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APPLICATION OF X-RAY FLUORESCENCE TECHNIQUE FOR DETERMINATION OF HEAVY METALS UPTAKE BY DIFFERENT SPECIES OF POPLAR

X-ray fluorescence (XRF) analysis of six metals: chromium, manganese, iron, nickel, copper and zinc (possible inhibitors of enzymatic hydrolysis of wood) in samples of two poplar species, Populus trichocarpa and Populus maximowiczii, was performed in order to check which of them collect more of the metallic inhibitors during tree growth and steam explosion pretreatment. The XRF point scan (for solid and ashed wood) and mapping (for stem cross-sections) options were used. Samples of the different parts – stem, branches, leaves and bark were studied. Steam explosion at 130°C, 160°C and 190°C was performed on both species and the influence of steam on the chosen metals content was analysed. On the basis of the results, P. trichocarpa is the species which accumulates a higher amount of the metals during tree growth and P. maximowiczii – during steam explosion.

Keywords: XRF, poplar, enzymatic hydrolysis, inhibitors, hot water pretreatment

Introduction

Poplar is a common fast growing tree which can be used for bio-fuel production. It may be useful as solid fuel [Barontini et al. 2014] as well as the raw material further processed to biogas [Galvagno et al. 2009] or liquid bio-fuels [Antczak et al. 2014]. This last application is probably the most complicated as processing contains many stages: preparation of raw material, hydrolysis, fermentation, and distillation are the most important of them. The hydrolysis stage may be

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improved on, as shown in the results of the studies presented in this paper. Nowadays biomass hydrolysis is preferably performed via the enzymatic process, although acidic hydrolysis is better known. Acidic hydrolysis causes a higher negative impact on the natural environment and that is why the application using enzymes has been focused on. Enzymatic hydrolysis is basically a well known process and has been used many times by different authors [e.g., Studer et al. 2011], but the high cost of the enzymes to be used makes the process unprofitable. That is why the studies on new, more efficient and less expensive enzymes are continually performed. On the other hand, there are also some parameters of the ligno-cellulosic material that can be improved to decrease the quantity of enzyme needed. These are, among others, the material disintegration degree, its porous structure or the initial chemical composition. The content of possible enzymatic hydrolysis inhibitors in raw material is also a very important factor which should be taken into account.

Poplar, like all other trees, contain many different low-molecular mass non-structural components. These are compounds such as fatty acids, terpenes, resins and others. Many of them are believed to be the inhibitors of the enzymatic hydrolysis [Chandel et al. 2013; Jönsson et al. 2013]. There is also another type of enzyme poison – heavy metals, which may be up-taken from the soil before tree-cutting, or during further material pretreatment. Iron, copper, chromium and nickel are mentioned in the literature [Chandel et al. 2013]. As the new species of poplar are artificially cultivated, there is also a need to check their ability to absorb specified metals. The choice of the best species for further processing will be easier, when there is more knowledge about their properties.

Some types of material pretreatment may also cause the metals content to increase. Steam explosion is one of them. Recently, this has been an increasingly popular method of ligno-cellulosic materials pretreatment [Tutt et al. 2014]. It consists of material treatment with steam at a temperature exceeding 100°C. As the conditions in the reactor are very severe, many different organic compounds may form from the wood during this treatment. Another question arises: could the reactor material pollute the treated ligno-cellulosic material with the aforementioned metals?

The aim of this paper was to investigate the differences in metal content and distribution in two species of poplar before and after steam pretreatment, and to check the usefulness of the XRF technique for this purpose. This is a fast technique which may be successfully used for comparative measurements of wood elemental composition [Zielenkiewicz et al. 2012].

Materials and Methods

Two common species of poplar, *Populus trichocarpa* and *Populus maximowiczii*, were analysed. Two and a half year old trees were cut (three specimens of each species). Samples of trunk (breast height), branch, bark and

leaves were collected from each tree. A Spectro Midex M XRF spectrometer was used to analyse the content of iron, copper, chromium and nickel which inhibiting action was stated. Trunk and branch were measured as wood pieces (without disintegration), bark and leaves were in the form of milled powder. Each sample was measured three times with the "point scan" method (each "point" was a 2 mm × 2 mm surface), the time of the x-ray exposure was 300 s. The results, in ppm, should be treated qualitatively and comparatively, because the XRF spectrometer calibration was originally prepared with respect to metallic types of samples.

Disks from a breast height of 2.5 year old stems of *Populus trichocarpa, Populus maximowiczii* and *Populus tremula* (as the reference material) were collected. Additional disks from 4 year old stems of *P. trichocarpa* and *P. maximowiczii* were collected to check the influence of tree age on the metals uptake. In order to analyse the distribution of chosen metals on the stem cross-section, the "mapping" option of the XRF spectrometer was used. The surface of the disk was divided into 2 mm × 2 mm squares, the time of x-ray exposure (each square) was 30 s. The values of so called impulse counts for each analysed metal, were the results for comparison and metals distribution determination.

