

Nutritional composition of Abies x borisii-regis Mattf. cones from Bulgaria

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Abstract. In this study, a proximate analysis and determination of the mineral content of *Abies x borisii-regis* cones from Bulgaria were carried out in connection with their potential use in the pharmaceutical industry. *A. x borisii-regis* cones showed to be rich in carbohydrates (79.06 g 100⁻¹ g⁻¹ dw), which were the most abundant macronutrients. The proteins in the *A. x borisii-regis* cones averaged 11.19 g 100⁻¹ g⁻¹ dw. The total energy content in the *A. x borisii-regis* cones is relatively high 1665.10 g⁻¹ dw. The obtained data on the content of trace elements in the cones of *A. x borisii-regis* show the highest content of Fe (137.03 mg kg⁻¹), followed by Mn (95.7 mg kg⁻¹) and Zn (70.06 mg kg⁻¹). The results of the proximate analysis of the cones of Tsar Boris fir, as well as the total content of trace elements, suggest the possibility of their direct use in pharmaceuticals and dietetics.

Key words: *Abies x borisii-regis* Mattf., proximate analysis, trace elements.

Introduction

The genus *Abies* is thought to include 52 recognized species, with an additional 58 unidentified species not yet included (WFO, 2023). This second-largest genus in the family Pinaceae is considered to be more complex than other genera in this family, and all of its species are distributed in the Northern Hemisphere (Nikolić et al., 2021). *Abies x borisii-regis* Mattf. (Pinaceae), commonly known as the Bulgarian fir, Tsar Boris fir, or Macedonian fir, is an endemic plant distributed in the southern part of the Balkan Peninsula. The species grows in humid bioclimates at altitudes of 700–1800 m in Bulgaria, North Macedonia, Serbia,

Kosovo, and northern Greece (Farjon, 2010; Farjon & Filer, 2013; Farjon et al., 2014).

Currently, in the flora of Bulgaria, *A. x borisii-regis* has the taxonomic status of a subspecies - *Abies alba* subsp. *borisii-regis* (Jordanov, 1963–1979). In the Flora Europaea (Chater, 1964), Flora Hellenica (Christensen, 1997) and Mountain Flora of Greece (Strid, 1986), *A. x borisii-regis* is treated as an independent species, a hybrid between *A. alba* and *A. cephalonica*, and this classification is based mainly on morphological characteristics distinctive to the three species (Farjon, 1989; Delcheva et al., 2010; Bella et al., 2015). *A. x borisii-regis* has a limited distribution in the southern part of the

Balkan Peninsula, but its range is not well defined in the literature. According to Flora Europaea (Chater, 1964) and Flora Hellenica (Christensen, 1997), it is distributed in the Balkan Peninsula from Southern Albania to the Thrace region. According to Flora of Bulgaria (Jordanov, 1963–1979), its distribution in Bulgaria is in the mountains in the southern part of the country (Slavyanka, Belasitsa, Southern Pirin, Middle and Western Rhodopes, Southwestern Rila) in an area of elevation between 900 and 1800 m. (Andreev, 1992; Chipev et al., 2015). The species takes part in the threatened habitat 9270 Hellenic beech forests with *A. x borisii-regis*. It is included in the Red List in Bulgaria under the category of Least Concern (LC) (Petrova & Vladimirov, 2009). The plant very rarely performs edifying functions. It occurs mainly as single individuals or small groups of trees in the composition of forests of common beech or coniferous forests of other species, and in places it forms a second floor (more often in white pine or black pine, as well as beech forests). Therefore, despite the significant distribution of the species, the considered habitat type is extremely rare in Bulgaria (Chipev et al., 2015). In Greece, this species, together with *A. cephalonica*, is protected *in situ* in various areas (Alizoti et al., 2011).

