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# THE POTENTIAL OF DIFFERENT LIME TREE (*Tilia* spp) GENOTYPES FOR PHYTOEXTRACTION OF HEAVY METALS

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The research of heavy metals contents (Pb, Mn, Zn, Ni, Fe) in soil in the area of the National Park „Fruška gora", along the highway M21 shows lower values for manganese, zinc and iron than the maximum allowed quantity prescribed by law. For nickel and lead it shows higher values than maximum allowed quantity. The heavy metals contents in leaves of lime tree in 12 analyzed genotypes are far below average values in accordance with ECCE with all genotypes except genotype 7 for lead and genotypes 7 and 8 for iron. The results of analysis of variance components show that out of four components (locality, genotype, locality x genotype and error) only the interaction between locality and genotype does not contribute to variance. The contents of Pb, Mn, Fe and Zn in leaves is primarily influenced by genotype while Ni contents may be considered a consequence

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of locality. The selection of genotypes which is able to uptake greater quantities of heavy metals than other genotypes may serve as a solid basis for phytoextraction of heavy metals as a technology by which heavy metals, metalloids and radionuclides are extracted from environment through usage of suitable species and plant genotypes able to uptake and accumulate the given pollutants in parts of plant tissue.

*Key words:* genotypes, heavy metals, lime tree

## INTRODUCTION

The presence of heavy metals in certain parts of biosphere is the consequence of a series of anthropogenic activities. While this presents a problem characterized with cumulative effect for humans, certain plant species have developed the ability to survive in habitats with increased concentrations of heavy metals. During evolution they have developed the whole series of adaptive mechanisms (plant morphology, specific physiological and biochemical processes or rather genotype attributes) which resulted in creation of constitutive tolerance on heavy metals on cellular level. Herbs are capable to accumulate higher quantities of heavy metals in their leaves than woody plants growing in the same habitats (ŠIJAČIĆ-NIKOLIĆ *el al.*, 2011). In general, trees are not adapted to increased concentrations of heavy metals in soil with very few metal tolerant ecotypes (DICKINSON *et al*., 1991; KAHLE, 1993).

Differences in uptake of mineral elements and especially heavy metals from soil depend before all on genetic individual characteristics of plant species, types, hybrid i.e. genotype (EPSTEIN, 1972; SARIĆ, 1981) and also on characteristics of root system, its capacity for absorption of ions and the level of evaporation and transpiration (ALLOWAY, 1995). A very important component in consideration of the issue of heavy metal uptake is the individual, species and interspecies diversity which is very often induced by climatic characteristics and humidity regime. As is the case with soil, the capacity of plants to accumulate heavy metals is limited.

It should be pointed out that in the field of specific effects of microelements on physiological and biochemical processes there are still today inexplicable facts on their indispensability and biological function; therefore these questions will still be actual issues for scientific research even in future. (KRSTIĆ, 2011)

Previous research which were dealing with the potential of different genotypes of woody plants for phytoextraction of heavy metals showed that tolerance is to a greater extent correlated with genotype i.e. hybrides than to species as taxonomic ranks (KRALJEVIĆ-BALALIĆ *et al*., 2009; BORIŠEV, 2010). LANDBERG and GREGER (1994) examined the accumulation of Cd and Zn in the series of willow clones in water solutions. Some of the clones were tolerant of both metals, some only of one. GREGER (1999) researched the capacity of Cd accumulation among 70 willow genotypes and determined the differences up to 43 times. PUNSHON and DICKINSON (1999) researched resistance of willows to treatment with Zn, Cd, Cu and Ni in water cultures and determined the specific characteristics of genotypes. NIKOLIC *et al*

(2008) determined that the level of heavy metals accumulation in plant tissue is determined by numerous biotic and abiotic factors out of which genotype specificity is one of the most dominant ones.

The aim of the research presented in this paper is the estimate of the potential of different genotypes of lime tree for uptake of pollutants and their accumulation in leaves i.e. phytoextraction.

## MATERIALS AND METHODS

The research was performed in the National Park „Fruška gora" where since 2005 there has been conducted a continuous monitoring of heavy metals concentrations in different plant species and soil (STANKOVIĆ, 2006). Silver Lime is the most dominant species in this area with its share of 37.6%. In certain ecological units and forest types Lime represents an edificator and more often being an accompanying species it has overpowered and repressed other edificators on a larger part of the area.

The selection of genotypes was performed on three localities along the highway M21 on the section of the road Irig - Iriski venac - Paragovo (12 km) in the length of 200-300 m and depth up to 10 m, both on left and right side of the road. The fourth locality is considerably far away from the road and in our previous research it represented the control locality (STANKOVIĆ, 2008). On each locality the leaves were collected from three different genotypes (trees) of lime, table 1.