In order to check the influence of steam pretreatment on the chosen metals content, additional measurements were performed. Samples of 2.5 year old *P. trichocarpa* and *P. maximowiczii* (cca. 5 g of each) were treated in water boiled in a hermetic stainless steel reactor (0.5 litres) up to 130°C, 160°C and 190°C using an oil bath. The time from the beginning of heating until the end of the experiment (rapid decompression) was 6 hours. Samples were rinsed with distilled water, dried at 105°C until a constant mass was reached and ashed for 6 hours at 600°C in a muffle furnace. The increase in temperature from 20°C to 600°C lasted an additional 2.5 hours. Ashing was performed in order to pre--concentrate samples (it was used for this purpose by Harju et al. 1997). The ash obtained was analyzed with the XRF "point scan" method, as above.

Results and discussion

The results of the content of particular elements in both poplar species are collected in figures 1-6. It was assumed that both poplar species had the same matrix for XRF analysis. Without this assumption comparison of the results obtained for *P. trichocarpa* and *P. maximowiczii* would be unjustified [Zielenkiewicz et al. 2012]. Results for wood samples, however, cannot be compared with bark and leaves, because these parts of the tree definitely form another type of matrix, especially after they were milled. In addition to the heavy metals mentioned earlier, described by Chandel et al. [2013] as hydrolysis inhibitors, manganese and zinc were analysed as additional elements. They are present in wood in high proportions and that is why their analysis is easier. Additionally these elements are important for the trees metabolism.

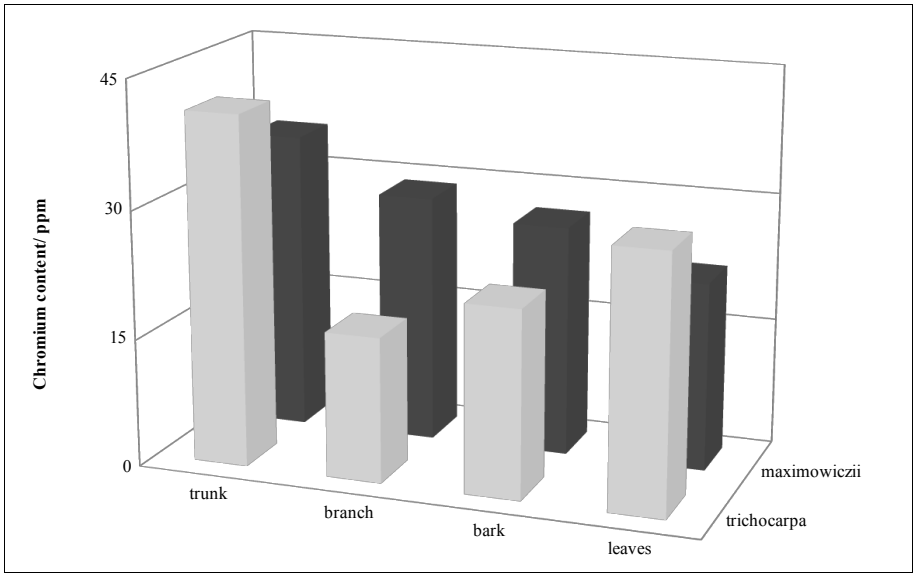

Fig. 1. Chromium content in analysed samples of *P. trichocarpa* and *P. maximowiczii*

The comparison of chromium content in both poplar species is presented in figure 1. *P. trichocarpa* contains more chromium in the trunk and leaves while *P. maximowiczii* – in the branches and bark. The most significant difference between the species is observable in branch samples. Chromium content is the highest in the trunk in both cases.

