBP groups with physiological differences. Willow clones of HBP group had a high level of antioxidant protection, the optimal parameters of activity of the photosynthetic apparatus, the high activity of enzymes of protein metabolism. As the results of this experiment, it was found that high productive clones of *Salix fragilis* x *alba* accumulate more of trace metals than low productive plants.

According to obtained results, the plants of the HBP and LBP groups from the territory of tailing dump also showed significant differences in biological productivity. No differences in the length of their root systems were reported.

From the literature data it is known that willows are able to develop a strong root system, up to 115 cm, from which 90 cm goes deep into the soil (Rytter R., Rytter L., 1998). This root system is resistant to hypoxia and contaminated soils (Kuzovkina, Volk, 2009). In fact, it is active also physiologically. For example, in the rhizosphere of the roots of willows there is a high content of organic acids (Gasecka et al., 2012), which can optimize the mineral nutrition of willow plants.

The revealed differences in the biometrical parameters of the above-ground organs indicate the inhibition of plant growth on the anthropogenically transformed territory. This mostly related to the assimilating surface area and the leaf water content level. The latter is of great importance for the willow as a hydrophilous plant.

The differences in the photosynthetic pigment content between the two groups of the studied plants were not significant. Comparison of the tested plants revealed that the SLA values were significantly higher in HBP group than in LBP plants. This fact also indicates higher photosynthetic activity of HBP plants.

In general, the pigment content of these willows can vary in wide range, depending on the species and growing conditions (floodplain forest communities, city): from (2.69 ± 0.02) to (8.31 ± ± 0.05) mg/g wet weight (Ivanova, Kostyuchenko, 2011). The tested plants of *Salix schwerinii* had minimal pigment content that it might be due to their low age.

Accumulation of chemical elements. The comparison of the two groups of the experimental plants growing in the tailing dump showed that there were no significant differences in the general accumulation of particular elements by plants of different physiological state. Only the following relatively small differences can be noted: the HBP plant group accumulated more Cd, Cu and Cr, whereas in the LBP group it was Fe, Co, Ni and Mn. However, these differences did not exceed 15–20 %. This means that in both groups of the experimental scheme, where the plants were growing in similar conditions concerning their chemical characteristics, the elements were coming into the plant root systems using the same mechanisms.

According to the literature data it is known that the mechanism of elements absorption by the roots includes passive and active ways. The ratio of the ways depends primarily on their concentration in the substrate: at low – active path are predominate (Cataldo et al., 1983), and at high – passive (Culter, Rains, 1974). The presence of two plant groups with different productivity but with similar values of the metal content provides grounds for assuming an active metabolic uptake in both groups of plants on industrial area.

The distribution of elements in the organs. The significant differences were revealed concerning the migration and distribution of these elements in the plant and their deposition in different organs. The comparison of the two plant groups growing in the territory of the pulp storage showed that the accumulation of such elements as Ni, Fe, Mn, Pb, Cu, Co depends on the plant functional status, its viability. All these elements in the HBP plant group accumulated in maximum concentrations in the roots (at 70 %), whereas in the LBP group their maximum concentrations were found in the leaves (at 70 %).

The elements absorbed by the active way can be transported through the apoplast and symplast to the endoderm and the basialis parts of the root (Wierzbicka, 1987; Nesterova, 1989; Seregin, 2001), where they can be either deposited or transported into the above organs via the xylem vessels with the transpiration stream (Salt, Rauser, 1995; Hart et al., 1998). It should be noted that Pb, Cu, Fe, Co are classified as metals with a high concentration in the roots, Ni, Mn – with a medium level of concentration in the roots (Stoltz, Greger, 2002; Vervaekea et al., 2003; Vandecasteele et al., 2005; Zhivotovsky et al., 2011).

In fact, in different species of willows, lead accumulates mainly in the roots (5–15 g/kg), than in xylem stems (70–40 mg/kg) and leaves (23–180 mg/kg) (Zhivotovsky et al., 2011). The average optimal level of accumulation of copper for willows is 20–30 mg/kg (Vervaekea et al., 2003). High level of copper amount has been found in the roots and shoots and low in the leaves (Stoltz, Greger, 2002). It is known that iron ions are accumulated mainly in the roots of willow (Vandecasteele et al., 2005). Accumulation of cobalt has been investigated willow poorly. In the works of Kulagin (1998) it was shown that cobalt (up to 3 mg/kg) is accumulated in the leaves of species *S. viminalis*, *S. alba*, *S. dasyclados*, growing in the contaminated area. According to recent studies nickel is deposited mainly in roots of willows at level of whole plant (Vandecasteele et al., 2005). In above-ground organs nickel is accumulated mainly in the leaves (Pulford, Watson, 2003). In condition of the contaminated soils only 1 % of nickel contained in a 10 cm layer of soil Scotland was transported into tissues and organs of willows (Salt et al., 1995). In literature there is lack of data about distribution of manganese in willows. It is known that in other plant species it is accumulated in leaves (Titov et al., 2011).

