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ORIGINAL PAPER

EVALUATION OF THE CONTENT OF INORGANIC ANIONS IN TREE SAPS

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Abstract

Tree saps were once commonly used in the countries of Northern, Central and Eastern Europe. Although once almost forgotten, their popularity has been growing recently as part of an interest in organic food and traditional medicine. Tree saps, tapped mainly from birch and maple trees, are drunk both fresh and fermented or are used as raw material for the production of food products, e.g. syrups. The aim of this study was to determine the content of inorganic anions in tree saps, as this parameter is often associated with toxicity of plant products. We measured the content of chlorides, nitrates and sulphates in the saps of six tree species with high pressure ion chromatography with the conductometric detection method. We detected the presence of chlorides, nitrates and sulphates in the studied tree saps. The highest concentration of nitrates was in the saps tapped from Norway maple (65.578 mg dm^{-3}) and boxelder (108.313 mg dm^{-3}). On the other hand, the highest concentration of sulphates was detected in the white willow tree saps (112.512 and 35.389 mg dm^{-3}), while the one of chlorides, in the hornbeam tree sap (47.257 mg dm^{-3}) and the Norway maple tree sap (41.143 mg dm^{-3}). The detected content of chlorides, sulphates and nitrates in the tree saps under study does not reach toxic levels. Therefore we conclude that tree saps do not pose any health threat to consumers regarding their content of inorganic ions. Additionally, we observed a high intra- and interspecies variability in the concentration of inorganic anions in saps. This observation should be taken into account while designing any quality monitoring protocol regarding tree saps.

Keywords: trees, tree saps, food analysis, food safety, anions, nitrates.

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INTRODUCTION

Gathering tree saps is a common tradition in many regions across the Northern Eurasia and North America. In Central Europe alone, as many as thirty different tree species were used to harvest saps. Among them, the most commonly used for tapping were the trees belonging to the birch (*Betulaceae*) and maple (*Aceraceae*) families (Svanberg et al. 2012, Papp et al. 2014). Maple saps were mostly processed into syrup or crystal sugar, yet easily perishable birch sap was consumed exclusively throughout the summer as a fresh or naturally fermented drink (Svanberg et al. 2012). Tree saps are valued for both nutritional and medicinal properties. As they are normally collected in the early spring, they were a welcome addition to the diet in the season, when food shortages were common in eastern and northern parts of Europe. In traditional medicine, birch sap has been used to treat kidney, stomach and liver diseases, kidney stones and gallstones in particular, as well as anaemia, pneumonia and a range of skin conditions (Svanberg et al. 2012, Papp et al. 2014).

Consumption of tree saps has been increasing significantly in recent years. It is a result of a renewed interest in traditional medicine together with a trend in searching for alternative and naturally occurring detoxifying products. Therefore, it is important to assess the safety and health benefits of tree sap consumption. For example, our group has recently published the results of analysis of tree saps in the context of metallic elements (Bilek et al. 2016), while others studied the elevated concentration of aliphatic compounds and heavy metals in saps of birch trees growing in polluted areas (Lewis et al. 2015).

Official guidelines for tapping trees focus on the selection of trees in proper location. Trees considered for tapping have to grow in areas far from sites of waste disposal or busy roads and free from any sources of air pollutants. Otherwise, collected saps may be contaminated with lead (Coli et al. 2009). Moreover, maple saps gathered in the proximity of roads, where table salt is used as a de-icing agent, were reported to contain significantly an increased concentration of chlorides. As a consequence, quality of such saps decreases and they have a characteristic salty flavour (Morselli, Whalen 1987).

Inorganic anions, such as chlorides or nitrates, may be present in tree saps at high concentrations as a result of their high content in soil (Schneider et al. 1996, White, Broadley 2001, Tagliavini, Millard 2005). The presence of inorganic anions should be naturally expected in saps, as they are necessary for the plant development (Grassi et al. 2002, Dürr 2009).

There are two main reasons why a high concentration of inorganic anions can be toxic. High levels of nitrates in the diet can cause a conversion of haemoglobin to methaemoglobin, which has a decreased oxygen-binding capacity. Furthermore they may be converted to nitrosamines, which are known carcinogens (EPA, EC 1997, EFSA 2008). On the other hand, high sulphate concentrations pose a risk of irritation to the gastrointestinal tract (WHO 2004).