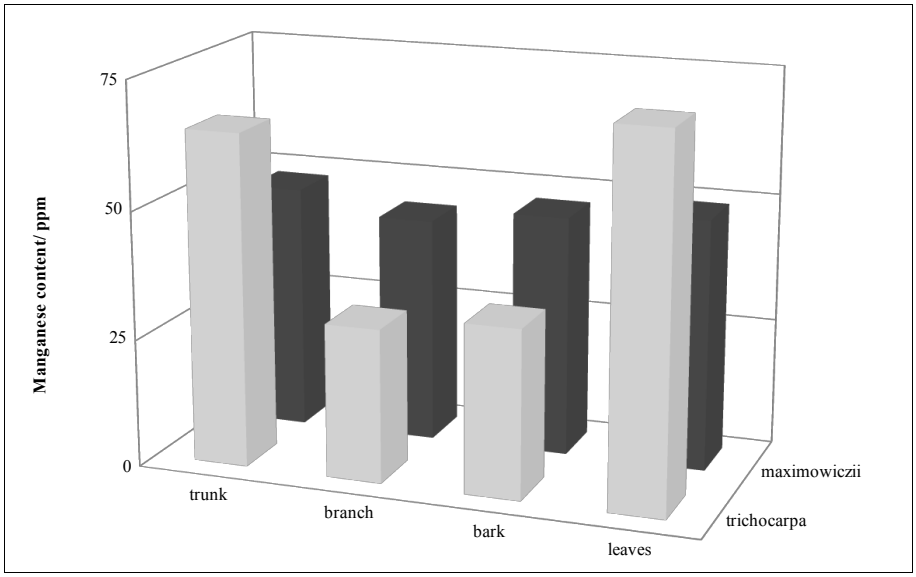

Fig. 2. Manganese content in analysed samples of *P. trichocarpa* and *P. maximowiczii*

The results of the manganese analysis are presented in figure 2. Manganese content in *P. trichocarpa* samples of trunk and leaves is higher (like in the case of chromium) in relation to *P. maximowiczii*. Visible differences are even more significant. The content of this metal is the highest in the leaves. Differences between values obtained for the trunk and branches are much more significant in the case of *P. trichocarpa* (like in the fig. 1).

Results of the iron analysis are presented in figure 3. Also, this time *P. trichocarpa* samples contain more iron in the trunk and leaves samples. The highest content of iron is found in leaves for *P. trichocarpa* and in the bark for *P. maximowiczii*. Again, the difference between values obtained for the trunk and branches is more significant in *P. trichocarpa* samples.

The results of nickel content in the analyzed samples are presented in figure 4. Its content in the trunk is higher in *P. trichocarpa*, as in previous cases, while values obtained for the leaves are similar in both species. The branches and bark of *P. maximowiczii* contain more nickel in relation to *P. trichocarpa*. There are similar relations between trunk and branches as in previous cases.

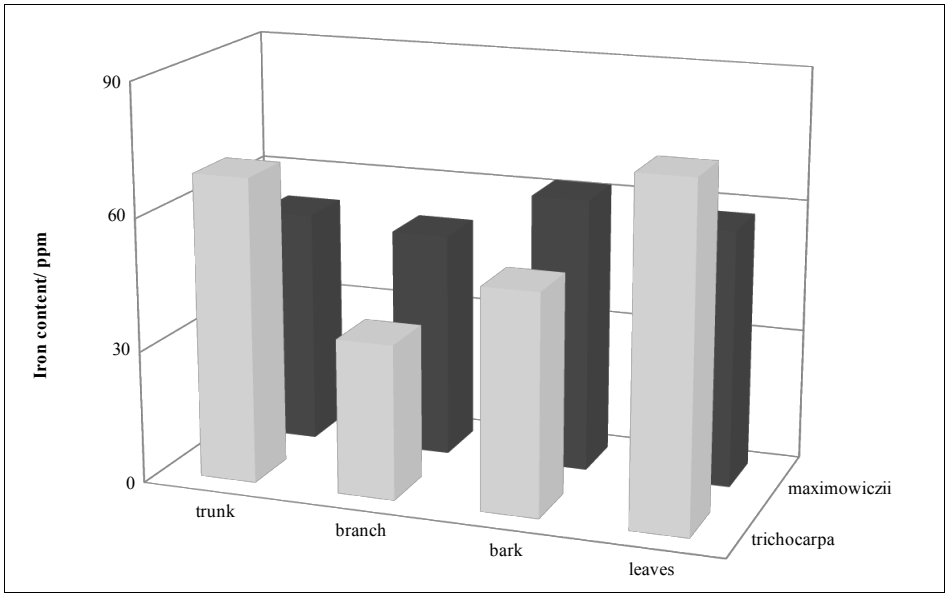

Fig. 3. Iron content in analysed samples of *P. trichocarpa* and *P. maximowiczii*

Copper content (fig. 5) in the trunk of *P. trichocarpa* is higher in relation to *P. maximowiczii.* In other samples values are higher for *P. maximowiczii.* The highest value in *P. trichocarpa* was reported for the trunk again, while in *P. maximowiczii* branches contain the highest amount of copper.

Similar results were obtained for zinc (fig. 6). Relations between particular values are parallel to copper.

