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Pilot landscape and design-based programme to foster student environmental literacy

Tayana PASSOS ROSA,[1] Zsombor BOROMISZA[2]

Department of Landscape Protection and Reclamation, Faculty of Landscape Architecture and Urbanism, Hungarian University of Agriculture and Life Sciences, Budapest, Hungary

e-mails: [1] Passos.Rosa.Tayana.2@phd.uni-mate.hu; [2] boromisza.zsombor@uni-mate.hu

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Abstract: This paper reports a pilot programme conducted in a Hungarian school to foster environmental literacy among students using landscape design principles. This programme utilized a design-based learning approach and incorporated soft skills to develop students' environmental understanding and engagement. In the period of four weeks, participants autonomously designed solutions for their schoolyard while following the design process. Data collection methods included questionnaires, interviews, observation, and analysis of the productions. The results indicated improved understanding of landscape design, increased awareness of the schoolyard, and enhanced soft skills among the participants. The findings provide insights for future programme iterations.

Keywords: design-based learning, future skills, landscape–human connection, environmental education, landscape design

1. Introduction

In the last 30 years, the UN and other organizations over the globe have been showing a growing concern about the climate crisis and the necessary measures to address this issue. Among many targets, the improvement and dedication to environmental education are considered core measures to tackle this challenge under the sustainable development perspective [1]. Environmental education is a lifelong, transdisciplinary process, where learning the natural systems, their networks, and the resulting issues should lead to agency supported by knowledge [2]. In this way, it should lead to forming environmentally literate people.

Likewise, achieving environmental literacy can be seen as the last step of the environmental education process ladder. In the first step, there is a rise in awareness, through concern for the environment. This is commonly followed by the pursuit of a broader environmental understanding, that is, the accumulation of environmental knowledge – reaching literacy when meaningful action towards the environment is realized. Consequently, environmental literacy can only exist in the presence of these three elements: concern, knowledge, and motivation [3, 4].

On account of being a long process, environmental education needs to start at an early age. Several authors support that schools are the most promising spaces to form involved citizens and create behavioural change [5, 6, 7], and the establishment of efficient and transformative environmental education in such settings is a critical imperative for ensuring the preservation and enjoyment of our natural heritage by present and future generations [6, 7]. The careful and thoughtful planning of public environmental education programmes holds the potential to significantly shape the future quality of life and environmental stewardship [6].

Conversely, environmental issues hold no simple answers [8]. The high complexity of the systems and their interactions makes it a wicked challenge, hard to be addressed by both educators and professionals. In this way, forming environmentally literate citizens involves transdisciplinarity, systems thinking, future skills, and resilience to deal with complex challenges.

Such intricacy requires a plastic methodology. The design process, known for its capacity to comprehend systems, is inherently interdisciplinary, encompassing perspectives from the physical, cultural, and social domains [9]. Design-Based Learning is a pedagogical approach that employs the design process as a means of instructing curriculum in an applied, hands-on manner [10]. In addition, the skill set of the 21st century is deeply rooted in the design process [9]. These skills are summarized as the 4 Cs: critical thinking, creativity, collaboration, and communication. The implementation of this set of skills in the educational setting has been advocated by influential educators, such as Paulo Freire, for at least a century [5].

The present paper describes a pilot programme applied in a school setting in Hungary. The main aim was to test the efficacy of the design process and landscape principles and ideals to the fostering of environmental education in a school community. And additionally, introduce landscape design practices to promote connectedness with the local environment and inspire advocacy for future sustainable developments [11]. The programme was student-centred, with the educator being a facilitator in the process. In this way, students should analyse and conceptualize solutions for the schoolyard autonomously and through practice. Additionally, the pupils had to explore how to exhibit aspects of sustainability and multi-functionality in their designs. By implementing carefully selected activities, it was expected to collect feedback and best practices to be updated and reassessed in future iterations of the project.

2. Materials and methods

The proposed methodology involves integrating landscape architecture and design concepts into the learning environment, with a focus on practical application. By directly addressing the proposed design challenges, students actively participate in the design process, creating a sense of ownership and connection to their surroundings. In addition, one of the principles of landscape architecture is to understand, read, feel, and transform a place [12], the transference of which skills to the new generations through practical activities can be a first step in creating a sense of belonging to a place to develop a chain of behavioural changes. Through this approach, participants strengthen their bond with the landscape and gain a deeper understanding of the factors at play and the potential effects of their proposed changes. Moreover, the integration of landscape architecture knowledge into real-world contexts serves to bridge the gap between theoretical concepts and practical applications.

A. The partner school

The study was carried out in partnership with the Polytechnic of Economics high school. Founded in 1991, it is a bilingual school that follows alternative pedagogical movements and continuously integrates modern pedagogical methodology into the daily teaching practice aligned with 21st-century skills. The school is maintained by a foundation, and the student community is formed mostly of upper and medium-income Hungarians [13]. The school is in District 9 of Budapest, on 3 Vendel Street, located in an urban, densely built area, surrounded by a municipality, residential buildings, and two other educational institutions.

