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# COMPARISON OF THREE TREE-RING SAMPLING METHODS FOR TRACE METAL ANALYSIS

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**Abstract.** Tree-ring growth analysis can provide information about tree development for forest inventory, environmental assessment, atmospheric and soil monitoring. Metal concentrations in wood increment correlate with metal concentrations in the environment and can indicate local environmental contamination sources. One of the most important steps of tree analysis is tree-ring sampling. To determine trace metal (TM) concentrations precisely in a separate ring, the possibility of random errors during wood sampling must be eliminated. This guides to choose a precise tree-ring sampling method. This paper examines three tree-ring sampling tools (common chisels, arched chisels and a plane) and compares TM concentrations (Mn, Zn, Ni, Cr, Cu, Pb,) in wood samples collected by each of the methods employed. Average values of metals in tree rings sampled with common chisels, arched chisels and plane were 17.0±0.16 mg/kg for Mn, 4.7±0.21 mg/kg for Zn, 0.49±0.20 mg/kg for Pb, 0.29±0.02 mg/kg for Cu, 0.21±0.02 mg/kg for Ni and 0.12±0.01 mg/kg for Cr and were in the range of typical concentrations in investigated region as well as for *Pinus sylvestris* L. The average values were calculated for the three methods used. Statistical analysis (Anderson–Darlin test, Kruskal–Wallis tests, median and percentage coefficient of variation) revealed no significant differences between metal concentrations determined in tree rings which were sampled using common chisels, arched chisels and planing. Various techniques – common chisels, arched chisels and plane – can be successfully used for tree-ring sampling, however, an increment borer provides possibility of sampling a non-felled tree.

**Keywords:** trace metals, chisel, wood samples, tree ring, plane.

## 1. Introduction

Tree-ring growth analysis can provide information about tree development for forest inventory, environmental assessment, atmospheric and soil monitoring. Tree-ring studies can show climate change patterns, e.g. those related with droughts, humid periods and accidents such as fires (Alexander 1999; McLaughlin 2002).

Decades of anthropogenic activities have led to higher amounts of trace metals (TMs) reaching forests (and solitary trees) in the form of dry and wet deposition. Synergetic effect of TMs with other industrial contaminants inhibits tree vitality and depresses tree nutritional functions (Ozolinius, Sujetovienė 2002, Rademacher 2003; Stravinskiene, Šimatonytė 2008; Stravinskienė, Erlickytė-Marčiulionienė 2009).

Tree root ability to take up TMs from the soil and atmosphere enables trees to be used as biological indicators, capable to accumulate TMs in tree rings and, thus, hold a record of environmental contamination data (DeWalle *et al*. 1995; Butkus *et al*. 2002, 2008; White, Tard 2002; Ozolinčius 2004). High correlation between concentrations of TMs in the soil and xylem rings has been reported (Larison, Helmisaari 1998). For example, increased lead (Pb) concentration in the annual growth rings of trees has been shown to relate with atmospheric Pb deposition of automobile exhaust emissions (Rolfe 1974; Kardell, Larsson 1978; Guyette *et al*. 1991; Latimer *et al*. 1996). Similarly, levels of manganese (Mn) in Scots pine stem are high in old annual rings and decrease towards the bark (Butkus *et al*. 2002; Baltrėnaitė, Butkus 2004). Nickel (Ni) is found in the trees at industrially polluted soils, areas affected by transport (Kirchner *et al.* 2008), and soils amended with sewage sludge (Katinas *et al.* 2002; Montse, Joan 2006). Copper (Cu) and zinc (Zn) are among elements commonly emitted from smelters and they are observed to be antagonistic. Zn concentration has been found to increase towards the bark in the studied trees (Larison, Helmisaari 1998).

Metal concentrations in wood increment correlate with metal concentrations in the environment and can indicate local environmental contamination sources. For instance, results from a previous investigation showed intense transfer of Zn and Cu from the soil to pine trees in two military training areas in Lithuania (Baltrėnaitė, Butkus 2006). The explanation given was that Zn and Cu were known as constituents of military activity; Zn was a component of bullets and Cu – a component of military transport emissions (Baltrėnaitė, Butkus 2004; Baltrėnas *et al.* 2005). Pines grown in three military training areas had

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about twice lower concentration of Cr, Mn, Pb and Ni in tree rings during the period 1989–1996, compared with 1978–1988 and 1997–2001, the periods known as the Lithuanian independence regeneration with decline of Soviet military activities and, therefore, less military transport emissions (Baltrėnaitė, Butkus 2006).