A. x borisii-regis is similar to *A. alba* in all its properties and applications (Nikolić, 2023). The various components of another species - *Abies marocana* Trab. (needles, twigs, and cones) have attracted attention in the field of pharmaceuticals due to the presence of active compounds (Zirari et al., 2024a,b), including carbohydrates, proteins, and lipids. These components play a key role in the human body and are used for various physiological, metabolic, and morphological processes (Prakash et al., 2021). Proximate analysis carried out for *A. marocana* revealed a variety of components in different parts of the plant, including moisture, ash, fiber, protein, carbohydrates, and fat. Cones and twigs were found to be rich in mineral elements. (Zirari et al., 2025). To our knowledge, similar studies on cones of *A. x borisii-regis* have not been carried out to date.

Conifers are particularly suitable for monitoring studies due to their wide distribution, adaptation to different habitats, and multi-year growth of needles, which allows the assessment of chemical element concentrations in different age ranges. (Sandulovici et al., 2024). Pollutants from the soil

are transported to the above-ground organs, eventually accumulating in the leaves (Siedlecka, 1995; Günthardt-Goerg et al., 2019). In addition, conifers have a remarkable ability to absorb various substances, including sulfur and heavy metals, from atmospheric emissions (Ceburnis & Steinnes, 2000; Viskari et al., 2000; Trimbacher & Weiss, 2004; Suchara et al., 2011). Some trace elements, including copper, manganese, iron, and zinc, are essential for plants and play important roles in metabolic processes (Påhlsson, 1989). However, when these elements are present in high levels in the soil, they become toxic to both plants and microorganisms. On the other hand, lead and cadmium, which are heavy metals that are not necessary for the functioning of plant organisms (Marschner, 1995; Cheng, 2003), can also be harmful. Therefore, to ensure the quality and safety of future plant-based pharmaceutical preparations, the presence of certain heavy metals has been investigated.

This study aims to perform a proximate and mineral content analysis of the cones of *A. x borisii-regis* Mattf. (Tsar Borisov fir), endemic to the southern parts of the Balkan Peninsula, as a potential opportunity for their use with pharmacological benefits. Similar published studies of the cones of *A. x borisii-regis* originating from Bulgaria have not been carried out to date.

Materials and methods

Plant material and samples preparation

The study was carried out on three-year-old mature cones of *A. x borisii-regis*. The cones were collected from one genotype from the floristic region of the Rhodopes (Western), between the villages of Golyamo Belovo and Yundola (Fig. 1). The material was air-dried between filter papers. Samples with an approximate weight of 5 g were used for the study.

Reagents

Reagents are qualified "AR" (Merck & Fluka). The starting standard solutions for ICP determination of Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb, and Cd at concentrations of 1000 mg l⁻¹ were supplied by Merck, Darmstadt, Germany. Water was deionized in a Milli Q system (Millipore, Bedford, MA, USA) to a resistivity of 18.2 MΩ cm. All plastic and glassware were cleaned by soaking in diluted HNO₃ (1/9, v/v) and rinsed with distilled water before use.