*Table 1.Layout of localities for selection of genotypes (1-12)*

| Locality | Locality description | Genotype label |
|---|---|---|
| 1 | Highway M21 at the entrance from Hopovo | 1,2,3 |
| 2 | Highway M21 at Iriski venac | 4,5,6 |
| 3 | Crossroad of entrance and downward road M21 at Paragovo | 7,8,9 |
| 4 | Old highway (control locality) | 10,11,12 |

Heavy metals contents in soil were determined on four different depths (KRSTIĆ *et al*., 2007, STANKOVIĆ *et al*., 2011) and on four different localities with different traffic frequency or different duration of automobile halts. The level of soil load with individual heavy metals was determined in accordance with BRUNE-ELLIGHAUS (1981): very low 1-5%; low 5-10%; middle 10-25%; high 25-50%, very high 50 -100% with reference to the maximum allowed value of heavy metals concentrations in soil (KNEŽEVIĆ *et al*., 2009, STANKOVIĆ *et al*., 2012.)

The soil on these localities shows selective absorption of elements in the manner that in most cases the first layers contain the highest heavy metals concentrations. And which metal is absorbed depends primarily on locality and in this case the distance from the road and traffic frequence.

Heavy metals concentrations in soil and plants are determined by atomic apsorption spectrophotometry AAS. The obtained data were processed by computer application STATISTICA 7, whereby we conducted Variance Analysis, LSD test and Variance Component Estimation for a Two-Way Random Factorial Design. On the basis of cluster analysis genotypes are grouped in clusters on the basis of statistical closeness of potential for phytoextraction of the analyzed heavy metals.

## RESULTS AND DISCUSSION

Table 2 shows heavy metals concentrations in soil by localities and soil depths. Considering the four analyzed localities it may be determined that soil on localities 1,2 and 3 is heavily loaded with lead (in accordance with BRUNE-ELLINGHAUS, 1981) which is most probably a consequence of high traffic frequence on these localities which is not the case with the control locality no.4. The measured quantity of lead and nickel in soil exceeds the maximum allowable quantities ($MDK_{Pb}$ = 100 ppm, $MDK_{Ni}$ = 50 ppm), which draws the attention to load of soil with these heavy metals. (Official gazette no.23/1993). The contents of Mn, Zn and Fe in soil on all researched localities is below critical values.

Taking into account 12 different lime genotypes, given in table 3a to 3e, it may be stated that the obtained differences among average values are statistically significant for all five analyzed heavy metals.

The average value of nickel content in leaves, considering the average of 12 genotypes amounts to 1.49 $mgkg^{-1}$. The genotypes 8, 10, 7, 12 and 11 show the greatest potential for phytoextraction of nickel. The nickel contents in leaves of those genotypes ranges from 2.91 to 2.18 $mgkg^{-1}$. The nickel contents in leaves of genotypes 6, 9, 1, 4 and 2 ranges from 0.52 to 0.68 $mgkg^{-1}$ and is almost three times lower than the average at the level of analyzed genotypes.

The average value of lead contents at the level of analyzed genotypes amounts to 2.57 $mgkg^{-1}$. The genotypes 7, 8, 3, and 10 show higher lead contents while all other genotypes show lower values than the average.

The average value of manganese contents in leaves, calculated at the level of analyzed genotypes amounts to 46.48 $mgkg^{-1}$. Significantly higher values in relation to this average are observed in the following genotypes: 1 (74.78 $mgkg^{-1}$), 8 (68.57 $mgkg^{-1}$) and 7 (64.99 $mgkg^{-1}$), while manganese contents in leaves of genotype 3 is up to four times lower and amounts to (12,12 $mgkg^{-1}$).

Iron is the element whose average value at the level of 12 analyzed genotypes amounts to 163.61 $mgkg^{-1}$. The following genotypes 8 (383.97 $mgkg^{-1}$), 7 (278.96 $mgkg^{-1}$) and 4 (196.77 $mgkg^{-1}$) contain significantly higher values than the average. The lowest iron contents are observed in the leaves of genotype 3 (102.65 $mgkg^{-1}$).

The average value of zinc contents at the level of analyzed genotypes amounts to 26.71 $mgkg^{-1}$. Higher values than the average were noted in genotypes

11, 12, 8, 7, 4, 5, and 10. The lowest value was evidenced in genotype 2 (12.72 $mgkg^{-1}$).