Variation in metal concentrations may indicate tree disease. For instance, it was reported that in Scots pine tree rings of 1959–1960, infected with root rot (*Heterobasidion annosum* (Fr.) Bref.), concentrations of Ni and chromium (Cr) were 4 and 7 times higher, respectively, than mean TM concentrations in non-affected wood. Possible defensive functions of TMs in a tree against biotic stress have been hypothesized (Baltrėnaitė, Butkus 2006; Poschenrieder *et al.* 2006).

One of the most important aspects of tree growth analysis is tree-ring sampling. To determine TMs concentration precisely in a separate ring, the possibility of random errors during wood sampling must be eliminated. This guides to choose a precise tree-ring sampling method.

Wood sampling procedures to determine wood physical properties, tree age, diseases are well documented (Antonova *et al.* 1995; Camarero *et al.* 1998; Horacek *et al.* 1999; Nelson *et al.* 2000; Schmitt *et al.* 2003; Stravinskienė 2005; Rossi *et al.* 2006). However, tree-ring sampling for TM analysis is still scarce. Nelson *et al.* (2000) reported three different wood sampling methods in paper and pulp production: (a) cutting cylinders from a stem and graining; (b) crushing large wood slivers, and (c) using wood chips. Correlation was calculated to be higher than 0.80 between pulp concentration and wood amount after all methods used (Nelson *et al.* 2000).

To examine wood structure non-specific tools (surgical bone needle, trap system needle, trephor) to sample micro-cores were reported (Rossi *et al.* 2006). Extracted micro-cores are 15–20 mm in length and the inner size of cutting tube is about 1.2–2.0 mm in diameter. The mass of such a micro-core varies from 25 to 50 mg and is sufficient for microscopic analysis but not for determination of TMs concentration and, especially, when separate tree rings are needed. Hence, the sampling method has to be cheap, easy to administer and provide sufficient amount of samples for further analysis.

This paper examines three tree-ring sampling tools (common chisels, arched chisels and a plane) and compares TMs concentrations (Mn, Zn, Ni, Cr, Cu, Pb,) in wood samples collected by each of the approaches employed (Godbold and Hüttermann 1985; Breckle 1991; Nies 1999).

## 2. Methods

### 2.1. Sampling site

The investigated single pine tree (30 m high, 0.4 m thick, 55 year-old) was located in southern Lithuania, 10 km from the town of Alytus (Fig. 1a, b; site coordinates: E024°02’56.9” N54°18’33.7”), which is widely known to have had an intense industry when Lithuania was a part of Soviet Union (1940–1991). According to geochemical investigations carried out in 1998–1999, the soils are known to contain dangerously high metals contamination derived from nearby refrigerator and textile factories (Kadūnas *et al.* 1999). The anthropogenic load to topsoil consists mainly of metals such as Zn, Pb, Cu, Cd, Ni, Cr, Hg, Ag, Sn and Mo (Kadūnas *et al.* 1999).

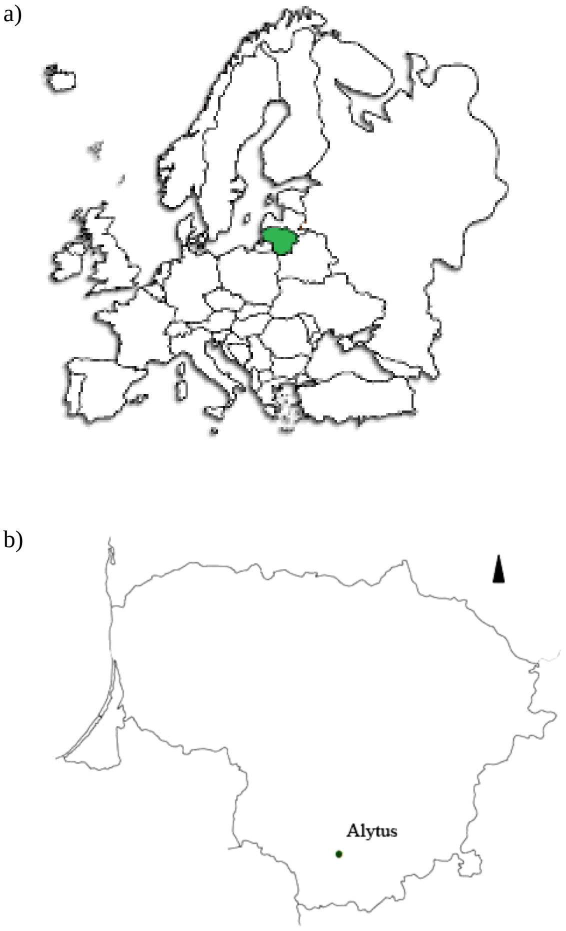

**Fig. 1.** Location of Lithuania in Europe (a); location of sampled tree (b)

Soil in the investigated area is sandy loam with a mean pH of 5.5. Landscape is hilly with an inclination of 3–5 degrees. Forest litter thickness varies from 4 to 5 cm; mean annual temperature is around 6.0 ºC. South-western wind with speed of 4.2 m/s prevails in the region. Mean annual precipitation is 619 mm. Pines prevail in Alytus region and cover 70–80% of the investigated forest area (Butkus, Beinaravičius 2005).

