

Research Article

Assessment of Albino Beech Supremacy to Pigmented Beech Proves to Be A Better Environmental Condition Bioindicator

Renata Gagić-Serdar*, Miroslava Marković, Ljubinko Rakonjac, Goran Češljarić and Bojan Konatar

Institute of Forestry, Kneza Višeslava 3, 11030 Belgrade, Serbia

Abstract

The research was conducted in southern Serbia, at an altitude of 600 meters, in a beech stand, on a dwarf albino specimen further usually called *Absiente pigmente fagus* or just *Absiente pigmente*. Soil sampling and analysis were carried out at depths of 0–10 cm, 11–20 cm, and 21–30 cm. Laboratory tests included determination of the textural composition of the soil, total humus content, total nitrogen, total phosphorus and potassium, the sum of absorbed base cations, determination of hydrolytic acidity, and both active and substitutional acidity. The soil was found to have a sandy textural composition, with a strong to very strong acidic reaction and a low degree of saturation with base cations. It was further determined to be highly humic, well-supplied with total nitrogen and plant-available potassium, but poorly supplied with phosphorus. Chemical composition analyses of the leaves were simultaneously conducted on both pigmented and non-pigmented leaves. The composition of 15 macro and microelements was examined. The results showed that their concentration in albino leaves was 30% – 50% higher, except in the case of Se. The presence of heavy metals such as cadmium, chromium, and nickel was significantly higher in the non-pigmented beech tissue compared to the green tissue (38.56% for Cd, 22.97% for Cr, and 44.89% for Ni). The research was conducted for the purpose of heavy metal extraction from the soil and green restoration, and it shall continue to this end.

Introduction

Biological monitoring of soil contamination, where higher plants serve as indicators, can be conducted at all levels of the organization of living systems, starting from the molecular level, through biochemical and physiological, cellular, individual, populational, species-related, biocenotic (ecosystem), to biome and biosphere levels. The advantage of biological indication compared to physicochemical methods of environmental pollution monitoring lies in the fact that living organisms can show the effect of the accumulation of pollutants over a longer period of time. On the other hand, physicochemical methods provide more precise data, but this data is only available at a specific moment in time. MAC - maximum allowable concentration is the concentration of pollutants that does not lead to changes in human health.

These maximum allowable concentrations are most often defined and prescribed by health organizations that focus solely on the human population. However, this does not necessarily mean that these are also the MAC for other living organisms.

The use of higher plants in the study of soil contamination is based on their ability to absorb metals (especially heavy metals) and other toxic substances from the soil and either transport them through their organism or accumulate them in leaves and other tissues. Some of these elements are essential for plants in physiological processes (such as manganese, zinc, copper, molybdenum, etc.). In natural conditions, on soils rich in heavy metals, especially above ore deposits, specialized species or genetically differentiated chemo-ecotypes develop, which indicate the presence of such metals or a specific

More Information

*Address for correspondence:

Renata Gagić-Serdar, Institute of Forestry, Kneza Višeslava 3, 11030 Belgrade, Serbia,
Email: serdarrenata79@gmail.com

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chemical element in the substrate. Generally speaking, plants adapted to such metal-rich soils are termed *Metallophytes* and can be indicators of specific mineral or ore deposits in different locations on Earth or within stands. Indicators of heavy metals include mosses, lichens, floating plants, henna, etc. Their mere presence indicates the existence and potential increase in the concentration of certain heavy metals in the soil in which they thrive. Plant species that are indicators of serpentine habitats, for instance, indicate soils that are low in calcium (Ca) and enriched with magnesium (Mg), as well as nickel (Ni), chromium (Cr), and cobalt (Co).

Potentially, any organic species can be used as a bioindicator of environmental conditions. A necessary prerequisite for this is the knowledge of the biology and ecology (idioecology) of each species used as a bioindicator. It is also necessary to know the ecological amplitude of the species for each environmental factor (temperature, humidity, light, soil pH, etc.). The ecological amplitude of each species for any environmental factor can vary greatly (Chatfield, et al. 2013). This principle also applies to the concentration of pollutants in the environment, as in the inorganic environment. Stenovalent organisms are those with a narrower ecological amplitude, and in the context of pollutants, they are more suitable for biological indication because they are used for qualitative analysis of environmental changes (e.g., lichens). Euryvalent organisms are those with a broader ecological amplitude, and in the context of pollutants, they are less suitable for biological indication because they are used for quantitative analysis of environmental changes (such as the abundance of organisms, population density, etc.). In this context, unlike lichens that disappear upon the occurrence of toxic elements, Albino beech acts as an absorber [1].