On the basis on the above mentioned it may be concluded that the greatest potential for phytoextraction is observed in genotypes 7 and 8 for all analyzed heavy metals while genotypes 2 and 3 show the lowest values of heavy metals contents in leaves in the greatest number of cases.

*Tabl 2. Concentrations of heavy metals in soil by localities and depths (*STANKOVIĆ*, 2006, 2008)*

| Soil depth | **Pb** ($mgkg^{-1}$) | **Mn** ($mgkg^{-1}$) | **Zn** ($mgkg^{-1}$) | **Ni** ($mgkg^{-1}$) | **Fe** ($mgkg^{-1}$) |
|---|---|---|---|---|---|
| | | **Locality 1** | | | |
| 0-5 cm | 230 | 640 | 250 | 99 | 24000 |
| 5-10 cm | 130 | 700 | 160 | 120 | 28000 |
| 10-20cm | 66 | 630 | 89 | 120 | 27000 |
| 20-40cm | 51 | 680 | 70 | 140 | 28000 |
| **Average value:** | **119,25** | **662,50** | **142,25** | **119,75** | **26750** |
| | | **Locality 2** | | | |
| 0-5 cm | 240 | 730 | 220 | 83 | 28000 |
| 5-10cm | 180 | 770 | 200 | 120 | 33000 |
| 10-20cm | 38 | 830 | 66 | 120 | 38000 |
| 20-40cm | 28 | 750 | 64 | 89 | 42000 |
| **Average value :** | **121,50** | **770** | **137,50** | **103** | **35250** |
| | | **Locality 3** | | | |
| 0-5cm | 84 | 700 | 63 | 95 | 26000 |
| 5-10cm | 130 | 740 | 96 | 79 | 24000 |
| 10-20 cm | 120 | 830 | 100 | 83 | 26000 |
| 20-40cm | 110 | 780 | 92 | 89 | 26000 |
| **Average value :** | **110** | **762,50** | **87,75** | **86,5** | **25500** |
| | | **Locality 4** | | | |
| 0-5 cm | 33 | 980 | 64 | 140 | 37000 |
| 5-10 cm | 28 | 920 | 58 | 150 | 37000 |
| 10-20 cm | 33 | 860 | 54 | 150 | 36000 |
| 20-40 cm | 26 | 900 | 51 | 150 | 37000 |
| **Average value :** | **30** | **915** | **56,75** | **147,50** | **36750** |

*Table 3a. Variance analysis and LSD test for heavy metals contents Ni in leaves in different genotypes (1-12) of lime tree in the area of the National Park „Fruška gora“*

| Ni | | | |
|---|---|---|---|
| Among genotypes | Mean Square | F-Ratio | P-Value |
| | 2,58472 | 11,21 | 0,0000 |

| LSD test | | |
|---|---|---|
| Genotype | Average value (mgkg$^{-1}$) | Homogenous groups |
| **6** | 0,52 | X |
| **9** | 0,61 | XX |
| **1** | 0,65 | XX |
| **4** | 0,66 | XX |
| **2** | 0,68 | XX |
| **3** | 1,02 | XX |
| **5** | 1,34 | X |
| **11** | 2,18 | X |
| **12** | 2,23 | X |
| **7** | 2,40 | X |
| **10** | 2,75 | X |
| **8** | 2,91 | X |
| Average values ECCE* 0,4-4 | | |

ECCE - Element Concentration Cadastres in Ecosystems (1994)

*Table 3b. Variance analysis and LSD test for heavy metals contents Pb in leaves in different genotypes (1-12) of lime tree in the area of the National Park „Fruška gora“*

| Pb | | | |
|---|---|---|---|
| Among genotypes | Mean Square | F-Ratio | P-Value |
| | 6,33486 | 6,83 | 0,0000 |

| LSD test | | |
|---|---|---|
| Genotype | Average value (mgkg$^{-1}$) | Homogenous groups |
| **12** | 1,14 | X |
| **2** | 1,40 | X |
| **1** | 1,67 | XX |
| **9** | 1,72 | XX |
| **11** | 1,91 | XX |
| **5** | 2,03 | XX |
| **6** | 2,04 | XX |
| **4** | 2,12 | XX |
| **10** | 3,04 | XX |
| **3** | 3,17 | XX |
| **8** | 4,38 | X |
| **7** | 6,21 | X |
| Average values ECCE* 0,1-5 | | |

ECCE - Element Concentration Cadastres in Ecosystems (1994)