A single pine tree was randomly selected (Wilson 2005) from a group of pines growing in the vicinity of the exposed industrial zone. Permission was gained and granted to fell but was restricted to one tree. This was sampled at the beginning of vegetation period – late April (Hill 2002; Trapp 2007).

## 2.2. Wood sampling methods

The investigated pine tree was chain-sawn and three sections (each 5 cm wide) were cut at three different heights: 1 m above the ground, in the middle of the tree-trunk and at 3/4 of the trunk height (Fig. 2). The bark layer was removed and sections were air-dried in the laboratory. The surface of each section was polished to reveal the tree-ring patterns and a marker was used to highlight the boundaries of sequent annual rings.

*Tree-ring sampling with common chisels (Fig. 3b).* Using common chisels wood rolls were split into slivers containing one ring in the tangental direction (Taylor *et al*. 2003; Butkus *et al*. 2007).

*Tree-ring sampling with arched chisels (Figs. 3a and 4).* The method is similar to that mentioned above. The difference is in chisels that are arched to repeat tree-ring curvatures (Fig. 5).

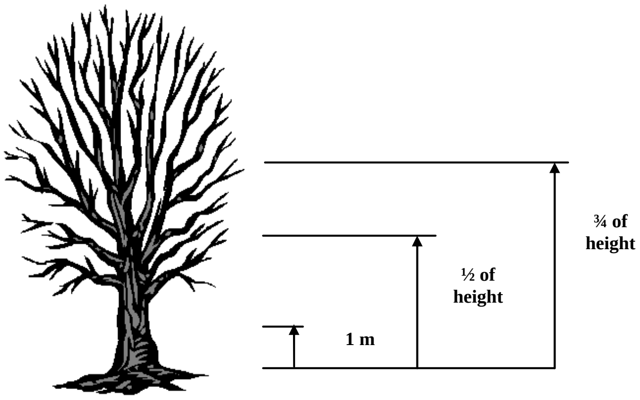

**Fig. 2.** Heights for cross-section sampling

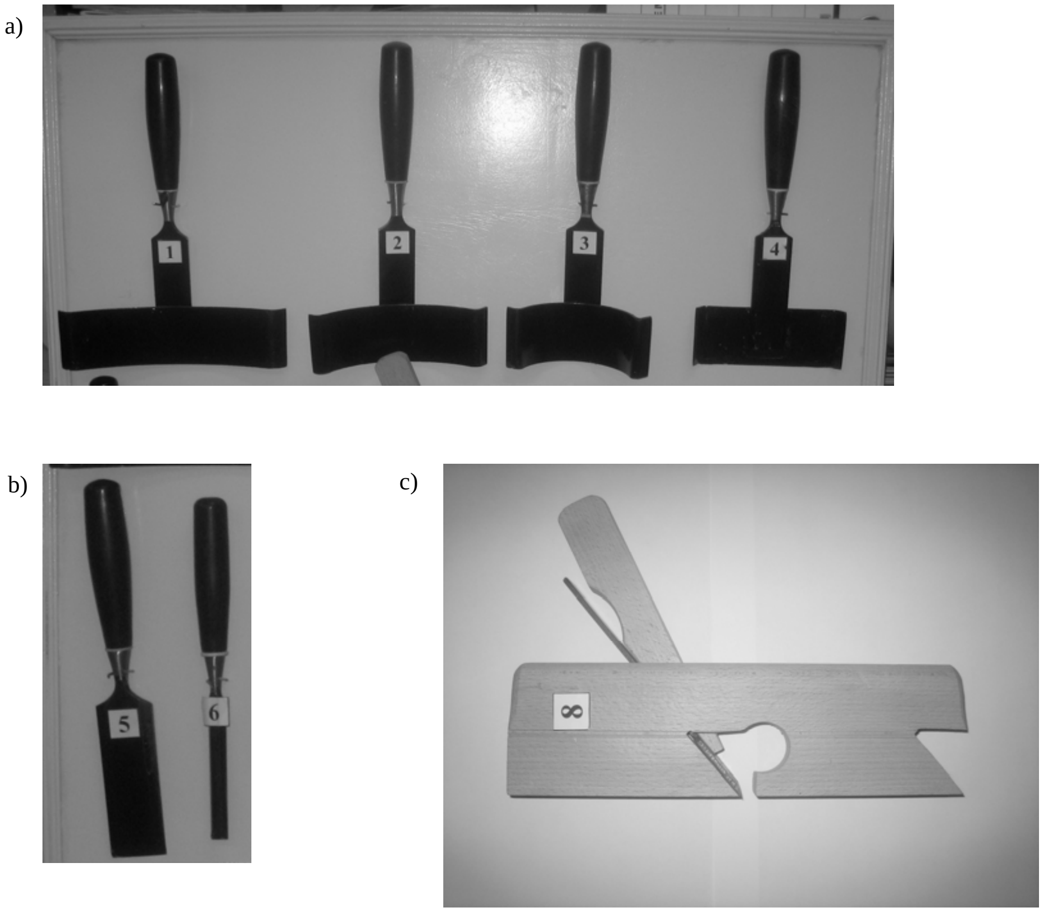

**Fig. 3.** Produced tree-ring sampling equipment: a) No 1, 2, 3, 4 – arched chisels; b) No 5, 6 – common chisels; c) No 8 – plane