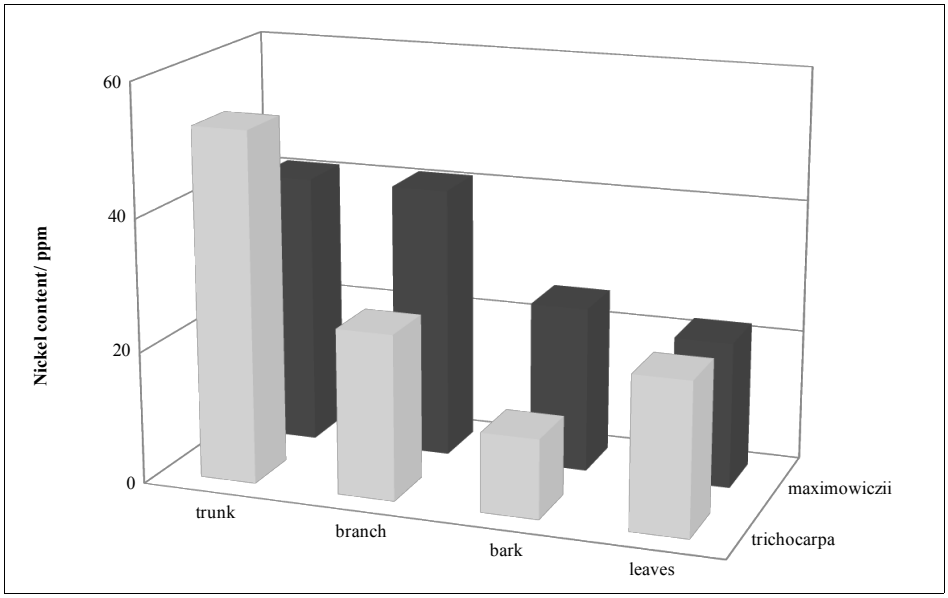

Fig. 4. Nickel content in analysed samples of *P. trichocarpa* and *P. maximowiczii*

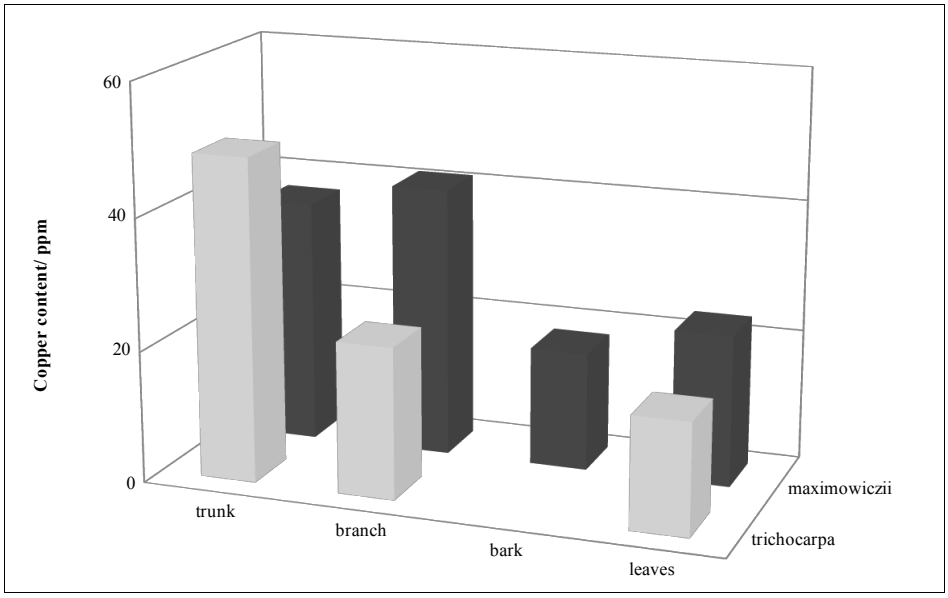

Fig. 5. Copper content in analysed samples of *P. trichocarpa* and *P. maximowiczii*

