

# State of Mineral Nutrition and Heavy Metals Distribution in Aboveground Biomass of Poplar Clones

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## Abstract

The state of mineral nutrition as well as accumulation and translocation of selected heavy metals As, Cr, Hg, and Pb were evaluated in poplar clones Robusta (*Populus x euramericana*) and I-214 (*Populus x euramericana*). These clones are characterized by fast growth and biomass production of relatively high quality. Soil water quantity and its dynamics is one of the most important factors for growth of poplar clones, in relation to their high transpiration and nutrient transport. The research was conducted in two locations in eastern and three in western Slovakia. The poplars were grown on alluvial plains continuously influenced by ground waters from surrounding rivers. Differences in As, Cr, and Pb concentrations were recorded among the locations only, whereas between these single-clones no differences were confirmed. Different accumulation patterns were found for some elements (Ca, K, Si) in poplars compared to those of softwood (spruce) and hardwood (beech) species. The mineral elements (Ca, K, Mg, Na, Si) were accumulated in above-ground parts as follows: stem > “small-wood”/bark > wood. Cr was the most represented risk element in biomass, while Hg reached the highest concentrations and in contrast to essential elements was accumulated predominantly in wood. A potential source of Hg was soil/water environment.

**Keywords:** biomass, heavy metals, mineral nutrition, poplar clones

## Introduction

The great advantage of poplar clones is mainly fast production of high-quality biomass. Trees grown in favorable stand conditions of temperate climatic zone reach up considerable dimensions already at the age of 10-15 years. Petráš and Mecko [1] state for this age category (e.g. clone I-214) in the highest site classes the average stand diameter ranged 40-50 cm and the mean annual increment in the tree aboveground biomass ranged 55-65 m<sup>3</sup>·ha<sup>-1</sup>. In comparison

with the other commercial woody plant species, the rate of growth and production is 5-10 times higher. Thanks to the very fast growth of poplar clones, their aboveground biomass can be utilized as an effective energy source. In this context, it is necessary to know their combustion heat values as well as chemical elements cyclically taken away from the growth environment of these clones and returning back in the form of wood ash.

Major factors influencing poplar growth are depth to water table and moisture-holding capacity of the soil. Poplar communities occur mainly in lowlands, in the area of alluvial deposits of water courses. These localities are permanently supplied with nutrients from frequently occur-

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ring floods and groundwater. Water consumption for transpiration to produce 1 kg of dry matter for poplars is as high as 500 L, 350 L for beech, and 170 L for pines [2].

The macro-elements (Ca, K, Mg, Na) and additional microelements (Si), are essential for plant nutrition, as parts of many enzymes, or they influence and regulate important biochemical processes. On the other hand, trace elements such as As, Cr, Hg, and Pb occur in plant organisms, although they do not have any physiological function and they are usually toxic. The toxicity of heavy metals consists mainly in generation complexes with organic compounds that can modify biological molecules leading to their correct function, and even cell extinction. In terms of uptake and accumulation of risk elements by poplars (including heavy metals) can maybe speculate in two ways:

- i) fast-growing tree species are able to accumulate lower amounts of heavy metals in their stems compared to slowly growing tree species, where time viewpoint may come into consideration.
- ii) fast-growing clones have a tendency to accumulate in stems of great quantities of heavy metals in connection with higher intensity of nutrient transport and high production in a relatively short time.

According to Laureysens et al. [3], who searched clonal variation in heavy metal accumulation and biomass production in a poplar coppice culture, it is a disadvantage that short-rotation clones have a tendency to receive relatively small amounts of heavy metals in comparison to hyper-accumulators. However, due to their total amount of produced biomass during a relatively short time, poplars and willows are integrated among potential wood species for phyto-remediation [4-7]. Mertens et al. [8] investigated the fact that the youngest and the most rapidly growing willows pre-accumulate the most cadmium. The fast growth of young poplars and willows can lead to an increase of heavy metal uptake while plants are young and to a decrease as the plants age. Wood species from the Salicaceae family are also known to adapt to grow in polluted areas [9].

However, not all tree species from this family are suitable for phyto-remediation strategies. Among poplar clones, we detected a high variability in accumulation of heavy metals and their distribution among individual parts and tree species organs [3, 10]. Roots and belowground parts of trees are often suitable indicators of heavy metals, which come from soil environment. Fast-growing tree species have been shown to be suitable candidates to uptake and store risk elements from soil environment in surrounding water-courses and lakes, in connection with their spreading and deeper root systems [11]. The transport of heavy metals in trees consists of absorption from soil solution by the plant's root surface and transport inside the roots and translocation to the shoots [12]. Transpiration is an important "moving power" of heavy metal transport from roots to leaves.