Methods

Soil samples were taken from a single pedological profile, from 3 layers: at depths of 0-10 cm, 11-20 cm, and 21-30 cm. Leaf samples were collected using standard methods from both the albino beech and a chlorophyll-containing beech specimen, both located in proximity.

The laboratory tests on the soil included the following methods: the textural composition was determined by the sedimentation method using sodium pyrophosphate as a peptizing agent [2]. Based on the mechanical composition, the soil texture class was determined using the Ferre triangle. The total humus content (organic carbon) was determined by wet combustion in a solution of potassium dichromate and sulfuric acid using the Tyurin method (Ratz, Škorić, 1969). The total nitrogen content in the soil was determined by digesting the sample in sulfuric acid in the presence of a catalyst to convert all forms of nitrogen into ammonium form. The ammonium form was distilled by steam distillation and captured in boric acid using the Kjeldahl method [3]. The content of plant-available forms of phosphorus and potassium was determined

using the AL-method according to Egner-Riehm (Džamić, et al. 1969). Phosphorus was determined by colorimetry, and potassium by flame photometry.

The sum of absorbed base cations was determined by extraction in hydrochloric acid at a concentration of 0.1 mol/l and titration with sodium hydroxide using the Kappen method [4]. Hydrolytic acidity was determined by extraction in sodium acetate at a concentration of 1 mol/l and titrated with sodium hydroxide at a concentration of 0.1 mol/l using the Kappen method [4]. The active and substitutional acidity of the soil solution (pH in H₂O and pH in KCl) was measured conductometrically (SRPS ISO 10390:2007 Soil Quality - Determination of pH value).

Results and Discussion

A group of metals, such as copper (Cu), cobalt (Co), iron (Fe), nickel (Ni), magnesium (Mg), molybdenum (Mo), chromium (Cr), selenium (Se), manganese (Mn), and zinc (Zn), are essential for various physiological and biochemical functions. However, some of these metals in high doses can have harmful effects on the body, while others, such as cadmium (Cd), mercury (Hg), lead (Pb), chromium (Cr), silver (Ag), and arsenic (As), can cause diseases even at very low concentrations. They are non-biodegradable, poorly soluble, and easily accumulate in living organisms. Their relative density is high, even exceeding 4x10⁶ mg/L. They can bind to vital cellular components such as nucleic acids, structural proteins, and enzymes.

In 86.67% of cases (13 out of 15) for all examined elements, albino beech has a higher percentage of microelements.

In the future, we need some tasks as first to do, if we want proper research development: Geno-fond preserving, seed formation following, followed by *in vitro* testing of its resistance to unfavorable conditions and symbiotic efficiency under controlled experimental conditions, all this in different conditions affecting. It is important that the new generation will save the attributes of the previous, which is the goal, in the end, some kind of intricate super plant processing. The response of plant types to various stress conditions and their effectiveness in atmospheric oxygen fixation in such conditions often differ greatly from that in the field.

In this study, the preparation of leaf samples for chemical composition analysis was performed using microwave digestion. The microwave digestion was conducted in a Milestone Ethos up Easy system. Approximately 0.2 g of dried sample was weighed (mass measured to four decimal places). The sample was then digested with 8 ml of concentrated nitric acid, HNO₃ (68%), and 2 ml of H₂O₂ (30%) at a maximum temperature of 200 °C. The preset microwave digestion program lasts a total of 30 minutes, with a maximum power of 1800W. During the first 15 minutes, the temperature gradually increases to 200 °C. After that, the temperature is maintained



at 200 °C for 15 minutes and then gradually cooled (Milestone Digestion application). After cooling to room temperature, the samples were filtered into a 100 ml standard flask and filled with deionized water to a final volume of 100 ml. A blank sample was prepared in the same way as the actual samples.

The samples prepared in this way were analyzed on a Perkin-Elmer ICP-MS. This technique, in which inductively coupled plasma is used as an ionization source and detection is performed by mass spectrometry, is known as Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The ICP-MS instrument utilizes a high-resolution mass spectrometer, which, by physically constraining the ion beam through a narrow slit of various dimensions and specific design, enables precise isotope focusing and supports three levels of resolution: low, medium, and high. By selecting the appropriate resolution for a specific element, maximum separation of the isotopes being measured is achieved. It is also possible to simultaneously determine 50 elements - multi-element analysis [5]. This instrument is characterized by high sensitivity and a wide linear range. It can be equipped with an autosampler, providing the capability for automation and measurement of a large number of samples [6].