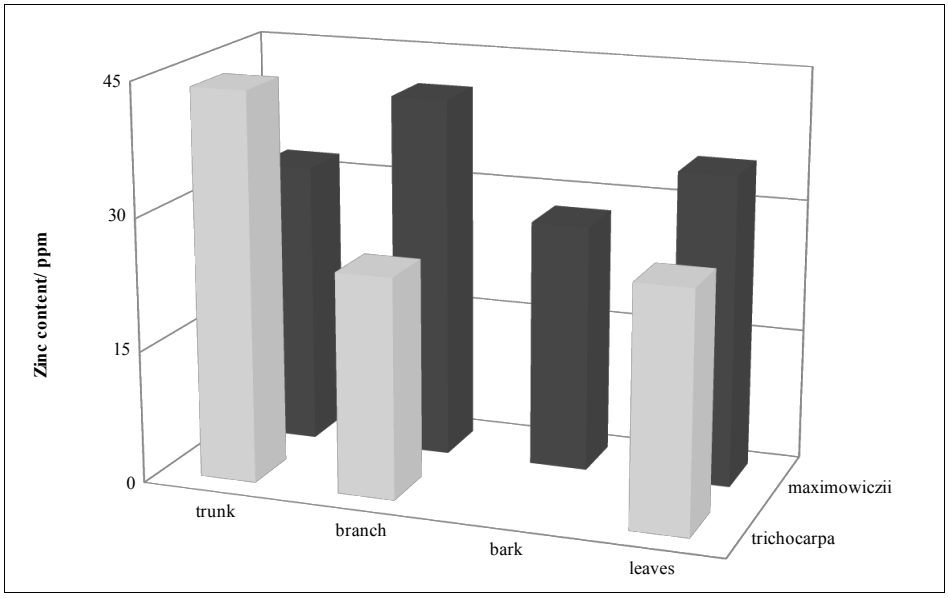

Fig. 6. Zinc content in analysed samples of *P. trichocarpa* and *P. maximowiczii*

There are many different observations that can be made. First of all, the content of each analysed element in trunk wood is higher for *P. trichocarpa* samples. A difference of even 37.5% in the case of manganese. In contradiction, all values for branch wood are higher in the case of *P. maximowiczii* samples (up to 70% for chromium). This may mean that *P. maximowiczii* trees accumulate heavy metals evenly within the whole volume of the plant – values for branch and trunk are similar for most of the analysed elements (in branch: lower for chromium, manganese and iron, higher for copper and zinc, exactly the same for nickel). Results for *P. trichocarpa* are always higher in trunk samples and the differences are significant. It may also indicate a more effective transport of nutrients to branches in *P. maximowiczii*.

The content of all analysed elements in the bark is higher in *P. maximowiczii* samples and for zinc it is almost three times higher than in *P. trichocarpa.* Results obtained for leaves are ambiguous. The contents of chromium, manganese and iron are higher in *P. trichocarpa* samples (even 47% higher in the case of manganese) but, contents of copper and zinc are clearly lower in this species while the content of nickel is almost the same in both of poplars. It does not correspond to differences observed in branches at all. Generally, leaves are the only part of the tree where no unequivocal tendency is observed.

An additional interesting observation is that the contents of nickel, copper and zinc in bark and leaves are much lower in relation to the contents of manganese and iron (in samples of *P. trichocarpa* bark contents of copper and zinc is even below the determination limit), while in trunk and branch, values of all elements are more or less on the same level. Values for chromium are of

a similar dependence, but, they are also significantly lower in relation to manganese and iron in branch samples.

The distribution of metals on a cross-section of both the studied species are presented in figures 7a-7f (2,5 and 4 year old) and *P. tremula* (2.5 year old) for comparison. Ranges of metal impulse counts correspond to different colours. Distribution of chromium is visible in figure 7a. A darker colour means a higher content of the element. It may be observed that the diameter of *P. tremula* is the lowest and of *P. trichocarpa* – the highest (each square dimension is 2 mm × 2 mm). The distribution of chromium is rather regular. *P. tremula* contains the highest amount of chromium on almost the whole surface of the cross-section, while 4 year old *P. trichocarpa* – the lowest amount (the darkest colour surface is much smaller than on other graphs).

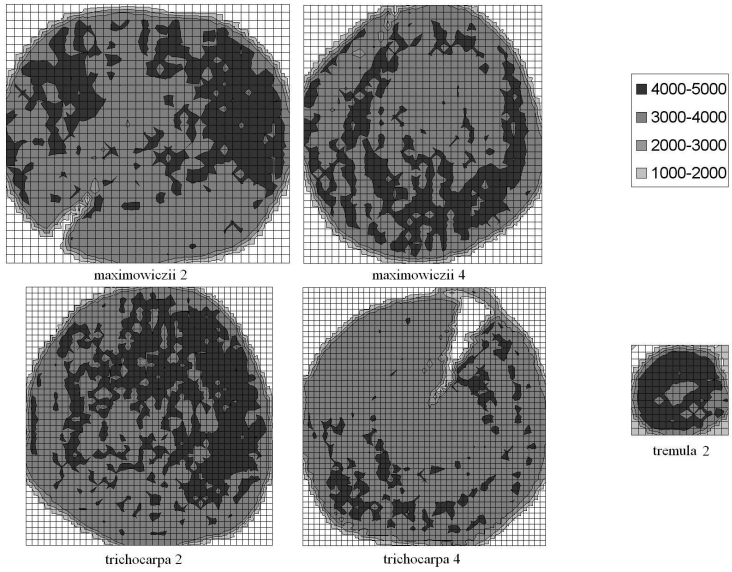

Fig. 7a. Distribution of chromium on the cross-sections of *P. maximowiczii,* (2.5 and 4 year old) *P. trichocarpa* (2.5 and 4 year old) and *P. tremula* (2.5year old) poplar with XRF mapping option (values of impulse counts)

Distribution of manganese is more regular in comparison to chromium (fig. 7b). Again, the *P. tremula* sample contains a higher amount of analysed metal than others. In the outer parts of other samples (especially 4 year olds), near the bark, darker surfaces may be observed. This is probably a phloem area, however, the difference in relation to the rest of the cross-section is insignificant.