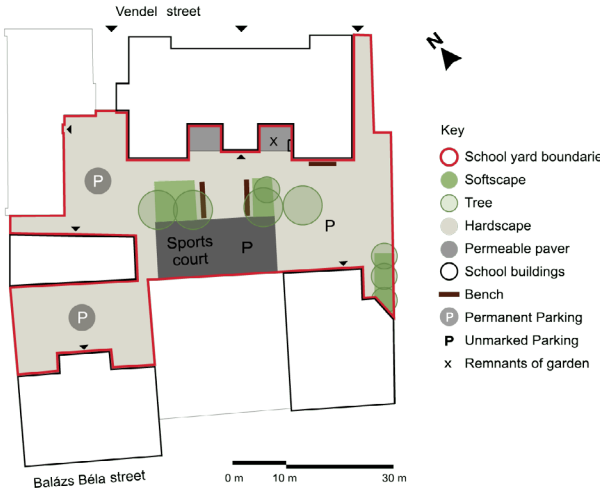

Figure 1. School grounds plan

During a guided visit, we could learn about the schoolyard structure (presented in *Figure 1*) and get to know the school community's assessment of the place. On this occasion, it was discussed that the schoolyard lacks social spaces for students, and there was a dissatisfaction with the sports court due to its appearance and location occupying a significant open space, as well as its secondary function as a parking area. The administration acknowledges the surplus of parking spaces, and there is a consensus on the need to enhance green areas in the yard. Additionally, the area is very enclosed by the surrounding space walls, resulting in limited sunlight and lacking attractive features for the students.

B. Programme outline

The design process is flexible and often adapted to fit the project to which it will be applied. For the development of the programme, a non-linear 6-step design process was followed, according to *Figure 2*. It also illustrates how the framework was expected to follow the process on each occasion. A total of four weekly meetings were held, with a planned duration of 1 hour and 30 minutes per session. The average attendance was 9 students, with ages between 14 and 17 years old.

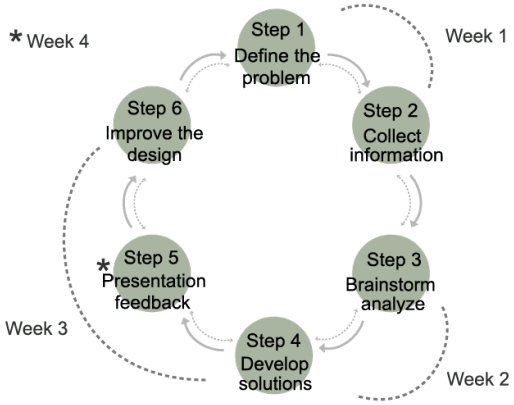

Figure 2. Design process and the following outline

On each occasion, except the last one, two or three activities were held, with the addition of homework. The final occasion was reserved for the student presentation of the results and the group assessment of the programme.

The data collection methodology employed in this study involved a mixed methods approach, incorporating various techniques such as questionnaires, interviews, observation, analysis of programme productions, and visual documentation.

In addition, a qualitative analysis was conducted to identify patterns and characteristics within the data, following specific assessment guidelines. This analysis encompassed aspects such as the timely completion and submission of assigned tasks, the effective application of the information received in previous meetings and assignments, improvements in students' understanding and perception of the schoolyard, advancements in their 4 Cs skills, and the progression and refinement of design concepts over time. The activities, homework, and their expected outcomes are described in the following.

On the first occasion, 9 students joined the activity.

Activity 1: Introduction and abstract relationship with the place: Each participant chooses one picture of objects related to the outdoors and explains their choice in order to initiate communication and interaction, thus creating a board showing students' relationship with the natural environment and landscape.

Activity 2: Never-before-seen design challenge [14]: Students individually create innovative concept 3D models for their ideal schoolyard. It should result in fast, small-scale models reflecting individual wishes for the schoolyard and highlighting functions that are currently lacking.

Activity 3: What can be done with the green: Participants suggest improvements based on pictures shown to them, the task being to replace an industrial element with a green element while keeping the same or similar function. Working individually or in groups, they propose creative and innovative solutions while enhancing critical thinking. In addition, pupils improve their understanding of green possibilities and acquire critical evaluation of mundane landscapes and an expanded sense of possibilities.

Homework: Students take pictures of their favourite and least favourite places in the schoolyard and propose improvements by sketching them. This assignment connects learnings from previous activities with the real-life environment. This results in the visual documentation of the schoolyard's values and problems and the integration of knowledge into students' daily lives.

Expected results of Session 1: The first week aims to establish a baseline understanding of the participants' relationship with the landscape and create a collection of innovative ideas related to the schoolyard. The activities also foster the use of the 4 Cs skills and improve knowledge in environmental education, landscape, and design. These outcomes lay the foundation for future weeks and further development of the participants' understanding and skills.

In the second week, four participants rejoined, while five participants were newcomers, totalling 9 participants.

Activity 1: Plan and post it: Students introduce and evaluate homework ideas using different coloured sticker notes. The expectations were to have a diverse collection of ideas that would provide insights into the schoolyard from different

perspectives. The students would then analyse and discuss different points of view, connecting them to the landscape and applying their acquired knowledge.

Activity 2: World Café [15]: Students participate in roundtable discussions on various topics related to the workshop in order to foster detailed discussions on key features of the workshop, such as the benefits of a greener schoolyard, the design process, and new functions or activities for the schoolyard, and to reinforce group communication, encourage contributions from all participants, and share learnings.

Activity 3: Design and landscape principles: This activity involved an explanation of design methods, landscape architecture principles, and design principles based on [16], [17], and [12]. Students then had to find examples of these elements within the schoolyard so as to connect theoretical knowledge with real-life examples, enhance their perception of design elements and behaviour in the schoolyard context.

Homework: In groups, participants were tasked with choosing a location in the schoolyard and creating a concept design for it. They had to test the feasibility of their ideas by acting on the experience of the proposed design, documenting it through photos, videos, and notes, and preparing a pitch presentation of the concept design with differentiated tools and mediums.

The expected outcomes of Session 2: This meeting aimed for an improved understanding of landscape design possibilities and an expanded awareness of schoolyard improvement opportunities. The students' work demonstrates the application of theoretical knowledge to real-life contexts, emphasizing the connections between landscape, design, and the environment. Additionally, the week aimed to promote communication and collaborative work among students, leading to more complex tasks and the integration of greenery with positive implications.