The goal of this study was to determine the content of inorganic ions (in particular chlorides, sulphates and nitrates) in saps collected from the trees belonging to six different species (i.e. silver birch, downy birch, hornbeam, Norway maple, boxelder and white willow).

The saps were collected in two different villages in Podkarpackie Province, Werynia and Niwiska, which are located 15 km from each other. In Werynia, the saps were tapped from trees belonging to six different species. All studied trees in Werynia shared the same remote localization. Therefore, they were exposed to the same set of growing conditions, which allowed us to exclude this factor as a possible source of variability. In Niwiska, the saps were tapped exclusively from silver birch trees. However, the trees selected for tapping were growing in four different locations. We used collected data to assess the safety of consumption of investigated tree saps. Moreover, we addressed the question of interspecies variability in the content of inorganic anions in tree saps of the studied species.

MATERIAL AND METHODS

The saps were collected in the area of two villages, Niwiska and Werynia, in Podkarpackie Province, in Poland. In Werynia, samples were gathered between 26th of February and 16th of March 2014, whereas in Niwiska, samples were gathered between 24th and 28th of February 2014.

In Werynia, samples of saps were collected from three trees belonging to each of the six studied species: silver birch (*Betula pendula* Roth.), downy birch (*Betula pubescens* Ehrh.), hornbeam (*Carpinus betulus* L.), Norway maple (*Acer platanoides* L.), boxelder (*Acer negundo* L.) and white willow (*Salix alba* L.). All sampled trees were growing at the grounds belonging to the Institute of Applied Biotechnology and Basic Sciences of the University of Rzeszow (Werynia, Podkarpackie Province, 50°25′ N, 21°83′ E). Samples were gathered in the area free from any agricultural activity, remote from any residential areas as well as from any major through roads. In Werynia, tapped trees, belonging to any of the studied species, grew maximally 15 m apart from each other to minimize the variability of growing conditions. Other sap gathering sites were localized in Niwiska (Podkarpackie Province, 50°22′ N, 21°64′ E). Niwiska is located 15 km away from Werynia. In Niwiska, saps were tapped exclusively from silver birch trees (*Betula pendula* Roth.). In total, 20 individual silver birch trees were tapped in four different localizations within Niwiska. Four sample gathering sites within Niwiska area were: a pasture situated 50 m away from a farmland and 20 m away from a rarely used country road (site A); a plot localized around 20 m away from an active farm (within this plot, we observed a small stream containing wastes from the nearby farm) (site B); a remote forest site, 1.5 km away from any infrastructure (site C); a plot in the neighbourhood of a cultivated field, 30 m away from a rarely used country road (site D). The tapped trees were localized no further than 15 m apart from each other within each of above localizations.

We established a protocol for sample collection based on previously published methods (BILEK et al. 2016). Collected samples were immediately frozen after collection and transported to a laboratory, where their analysis was performed directly after thawing. Undiluted samples were degassed for 10 min and sterilized with syringe filters, MCE 0.45 μm (Alchem, Rzeszów, Poland) prior to chromatographic analysis. The content of inorganic anions was analyzed in the collected tree saps according to previously published methods (BILEK et al. 2015). In case the measured concentration values lay outside of the specified linearity range, the samples were diluted and the analysis was repeated.

Results were analysed with statistical analysis software – Statistica10 (StatSoft, Inc.). Two-way analysis of variance (ANOVA) and Least Significant Difference (LSD) tests were performed with the statistical significance level set at 5% (α = 0.05).

RESULTS AND DISCUSSION

We detected chlorides, nitrates and sulphates in the studied tree saps collected in Werynia. The concentration of chlorides was low and did not differ significantly in the sap samples from downy birch, silver birch and white willow. The highest content of chlorides was found in saps of Norway maple and hornbeam (Figure 1). On the other hand, we measured a low concentration of nitrates in the saps of silver birch, downy birch, hornbeam and white willow, while the highest concentration of these anions was detected in the samples from boxelder, followed by Norway maple (Figure 2). In regard to the content of sulphates, in samples collected in Werynia, we detected a significantly higher concentration of these ions in the samples from white willows in comparison with other studied species (Figure 3).

Interestingly, we detected a high variability in the content of studied inorganic anions between individual trees of the same species (Table 1).