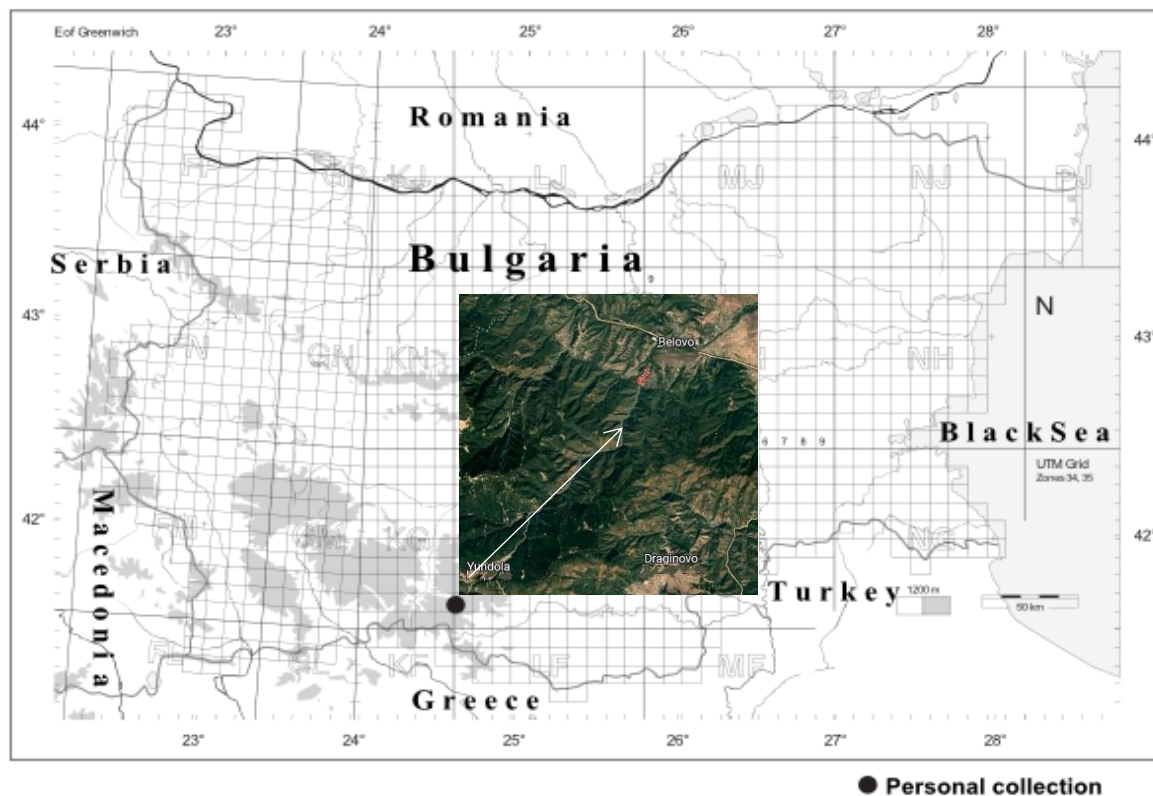


Fig. 1. Plant material collection area.

Proximate composition analysis

Determination of crude protein

The protein content was determined using Folin phenol reagent. 0.5 g of the powdered sample of *A. x borisii-regis* cones. The sample was extracted with 50 cm 2% NaCl in a water bath at 60°C for 1 hour. The extract was filtered, and 50.0 cm 3% copper acetate monohydrate was added to the filtrate to precipitate the protein. The precipitated protein was then centrifuged and dissolved in 50 cm.

Carbohydrates

Total carbohydrate was determined by 2 g of each sample in 50 ml of distilled water, of which 0.2 ml was diluted tenfold. To 1 ml of the resulting solution and serial dilutions of glucose stock (10 mg 100 ml⁻¹) solution, 4 ml of a throne reagent was added, and the absorbance of the solutions was measured by a spectrophotometer at 620 nm against a reagent blank.

Determination of crude fat

Crude fat was determined by extracting 2 mg moisture-free samples with petroleum ether in a Soxhlet extractor, heating the flask on a sand bath for about 6 h till a drop taken from the drippings

left no greasy stain on the filter paper. After boiling with petroleum ether, the residual petroleum ether was filtered using Whatman No 40 filter paper, and the filtrate was evaporated in a pre-weighed beaker. An increase in the weight of the beaker gave the crude fat.

Ash content

A powdered sample (3 g) of *A. x borisii-regis* cones was ashed in a Gallenkamp furnace in a pre-ignited and cooled crucible of known weight at 55°C for 6 h fairly cooled crucibles were put in desiccators and weighed.

Energy

Total energy was calculated according to the following equation: Energy (kJ) = 17 (g protein + g carbohydrate) + 37 (g lipid).

Moisture content

The fresh weight of each *A. x borisii-regis* cone sample was taken using a chemical balance. These samples were then oven dried separately at 105°C for 24 h. The loss in weight obtained after drying was regarded as the moisture content.