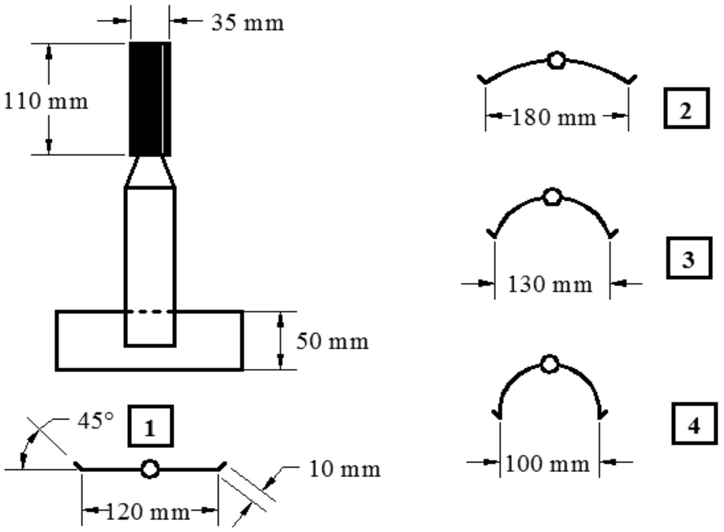

**Fig. 4.** Tree-ring sampling tools: No 1 – common chisel, No 2, 3, 4 – differently arched chisels (Beinaravičius 2005)

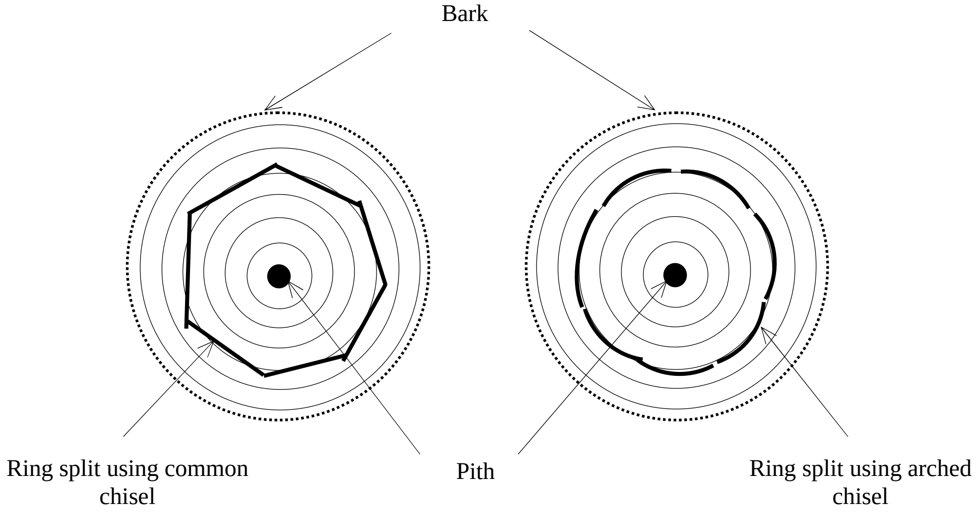

**Fig. 5.** Schematic view of ring splitting using two tools: common and arched chisels

Arched chisels are developed to separate tree rings of wooden plants of 10–80 years. A flat one is used to remove the bark and separate rings from rolls larger than 500 mm in diameter. To reduce risk of higher random errors the height of the roll cannot exceed 5 cm. Chisel blades were extended by welding plates on their surface produced from particularly hard steel which is resistant to long-term use and deformation. The leaning angle of a blade is $25^o$ and the height of a sharpened blade is 10 mm. A number of differently arched chisels were produced to fit ring curvature and reduce the risk of sampling error during the ring separation procedure. Chisel blades were extended with 10 mm of length-limiting extensions, perpendicular to a blade, which do not allow a ring to deviate and makes ring samples of a more accurate shape. Later rings should be sampled with chisels of a larger diameter and less arched, and rings close to the pith should be separated using chisels of a smaller diameter and higher arched (Fig. 3).

*Tree-ring sampling with a plane.* Separate ring samples were formed out of chips after planing away separate rings. Mass of one-specimen samples using common and arched chisels and a plane was in the range of 200–300 g (Fig. 3c).

Tree-sampling tools were produced at the Department of Environmental Protection (Vilnius Gediminas Technical University, Lithuania).

## Preparation of wood samples

After dissection (by the three techniques) the wooden samples were ground with a Retsch grinding machine RM 200 to 3–5 mm particles and prepared for TM analysis by digestion. Wood samples of 0.5 g each were taken and mixed with 8 ml of $HNO_3$ (65%) and 2 ml of $H_2O_2$

(30%). Then they were poured into specific vessels and put into a microwave digester *Milestone ETHOS* to be digested for 31 min. The solutions were poured into 50 ml flasks and diluted with deionized water to the mark of 50 ml. A parallel procedure was used for the five blanks (Soon, Abboud 1993).