In saps collected from the trees growing in Werynia, the observed differences were statistically significant. It is striking as all the sampled trees were of comparable age, shared the same growing conditions and were tapped in the same moment of the sap collecting season. We detected more consistent chlorides content in the saps from individual silver birch trees. Moreover, sulphates content was comparable in two out of three individual

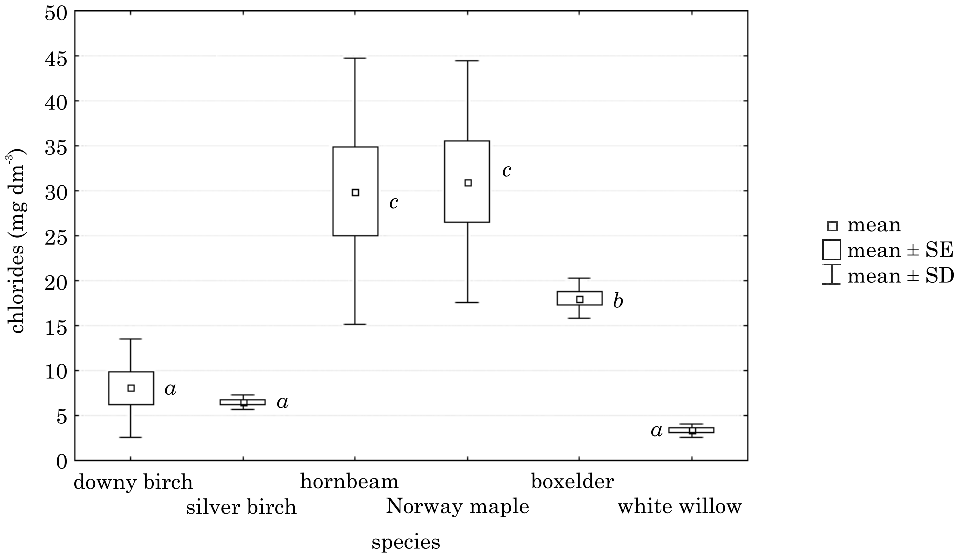

Fig. 1. Content of chlorides in the tree saps (Werynia). Same letters indicate lack of statistically significant differences between studied species (α = 0.05)

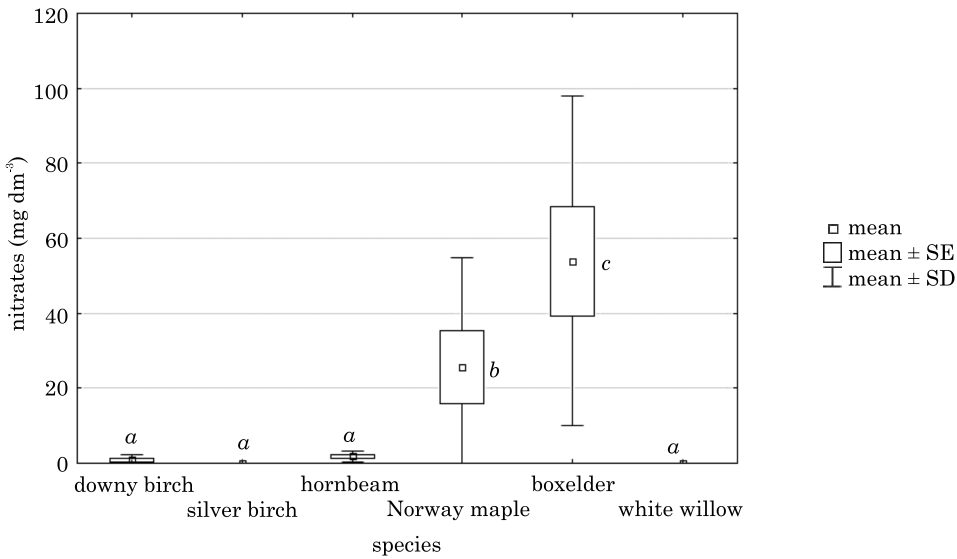

Fig. 2. Content of nitrates content in the tree saps (Werynia). Same letters indicate lack of statistically significant differences between studied species (α = 0.05)

downy birches and boxelders. Finally, the content of nitrates remained below the detection threshold for many studied samples.