Cadmium is metal that was studied very actively in willows in connection with the phytoremediation. It is known that willows accumulate of cadmium both in the roots and the leaves (Dickinson, Pulford, 2005). In hydroponic growing willows cadmium accumulates mainly in the roots (Cosio et al., 2006). In above ground organs of willows cadmium accumulates in the leaves at level 20–40 % (Pulford, Watson, 2003). In the several studies (Stoltz, Greger, 2002) it was shown that in willows zinc is accumulated in roots, stems and leaves at the same level. According to Pulford, Watson (2003) in the above-ground organs of willow zinc is accumulated mainly in the leaves and bark. According to our data the Cd and Zn were accumulated mainly in the bark of stem of willows.

The obtained result concerning the active transport of Pb, Cu, Fe, Co into the leaves in willows with suppressed functional activity can be associated with the insufficient synthesis of proteins (little energy for their synthesis), accumulation of these elements in the roots and/or higher apoplast activity of this plant group. Willows can be classified among species with enhanced water exchange and high transpiration intensity, and notably, with apoplast involvement (Ivanova, 2003; Gamaley, 2004; Wikberg, 2006; Wikberg, Ören, 2007; Terebova et al., 2013). For this reason, the transportation of metals to the leaves, especially in the context of sufficient moistening and with the involvement of apoplast system is advantageous from the point of view of energy and serves as fast transport of elements, involving Ni, Fe, Mn, Pb, Cu, Co, which is especially relevant and is found in plants with suppressed functional activity.

Compared with the data from other sources, the willow leaves in the pulp storage accumulate Ni, Fe and Mn in high concentrations, close to the accumulation level of these elements by the leaves in other conditions of contaminated soils (Pulford et al., 2002; Meers et al., 2007). Concentration data of other metals in the leaves is usually lower than in tested plants cultivated on contaminated soils, that can be connected with a shorter growing period (one year) or a low contamination level of the pulp storage on the study area.

After a year of growing plants on the contaminated area, accumulation of metals in significant quantities was found in comparison with the initial cuttings. The maximum level of increasing was fixed for main pollutants – iron (in 68 times) and nickel (in 24 times).

Besides, it should be noted, that willows absorb most actively such elements as Mn, Zn, Cd, Cu, Pb and Co; less actively – Fe, Ni and Cr. More active uptake of metals from the substrate of tailing dump leads to becoming of concentration of Cr, Pb, Cu and Cd close to background values in uncontaminated substrate.

CONCLUSION

Species *Salix schwerinii* can be used as a phytoremediant on the contaminated territories of the mining enterprise. Heavy metals are accumulated from contaminated soil of Joint Stock Co. «Karelsky Okatysh» by the roots in tested plants of HBP group, while plants of the LBP group accumulate them mostly in leaves. The higher functional activity of the healthy plants (HBP) is able to ensure the utilization of heavy metals in the root system, preventing them from transportation to the aboveground organs. In fact, for phytoremediation of contaminated areas, using the plants which accumulate metals in roots is preferred. Thus, it is important to select high-quality plant material, which has high functional activity in the conditions of water-and-soil contamination, and to create optimal conditions for the mineral nutrition of phytoremediant plants.

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ПОГЛОЩЕНИЕ ТЯЖЕЛЫХ МЕТАЛЛОВ ДЕРЕВЬЯМИ ИВЫ *Salix schwerinii* E. Wolf НА ЗАГРЯЗНЕННЫХ ТЕРРИТОРИЯХ ГОРНОРУДНОЙ ПРОМЫШЛЕННОСТИ НА СЕВЕРО-ЗАПАДЕ РОССИИ

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Работа выполнена на техногенных территориях ОАО «Карельский окатыш» (Россия, Республика Карелия, г. Костомукша). В качестве фиторемедианта использованы растения вида *Salix schwerinii* E. Wolf (Финляндия), которые выращивали на наиболее загрязненной территории комбината – пульпохранилище (основное загрязнение по никелю, железу). Растения ивы после года выращивания разделили на две группы: здоровые с высокой продукцией биомассы (HBP) и ослабленные с низкой продукцией биомассы (LBP). Корневая система HBP и LBP не имела различий, но надземная масса была более развита у HBP растений ив. Содержание фотосинтетических пигментов у обеих групп ив было невысоким – на уровне (1.62 ± 0.10) мг/г сырой массы. Показатель SLA (specific leaf area) был равен 1.53 мм^2/мг у HBP и 1.21 мм^2/мг – у LBP групп. Около 50–90 % Ni, Fe, Mn, Co, Cu, Cr поглощаются корнями и листьями ив. Все эти элементы у растений группы HBP накапливались в максимальных количествах в корнях (до 70 %), а в группе LBP – в листьях (до 70 %). Pb распределился по 20–30 % в корнях, листьях и коре, Cd, Zn – на 50–60 % в коре. Коэффициенты биологического поглощения тяжелых металлов растением ивы составили следующий ряд: Zn (8) > Mn (6–8) > Cd (4–6) > Cu (4–5) > Pb (3) > Co (1) > Ni (0.6) = Cr (0.5–0.7) > Fe (0.2).

Ключевые слова: *ива Salix schwerinii, фиторемедиация, тяжелые металлы, фотосинтетические пигменты, биометрические параметры, удельная площадь листа, Республика Карелия*.