On the third occasion, there were present three participants who attended all previous sessions, five returned after missing the second week, and three of the five newcomers from week 2 returned, totalling 11 students.

Activity 1: Presentation and six thinking hats [18]: Students present their findings and pitch their ideas. After each presentation, a "hat session" is conducted where the ideas are analysed from different perspectives using the Six Thinking Hats method. The expectations are to improve the students' production by providing meaningful insights, addressing problems, presenting viable solutions, and reinforcing empathy and critical thinking skills.

Activity 2: Prototype: Students work in one group to merge or further develop the ideas from the previous activity and create a final solution. They prototype their idea, considering the strengths and weaknesses identified in the analysis from the previous activity. The expected outcome is for students to collaborate, apply advanced communication and collaboration skills, and project their knowledge to create an intervention that positively impacts the schoolyard environment.

Homework: As a homework, students are expected to finalize their prototypes, if necessary, and prepare a pitch to sell their ideas to the school community. They

can use various creative tools. The goal is to engage and inform stakeholders by presenting the results in an engaging and informative manner.

Expected outcomes of Session 3: The aim was to advance the concepts to be more realistic and impactful, to merge or further improve the concepts based on feedback. The proposals should reflect the students' growth and improvement throughout the workshop, incorporating the 4 Cs and addressing the needs and functions of the schoolyard environment.

On the final day, there were present two participants that joined all the previous sessions, four of those who returned after missing the second week, and three of the five newcomers from week 2 returned a second time, thus 9 being the total number of attendants.

Activity 1: Final analysis of the pitches: In this activity, each group presents an improved version of their ideas from the previous week, and the pitches are analysed based on various criteria as suggested by [19]. The expectation is to gain a realistic understanding of how the concepts can be realized and to identify the next steps to make them a reality.

Activity 2: Right for yes, left for no: Students answer questions related to the workshop by positioning themselves between two walls, each representing agreement or disagreement with the statement, their responses being represented on a Likert scale. The purpose is to gather visual responses to important questions about the workshop experience, the landscape, and the results. This activity aims to confirm the effectiveness of the programme and to assess the students' understanding of and feelings about the workshop.

Activity 3: Focus groups: Participants are divided into small groups to discuss specific questions related to their learnings, favourite and least favourite parts of the workshop, expectations, and satisfaction with the results. The goal is to encourage open and honest sharing among the participants and gain insights into the most effective aspects of the workshop and the students' overall satisfaction.

Activity 4: Summary in one word: Students are asked to write and show one word that summarizes their feelings during the entire experience, finishing with a quick sentence to explain their choice. They are expected to learn in simple terms what the most memorable moment, aspect, or learning from the workshop was from each participant's point of view.

Expected outcomes of Session 4: The main expectation was to see the development and evolution of the students' works, as well as the application of ideas gathered during the process and the integration of newly acquired knowledge. The goal was to identify strengths, weaknesses, and the next steps necessary for future implementation. It sought to answer questions about the valuable information learned by the students, their overall experience and feelings, the challenges faced, and the outcomes achieved. The evaluation also aimed to assess the students' awareness of what they had learned and to identify areas for programme improvement.

3. Results and discussions

The four-week workshop aimed to engage students in the process of landscape design and promote their understanding of the schoolyard environment. As a product of the programme, instead of one final concept created by the entire group, four concepts were created by the students and evaluated during the session based on feasibility, sustainability, and multifunctionality criteria [19]. Overall, the productions showed that the proposals incorporated diverse green elements, considered user needs and emotions, and showcased various functions for the schoolyard. Most importantly, the participants demonstrated a strong motivation to improve the schoolyard and a deeper understanding of key learning outcomes related to integrating green elements, connecting with the landscape, and developing soft skills. The final concepts were presented at an event hosted by the school. Each group created a poster for an exposition, involving the community and informing them about the students' ideas for the schoolyard.

On the other hand, limitations were experienced, mostly connected to the students' engagement with homework, which hindered the programme and prevented the successful completion of some activities that relied on this production. Communication in English seemed to be a factor of concern, but it did not have a significantly negative impact.

This section highlights the assessment with the students and the lessons learned for future applications of the programme in different settings. The fourth week of the workshop focused on providing a final overview of the workshop's production and gathering feedback from the students to evaluate its impact.

The results from the *Right for Yes, Left for No* activity (*Figure 3*) indicated that the students had a positive experience overall. They demonstrated good communication and collaboration skills, felt connected to their surroundings, and showed an understanding of the use of green elements and the design process. However, there were mixed responses regarding critical thinking and learning about landscape architecture, suggesting potential gaps in the students' expectations of learning or their perception of their own development during the workshop.

The focus groups provided an opportunity for students to share their thoughts in smaller groups. They discussed the main learnings from the workshop, their favourite and least favourite parts, whether the workshop had met their expectations, and their opinions on the workshop's results. The discussions (shown in *Table 1*) revealed that the students enjoyed the workshop, found it fun and positive, and they expressed a desire to continue working on the concepts they developed. The students also acknowledged that they had learned how to spark change for a sustainable future, which represents a proper step in the improvement of environmental literacy.