We measured the content of chlorides, sulphates and nitrates in the saps collected from twenty silver birch trees growing in four different locations in

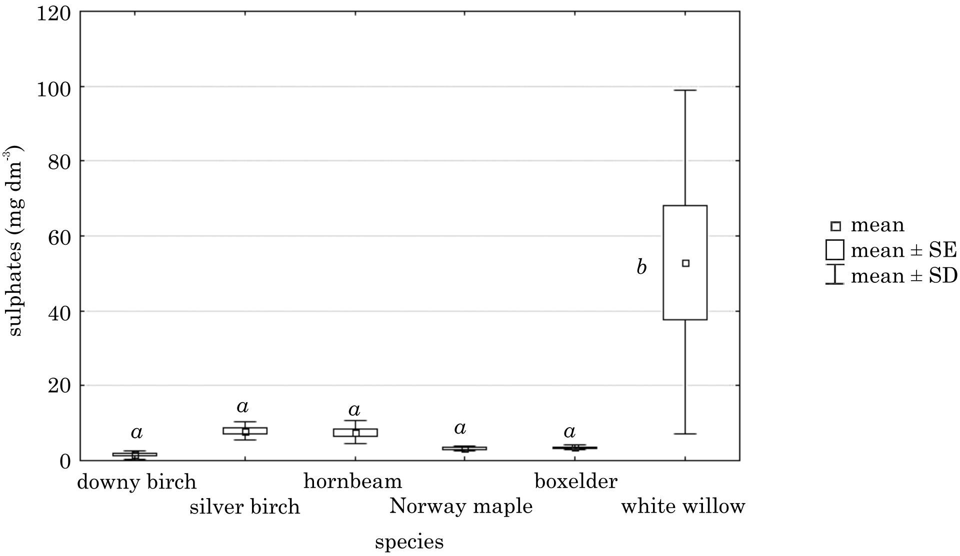

Fig. 3. Content of sulphates in the tree saps (Werynia). Same letters indicate lack of statistically significant differences between studied species (α = 0.05)

Table 1

Content of inorganic anions in saps from trees growing in Werynia

Species		Mean inorganic anion content (mg dm^{-3}) ±SD (n = 2)		
		chlorides	nitrates	sulphates
Hornbeam *Carpinus betulus*	1.	$47.257^{c} \pm 0.008$	nd	$9.963^{c} \pm 0.061$
	2.	$13.041^{a} \pm 0.064$	$2.777^{a} \pm 0.001$	$3.698^{a} \pm 0.003$
	3.	$29.406^{b} \pm 0.059$	$2.765^{a} \pm 0.002$	$9.144^{b} \pm 0.014$
Boxelder *Acer negundo*	1.	$15.454^{a} \pm 0.08$	$108.313^{c} \pm 0.095$	$3.106^{a} \pm 0.008$
	2.	$20.505^{c} \pm 0.054$	$45.416^{b} \pm 0.065$	$4.25^{b} \pm 0.005$
	3.	$18.093^{b} \pm 0.015$	$8.067^{a} \pm 0.112$	$3.30^{a} \pm 0.015$
Silver birch *Betula pendula*	1.	$5.918^{a} \pm 0.02$	nd	$10.228^{c} \pm 0.015$
	2.	$7.592^{b} \pm 0.015$	nd	$8.731^{b} \pm 0.006$
	3.	$6.081^{a} \pm 0.001$	nd	$4.863^{a} \pm 0.012$
White willow *Salix alba*	1.	$2.605^{a} \pm 0.013$	nd	$112.512^{c} \pm 0.015$
	2.	$4.317^{c} \pm 0.015$	nd	$10.957^{a} \pm 0.046$
	3.	$3.182^{b} \pm 0.002$	nd	$35.389^{b} \pm 0.026$
Downy birch *Betula pubescens*	1.	$15.394^{c} \pm 0.048$	nd	$2.403^{a} \pm 0.014$
	2.	$4.888^{b} \pm 0.003$	nd	nd
	3.	$3.886^{a} \pm 0.005$	$2.782^{a} \pm 0.012$	$2.308^{a} \pm 0.005$
Norway maple *Acer platanoides*	1.	$13.177^{a} \pm 0.021$	$5.451^{a} \pm 0.021$	$2.468^{a} \pm 0.011$
	2.	$38.718^{b} \pm 0.019$	$7.275^{b} \pm 0.025$	$4.163^{c} \pm 0.027$
	3.	$41.143^{c} \pm 0.038$	$65.578^{c} \pm 0.043$	$3.011^{b} \pm 0.013$

The mean values within the same species followed by the same letters do not differ significantly (α = 0.05); nd – not detected

Niwiska. We detected significant variation in the content of chlorides among both individual trees and among different locations where the trees were growing (Figure 4). The lowest concentration of chlorides was detected in