Risk elements do not enter into the plant only through the root system, but also from atmospheric deposition through leaf surfaces. Some studies have shown that assimilatory organs of plants grown in polluted areas can be referred to as cumulative bio-monitors of many metallic

elements. A high percentage of deposited elements is not imported into leaf tissues, but is caught on the surface or in wax cuticula [13].

Results with high scientific value were obtained by analyzing bark samples of species like elm, willow, poplar, pine, and olive, etc. However, published scientific results vary regarding accumulation bark potential compared to other plant organs [14]. Elements like Hg and Cd mainly originate from atmospheric deposition or, in the case of poplars, from flooding water: in large measure they are absorbed in tree bark in relation to its structure and porosity. Siwik et al. [15] found much higher mercury concentrations in bark of lakeshore species in comparison to "inland" woody species such as maple and oak. On the contrary, mercury concentrations in assimilatory organs of maples were much higher compared to poplars. Differences among species in bark and wood morphology, physiology, and chemical composition may markedly influence heavy metal uptake and their distribution. A key role in this case may also be played by higher incidences of bark lenticels in wood species growing in periodically inundated areas [16].

In terms of possible applications of these wood species for phyto-extraction techniques (as well as for bio-fuels), additional research in terms of elements uptake, translocation, and tolerance to heavy metals among poplar clones are needed. The aim of this paper is:

- a) to quantify content of basic chemical elements and selected heavy metals As, Cr, Hg, and Pb in above-ground biomass of *Populus x euramericana* clone Robusta and *Populus x euramericana* clone I-214
- b) to determine the elements partitioning among wood, "small-wood," and bark
- c) to analyze the variability of these elements with the most important factors that influence them
- d) to examine the utility of wood and bark as a means of monitoring heavy metals in the environment.

## Experimental Procedures

The experimental material was obtained from trees cut within the research on density of the basic biomass components in the poplar clones [17]. Three individuals were of Robusta clone and a further two of I-214 clone. All the trees grew in forest stands of the Danube Lowland and the East-Slovak Lowland at an altitude of about 100 m a.s.l. Soil substratum was formed by alluvial deposits of different mineral composition and granularity originating from nearby rivers: the Danube, the Váh, and the Latorica rivers for Robusta clone, and the Danube and the Ondava rivers for I-214 clone (Fig. 1).

All the poplar stands were under impression of ground water from surrounding rivers. The age of cutting trees was in the range of 20-40 years, diameter at breast height (DBH) of 25-60 cm and height of 25-40 m.

Four samples of wood with bark were cut off from each tree. The first sample was from trunk base, the second from the middle part of the trunk (for about under crown), and the third one from the crown. All three samples were divid-

ed into wood and bark and were mixed into the individual sample for wood and the individual one for bark. From “small-wood” was taken only one sample, where wood and bark were analyzed together. “Small-wood” refers to branches of tree crowns with diameter < 7 cm. The total number of samples was 15; five of them for each biomass component, i.e. wood, bark and “small-wood.” The absolute weight of each sample was in the range 200-500 g. All samples were dried at 105°C and milled.

Each sample of 5 g was ashed in electric muffle furnace at the temperature of 550°C, according to the standard procedures Tappi T 211. The analysis of ash composition was done according to Tappi T 266. Poplar ash was dissolved in HCl (6 mol·L<sup>-1</sup>). The resulting solution was filtered and the contents of Ca, K, Mg, and Na were determined by atomic absorption spectrometry (F-AAS) with a SOLAAR M6 device. The Si content was determined as SiO<sub>2</sub> by gravimetric method.

The concentrations of As, Cr, and Pb were determined by atomic absorption spectrometry using a graphite furnace (GF-AAS) with a SOLAAR M6 device. Before self determination of heavy metals, the samples were decomposed in 20 ml of highly pure HNO<sub>3</sub> using microwave digestion. The solutions were diluted with ultra-pure water to a 50 ml volume. The content of Hg was determined in solid samples (of 0.1 g each) by AAS with a TMA 254 device.