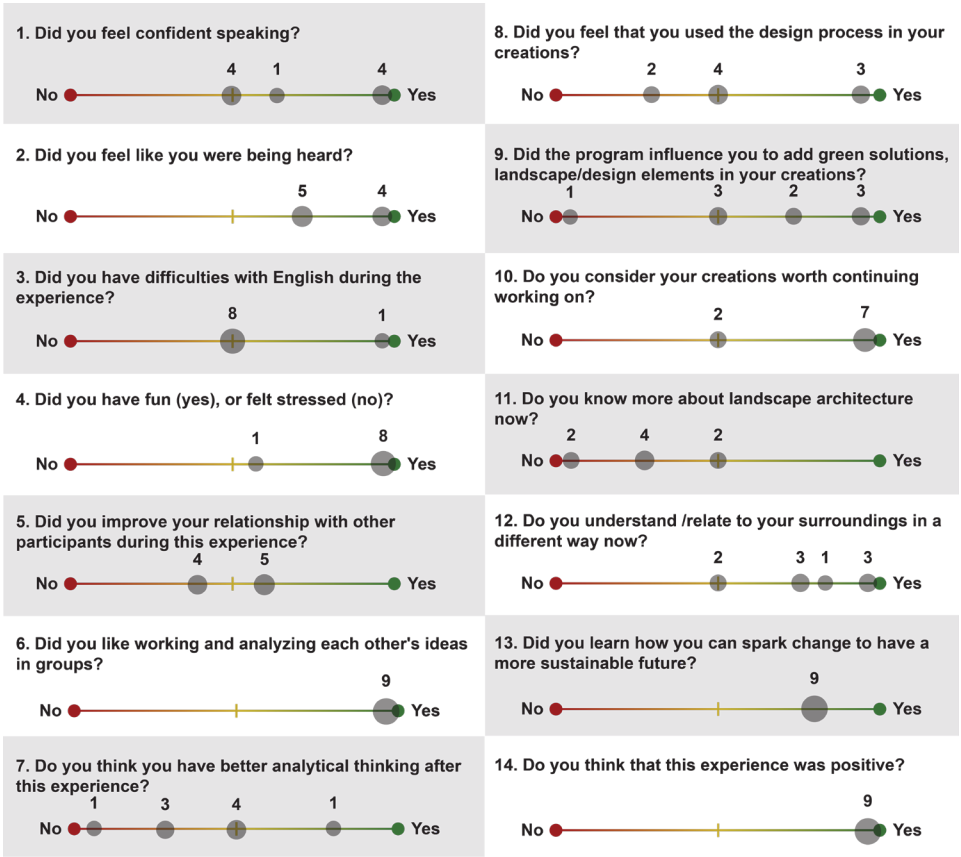

Figure 3. Results from *Right for Yes, Left for No*

Table 1. Results of the focus groups

1. Main learnings, conclusions	*2. Most enjoyed and less enjoyed aspects*	
How to analyse and improve ideas. How to use a process to create. Growing ideas, improvement. Communication, expressing ideas. Even small places have many opportunities and possibilities. Even great ideas might not be realized.	Most enjoyed	Less enjoyed
	Prototyping (3D). Collaboration. Communication. Creativity under pressure/innovation. Useful process.	World café activity (too theoretical). Short time. Lack of material. Not going straight to the action.
3. Expectations for the workshop	*4. Rating the workshop production*	
To learn more about the landscape. More time in the yard, field search. Going beyond the conceptual phase. Engagement of the school community.	Liked the productions. Considered the whole process useful. Considered it a creative activity performed under pressure and found it positive. Applied the process consciously.	

Furthermore, based on the results from the final week, several lessons can be learned for future iterations of the programme. Firstly, it is crucial to communicate the workshop's goals and objectives clearly to the students to align their expectations with the intended outcomes. This can help bridge any gaps between what students expect and what the workshop aims to achieve.

The cumulative character had a positive feedback with regard to using green elements and applying the design process. Future iterations of the programme should continue to emphasize these aspects to enhance students' understanding and practical skills. Additionally, the programme provided opportunities for students to develop their communication and collaboration skills through group work and discussions. These aspects should be further emphasized and integrated into the programme, as they are highly appreciated by the participants and are valuable skills for future professionals in any field.

The results indicated that English proficiency posed a challenge for some students, particularly in fully understanding certain tasks. Future workshops should consider providing additional support or alternative communication methods to ensure that language barriers do not hinder students' participation and learning. And, finally, the closing week's assessment activities allowed for student perspectives to be heard and considered. It is essential to create space for students to reflect on their experiences and provide feedback, as this can inform programme improvements and enhance overall satisfaction.

4. Conclusions

In conclusion, the pilot landscape and design-based programme offered some improvement in student environmental literacy in the given environment and conditions provided by integrating landscape architecture and design concepts into the context. The programme engaged students in the design process, promoted their understanding of the schoolyard environment, and developed their future skills. The results and discussions highlighted the positive impact of the programme, including the generation of innovative ideas, the integration of theoretical knowledge with practical applications, and the promotion of communication and collaboration. These outcomes emphasize the importance of schools as spaces for environmental education and the potential of design-based approaches in shaping environmentally literate students. Further research and implementation of similar programmes are being conducted to create an iterative guideline to have programmes more impactful and to explore different social groups and cultural backgrounds.

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Impacts of agroforestry practices on the physico-chemical, water and soil fertility properties in semi-arid environments: The case of Bordjias Plain (Mesra, North-West Algeria)

Fettouch DALILA,[1] Berkane IBRAHIM,[2] Boulenouar HOUARI,[2] Larid MOHAMED[3]

[1] Department of Agricultural Sciences, Laboratory GCESE, University Abdelhamid Ben Badis (UABM) Mostaganem, Algeria; e-mail:dalila.fettouch@yahoo.com

[2] Department of Agricultural Sciences, University of Relizane, Algeria; e-mail: boulenouarhouari21@gmail.com

[3] University Abdelhamid Ben Badis of Mostaganem (UABM), Algeria; e-mail: laridsid@yahoo.fr

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Abstract: All problems related to conventional agriculture are a reference to the search for sustainable solutions. In this context, a comparative study between three agroforestry practices (AFP): *Citrus sinensis, Olea europaea, and Punica granatum, each associated with Vicia faba* (Fabaceae), was conducted on Bordjias Plain. In order to study the impacts of each association on the physico-chemical properties and water and soil fertility, the soil samples were taken from two profiles (P1 under the tree and P2 between trees), through three horizons (Sh, Mh and Lh), before and after the establishment of culture. The results of the analyses show that moisture variability appears to be influenced by the type of soil and the woody species. By increasing the organic matter content, these associations improve the structural stability of the soil, accelerating the process of dissolution of calcium carbonate, changing the pH, EC, P2O5, CEC, and ion exchange in soil. The statistical approach (ANOVA) allowed us to bring out the positive impact of agroforestry practices on soil fertility.