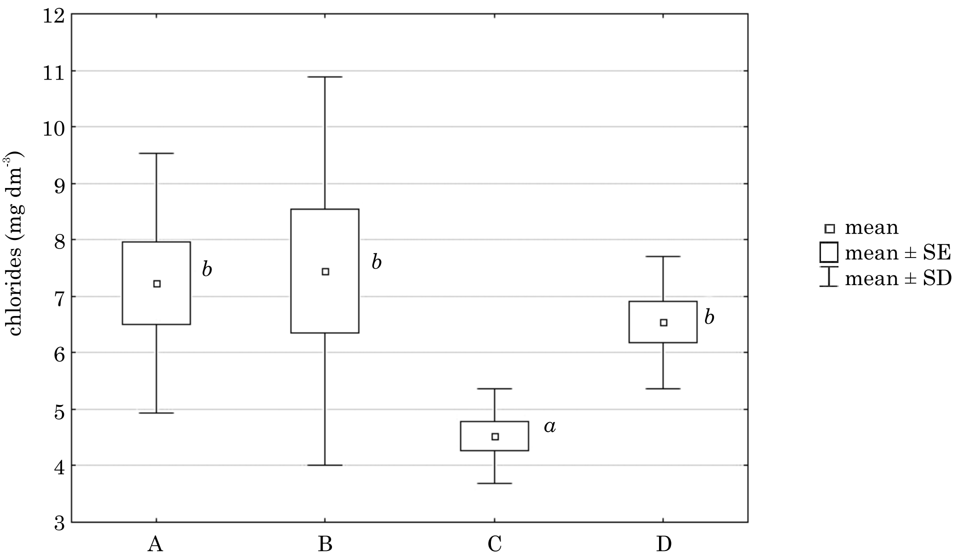

Fig. 4. Content of chlorides in saps from silver birch trees (Niwiska): A – a site close to a pasture, B – a site close to a farm, C – a site in the middle of a forest, D – a site close to a field (for details see Materials and Methods). Same letters indicate lack of statistically significant differences between studied locations (α = 0.05)

saps from trees growing in the middle of a forest (site C), far from agricultural areas and roads, whereas the highest concentration of chlorides was detected in saps tapped from trees growing in the proximity of a pasture or a farm (sites A and B). Moreover, we observed the lowest variability in the chlorides content in saps from trees growing in the forest (site C) and the highest variability among trees growing in the proximity of a farm (site B).

As far as the concentration of sulphates is concerned, we did not detect any significant differences between trees growing in four studied locations (Figure 5). Nevertheless, we observed significant variability in the content of sulphates in saps from trees growing in all of these locations. Interestingly, the saps tapped from trees growing in close proximity to farms (site B), which were characterized by the highest variability in the content of chlorides, showed the lowest variability in the content of sulphates. On the other hand, the highest variability in the content of sulphates was detected among the trees growing in the proximity to a field and a road (site D).

It is noteworthy that nitrates were not detected at all in the saps from silver birch trees in any of the studied sites in Niwiska. It is surprising, as the trees growing in the proximity to a field, a farm or a pasture are potentially exposed to elevated levels of nitrates from anthropogenic sources, such as mineral fertilizers or animal faeces. The variability between silver birch