Data were subjected to the generalized analysis of variance (ANOVA) for a statistical examination of the effects produced by clone, biomass fraction, and location:

- Clone, 2 levels: Robusta and I-214
- Biomass fraction, 3 levels: wood, bark, “small-wood”
- Location, 4 levels: the Danube River, the Váh River, the Latorica, and the Ondava rivers.

Statistical analyses were performed using “QC.Expert” statistical software [18]. The result of analysis of variance is a comprehensive analysis assessing the complex influence of all factors together, as well as contribution of the individual factors affecting the amounts of the studied elements. The differences between the average values at the particulate levels were tested using Student’s t-test. The level of significance was set to P=0.05 for all analyses.

## Results and Discussion

The overall influence of all three factors was statistically significant for all elements except potassium content (Table 1). The factor biomass fraction (wood, bark and “small-wood”) was found statistically significant in the case of ash amount and in the case of Ca, Mg, Na, Si, and Hg contents. The growing location had significant influence (95% probability) only in Pb, Cr, and As. The results showed that the poplar clone had no effect on examined element contents in aboveground biomass.

The average contents of the examined elements and their standard deviations were calculated for single levels of both factors (Fig. 2). The highest ash content, approximately 5.21±0.79%, was found in bark. Wood has only 1.23±0.45% of ash content. Its proportion is statistically significantly lower compared to bark and “small-wood.” The proportions of macro-elements Ca, K, and Mg are markedly lower (Fig. 2a) and the minimum proportions, approximately only a few centesimal or millesimal %, were found in the case of Na and Si (Fig. 2b). T-tests revealed significant differences (95% probability) in proportions of ash, Ca, Mg, and Na between the trunk wood (lower values) and bark and also “small-wood” (higher values). Their proportions in bark and “small-wood” do not differ. The differences in order of nutrients accumulation between bark and wood were detected for elements Ca and K. Calcium was accumulated mainly in bark, and potassium did in wood.

The mineral nutrient supply in wood, bark and “small-wood”, except Ca, was markedly lower in comparison to assimilatory organs of I-214 poplar clone in Italy [19]: 1.3 times for Ca; 3 times for K; 4.9 times for Mg and as much as 29 times for Na. Compared to concentration levels of these elements in poplar stems in Italy, the differences were already lower: twice for K and Mg, 4 times for Na, no differences for Ca. Regarding the previous findings, accumulation and translocation patterns of essential elements for given poplar clones can be established in the following decreasing order: leaves > stem > “small-wood” / bark > wood.

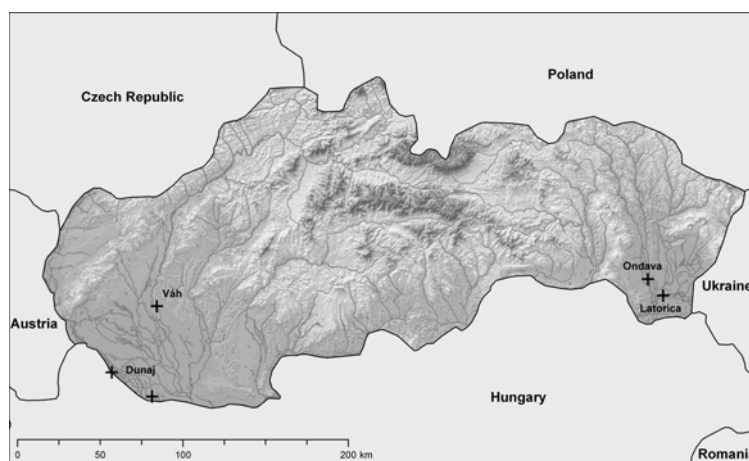


Fig.1. Studied locations situated in forest ecosystems along the rivers Dunaj and Váh (Western Slovakia), Ondava and Latorica (Eastern Slovakia).

Table 1. Generalized analysis of variance for the analyzed elements.