Digestion procedures

Multiwave 3000 closed vessel microwave system (maximum power was 1400 W, and the maximum pressure in Teflon vessels - 40 bar) was used in this study. *A. x borisii-regis* cones samples (0.25 g) were digested with 6 ml of HNO₃ (65%) and 1

ml of H₂O₂ (30%) in microwave digestion system for 23 min and diluted to 25 ml with deionized water. A blank digest was carried out in the same way. All sample solutions were clear. Digestion conditions for the microwave system are given in Table 1.

Table 1. Microwave acid digestion programme.

Step	Ramp time, (min)	Hold time (min)	Cooling period (min)	Pressure (MPa)	Temperature (°C)
1	10	10	5	0.758	110
2	10	10	5	1.023	150
3	20	10	5	0.758	190

Analytical procedure

Quantitative determination of the concentration of the studied trace elements (Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb, Cd) was carried out in the mineralized samples by Multi-Quadrupole ICP-MS.

Accuracy and precision

In order to validate the method for accuracy and precision, the certified reference materials (CRM) - Virginia Tobacco Leaves (CTA-VTL-2) were analysed for the corresponding elements. The results are shown in Table 2. For the evaluation of the correctness of the results, three generally accepted criteria are used as follows:

(1) $D = X - X_{CRM}$, where X is the measured value, and X_{CRM} is the certified value. When D is within the borders of $\pm 2\sigma$, where σ is the standard deviation from the certified value, the result is considered to be good; when it is $-3\sigma \leq D \leq 3\sigma$, satisfactory, and beyond these limits, the result is unsatisfactory.

(2) $D\% = D / X_{CRM} \times 100$. When the values of $D\%$ are in the limits $\pm 200\sigma / X_{CRM}$, the result is considered to be good; when the value is in the limits $\pm 200\sigma / X_{CRM}$ and $\pm 300\sigma / X_{CRM}$, satisfactory; and when it is out of the limits $\pm 300\sigma / X_{CRM}$, the result is unsatisfactory.

(3) $Z = X - X_{CRM} / \sigma$. When $Z \leq 2$, the result is considered to be good; when $2 \leq Z \leq 3$, satisfactory; when $Z > 3$, unsatisfactory.

For evaluation of the accuracy of the digestion and measuring procedures, we have used the R criterion, showing the extent of extraction of the element in percent from the certified value. When the measured value X is within the borders of $X_{CRM} \pm U_{CRM}$, where U_{CRM} is the uncertainty of the certified value, we accept an extent of extraction to

be 100%. In all the remaining cases, the extent of extraction is equal to $X / X_{CRM} \times 100$. As can be seen from the tables, the results obtained for all certified materials yield a recovery of 100% for both elements.

Results and Discussion**Chemical composition of *A. x borisii-regis* cones**

Nutrient composition of *A. x borisii-regis* cones is presented in Table 3. *A. x borisii-regis* cones showed to be rich in carbohydrates (79.06 g 100⁻¹ g⁻¹ dw), which were the most abundant macronutrients. For comparison, the carbohydrate content of *A. marocana* cones averages 64.35 g 100⁻¹ g⁻¹ dw (Zirari et al., 2025) and of *Pinus nigra* cones averages 67.8 g 100⁻¹ g⁻¹ dw (Robert et al., 2003). The biological role of carbohydrates is related to providing the necessary energy. (Achi et al., 2017). They are responsible for fueling various cells in the body, including those in the brain, muscles, and blood (Thomas & Krishnakumari, 2015).

The proteins in the *A. x borisii-regis* cones averaged 11.19 g 100⁻¹ g⁻¹ dw. Studies of Zirari et al. (2025) on *A. marocana* show markedly higher protein content of the cones (2.584 ± 0.022%) in comparison to the protein content of the twigs (1.753 ± 0.027%). According to Bwai et al. (2014), plants are considered good protein sources if their protein caloric value is more than 12%. In this regard, the cones of the Tsar Boris fir can be defined as a moderate source of protein.