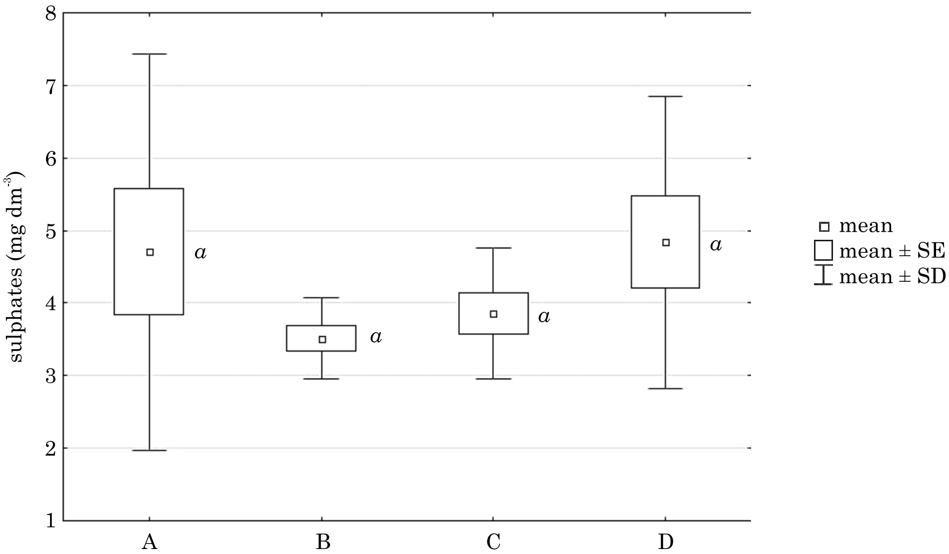

Fig. 5. Content of sulphates in saps from silver birch trees (Niwiska): A – a site close to a pasture, B – a site close to a farm, C – a site in the middle of a forest, D – a site close to a field (for details see Materials and Methods). Same letters indicate lack of statistically significant differences between studied locations (α = 0.05)

saps collected in Niwiska was comparable with the variability observed between saps collected in Werynia (compare Table 1 and Table 2).

Based on the measured values of concentrations of inorganic ions in saps collected from individual trees growing in close proximity to each other in both Werynia and Niwiska, we conclude that there is high intrinsic variability in the concentration of measured inorganic ions in the saps of the studied species. In comparison, HARJU and HULDÉN (1990) studied the content of metallic elements in saps of birches growing in increasing distances from a geological fault that was rich in metal sulfides. They noted that the content of metallic elements is the highest in saps of the trees growing in the immediate vicinity to geological faults (HARJU, HULDÉN 1990). On the other hand, MORSELLI and WHALEN (1987) found significant differences between the trees growing in the middle of a forest and those growing at the edge of it, close to roads where sodium chlorides is used for de-icing. The above observation remains in agreement with our studies as we consistently detected low concentration of chlorides in saps from silver birches growing in the middle of a forest in Niwiska. Such differences are also observed by plant physiologists who reported the content of chlorides and nitrates in the tree saps to be dependent on the content of these anions in soil (SCHNEIDER et al. 1996, WHITE, BROADLEY 2001, TAGLIAVINI, MILLARD 2005). However, in the first part of our study (in Werynia), the environmental and climate conditions were shared by all tapped trees, therefore our study emphasizes the significance of intrinsic inter-individual variability.

Table 2

Content of inorganic anions in saps from silver birch trees growing in Niwiska

Site		Mean inorganic anions content (mg dm^{-3}) ± SD (n = 2)		
		chlorides	nitrates	sulphates
A	1.	$4.973^{a} \pm 0.024$	nd	$2.711^{a} \pm 0.154$
	2.	$5.337^{b} \pm 0.091$	nd	$2.768^{a} \pm 0.134$
	3.	$7.482^{c} \pm 0.001$	nd	$2.713^{a} \pm 0.078$
	4.	$7.279^{c} \pm 0.168$	nd	$6.223^{b} \pm 0.132$
	5.	$11.083^{d} \pm 0.179$	nd	$9.124^{c} \pm 0.166$
B	1.	$12.957^{e} \pm 0.126$	nd	$3.256^{a} \pm 0.017$
	2.	$8.624^{d} \pm 0.570$	nd	$3.418^{a} \pm 0.039$
	3.	$3.235^{a} \pm 0.082$	nd	$4.504^{b} \pm 0.024$
	4.	$6.758^{c} \pm 0.008$	nd	$3.238^{a} \pm 0.383$
	5.	$5.649^{b} \pm 0.179$	nd	$3.158^{a} \pm 0.311$
C	1.	$4.447^{b} \pm 0.235$	nd	$3.696^{b} \pm 0.473$
	2.	$5.016^{c} \pm 0.086$	nd	$2.707^{a} \pm 0.044$
	3.	$3.096^{a} \pm 0.050$	nd	$5.309^{c} \pm 0.082$
	4.	$4.667^{bc} \pm 0.035$	nd	$3.980^{b} \pm 0.088$
	5.	$5.390^{c} \pm 0.204$	nd	$3.616^{b} \pm 0.181$
D	1.	$4.637^{a} \pm 0.039$	nd	$5.825^{d} \pm 0.014$
	2.	$6.662^{c} \pm 0.165$	nd	$3.262^{b} \pm 0.097$
	3.	$6.167^{b} \pm 0.087$	nd	$7.857^{e} \pm 0.404$
	4.	$7.698^{d} \pm 0.051$	nd	$2.469^{a} \pm 0.047$
	5.	$7.531^{d} \pm 0.074$	nd	$4.784^{c} \pm 0.155$