ICP-MS was used to determine Al, Na, Fe, Ni, Mn, Zn, Mo, B, Se, Cu, Cr, As, Cd, Hg, and Pb. The parameter conditions used in ICP-MS are summarized in Table 1. Blank samples were analyzed and subtracted from the sample measurements before the results were calculated. Each sample was measured three times, and the average value was used.

Laboratory

There is a possibility that common stunted development of multiple organelles in cells with defects occurs in Albino beech, but autophagy has somehow been avoided, as indicated by Jung, et al. [7]. It is likely that in Albino individuals without healthy chloroplasts, this space is physiologically filled with toxins or metals, with the possibility that these concentrations are necessary for their unimpeded functioning (possibly as a form of defense). These are bold hypotheses that are yet to be proven, but there is a reasonable suspicion that in due course they will be confirmed as facts, supporting the concept of obligate parasitism in plants. Most of these conclusions would more precisely explain the reasons that make the Albino beech different from the ordinary beech.

The appearance of the leaf mass of the dwarf Albino individual *Fagus moesiaca*, alongside the leaf mass of a

chlorophyll-containing individual of the same species in proximity (on which the research was conducted), is shown in Figure 1.

The height of the Albino beech is approximately 50 cm; the individual is mature, as it bears fruit, but its exact age has not been determined.

The reaction of the soil solution in the surface layer is acidic (Graph 1, Tables 1-4).

With the soil depth, the pH value decreases further, and the reaction becomes strongly acidic. The total absorption capacity of the soil surface layer is high due to the high humus content in the soil. With increasing depth, the total absorption capacity decreases. Based on the degree of saturation of the absorption complex with basic cations (positively charged metal ions), the soil is classified as dystric throughout its depth. Based on the humus content, the surface soil layer (0–10 cm) belongs to the class of highly humus-rich soils. The examined soil belongs to sandy loam throughout the depth, according to its textural class (Table 2). The dominant textural fraction is fine sand, followed by silt, with clay being the least represented (Figure 2).

The second analyzed layer (11–20 cm) lies at the transition

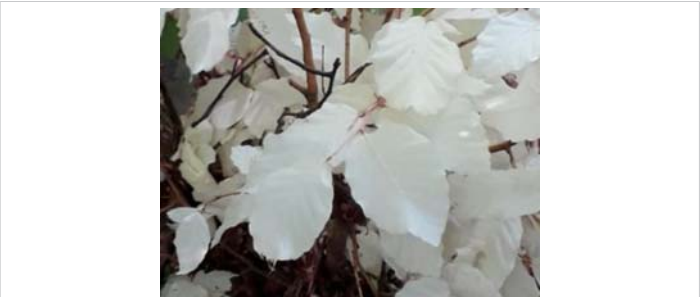
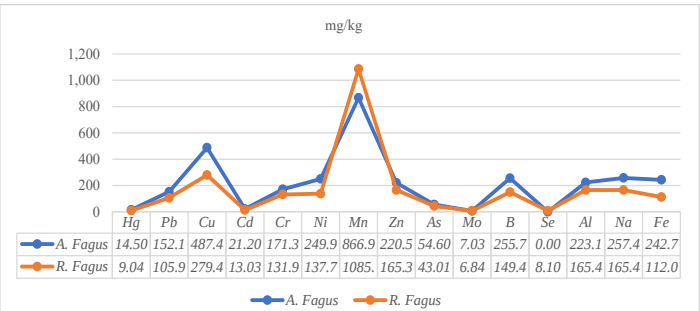


Figure 1: Leaves of Albino *Fagus moesiaca* (Absinthe pigment) in April 2024.



Graph 1: Graphical representation of macro and microelement content in leaves with and without chlorophyll.

Table 1: Chemical properties of the soil

Sample No.	Depth cm	pH		Absorptive complex							C/N	Affordable	
				T	S	T-S	V	Y _i	Humus	N		P ₂ O ₅	K ₂ O
		H ₂ O	KCl	cmol/kg	cmol/kg	cmol/kg	%	cm ²	%	%		mg/100g	mg/100g
1	0-10	5.02	4.14	36.83	16.35	20.48	44.38	31.52	6.59	0.58	6.56	4.51	30.70
2	11-20	4.93	3.95	22.85	5.06	17.79	22.14	27.37	5.15	0.43	6.98	3.46	32.00
3	21-30	4.64	3.49	24.9	5.37	19.42	21.65	29.88	3.31	0.30	6.41	1.55	20.80

Table 2: Graphical representation of particle size distribution from powder to coarse sand, from soil samples taken from three horizons in a profile excavated beneath the beech tree *Absinte Pigmente*.