### 2.3. Analysis of trace metal concentrations

A total of TMs was determined by flame atomic absorption spectrophotometer (FAAS). When they were below detection limits, graphite furnace atomic absorption spectrophotometer (GFAAS) was employed. Detection limits of Mn, Ni, Zn, Pb, Cu and Cr were 2.0; 90; 0.5; 10; 1.0 and 3.0 (μg/l) by FAAS and 0.01; 0.1; 0.001; 0.05; 0.02 and 0.01 (μg/l) for GFAAS.

### 2.4. Data analysis

Anderson-Darling tests were carried out to observe the normality of the elemental data for each tree-sampling technique. On the basis of that, nonparametric Kruskal–Wallis test was employed to investigate differential data of the methods. Median data and percentage coefficient of variation (CoV) were also determined.

## 3. Results and discussion

The results of TMs in wood showed no elevated values (Fig. 6) and were in the range of concentrations found in pine trees in the same region: Ni – 0.1–3.50 mg/kg; Cr – 0.1–1.50 mg/kg; Cu – 0.25–3.00 mg/kg; Mn – 10–160 mg/kg; Zn – 2–75 mg/kg, Pb – 0.05–2.80 mg/kg (Butkus, Baltrėnaitė 2007). Cu and Cr concentrations were in the range of values found in similar growing conditions abroad: Cr – 0.12–0.46 mg/kg; Cu – 0.15–1.02 mg/kg (Padilla, Anderson 2002). The highest concentrations in pinewood were typical of Mn and Zn (1.695±0.161 mg/kg and 0.471±0.206 mg/kg, respectively) followed by Pb (0.487±0.202 mg/kg) and Cu (0.290±0.021 mg/kg). The lowest concentrations were found for Ni (0.210±0.023 mg/kg) and Cr (0.117±0.010 mg/kg). Mn and Zn accumulation in tree xylem is considered to be associated with tree growth (Baes, McLaughlin 1984), hence, high amounts of Mn and Zn compared with the rest of the investigated metals indicated healthy tree growth conditions. The majority of Pb in tree wood comes from atmospheric pollution either directly through aerial interception or indirectly through the uptake of a highly Pb-contaminated soil (Bindled *et al.* 2004) which was not the case on the investigated site. Thus the source of Pb was likely the aerial uptake. Cu and Ni reach wood xylem by root uptake from acidic soils or the bark. Pb, if entered through the bark, is transported radially to a lesser extent and more accurate record changes in trace metal deposition (Watmough, Hutchison 1999). Zn, Cu and Cr are known to relate with the amount of air pollutants deposited on the stand (Pärn 2001).