Graphs with the distribution of iron are collected in figure 7c. The distribution is regular and the levels of impulse counts values are similar for all species. Distribution is more differentiated for only one sample, 4 year old *P. trichocarpa*. Increased iron content in the phloem section may be caused by the cutting saw.

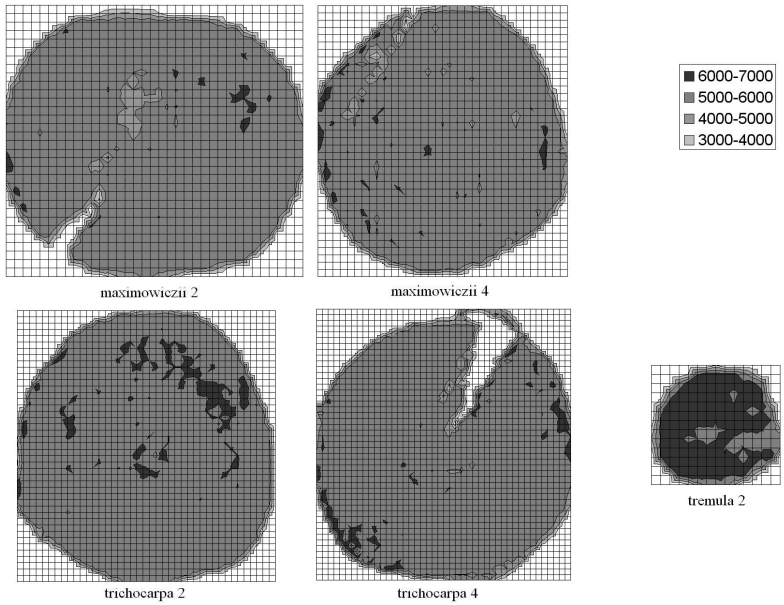

Fig. 7b. Distribution of manganese on the cross-sections of *P. maximowiczii,* (2.5 and 4 year old) *P. trichocarpa* (2.5 and 4 year old) and *P. tremula* (2.5 year old) poplar with XRF mapping option (values of impulse counts)

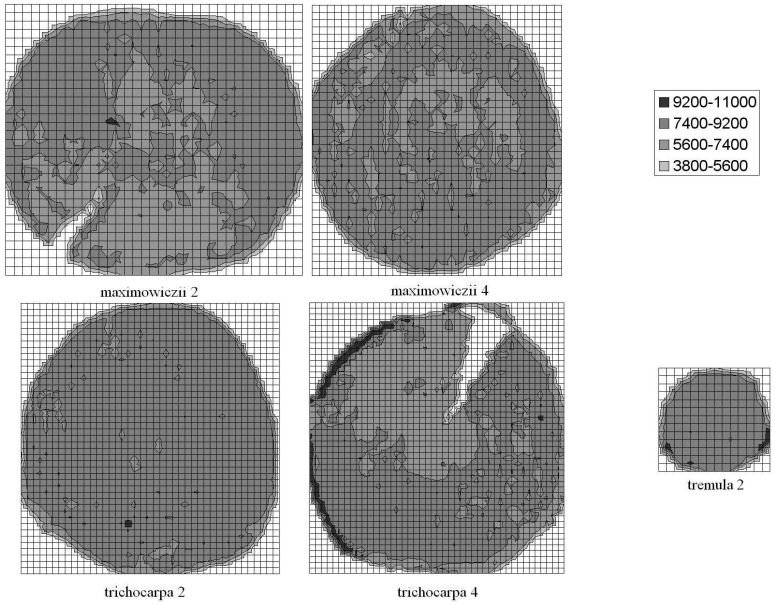

Fig. 7c. Distribution of iron on the cross-sections of *P. maximowiczii,* (2.5 and 4 year old) *P. trichocarpa* (2.5 and 4 year old) and *P. tremula* (2.5 year old) poplar with XRF mapping option (values of impulse counts)

The distribution of nickel (fig. 7d) is rather regular. Some differences are observed (excluding the *P. tremula* sample) but no true pattern could be found. Similar observations may be denoted in the case of copper distribution (fig. 7e). Some increase in the copper content is visible in the phloem area (only for *P. maximowiczii*).