Seq. No. of Sample	Soil depth	Coarse sand	Fine sand	Dust/Powder	Clay	Total Sand	Total Clay	Texture class
	cm	%	%	%	%	%	%	
1	0-10	11.12	43.80	33.97	11.12	54.92	45.08	Sandy clay loam
2	11-20	10.52	44.35	34.52	10.62	54.86	45.14	Sandy clay loam
3	21-30	10.58	42.31	35.31	11.80	52.89	47.11	Sandy clay loam

Table 3: Content and concentration differences of elements in the leaves of green and albino beech (mg/kg). Albino *Fagus* - Albino leaf, Regular *Fagus* - Green leaf.

#	Hg	Pb	Cu	Cd	Cr	Ni	Mn	Zn	As	Mo	B	Se	Al	Na	Fe
1. A. <i>Fagus</i>	14.50	152.19	487.45	21.20	171.30	249.91	866.93	220.59	54.60	7.03	255.79	<LD	223.11	257.42	242.72
2. R. <i>Fagus</i>	9.04	105.90	279.43	13.03	131.96	137.73	108.59	165.32	43.01	6.84	149.49	8.10	165.46	165.41	112.00
% AF/RF	37.70	30.42	42.67	38.56	22.97	44.89	-25.22	25.06	21.23	2.70	41.56		25.84	35.74	53.85

Table 4: ICP-MS parameter conditions for testing the chemical composition of leaves.

Power of a radiofrequency (RF) generator (W)	1600
Auxiliary gas flow (l/min)	1.2
Nebulizer gas flow (l/min)	15
Peristaltic pump speed (rpm)	35
Sample intake time (s)	20

**Figure 2:** Dwarfism – dwarf growth of the Albino beech *Fagus moesiaca*, Absinthe pigmente (Orig.)

between moderately humus-rich and highly humus-rich soils, while the third layer (21–30 cm) belongs to the class of moderately humus-rich soils. The high humus content provides a high nitrogen content throughout the analyzed depth. The carbon-to-nitrogen ratio is low, relatively unremarkable, which enables rapid mineralization of the organic matter and a quick transformation of organic nitrogen forms into mineral forms accessible to plants. The content of phosphorus accessible to the plants is within the low range throughout the depth. Higher amounts of phosphorus are noted in the surface layer, a result of biological accumulation, and this content decreases with soil depth. The soil is well-supplied with easily accessible potassium for plants throughout the depth.

Given the light-textured composition, the soil is well-permeable for water and well-aerated [8], meaning there are no obstacles for plant development.

Beech can be classified as a hyperaccumulative species, serving as a bioindicator of toxic elements. It can be used for the extraction of heavy metals from soil and for green restoration. This is a highly useful and cost-effective method

for removing heavy metals and other harmful substances from soil, water, and air, as well as for eco-remediation, especially in reforestation efforts in places where forests have disappeared due to a climate-related crisis (Haccius, 1978).

To date, the presence of about 70 elements has been identified in plant tissues, which directly or indirectly affect all life processes in plants. During growth and development, plants absorb the necessary macro and microelements, reflecting the amount of bioavailable metals [9]. Plants play an important role in the cycling of heavy metals in nature. The distribution of heavy metals in nature is widespread, and due to contamination of the atmosphere, soil, and water, they can easily penetrate food chains.

A lower content of microelements is found in manganese, which is 25.22% lower compared to common beech at the same location, and this is especially pronounced with selenium, which is completely absent in the albino variety.

In albino beech, the concentration of mercury is 37.7% higher, lead is 30.4% higher, and copper is 42.67% higher. Heavy metals like cadmium, chromium, and nickel are noticeably more present in the Albino beech (38.56% Cd, 22.97% Cr, and 44.89% Ni). Boron content in the leaf tissue of albino beech is 41.56% higher than in chlorophyll-containing beech. For most other elements, concentrations are approximately one-fifth higher in the Albino beech, except selenium, which is crucial for the physiology of all organisms but is absent in the pigment-free beech.