Keywords: agroforestry, fertility, soil, environments semi-arid, Bordjias Plain

1. Introduction

Soil, a non-renewable resource with potentially rapid rates of degradation and extremely slow processes of formation and regeneration [1], is subject to

unprecedented natural and anthropogenic pressures and can no longer perform its functions perfectly [9].

The association of trees, crops, and/or sometimes farms on the same plot is assimilated to the notion of agroforestry practices (AFP), which are often very effective both in terms of production and environmental protection [2]. These land-use patterns have existed in Algeria for centuries, where, however, the role of trees in different farming practices has been neglected by farmers. It is in this context that we conducted this study on the impact of AFP on the physico-chemical, water and soil fertility properties in order to determine the changes undergone at the soil level due to the presence of trees (*Citrus sinensis*, *Olea europaea*, and *Punica granatum*) in association with a crop (*Vicia faba*).

2. Materials and methods

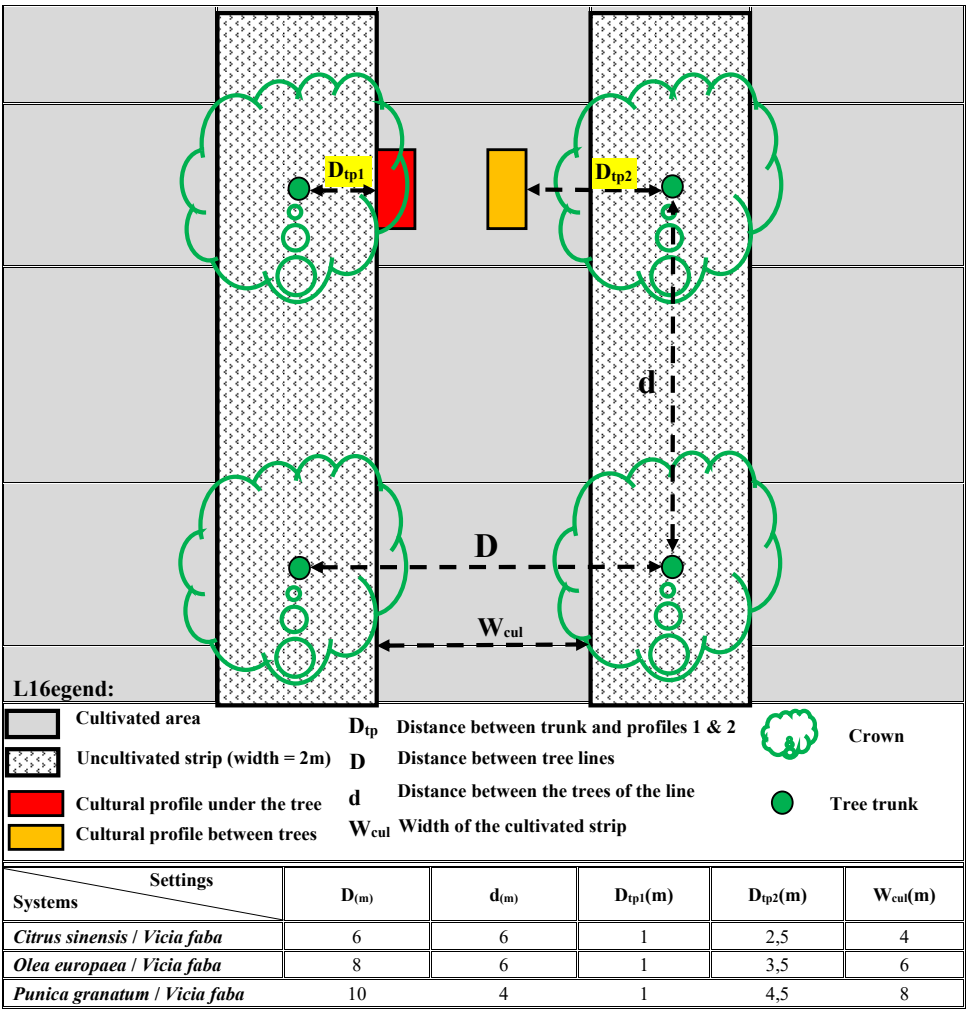
2.1. Presentation of the study area

The study area is part of Bordjias Plain. This plain is located northwest of Algeria. It is bounded on the east by the mountains of Ennaro, Zaimia, and Beiod, on the west by Macta Plain, on the north by Mostaganem Plateau, and on the south by Habra Plain (see *Fig. 1*). The study area is characterized by a semi-arid Mediterranean climate with a long drought period, an annual average reference evapotranspiration of 1,171.32 mm, and an average annual rainfall of 378.13 mm, insufficient to meet the water needs of crops. The land cultivated in this zone occupies a large area of approximately 3,800 ha, i.e. 70% of the total area is market gardening. It is followed by cereal farming (15%), fruit growing (10%), and fodder [3]. Tree-crop associations are becoming more widespread in orchards for economic rather than environmental purposes.