The overall ANOVA						ANOVA for individual factors			
Source of the Variability	Degrees of Freedom	Sum of Squares	Mean Square	F-statistic	p-value	Factors	Sum of Squares	F-statistic	p-value
Ash %									
Total	14	48.9836	3.4988			Clone	1.335	0.36	0.556
Explained	0	46.1594		17.34	0.000	Locality	3.032	0.24	0.866
Residual	14	2.8241	0.2017			Biomass	43.101	43.96	0.000
Ca %									
Total	14	6.3888	0.4563			Clone	0.213	0.45	0.514
Explained	0	5.9852		15.83	0.000	Locality	0.341	0.21	0.890
Residual	14	0.4036	0.0288			Biomass	5.634	44.76	0.000
K %									
Total	14	0.2165	0.0155			Clone	0.001	0.05	0.828
Explained	0	0.0759		1.54	0.233	Locality	0.042	0.87	0.479
Residual	14	0.1406	0.0100			Biomass	0.033	1.08	0.367
Mg %									
Total	14	0.0290	0.0021			Clone	0.000	0.01	0.932
Explained	0	0.0227		4.63	0.004	Locality	0.002	0.28	0.838
Residual	14	0.0063	0.0004			Biomass	0.021	14.45	0.000
Na %									
Total	14	0.0004	0.0000			Clone	0.000	0.34	0.567
Explained	0	0.0003		4.54	0.004	Locality	0.000	0.92	0.458
Residual	14	0.0001	0.0000			Biomass	0.000	8.25	0.004
Si %									
Total	14	0.0024	0.0002			Clone	0.000	0.55	0.469
Explained	0	0.0017		3.30	0.018	Locality	0.001	0.98	0.431
Residual	14	0.0007	0.0001			Biomass	0.001	4.97	0.023
Pb mg/kg									
Total	14	0.1108	0.0079			Clone	0.001	0.17	0.690
Explained	0	0.0794		3.53	0.013	Locality	0.067	5.64	0.010
Residual	14	0.0314	0.0022			Biomass	0.012	0.74	0.493
Cr mg/kg									
Total	14	0.2482	0.0177			Clone	0.001	0.03	0.857
Explained	0	0.2018		5.35	0.002	Locality	0.183	10.36	0.001
Residual	14	0.0464	0.0033			Biomass	0.018	0.48	0.627
As mg/kg									
Total	14	0.0023	0.0002			Clone	0.000	1.07	0.319
Explained	0	0.0017		4.36	0.005	Locality	0.001	7.14	0.004
Residual	14	0.0005	0.0000			Biomass	0.000	0.69	0.520
Hg mg/kg									
Total	14	0.0049	0.0003			Clone	0.000	0.27	0.613
Explained	0	0.0031		2.72	0.039	Locality	0.001	0.47	0.710
Residual	14	0.0018	0.0001			Biomass	0.002	5.14	0.021

Bublinec [20] dealt with the issue of mineral nutrition in beech and spruce ecosystems in Slovakia. Compared to our results, Ca concentrations in poplar bark were similar to those in spruce, and lower as in beech. On the contrary, Ca concentrations in poplar wood were 2 to 2.5 times higher, as for spruce and beech. The concentrations of K were much higher in both bark and wood of poplars. On the other hand, Si occurred in wood, bark and "small-wood" of poplars in markedly lower concentrations. The contents of Mg and Na were about equal for all three wood species, except for Mg contents in spruce wood, which was 3 times lower.

To assess the heavy metal partitioning, the statistical differences were recorded only in the case of mercury (Fig. 3). The wood contains significantly higher mercury amounts than bark or "small-wood." The literature records on Hg concentrations in wood are rare and the published values are found to be at lower concentrations compared to bark or leaves [3, 15, 21, 22]. On the other hand, Pulford et al. [23] recorded, analogous to our case, higher concentrations of some heavy metals in willow wood. Uptake of mercury from soil environment by vascular plants is rather limited, with mercury concentrations in biomass being significantly lower than in soils, where roots represent important adsorption sites and barriers for Hg input toward above-ground biomass [24, 25]. In the case of mercury originated in atmosphere, the input into plant organism via assimilatory organs and bark is dominant. Grigal et al. [24] and Zhang et al. [22] suppose