The total energy content in the *A. x borisii-regis* cones is relatively high, 1665.10 ± 8.67⁻¹ g⁻¹ dw. According to the research of Zirari et al. (2025), the energy content of the twigs on *A. Marocana* (329.490 ± 2.022 kcal 100⁻¹ g⁻¹ dw) surpasses

that of the cones ($324.692 \pm 3.424 \text{ kcal } 100^{-1} \text{ g}^{-1} \text{ dw}$). These results are in accordance with the outcomes documented by Pathak & Kapil (2004) for *Picea abies* needles.

The moisture content of the cones of *A. x borisii-regis* was 8.73 % of dw. In their research on *A. marocana*, Zirari et al. (2025) point out $12.142 \pm 0.127\%$ moisture content in the cones. The recorded moisture content closely approximated the moisture content of pine wood as documented by Jones et al. (1985). The moisture content of the cones of *A. x borisii-regis* was 8.73% of dw. The lower moisture level in the cones of Tsar Boris fir is a prerequisite for extending the shelf life of the plant material (Kris-Etherton et al., 2002).

The ash content in plant organs is an indicator of the concentration of mineral substances. The cones of *A. x borisii-regis* have 7.50% of dw ash content. In comparison, in *A. marocana*, the ash content in the cones was $7.640 \pm 0.788\%$ (Zirari et al., 2025). The fat content in the Tsar Boris fir cones is similar to that of the Moroccan fir, 4.63% of dw and $4.052 \pm 0.046\%$ respectively. Studies by Onwuka (2005) on *A. alba* and *Picea abies* found fat concentrations reaching $11.027 \text{ mg g}^{-1} \text{ dw}$ and $12.810 \text{ mg g}^{-1} \text{ dw}$ in the heartwood, respectively. Lipids play a key role in the human diet. They are a valuable source of energy, facilitate the transport of fat-soluble vitamins, contribute to essential cellular functions, and provide insulation and protection for internal tissues (Ejelonu et al., 2011). A proper diet should provide 1%–2% lipids (Emebu & Anyika, 2011).

The results of the approximate analysis of the cones of the Tsar Boris fir show a potential opportunity for their use as a valuable source of impor-

tant nutrients and energy, making them suitable as a nutritional supplement to enrich human diet.

The results for the efficiency of microwave mineralization for Fe, Ni, Cr, Cu, Co, Zn, Mn, and Cd determination in the certified referent material - Virginia tobacco CTA- VTA-2 are displayed in Table 3. The results from the descriptive analysis of the concentration of Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb, and Cd in *A. x borisii-regis* samples are presented in Table 4.

The obtained data on the content of trace elements in the cones of *A. x borisii-regis* have the following descending order of element concentrations: $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Ni} > \text{Co} > \text{Cd} > \text{Pb} > \text{Cr}$. The accumulation of metals in plants and their distribution depend on the physiological processes of evaporation and transpiration (Fargašová, 2009). According to Kmet (1996), they can be physiologically controlled by the plant. Nikolov et al. (2003) indicate the following descending order of element concentrations in *A. x borisii-regis*: $\text{Cr} > \text{Ni} > \text{Pb} > \text{As} > \text{Cd}$. According to Nechita et al. (2025), Norway spruce had a higher accumulation capacity of Cr, Ni, Fe, Zn, and Cu. The authors point out that in *Picea abies* leaves, the metals show a descending order of mean concentrations as follows: $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu}$ and $\text{Sr} > \text{Cr} > \text{Ni} > \text{Li} > \text{As} > \text{Cd}$. Together with minerals, toxic metals (Hg, Cu, Pb, Ni, Zn, As) are assimilated, affecting plant physiology (Montoya et al., 2020). Micronutrients are essential for numerous physiological processes and play a crucial role in human nutrition (Shahar et al., 2023). Minerals play a crucial role in supporting the proper functioning of tissues and act as secondary messengers in various biochemical processes (Martínez-Gómez et al., 2022).