*Table 3c. Variance analysis and LSD test for heavy metals contents Mn in leaves in different genotypes (1-12) of lime tree in the area of the National Park „Fruška gora“*

| Mn | | | |
|---|---|---|---|
| Among genotypes | Mean Square | F-Ratio | P-Value |
| | 955,03 | 9,59 | 0,0000 |
| **LSD test** | | | |
| Genotype | Average value (mgkg$^{-1}$) | Homogenous groups | |
| **3** | 12,12 | X | |
| **6** | 25,12 | XX | |
| **11** | 37,96 | XX | |
| **9** | 38,57 | XX | |
| **4** | 41,44 | XX | |
| **2** | 44,21 | X | |
| **10** | 46,27 | X | |
| **12** | 50,34 | XX | |
| **5** | 53,37 | XXX | |
| **7** | 64,99 | XXX | |
| **8** | 68,57 | XX | |
| **1** | 74,87 | X | |
| Average values ECCE* 1-700 | | | |

ECCE - Element Concentration Cadastres in Ecosystems (1994)

*Table 3d. Variance analysis and LSD test for heavy metals contents Fe in leaves in different genotypes (1-12) of lime tree in the area of the National Park „Fruška gora“*

| Fe | | | |
|---|---|---|---|
| Among genotypes | Mean Square | F-Ratio | P-Value |
| | 21657,5 | 38,45 | 0,0000 |
| **LSD test** | | | |
| Genotype | Average value (mgkg$^{-1}$) | Homogenous groups | |
| **3** | 102,65 | X | |
| **10** | 105,41 | X | |
| **9** | 117,48 | X | |
| **11** | 119,08 | X | |
| **6** | 125,19 | X | |
| **1** | 127,29 | X | |
| **2** | 130,20 | X | |
| **5** | 134,97 | X | |
| **12** | 141,29 | X | |
| **4** | 196,77 | X | |
| **7** | 278,96 | X | |
| **8** | 383,97 | X | |
| Average values ECCE* 5-200 | | | |

ECCE - Element Concentration Cadastres in Ecosystems (1994)

*Table 3e. Variance analysis and LSD test for heavy metals contents Zn in leaves in different genotypes (1-12) of lime tree in the area of the National Park „Fruška gora“*

| Zn | | | |
|---|---|---|---|
| Among genotypes | Mean Square | F-Ratio | P-Value |
| | 280,059 | 6,55 | 0,0001 |

| LSD test | | |
|---|---|---|
| Genotype | Average value (mgkg$^{-1}$) | Homogenous groups |
| **2** | 12,72 | X |
| **9** | 14,74 | XX |
| **1** | 16,35 | XXX |
| **6** | 17,13 | XXX |
| **3** | 25,67 | XXX |
| **10** | 27,12 | XX |
| **5** | 28,77 | XX |
| **4** | 30,29 | XX |
| **7** | 33,01 | XXX |
| **8** | 33,78 | XXX |
| **12** | 39,62 | XX |
| **11** | 41,34 | X |
| Average values ECCE* 15-150 | | |

ECCE - Element Concentration Cadastres in Ecosystems (1994)

The results of the analysis of variance components presented in table 4 show that out of 4 components the interaction between locality and genotype is the only component that does not contribute to variance. Out of the other three non zero components the component of locality is very interesting for the variable Mn in leaves since it shows negative value. This is one of the unwanted effects of the estimate of variance components and therefore in practice these values are usually replaced by zero or presented as zero components. With that regard, for the component locality in relation to manganese contents we can also say that is does not contribute to variance.

*Table 4 Components of Variance calculated by Mean Squares, Type: 1*

| | Ni (leaf) | Pb (leaf) | Mn (leaf) | Fe (leaf) | Zn (leaf) |
|---|---|---|---|---|---|
| {1}LOCALITY | 0,504172 | 0,467792 | -67,4368 | 2686,642 | 30,73750 |
| {2}GENOTYPE | 0,372244 | 1,419694 | 340,3322 | 4833,264 | 53,96081 |
| 1X2 | 0,000000 | 0,000000 | 0,0000 | 0,000 | 0,00000 |
| Error | 0,230479 | 0,927562 | 99,5600 | 563,212 | 42,72962 |

On the basis of the above elaboration it may be determined that the contents of Pb, Mn, Fe and Zn in leaves of different genotypes are primarily influenced by genotype. Ni contents in leaves of different lime genotypes is a consequence of locality which may be explained by higher values of this element in soil than the critical values on all four analyzed localities. The interaction of genotype and locality does not influence heavy metals contents in leaves of the analyzed genotypes.