Vascular plants serve as indicators of absorption capacity and hyperaccumulators of heavy metals from their surrounding environment, including air, water, soil, and even other plants they come into contact with. They can thus precisely indicate the presence and intensity of various pollutants (heavy metals, chemical substances, etc.) in the air and soil, in both natural ecosystems and urban areas. In biomonitoring of heavy metals, tree leaves and bark are most commonly analyzed, though examination of rhizomes and soil is also recommended for a comprehensive assessment of pollution. The accumulation of toxic elements in plants at higher concentrations indicates a relative increase and spread of habitat pollution.

The distribution of certain heavy metals in the plant organs of the examined plant is specific and also dependent on its genetic characteristics, so in addition to studying the quantity of absorbed metals, it is necessary to perform genome sequencing of both plants. Although these analyses are not yet complete, it is evident that both strains of the examined beeches (albino and common) exhibit horizontal transfer of some auxiliary genes (Figure 3).

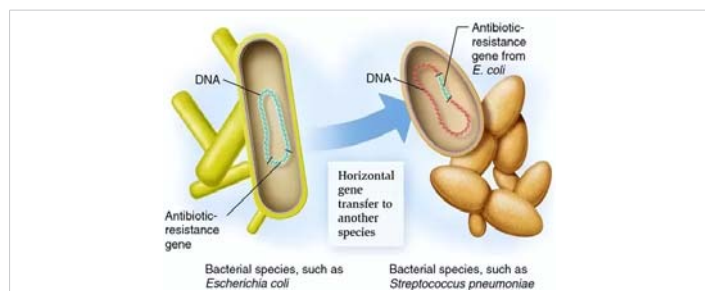


Figure 3: Horizontal transfer of genes, described on two bacterial species [10], <https://qr.ae/p2I5Px> (accessed October 21st, 2024).

This is a common phenomenon between different types (strains) of the same species, especially within the same species. Horizontal gene transfer (HGT) is the reason for the development of new traits, as seen here with two typologically distinct individuals likely originating from the same parent. In the case of the two examined *Fagus* individuals, it would be beneficial to investigate and confirm this further [10].

For such an exceptional organism as the albino beech to survive in a new biome, the outcome and duration of any natural process ultimately lead to gradual, necessary adaptation and various modifications that enhance its survival capacity [2]. The unique characteristics of the *Absiente Pigmente* beech, beyond those visible to the naked eye, became apparent immediately in the laboratory. Through its roots and via the xylem, the albino plant managed to deposit in its leaves high concentrations of both the common elements found in assimilative tissues and of heavy metals. One possibility is this root-based accumulation; an alternative explanation might be the direct absorption of these elements through the leaf tissue, though this is less likely.

Conclusion

The beech tree belongs to the group of hyperaccumulators and can also be classified as a bioindicator of heavy metals, particularly lead. This can be used for the extraction of heavy metals from the soil and for environmental remediation, serving as a very useful and economical method of removing heavy metals and other harmful substances from soil, water, and air. For this purpose, we will attempt to propagate it through tissue culture, as its seeds are likely sterile.

The content of heavy metals - cadmium, zinc, and lead in the observed plant species will vary depending on the plant's further survival under scenarios of meteorological and climatic

crisis, possible disruptions of the conditions in the habitat, potential changes in soil pH levels, erosion, interactions between the observed elements, and other ecological factors.

Interestingly enough, the average values of lead are 30% higher in the albino form than in the leaves of the green plant, and it is necessary to determine if these exceed the threshold values in both samples, as well as to establish the average values of lead, cadmium, and mercury (Hg) in the above-ground parts. It will be very important to find the mean values of all disputed elements in characteristic concentrations for beech and then make comparisons, particularly about cadmium and zinc.

The first step in selecting a type of beech that could be used as a bioaccumulator is certainly its identification, followed by *in vitro* testing of its resistance to unfavorable conditions and symbiotic efficiency under controlled experimental conditions. The response of plant types to various stress conditions and their effectiveness in atmospheric oxygen fixation in such conditions often differ greatly from those in the field. Therefore, as the final phase in finding a quality absorption type of assimilation tissue, long-term field experiments are recommended, ideally conducted on different soil types (Montalbán *et al.*, 2013).

Plants obtained this way would become future bio-collectors or living collectors - bioaccumulation units for gathering phytotoxic waste from the surrounding environment, resulting from human neglect. At this time, this is only a hypothesis, but one far more valuable than many recognized or confirmed findings in the stands of Serbia. A plant this unique, without a clear explanation of its origin and all its characteristics, has not been documented in a long time. It is entirely singular in its photosynthetic capacity, which forms the basis of every plant food chain. Nonetheless, as a significant and extraordinary potential, everything related to this plant is treated with special care from the very beginning, and we face the challenging task of creating limitless possibilities from micro-conditions - a goal that is by no means simple.