Table 2. Effectiveness of microwave mineralization in the determination of Fe, Ni, Cr, Cu, Co, Zn, Mn, Pb, and Cd in Virginia Tobacco-CTA-VTA-2 certified reference material (n = 3).

Element	Certified value	Observed value Microwave digestion	Recovery (%)
Fe	1083 ± 33	1160 ± 45	103
Ni	1.98 ± 0.21	1.92 ± 0.17	98.1
Cr	1.87 ± 0.16	1.83 ± 0.14	93.6
Cu	18.2 ± 0.8	18.1 ± 0.7	99.4
Co	0.429 ± 1.4	0.420 ± 0.02	98.0
Zn	43.3 ± 2.1	44.1 ± 1.6	101.8
Mn	79.7 ± 2.6	77.5 ± 2.1	97.2
Pb	22.1 ± 1.2	23.0 ± 0.8	104
Cd	1.52 ± 0.17	1.50 ± 0.05	98.7

Table 3. Moisture (% of DW*), macronutrients (% of DW*), and total energy (kJ/100g of DW) in the *A. x borisii-regis* cones.

Components	\bar{X}	SD	-95% Confid.	+95% Confid.
Moisture	8.73	± 0.39	7.77	9.68
Ash	7.50	± 0.68	5.81	9.19
Crude protein	5.75	± 0.07	5.59	5.91
Crude fat	4.63	± 0.15	4.27	4.99
Total carbohydrates	82.12	± 0.89	79.91	84.33
Total energy	1665.10	± 8.67	1643.56	1686.64

Each value is expressed as mean ± SD (n = 3). Means with different letters within a row are significantly different (p < 0.05) levels by Duncan's Multiple Range Test.

* DW - dry weight

Table 4. Concentration of trace elements in *A. x borisii-regis* samples collected from Rhodopes (Western) mountain, Bulgaria (n = 3)

Element	\bar{X} mg kg ⁻¹	SD mg kg ⁻¹	- 95% Confid.	+ 95% Confid.
Fe	137.03	± 13.56	103.35	170.71
Ni	14.78	± 1.21	11.79	17.78
Cr	0.17	± 0.04	0.07	0.27
Cu	25.79	± 2.08	20.61	30.97
Co	1.63	± 0.32	0.84	2.42
Zn	70.06	± 6.92	52.86	87.26
Mn	95.70	± 10.24	70.27	121.13
Pb	0.22	± 0.13	-0.09	0.54
Cd	1.58	± 0.17	1.15	2.02

The cones of the King Boris fir showed the highest content of Fe (137.03 mg kg⁻¹). Fe plays a role in energy transfer within the plant and also regulates the oxidation of biomolecules (Do et al., 2014). Iron is an element that controls the physiological functions of enzymes. Due to its involvement in numerous metabolic processes, iron is essential for the normal functioning of the human body (Kumari et al., 2017). Iron is vital for the synthesis of hemoglobin (Parkash et al., 2015). It acts as a catalyst for specific enzymes such as cytochrome oxidase and is an essential element in proteins (Do et al., 2014).

In second place in terms of concentration in the cones of *A. x borisii-regis* is Mn (95.7 mg kg⁻¹). Mn is a nutrient with physiological implications since it acts as an activator and cofactor of various metalloenzymes (Schmidt & Husted, 2019).

The third trace element with high concentration in the cones of *A. x borisii-regis* is Zn (70.06 mg kg⁻¹). Zn is mainly valued as a plant nutrient that enhances their resistance to drought stress by re-

gulating physiological and molecular functions, such as cell membrane stability, stomatal regulation, and water use efficiency, as well as a stimulator of antioxidant activity (Umair et al., 2020; Kaur & Garg, 2021). Zinc is a trace element present in all living organisms. Zinc concentrations range from 10 to 100 ppm. It plays a fundamental role in the generation of chlorophyll in plant cells and is involved in many metabolic processes. It also ensures the synthesis of enzymes. Zinc content correlates with magnesium. Excess zinc can lead to reduced concentrations of manganese and copper. Conversely, a lack of phosphate can also cause reduced zinc content in plants. Zinc deficiency in plants results in stunted cell growth (Raven & Jonson, 1986). Zinc variability correlates with seasonal changes, similar to K, Cr, and Mn. For the human body, zinc is essential for protein synthesis, cell differentiation, DNA replication, reproductive processes, and immune function (Ameen et al., 2021).