Figure 1. Situation of the study area

2.2. Methodological approach

In this study, we opted for an experimental approach that aims at studying the soil parameters in relation to some AFPs practised in the study area. Cultural profiles (100, 50, and 50 cm) were dug at a distance of 1 m from the trunk of the tree and between the trees (see *Fig. 2*). The soil samples taken from each horizon were subjected to physico-chemical and water analyses, the results of which were statistically processed (ANOVA).



Settings / Systems	$D_{(m)}$	$d_{(m)}$	D_{tp1}(m)	D_{tp2}(m)	W_{cul}(m)
Citrus sinensis / *Vicia faba*	6	6	1	2,5	4
Olea europaea / *Vicia faba*	8	6	1	3,5	6
Punica granatum / *Vicia faba*	10	4	1	4,5	8

Figure 2. Experimental apparatus

3. Results and discussions

3.1. Particle size analyses

Based on the respective values of the clay, silt, and sand fractions projected onto the USDA textural triangle (see *Fig. 3*), the studied soils belong to the sandy loam and sandy-textured textural classes for the first two sites, respectively, and to experimental and silty-sandy loam-clay-sandy for the third site.

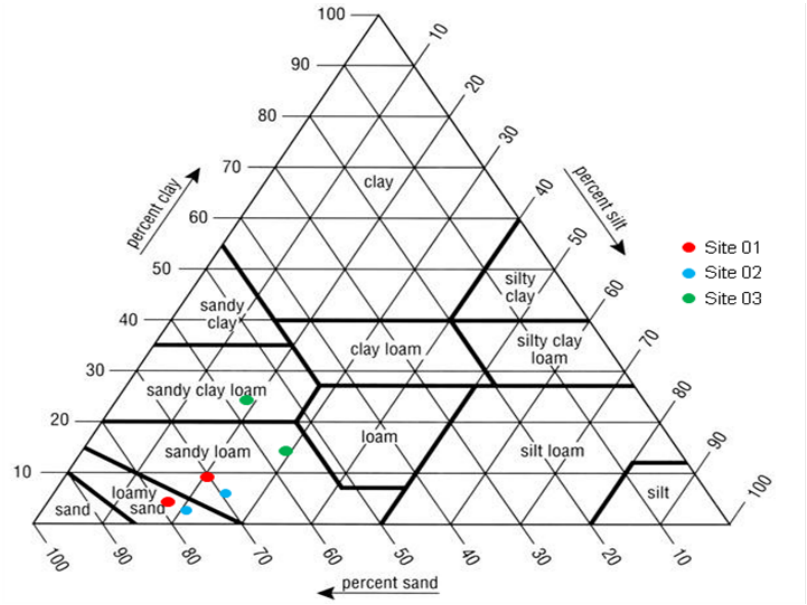


Figure 3. Soil textural triangle

3.2. Statistical data processing (ANOVA)

The interpretation of the results is essentially based on the judgment of the Fisher–Snédécor (F) test, which reflects the global effect of the factor on the different variables characterizing soil fertility and on the Newman–Keuls test, which allows to highlight the place (represented by letters A, AB, B, C, etc.) of each modality of the factor in question relative to another one in the explanation of the overall effect. Three factors (AFP), each with two modalities, were considered in the analysis: the treatment (S1-S2-S3), the plot (P1-P2) with 03, and horizons (Sh-Mh-Lh). The levels of significance achieved are determined from the levels of probabilities, namely:

– Very highly significant (VHS) for a probability level of 0.00001,
– Highly significant (HS) for 0.0001,

– Very significant (VS) for 0.001,
– Significant (S) for 0.05 (critical threshold),
– Not significant (NS) for a level greater than 0.05.

Table 1. Impact of agroforestry practices on the physico-chemical and water properties of soils

Factors	**Agroforestry practices**				**Profiles**			**Horizons**			
Variables	**S_1**	**S_2**	**S_3**	**F**	**P_1**	**P_2**	**F**	**Sh**	**Mh**	**Lh**	**F**
BI	0.00	0.00	0.00	**3.83**	0.00	0.00	**0.13**	0.37	0.50	0.20	**11.08**
	/	/	/	**NS**	/	/	**NS**	**AB**	**A**	**B**	**S**
BD	0.00	0.00	0.00	**2.04**	1.56	1.68	**12.60**	1.52	1.62	1.72	**12.09**
	/	/	/	**NS**	**B**	**A**	**S**	**B**	**AB**	**A**	**S**
EC	0.04	0.04	0.10	**28.64**	0.00	0.00	**0.77**	0.00	0.00	0.00	**0.29**
	B	**B**	**A**	**VS**	/	/	**NS**	/	/	/	**NS**
pH	7.14	7.24	7.68	**22.73**	0.00	0.00	**0.72**	0.00	0.00	0.00	**1.54**
	B	**B**	**A**	**VS**	/	/	**NS**	/	/	/	**NS**
OM	0.00	0.00	0.00	**1.03**	0.00	0.00	**0.03**	0.00	0.00	0.00	**1.15**
	/	/	/	**NS**	/	/	**NS**	/	/	/	**NS**
C/N	0.00	0.00	0.00	**0.84**	0.00	0.00	**1.03**	0.00	0.00	0.00	**0.29**
	/	/	/	**NS**	/	/	**NS**	/	/	/	**NS**
P_2O_5	105.33	63.68	246.47	**93.20**	0.00	0.00	**0.86**	236.13	95.24	84.10	**72.91**
	C	**B**	**A**	**VS**	/	/	**NS**	**A**	**B**	**B**	**VS**
$CaCO_3T$	0.64	0.64	8.99	**207.77**	0.00	0.00	**4.31**	1.77	2.76	5.73	**38.03**
	B	**B**	**A**	**HS**	/	/	**NS**	**B**	**B**	**A**	**VS**
$CaCO_3A$	0.25	0.23	3.13	**16.66**	0.00	0.00	**3.04**	0.00	0.00	0.00	**0.31**
	B	**B**	**A**	**S**	/	/	**NS**	/	/	/	**NS**
CEC	4.25	3.25	10.54	**12.08**	0.00	0.00	**0.01**	0.00	0.00	0.00	**0.16**
	B	**B**	**A**	**S**	/	/	**NS**	/	/	/	**NS**
Exchangeable cations **Ca**	0.00	0.00	0.00	**0.70**	0.00	0.00	**3.47**	0.00	0.00	0.00	**0.34**
	/	/	/	**NS**	/	/	**NS**	/	/	/	**NS**
Mg	0.80	0.47	3.32	**1440.53**	0.00	0.00	**6.74**	0.00	0.00	0.00	**3.45**
	B	**C**	**A**	**HS**	/	/	**NS**	/	/	/	**NS**
K	0.00	0.00	0.00	**1.70**	0.00	0.00	**0.04**	0.00	0.00	0.00	**1.03**
	/	/	/	**NS**	/	/	**NS**	/	/	/	**NS**
Na	0.22	0.13	0.57	**8.01**	0.00	0.00	**1.48**	0.00	0.00	0.00	**1.30**
	B	**B**	**A**	**S**	/	/	**NS**	/	/	/	**NS**
H	1.13	5.24	3.68	**18.71**	1.12	0.61	**7.25**	1.02	1.93	2.57	**11.72**
	C	**A**	**B**	**S**	**A**	**B**	**S**	**C**	**B**	**A**	**S**