*Statistical comparison*

Nonparametric Kruskal–Wallis *H*–tests were performed on each of the elemental parameters using the three tree-sampling techniques (Fig. 7). All the calculated *H*–values fail to exceed the critical *H*–value (at p <0.05), indicating that there are no statistically significant differences between the elemental data of different tree-sampling techniques (Table).

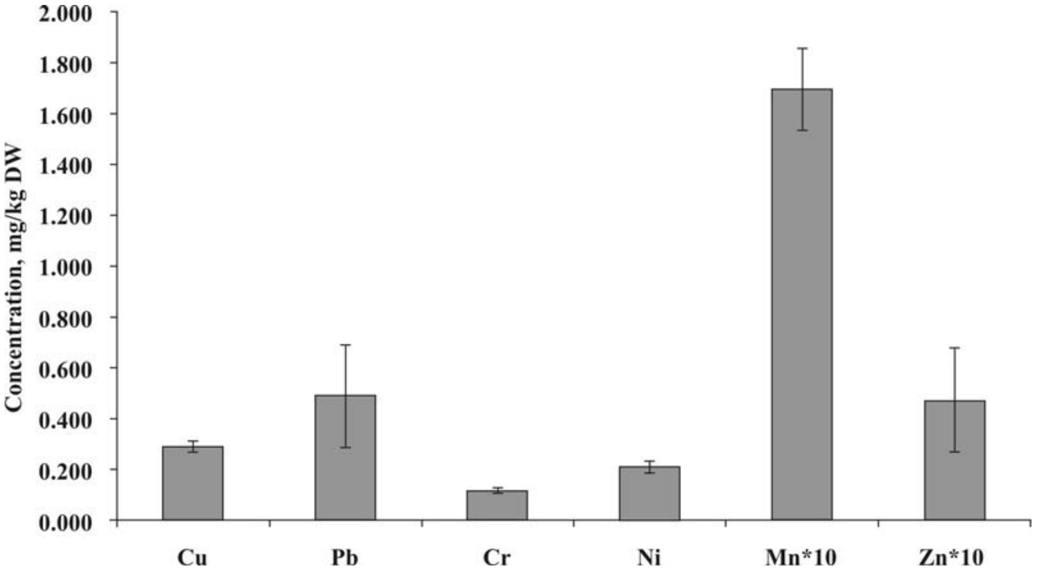

**Fig. 6.** Median data of metal concentrations in wood (mg/kg dry weight) (n = 24)

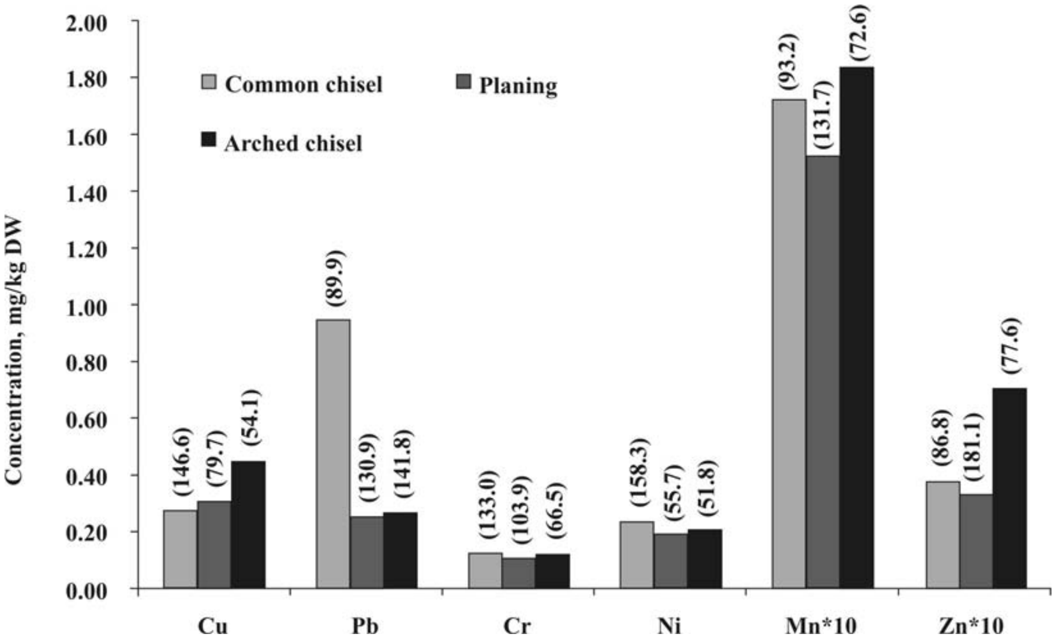

**Fig 7.** Median data of metal concentrations in wood (mg/kg dry weight) and the percentage coefficient of variation (in parenthesis) of the elemental data for each tree-sampling technique (n = 8)