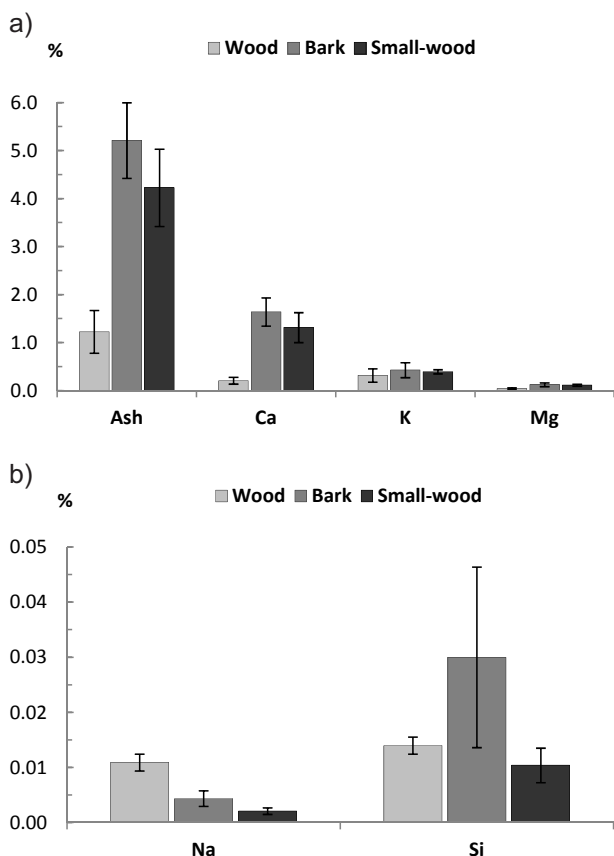


Fig. 2. Mean values and standard deviation of contents of essential elements in woody biomass of poplar clones: a) ash, Ca, K, and Mg contents, b) Na and Si contents.

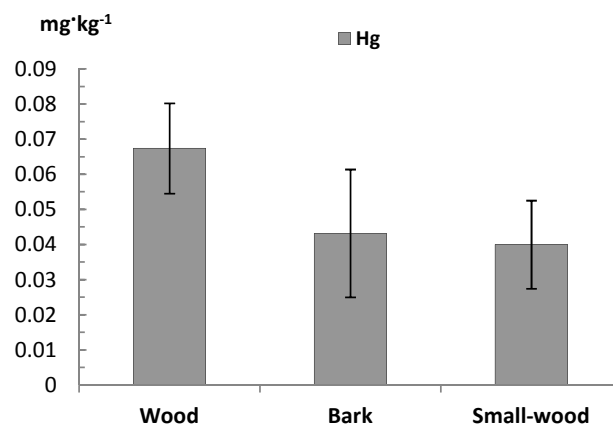


Fig. 3. Mercury contents in different parts of poplar woody biomass.

that mercury accumulated in wood originates mainly from the atmosphere. They further suggest that Hg enters the wood of trees either by lateral movement from bark or from Hg transported down the phloem from the leaves. On the contrary, according to Lindquist et al. [26], mercury pre-accumulated in the leaves is translocated into other plant parts only to a very limited extent.

Mercury is also known for its affinity to humus particles and water sediments, where is deposited in increased measure and is transformed in organic form as methylmercury (MeHg). MeHg, in contrast to Hg-II, easily and rapidly exchanges one thiol group for another, which explains why methylmercury spreads more easily through internal plant tissues than inorganic Hg-II, which has a greater tendency to be retained at the point of entry [27, 28].

In regard to previous reflections, it may be supposed that mercury accumulated in wood of Robusta and I-214 poplar clones growing on alluvial deposits originates from soil-water environment and not of atmospheric deposition. We further assume that Hg is entering the trunks of trees by the roots, or by the lateral movement of Hg deposited on the outside of the tree bark, especially in the lower part influenced by floodwater. The determination of heavy metal concentrations in poplar leaves and soil environment, as impulse of future research, would contribute to reliable source estimation of this element and entry points into plant system.

The mercury concentration levels for given poplar clones (wood: 0.069 mg/kg, bark: 0.043 mg/kg, "small-wood": 0.041 mg/kg) were found to be higher in comparison to concentrations (<0.004-0.019 mg/kg) determined by Siwik et al. [15] for poplars, willows, oaks, and maples growing in contaminated and also relatively unpolluted areas surrounding Kingston, Ontario. However, these data were at the lower end of literature values for tree Hg concentrations. Zhang et al. [22] found spruce wood Hg concentrations between 0.013 and 0.037 mg/kg in Quebec, Canada, and Nóvoa-Muñoz et al. [29] obtained a range of 0.008-0.016 mg/kg for wood of oak, birch, and pine near a coal-fired power plant in Spain. Maňková [30] published average Hg values between 0.07-0.08 mg/kg in *Picea abies* needles and *Fagus sylvatica* leaves within the frame of Slovakia.