3.2.1. Effect of AFPs on soil properties

AFPs do not have an overall effect on the following physico-chemical parameters (see *Tab. 1*): BI, BD, OM, and C / N. However, these practices have a significant effect on the BI at different horizons of the soil. The increase in the rate of organic matter in the soil explains the decrease in the soil fertility index.

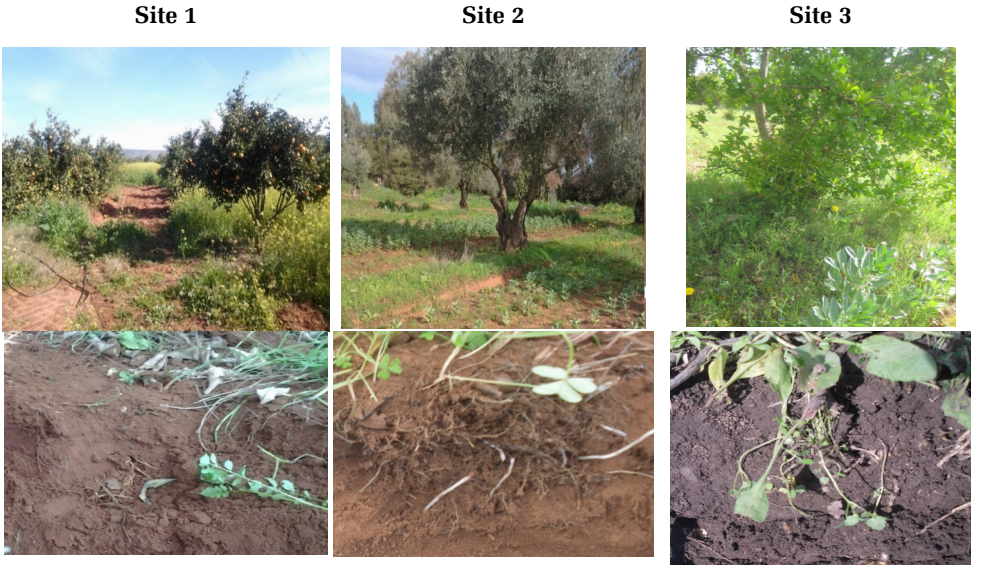
The position of the profile and the change of the soil horizon seem to have a significant effect on bulk density. Variations in bulk density with different horizons of the profiles appear to be strongly influenced by the position of the profile at the tree trunk. It is observed that the further away one gets from the tree, the more the soil becomes compact. The settlement occurs between the tree lines due to the passage of farmers, animals, and gear. The area of influence of the tree is about 3 to 5 m in diameter.

– AFPs have a very significant effect on EC and soil pH. Thus, the system characterizing the woody species *Punica granatum* has a greater overall effect compared to other woody species (*Citrus sinensis* and *Olea europaea*). Maintaining the pH of the soil between some limits close to neutrality (6 to 7) is necessary for the nutrients of the soil to be well assimilated by the roots of the plants. pH is a parameter that plant roots can directly alter through multiple processes, mainly including root respiration and excretion of root substances from trees and crops [6]. Pousset [7] shows that the biological activity always tends to bring the pH towards neutrality, in the neighbourhood of 7. EC reveals nothing of the nutritive elements that are present, nor of their quantity, but it gives an overall idea of the nutritional value of the solution. Generally, crops and trees have an influence on EC in poor soils (sandy soils) as nutrients [4].

– AFPs have a very significant overall effect on P_2O_5 in different horizons of the soil. Also, the system characterizing the species *Punica granatum* has a greater effect compared to other woody species. In the presence of calcium in the soil, iron, aluminium, etc., phosphorus combines to form phosphates, insoluble compounds. Phosphorus is no longer available for the plant; it is no longer assimilable [5]. Thus, these practices have a significant to highly significant effect on total limestone. The variability of limestone seems to be much more influenced by the system characterizing the species *Punica granatum* and by the horizon of the soil. The limestone content varies from one soil to another because this parameter (limestone) characterizing each horizon behaves differently depending on the nature of the parent material and the root and the biological activity of each experimental site applied to it (see *Fig. 4*).