According to Grešíková & Janiga (2017), Zn, Mn, and Ni are well-distributed to the entire plant

and accumulated both in the roots and in the aboveground plant parts (Fargašová, 2009). According to Paluch & Gruba (2010) and Augusto et al. (2002), the foliage of hardwood species usually has higher concentrations of N, K, Ca and Mg than does that of coniferous species.

The study of Zirari et al., (2025), on *A. marocana* shows the content of Fe 38.66 mg kg⁻¹ in cones and 53.5 mg kg⁻¹ in twigs. The content of Zn was 23.66 mg kg⁻¹ in twigs and 13.98 mg kg⁻¹ in cones. The Mn was 8.2 mg kg⁻¹ in twigs and 25.58 mg kg⁻¹ in cones. There are similar results in the research of Lima Rojas (Vunchi et al., 2011) on the mineral composition of bark, wood, and heartwood of *A. religiosa*.

Of the other trace elements found in the cones of King Boris fir, Cu and Ni have a relatively high content (25.79 mg kg⁻¹ and 14.78 mg kg⁻¹, respectively). For comparison, in the leaves and wood of *P. abies* Nechita et al. (2025) found 9.26 mg kg⁻¹ and 1.78 mg kg⁻¹ Cu and 16 mg kg⁻¹ and 2.22 mg kg⁻¹ Ni, respectively. Cu is essential for enzymatic activities, chlorophyll production, respiration, antioxidant systems, and signal transduction (Chen et al., 2022). Nickel (Ni), in small amounts, is beneficial for regulating metabolism through enzymatic activity.

In the cones of *A. x borisii-regis*, we found a relatively low chromium content - 0.17 mg kg⁻¹. In *P. abies*, Nechita et al. (2025) found 46 mg kg⁻¹ and 4.05 mg kg⁻¹ Cr contents in the leaves and wood, respectively. Cr is taken up by plants through phosphate or sulfate transporters (Srivastava et al., 2021). Several toxic effects of Cr on plant physiology, biochemistry, molecular traits disrupting the cell cycle, enzyme activity, nitrogen assimilation, and antioxidant activity, were previously reported (He et al., 2021; Ali et al., 2023; Zulfiqar et al., 2023).

Of the remaining trace elements, Cd can be noted, which in the cones of King Boris fir is 1.58 mg kg⁻¹, while in *P. abies*, its content varies from 0.24 mg kg⁻¹ in the leaves to 0.09 mg kg⁻¹ in the wood (Nechita et al., 2025). Cd reduces Fe and Zn uptake in plants (Xu et al., 2017).

Macro- and microelements, such as calcium, magnesium, manganese, and phosphorus, are part of the composition of human bones and teeth. They help transmit nerve impulses, produce various hormones, and serve as a means of regulating the regular heartbeat (Zirari et al., 2024a). A

large part of microelements maintains the proper functioning of tissues and acts as secondary mediators in various biochemical processes (Martínez-Gómez et al., 2022). The majority of microelements, such as copper, iron, manganese, magnesium, and zinc, serve as vital components in numerous enzymes, playing a key role in their structural composition (Gharibzahedi & Jafari, 2017).

Conclusions

The results of the proximate analysis of the *A. x borisii-regis* cones show a potential opportunity for their use as a valuable source of important nutrients and energy, which makes them suitable as a nutritional supplement for enriching the human diet.

The total content of trace elements determines the cones of Tsar Boris fir as environmentally friendly and a suitable additive for pharmaceutical products related to maintaining and improving human health.

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