**Table.** Kruskal–Wallis *H*–test analyses with p–values (in parenthesis) from the sample populations of the three sampling techniques for each elemental parameter (n = 8)

| TM | H–value | TM | H–value |
|---|---|---|---|
| Cu | 1.22 (0.544) | Ni | 0.65 (0.724) |
| Pb | 2.90 (0.235) | Mn | 1.37 (0.505) |
| Cr | 0.59 (0.743) | Zn | 4.45 (0.108) |

Critical *H*–value $p < 0.05$ = 9.49

### *Practical comparison of the techniques*

Practical comparison of the methods is further carried out according to the main four aspects: time consumed for sampling, method costs, skills required to conduct sampling and risk of errors. After comparison the overall evaluation is presented.

Time consumption for sampling is one of the most important factors. Rapid sampling methods always win over those that need time. Chisels and a plane can be used only for a felled tree. There are suggestions to use an increment borer to take a tree-ring sample, but the mass of a sample is rather small (~0.1 g) and for a sufficient amount (at least 5 g) of a ring sample, sampling must be repeated.

Planing of tree rings takes approximately one hour, plus one further hour is needed to screw and split rings from the wood bore. Since statistically at least three rolls are taken from a tree-trunk, the sampling procedures will last for about 10 hours when using any of the tools. Therefore, in terms of time, all the three methods are comparatively similar.

Sampling costs are usually related with the costs of sampling equipment. Common chisels and a plane are the most typical instruments used in household, especially by furniture-makers. Arched chisels were specially created and patented (Pranas Baltrėnas, Donatas Butkus, Rimantas Beinaravičius LT 5358 B), therefore, it is is more difficult to acquire them.

Higher costs can be associated with a sufficient number of sampled trees. Each felling of a tree is a matter of permit and costs.

Complexity of tool application is of significant importance taking into account potential mistakes and influence on the quality of results. Tree felling and splitting into rolls require technical assistance. At least three people must be employed in sampling. A saw, one-man force and tree-cutting skills are required. Practice has shown that planing rather than chisel use is more skill-based. The chisel method requires more human force.

Invention of arched chisels was based to repeat concentric circles of tree-rings when splitting. This is extremely useful for rings having different curvatures in the southern and northern parts of a tree-trunk. This feature makes arched chisels superior to common ones because splitting a part of adjacent tree-ring wood is less probable. Furthermore, probability of a random error is then less possible.

The requirement for ring sampling is to take samples from different heights of the investigated tree (1 m above the ground, in the middle of the tree-trunk and at 3/4 of the trunk height). This enables to compare TM concentrations at different trunk heights or homogenize wood of the same rings to determine mean TM concentrations along the trunk. In such a case only cutting down of a tree is possible and thus the use of all the above-mentioned methods then might be applied.

Further sample processing includes decomposition for which a mass of 0.1–0.5 g of wood sample is required. All the mentioned methods can be used according to wood sample mass.

*Related future research areas*

This study has shown that various techniques can be successfully used to split the tree-rings of pine trees. However, the main disadvantage of all the mentioned methods is that a tree must be felled before further sampling. Bearing in mind that metal analysis requires a representative number of samples to produce reliable results, felling of a big number of trees may require time and labour. An increment borer, which becomes more popular for tree-ring sampling also for metal analysis provides possibility of sampling a non-felled tree. In this case new risk, e.g. too small mass of sample, boring skills, must be considered.

## 4. Conclusions

1. Average values of metals in tree rings sampled with common chisels, arched chisels and a plane were 17.0±0.16 mg/kg for Mn, 4.70±0.21 mg/kg for Zn, 0.49±0.20 mg/kg for Pb, 0.29±0.02 mg/kg for Cu, 0.21±0.02 mg/kg for Ni and 0.12±0.01 mg/kg for Cr and were in the range of typical concentrations in the investigated region for *Pinus sylvestris* L. as well.
2. Statistical analysis (Anderson–Darlin test, Kruskal–Wallis tests, median data and percentage coefficient of variation) revealed no significant differences between metal concentrations determined in tree rings which were sampled using common chisels, arched chisels and planing.
3. Various tools – common chisels, arched chisels and a plane – can be successfully used for tree-ring sampling, however, an increment borer provides possibility of sampling a non-felled tree.