Different letters in columns denote significant differences between individual trees at the significance level α = 0.05, nd – not detected; A – a site close to a pasture; B – a site close to a farm; C – a site in the middle of a forest, D – a site close to a field (for details see Materials and Methods)

In comparison with the previously published work of Kūka et al. (2013), concerning the content of inorganic anions in two selected tree species, our results show much larger interspecies differences in the content of nitrates (for all studied species in Werynia). Interestingly, those authors report that nitrate-nitrogen concentration in sap samples collected at two different locations from maple and birch trees was consistent for any given species, 3.4 mg dm^{-3}, i.e. 15.062 mg dm^{-3} of nitrates respectively (Kūka et al. 2013). Moreover, we measured a lower total concentration of sulphates in the collected tree saps of birches and maples than the one reported by the Kūka et al. (2013). Additionally, the chloride concentration in maple tree saps was higher in our study while the concentration of chlorides in the birch tree saps was comparable.

Based on the measured concentration of inorganic ions, we addressed the question about the safety of their consumption. The WHO (World Health Organization) recommends the concentration of chlorides in drinking water to remain below 250 mg dm^{-3} (WHO 2003). In our study, we detected the highest concentration of chlorides (47.257 mg dm^{-3}) in the sap collected from one individual hornbeam tree. In comparison, the concentration of chlorides remains much lower in commercially available mineral water, from 1.28 to 25.7 mg dm^{-3}, and soft drinks, from 3.33 to 7.47 mg dm^{-3} (Pasternakiewicz et al. 2014). Tree saps are therefore a richer source of chloride ions but remain well within the recommended safety range. The WHO reports that sulphates can irritate the gastrointestinal tract in infants if present in a concentration above 630 mg dm^{-3} (WHO 2004). We detected the highest sulphate content of 112.512 mg dm^{-3} in the sap of one of the studied white willows. This is a safe yet high sulphates content, as mineral waters contain usually from 0.78 to 88.55 mg dm^{-3} and soft drinks from 3.26 to 23.09 mg dm^{-3} of sulphates (Pasternakiewicz et al. 2014, Bilek et al. 2015). The concentrations of nitrates found in the two tree saps from Werynia, i.e. 65.578 mg dm^{-3} in Norway maple sap and 108.313 mg dm^{-3} in boxelder sap, exceed the allowed limit of nitrates in drinking water, as recommended by the Ministry of Health Regulation, i.e. 50 mg dm^{-3} (*Regulation* ... 2010). In comparison, mineral water contains from 0.62 to 6.66 mg dm^{-3}, whereas soft drinks contain from 1.31 to 5.1 mg dm^{-3} of nitrates (Pasternakiewicz et al. 2014, Bilek et al. 2015). However, the EFSA (European Food Safety Authority) and JECFA (The Joint FAO/WHO Expert Committee on Food Additives) define the maximal acceptable intake of nitrates for adults to remain below 3.7 mg kg^{-1} (EFSA 2008). Thus, according to these health standards, an adult weighing 70 kg, can drink safely more than two litres of tree sap with the highest recorded concentration of nitrates. Tree saps can be therefore considered a significant source of nitrates, yet they do not pose any health threat. However, the fact that we did not detect any nitrates in birch saps collected in Niwiska emphasizes the need for testing individual batches so that exact information about anion content could be provided to consumers.

We conclude that regarding the content of chlorides, sulphates and nitrates, tree saps do not pose any health threat to consumers. However, the seasonal variation in the content of anions in tree saps should be addressed further, as it was done in studies by Kalio and Ahtonen (1987*a*, *b*) and Ahtonen and Kalio (1989) in regard to organic acids, sugars and amino acids or mineral substances by Harju and Huldén (1990).

CONCLUSIONS

1. The content of chlorides, sulphates and nitrates in the tree saps under study does not reach toxic levels. Tree saps do not pose any health threat to consumers regarding their content of inorganic ions.

2. High intra- and interspecies variability in the concentration of inorganic anions in saps was observed. This observation should be taken into account while designing any quality monitoring protocol regarding tree saps.

3. Silver birch tree sap, most popular tree sap in Poland, is characterized by the absence of nitrates and a low concentration of sulphates, irrespective of the gathering sites of sap. In turn, the lowest concentration of chlorides, which was detected in saps from trees growing in the middle of a forest, shows that the most favourable tree sap collection sites occur away from anthropogenic impact.

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