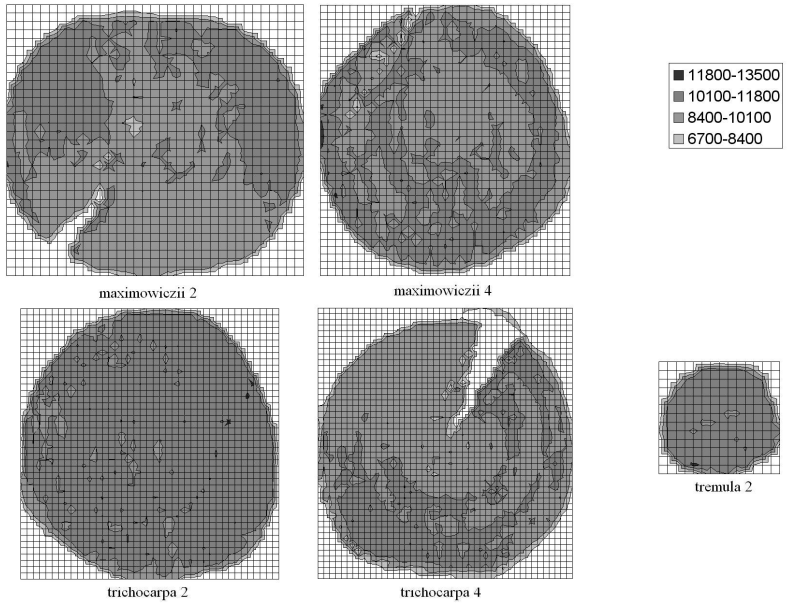

Fig. 7d. Distribution of nickel on the cross-sections of *P. maximowiczii,* (2.5 and 4 year old) *P. trichocarpa* (2.5 and 4 year old) and *P. tremula* (2.5 year old) poplar with XRF mapping option (values of impulse counts)

An increase of zinc content (fig. 7f) in the phloem area is observed for all samples excluding *P. tremula*. The distribution of this element is regular in other parts of the samples.

In summary, mapping measurements of stem cross-sections collected from breast height does not give any information about the possible areas in stem, where metals are accumulated. Some increases in the phloem area are insignificant in character. Older samples seem to contain lower amounts of the analysed metals.

Table 1 shows the contents of the studied elements in the ashes is the ashes of samples. Both species were first submitted for steam explosion at three different temperatures: 130°C, 160°C and 190°C. Reference samples of ash without steam treatment were also measured. First of all, differences between data observed in table 1 and figures 1-6 should be explained. Solid wood and ash are definitely different matrixes for XRF measurements. That is why there is no justification for any attempt at comparison of these two sets of results.

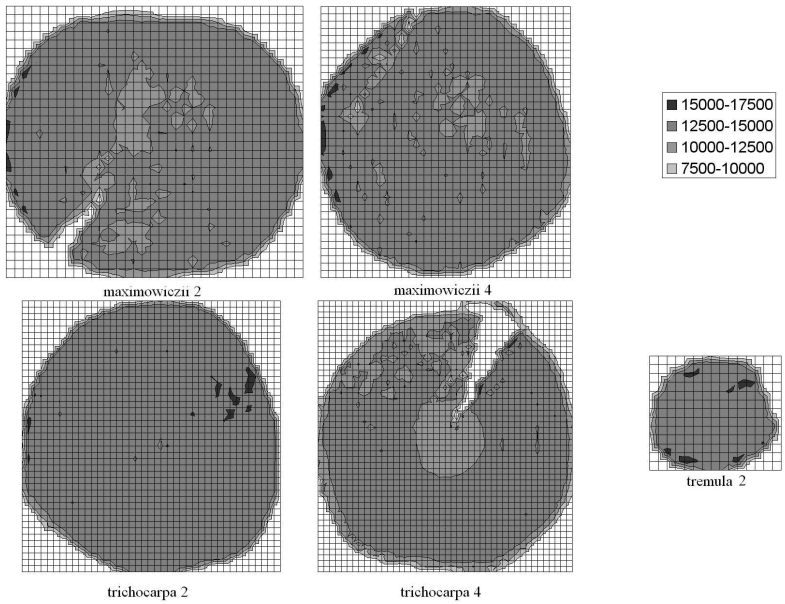

Fig. 7e. Distribution of copper on the cross-sections of *P. maximowiczii,* (2.5 and 4 year old) *P. trichocarpa* (2.5 and 4 year old) and *P. tremula* (2.5 year old) poplar with XRF mapping option (values of impulse counts)