- It is likely that In Albino individuals without healthy chloroplasts, this space is physiologically filled with toxins or metals, due to our research
- There's a possibility that these odd substance concentrations are necessary for their unimpeded functioning (possibly as a form of defense), or just to be found in
- The advantage of biological indication is that living organisms can show the effect of the accumulation of pollutants over a longer period of time, which makes their use for ecoremediation
- Physico-chemical methods of environmental pollution monitoring lie in this fact



- Albino *Fagus moesiaca* (*Absent Pigment*) found in Serbia showed this attribute on a massive scale
- As individual as a literal individual, unjustifiably anthropocentric - the *Fagus* tree was always called „Albino“ with a capital letter. In other cases, as a trait - with a lowercase letter (if there is ambiguity)!

The concentrations of macro and microelements in the leaves of albino and green beech, measured by atomic absorption and expressed in mg/kg, are shown in Table 3 and Figure 3. Also, numerous, specifically the heavy ones, in obvious ratios and easily measured percentages, are significantly higher in all of the plant tissues. The logical next step would be to precisely measure how and in what open science sources, that could be reproduced, multiple or even cloned, to be made just, to be understood, and introduced to the polluted world. At the beginning. Further, the genome of this organism must be put under mapping, in a search for any answers it could provide us. Until that, while vegetative dormancy lasts, consideration of the possibility, for example, in vitro providing offspring, is absolutely the priority.

One of many intriguing facts is certain for sure, this phenomenon and mysterious albino, also a very woody, not herbaceous plant, are practically like some heavy elements “living sponge”. Elaboration is far and more complex, but in the polluted and uncertain future, some living organism with such “random” attribute and ability to multiply could be seen, but again, not by meets the eay, yet by its enormous potential as an answer, source, wish came true for generic but so unique, planet salvation.

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Declarations

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interest, ethics-related approvals are not in any shape or form applicable to this research. In short, authors are completely free to publish their findings as they seem fit to do so:

Data availability: It has been understood, of course, in advance that and authors can deliver metadata if those are needed at any time

Materials: Considered that as such, as are N/A

Author's contribution: All start ideas begin with plant finding, then any processes and laboratory procedures are equally shared between them.

All authors had certified next: They are not affiliated with any other organisation, We are absolutely without any connection to other job topic-related affiliations, particularly those that have ever even been possible without solid agreement. Those people are not or were not in any way involved within any organization or entity bringing through any financial interest or non-financial interest in the subject matter, which also covers using not ours, but some kind of material discussed or mentioned in this manuscript.

Everything has already been elaborated on previously, in quite detail, and tendentiously truthfully, in the Element-Chapter called Funding.

Chapter called Funding: Founded by the government of the Republic of Serbia, as non non-profit organization, there are 7 decades of providing continuous and quality control of the overall health state of Serbian forests, nurseries, and practicing the best expertise insight in the private and public domain. Legal framework and process of compliance with EU and neighboring countries is monitored and proactively guided by the Institute of Forestry, providing legislative bodies with expert advice and know-how!

Ethics approval and consent to participate. All authors have read it and approved the final manuscript submission, with special satisfaction in the team's and so far, always successful work.

Data availability: The datasets used or analyzed during the current study will always be available from the corresponding author upon request you could demand.

Code availability: It is understandable that such of thing we are pursuing like some kind of “disclosure” agreements. We consider our team's ideas as the property of many (team members).

Author contribution: Dr RGS and Dr MM, with constant supervision of director Dr LJR, mainly contributed to the study conception and design, but also in data collection. Material preparation and analysis were performed by both Dr GČ and Master BK. The First Draft of the Manuscript Was Written by these three people as a team. All authors are clearly commented on this, maybe, and for a long time, and

draft versions of the manuscript are more than ready. Here, organization is particularly important, and it was recognized as the importance of protecting this special plant in the field with a metal wire cage, around a wooden structure, in a unique secret place in the field, and sampling gates to protect it primarily from wildlife, also by BK, and in fieldwork, far from civilization. Similar phenomenon (only one in Switzerland – is kept under false coordinate).

Word of the corresponding person

By Elements Attracted Albino in Shortly: Everything important or relevant, not related directly to results, has already been elaborated in separate chapters, according to guidance for authors previously, in quite detail, and tententiously truthfully, again, but this time more clearly in the Chapter called Funding.

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