This is also confirmed by dendogram of cluster analysis for 12 different lime genotypes prepared on the basis of heavy metals contents (Ni, Pb, Mn, Fe and Zn) in leaves and presented in chart 1. Grouping of genotypes is also observed independently of the locality the individual genotypes are growing. The genotypes 2 (locality 1) and 9 (locality 3) are grouped with genotype 6 (locality 2) on the shortest distance which corroborates the fact that phytoextraction of heavy metals is an individual characteristic of each genotype. This is also confirmed by another example of grouping genotypes 5 and 12 which make a homogenous group and grow in different localities. The specific feature of genotypes 7 and 8 which are stated to have the greatest potential for phytoectraction of heavy metals is reflected in their manner of grouping: on the greatest distance in comparison to all other analyzed genotypes.

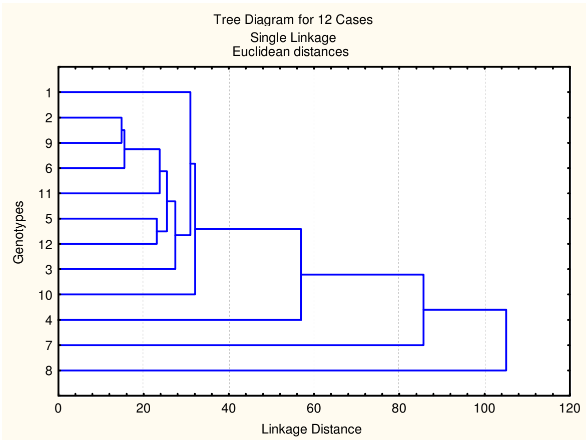


Chart 1. Dendrogram of cluster analysis for 12 different lime genotypes prepared on the basis of heavy metals contents (Ni, Pb, Mn, Fe i Zn) in leaves.

## CONCLUSION

On the basis of the results of the conducted research it may be noted that heavy metals concentrations in soil on 4 analyzed localities, along the highway M21 within the National park „Fruska gora" are below critical values for manganese, zinc and iron. The values above critical values are evidenced for nickel on all four analyzed localities and for lead on localities 1, 2 and 3. The increased concentration of lead in soil along the highway may be considered the consequence of the intensive traffic in that area. Heavy metals contents in lime tree leaves in 12 analyzed genotypes is far below average values according to ECCE in all genotypes except genotype 7 for lead and genotypes 7 and 8 for iron.

The conducted research shows that the contents of Pb, Mn, Fe and Zn in leaves are primarily influenced by genotype. Ni contents in leaves of different lime genotypes is the consequence of locality which may be explained by higher values of this element in soil than the maximum allowed quantities on all four analyzed localities. The interaction of genotype and locality has no influence on heavy metals contents in leaves of the analyzed genotypes.

The selection of genotypes able to uptake higher quantities of heavy metals than the others may serve as a good basis for phytoextraction as a technology by which heavy metals, metalloids and radoinuclids are removed from environment by using suitable species and plant genotypes able to uptake and accumulate the given pollutants in parts of their plant tissue which may be removed from the polluted area in the easiest way.

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# POTENCIJAL RAZLIČITIH GENOTIPOVA LIPE (*Tilia* spp) U FITOEKSTRAKCIJI TEŠKIH METALA

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Istraživanja sadržaja teških metala (Pb, Mn, Zn, Ni, Fe) u zemljištu na području NP „Fruška gora", duž magistralnog puta M21, pokazuju manje vrednosti od zakonom propisanih za mangan, cink i gvožđe. Vrednosti iznad maksimalno dozvoljene količine konstatovane su samo za nikl. Sadržaj teških metala u listovima lipe, kod 12 analiziranih genotipova, je daleko ispod prosečnih vrednosti prema ECCE kod svih genotipova osim kod genotipa 7 za olovo i genotipa 7 i 8 za gvožđe. Rezultati analize komponenti varijanse, pokazuju, da od četiri komponente (lokalitet, genotip, lokalitet x genotip i greška) interakcija između lokaliteta i genotipa jedina ne doprinosi varijansi. Na sadržaj Pb, Mn, Fe i Zn u listovima, prvenstveno utiče genotip, dok se sadržaj Ni u listovima može smatrati posledicom lokaliteta. Selekcija genotipova koji su sposobni da usvajaju veće količine teških metala od ostalih, može poslužiti kao dobra osnova fitoekstrakciji kao tehnologiji kojom se teški metali, metaloidi i radionuklidi uklanjaju iz životne sredine korišćenjem pogodnih vrsta i genotipova biljaka, sposobnih da date polutante usvoje i akumuliraju u delovima biljnog tkiva.

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