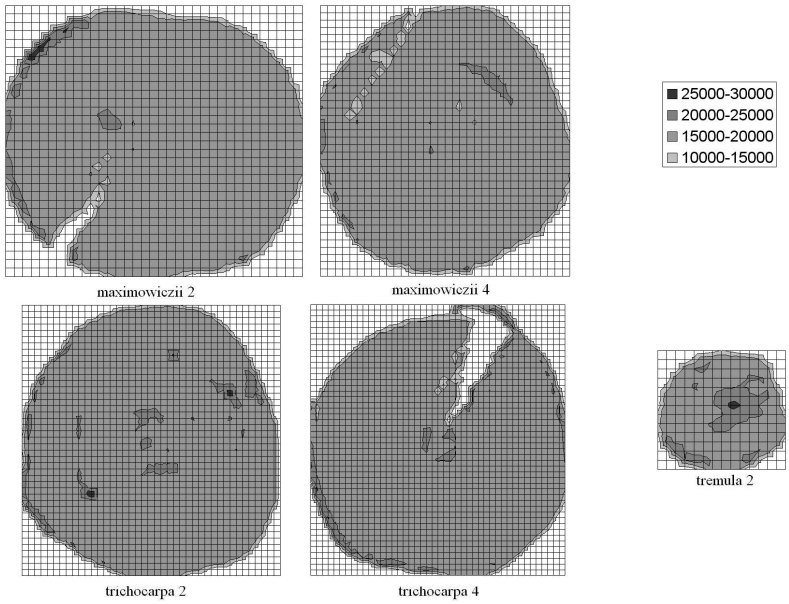

Fig. 7f. Distribution of zinc on the cross-sections of *P. maximowiczii,* (2.5 and 4 year old) *P. trichocarpa* (2.5 and 4 year old) and *P. tremula* (2.5 year old) poplar with XRF mapping option (values of impulse counts)

Table 1. Average contents (in ppm) of chosen metals in ashed samples of *P. trichocarpa* and *P. maximowiczii* after steam treatment in 130, 160 and 190°C

Element	Cr	Mn	Fe	Ni	Cu	Zn
Trich	11	120	56	–*	12	160
Trich 130	128	172	2383	14	74	319
Trich 160	17	120	1373	16	33	174
Trich 190	32	126	1464	40	63	208
Max	–	47	73	–	19	170
Max 130	–	41	503	–	58	271
Max 160	–	25	1500	–	46	172
Max 190	26	91	6002	19	40	1201

*Below determination level.

Comparing reference samples of *P. trichocarpa* with samples after steam explosion at 160°C and 190°C, it may be observed that the contents of all the analysed elements increase with temperature. The increase is insignificant for Mn and Zn but, the content of others raises fivefold (Cu) or even almost 30fold (Fe). The values for all metals, however, (excluding nickel) are the highest in the case of 130°C treatment. This is quite a surprising result and in the context of the rest of the positions should be probably acknowledged as an error.

Results for *P. maximowiczii* samples are not so unequivocal. Treatment at 190°C causes the highest increase in content of all metals excluding copper. Iron content is almost 100 times higher and zinc content is about sevenfold higher than in reference samples without treatment. The influence of lower treatment temperatures is also visible but the dependence is not obvious. Comparing the results for both species, it may be stated that the influence of 130°C and 160°C on studied elements content is quite similar. Treatment at 190°C causes significantly higher increases of iron and zinc content in the *P. maximowiczii* sample.

Conclusion

More heavy metals are accumulated in the trunk of *P. trichocarpa* compared to that of *P. maximowiczii*. As the trunk contains most of the poplar mass at this age, *P. trichocarpa* probably generally collects higher amounts of heavy metals, including hydrolysis inhibitors such as chromium, nickel, copper and iron. Thus, if other important parameters for material processing (to bio-fuels) of these species are similar, it can be concluded that *P. maximowiczii* is recommended for further processing.

Mapping studies suggest that older samples may contain lower amounts of metallic inhibitors.

Additional XRF analysis of all samples after ashing could be the verification of the presented comparison. Although laborious, ashing is a good method for the samples pre-concentration. It unifies the matrix and raises the certainty that more parallel results for both analysed species could be obtained. In addition, more comparable results will be obtained for different wood fractions (wood, bark and leaves).

The content of metals for ashed samples after steam explosion was found to be much higher than without treatment. This may lead to the conclusion that decomposition of the material at 190°C steam treatment in the case of *P. maximowiczii* caused a higher amount of aggressive compounds to formresulting in a much higher uptake of iron and zinc.

XRF is a good tool for comparative analysis because it is a fairly fast technique and does not demand laborious sample preparation. It enables complex analysis including comparative content and distribution measurements of the studied elements.

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