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## MEDIENOS RIEVIŲ ĖMINIŲ ĖMIMO SUNKIŲJŲ METALŲ ANALIZEI METODŲ PALYGINIMAS

**E. Baltrėnaitė, D. Butkus, C. A. Booth**

Santrauka

Medienos metinių rievių analizė suteikia informacijos apie medžio vystymąsi, svarbios inventorizuojant miškus, vertinant aplinkos poveikį, atliekant aplinkos oro ir dirvožemio monitoringą. Sunkiųjų metalų koncentracijos metinėse rievėse susijusios su metalų koncentracijomis aplinkoje ir gali nusakyti vietinės aplinkos taršos lygį. Vienas iš svarbių medienos analizės etapų yra metinių rievių ėminių ėmimas. Siekiant tiksliai nustatyti sunkiųjų metalų koncentraciją metinėje rievėje svarbu išvengti atsitiktinių paklaidų. Šiame darbe aptariami trys metinių rievių ėminių ėmimo metodai (įprastiniai kaltai, lenktieji kaltai ir obliavimas) ir lyginamos pagrindinių sunkiųjų metalų (Mn, Zn, Ni, Cu, Cr, Pb) koncentracijos ėminiuose, paimtuose kiekvienu iš minėtųjų metodų. Vidutinės sunkiųjų metalų koncentracijos, nustatytos šiais metodais, siekė 17,0±0,16 mg/kg Mn, 4,7±0,21 mg/kg Zn, 0,49±0,20 mg/kg Pb, 0,29±0,02 mg/kg Cu, 0,21±0,02 mg/kg Ni ir 0,12±0,01 mg/kg Cr ir buvo panašios į koncentracijas, aptiktas *Pinus sylvestris* L. medienoje nagrinėjamoje teritorijoje. Apskaičiuotos vidutinės vertės. Atlikus statistinę analizę (Anderson ir Darlin testas, Kruskal ir Wallis testas, variacijos koeficientas) tebuvo tik nereikšmingas sunkiųjų metalų koncentracijų metinėse rievėse skirtumas, ėminius ėmus aptartais metodais. Įvairūs metodai – įprastiniai kaltai, lenktieji kaltai ir obliavimas – gali būti sėkmingai taikomi metinių rievių ėminiams imti, tačiau „amžiaus“ grąžtu ėminius galima imti nenukertant medžio.

**Reikšminiai žodžiai:** „amžiaus“ grąžtas, kaltas, medienos ėminiai, metinė rievė, obliavimas, sunkieji metalai.

## СРАВНЕНИЕ МЕТОДОВ ВЗЯТИЯ ПРОБ ГОДИЧНЫХ КОЛЕЦ ДРЕВЕСИНЫ ДЛЯ АНАЛИЗА ТЯЖЕЛЫХ МЕТАЛЛОВ

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Резюме

Анализ годичных колец древесины предоставляет информацию о развитии дерева, что важно для инвентаризации леса, оценки воздействия на окружающую среду и мониторинга воздуха и почвы. Концентрации тяжелых металлов в годичных кольцах древесины зависят от их концентрации в окружающей среде и могут свидетельствовать об уровне загрязнения местности. Одним из важных этапов анализа древесины является взятие проб годичных колец. Для точного определения концентрации тяжелых металлов в годичных кольцах древесины важно избежать случайных погрешностей. В статье анализируются три метода взятия проб годичных колец древесины (дробление обычным долотом, круглым долотом и строгание) и сравниваются концентрации основных тяжелых металлов (Mn, Zn, Ni, Cu, Cr, Pb) в пробах, взятых каждым из названных методов. Средние концентрации тяжелых металлов при их измерении указанными методами достигали 1,695±0,161 мг/кг для Mn, 0,471±0,206 мг/кг для Zn, 0,487±0,202 мг/кг для Pb, 0,290±0,021 мг/кг для Cu, 0,210±0,023 мг/кг для Ni и 0,117±0,010 мг/кг для Cr. Эти концентрации тяжелых металлов аналогичны концентрациям в древесине сосны (*Pinus sylvestris* L) на данной исследуемой территории. При помощи статистического анализа [тест Андерсона–Дарлина (Anderson–Darlin), тест Крускаля–Валиса (Kruskal–Wallis), коэффициент вариации] выявлена лишь незначительная разница между концентрациями тяжелых металлов в годичных кольцах древесины, взятых уже названными методами. Методы дробления обычным долотом, круглым долотом и строгания могут успешно применяться для взятия проб годичных колец древесины, однако сверло „века“ предоставляет возможность взятия пробы без спиливания дерева.

**Ключевые слова:** сверло „века“, долото, пробы древесины, годичные кольца, строгание, тяжелые металлы.

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