The concentration levels of As, Cr, and Pb in above-ground woody biomass of studied tree species do not run over the limits and they correspond with default values that are registered for broadleaves (oak, beech, birch, poplar) in Europe (in mg/kg): 0.02 for As, 0.6 for Cr, 0.1-10 for Pb [31].

Ultra-low lead concentrations were recorded, on average 0.05-0.2 mg/kg. It is possible to conclude that poplar wood is not the most suitable indicator of environmental pollution by this element. Lead belongs to the least mobile elements in soil, where it is strongly fixed to clay fraction and organic matter, and Fe and Mn oxides, with a minimal uptake. However, if lead enters the plant through the soil environment, it is typically accumulated in the roots and its transport to above-ground plant parts is reduced. Jamnická et al. [32] showed transfer coefficients (TC) of Pb between soil and plants of beech ecosystems only on the level of 0.06-0.062. Kuklová et al. [33] found relatively low TC for lead between plants and soil in spruce ecosystems. The wood Pb concentrations below detection limits (0.1 µg/l) were observed in beech trees in Slovakia in the surroundings of Bratislava city [20], as well as for poplar clones in a Belgium region moderately contaminated by heavy metals [3]. On the contrary, much higher amounts of Pb were found in bark and assimilatory organs, which have a greater potential to be suitable indicators of Pb originating from atmospheric deposition. Bark Hg of poplar clones may be assumed to be of water sediment origin, in connection with frequent inundation of researched stands.

Growth location was a significant factor only for As, Pb, and Cr (Fig. 4). The highest amounts of these elements were recorded in biomass of poplar trees growing on the East-Slovak Lowland. They are statistically significantly higher compared to poplars growing in western Slovakia in the surroundings of the Danube and Váh rivers. The three types of regions with different environmental quality are indicated on the territory of Slovakia. The region with considerably disturbed environment is in eastern Slovakia, in the surroundings of the Latorica and the Ondava rivers [34].

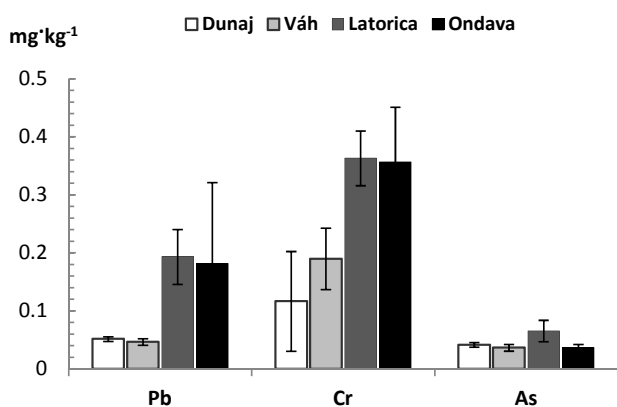


Fig. 4. Mean values and standard deviation of heavy metal concentrations in woody biomass of poplar clones grown at different locations.

## Conclusions

We did not confirm differences in mineral nutrient reserves and heavy metal contents between the I-214 and Robusta poplar clones. As for the individual element contents in individual aboveground biomass fractions, significantly higher values were found in the bark. The nutrient reserves followed the sequence Ca>K>Mg>Si>Na in bark, K>Ca>Mg>Si>Na in wood. Significant differences in Mg and Si contents were detected between poplars – species growing close to watercourses and beech and spruce – species growing in mountain forests.

The accumulation of heavy metals in woody biomass of poplar clones followed the sequence: Cr>Pb>As>Hg in bark and in “small-wood”; Cr>Pb>Hg>As in wood. The concentrations of heavy metals As, Cr, and Pb reached the background values, with higher amounts recorded in eastern Slovakia. It was revealed that poplar wood is not a suitable indicator for environmental damage by lead in natural conditions. On the other hand, relatively high concentrations were recorded for mercury (especially in wood), so we can conclude that these clones, creating their biomass quickly and in high amounts, may be promising for phytoremediation. It is probable that the mercury found in the wood had been accumulated from the soil-water environment on alluvial plains when the poplar trees were growing. The accumulated mercury presents a certain risk of release after aboveground biomass combustion. This fact is necessary to take into account when considering the use of poplar clones as a bio-energy source.

It is key to tackle the issue more thoroughly – to decide whether the given wood is suitable for use in phyto-remediation strategies or as a biofuel – under condition that the over-accumulation of risk elements is avoided by growing the poplar clones in pollutant-free soils. The next research concerning this problem should be focused on the soil environment and the root system.

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