– AFPs have a significant overall to highly significant influence on CEC and exchangeable cations (Mg, Na). The system characterizing the woody species *Punica granatum* has a greater effect compared to other woody species. The variability of moisture seems to be strongly influenced by the type of the woody

species (*Punica granatum*, *Citrus sinensis*, or *Olea europaea*) and by the position of the profile with respect to the trees for the three horizons of the profiles (Sh, Mh, Lh). It is better felt in the second site than in the first and third ones. The low water content at the surface horizon (Sh) appears to be related to soil texture and evaporation.



Photos by D. Fettouch, 2015

Figure 4. Effects of AFP on the root activity of each experimental site

Impact of AFP on soil ion balance

AFPs have a significant overall to very significant influence on the ionic balance of profiles (see *Tab. 2*). The system characterizing the woody species *Punica granatum* has a greater overall effect on the ionic balance and much more on the bicarbonates compared to other woody species because soluble calcium in the form of calcium bicarbonate Ca $(HCO_3)_2$ is more important, as the level of active limestone is high in the soil [5]. [8] shows a large variation in the composition of mineral exudates released by trees according to the species; these results show that apart from Na^+, it is K^+ and Ca^{2+} that are released in greater quantities and dominate the cationic moiety, while the anionic moiety is predominantly Cl^- and SO_4^{2-}.

Table 2. Impacts of agroforestry practices on soil ion balance

Factors	Agroforestry practices				Profiles			Horizons			
Variables	S_1	S_2	S_3	F	P_1	P_2	F	Sh	Mh	Lh	F
Cl^-	1.74	1.16	1.08	**19.62**	1.66	0.99	**51.69**	0.00	0.00	0.00	**0.68**
	A	**B**	**B**	**S**	**A**	**B**	**VS**	/	/	/	**NS**
HCO_3^-	0.70	0.75	1.51	**83.61**	0.00	0.00	**0.24**	0.00	0.00	0.00	**1.25**
	B	**B**	**A**	**VS**	/	/	**NS**	/	/	/	**NS**
SO_4^{2-}	0.00	0.00	0.00	**1.51**	0.00	0.00	**0.45**	0.00	0.00	0.00	**0.20**
	/	/	/	**NS**	/	/	**NS**	/	/	/	**NS**
Ca^{2+}	1.09	1.17	1.66	**10.87**	0.00	0.00	**0.87**	1.61	1.18	1.13	**7.70**
	B	**B**	**A**	**S**	/	/	**NS**	**A**	**B**	**B**	**S**
Mg^{2+}	0.79	0.54	1.18	**20.04**	0.00	0.00	**0.67**	0.00	0.00	0.00	**6.52**
	B	**B**	**A**	**S**	/	/	**NS**	/	/	/	**NS**
Na^+	1.34	0.90	2.40	**12.66**	0.00	0.00	**0.35**	0.00	0.00	0.00	**0.56**
	B	**B**	**A**	**S**	/	/	**NS**	/	/	/	**NS**
K^+	0.00	0.00	0.00	**5.16**	0.00	0.00	**1.26**	0.00	0.00	0.00	**0.58**
	/	/	/	**NS**	/	/	**NS**	/	/	/	**NS**

Conclusions

As a result of this study, it appears that variations in moisture and bulk density are influenced by soil type and woody species. Variations in bulk density appear to be strongly influenced by distances to the tree where the soil becomes compact as one moves farther away. pH is a parameter that tree roots and crops can alter directly. This effect on pH varies with species and soil type. AFPs have an influence on EC in soils that are poor in nutrients (sandy soils). This influence may be due to the phenomenon of macro-element rooting. Thus, these practices have an effect on the beating index by increasing the rate of organic matter in the soil horizons. Limestone is much more influenced by the nature of the parent material of each site.

AFPs have an influence on CEC, exchangeable cations, and soluble ions in the soil solution due to rooting phenomena. The study showed that a significant increase in CEC is observed near trees. From these results, we consider that the effect of these practices on soil properties is not negligible in our study area. The introduction of trees in cultivated plots gradually and durably modifies the agricultural landscape. Therefore, the return of trees to agricultural communities is an essential concern in order to ensure the sustainable rural management of soil fertility.

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Lactic acid bacteria (*Leuconostoc mesenteroides*) as bioprotective agents against some pathogenic fungi in common bean

Mohamed DJELILATE,[1,2] Lahouaria Mounia MANSOURI,[4] Anas MAMI,[1,3] Abdenour KHELOUFI*[4]

[1] Laboratory of Applied Microbiology, Faculty of Sciences, University of Oran, 31100 Oran, Algeria; e-mails: djelilatemohammed31@gmail.com; anas.mami@yahoo.fr

[2] Department of Biology, Faculty of Science and Technology, University of Relizane, 48000 Relizane, Algeria; e-mail: djelilatemohammed31@gmail.com

[3] Department of Life and Environment, University of Sciences and Technology of Oran, 31000 Oran, Algeria; e-mail: anas.mami@yahoo.fr

[4] Department of Ecology and Environment, Faculty of Natural and Life Sciences, University of Batna 2, 05078 Batna, Algeria; e-mails: l.mansouri@univ-batna2.dz; a.kheloufi@univ-batna2.dz

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Abstract. The common bean (*Phaseolus vulgaris* L.) is the most important edible food legume in the world. However, its cultivation encounters several phytopathogens. Our study aims to improve food quality and safety through more natural protection methods such as the use of microorganisms that allows us to avoid and minimize pesticide risks. For this purpose, we tested the antagonistic capacity of lactic acid bacteria isolated from raw goat's milk against four fungal strains isolated from *P. vulgaris* var. *djedida*. In this study, lactic acid bacteria (LAB) (*Leuconostoc mesenteroides*) were screened in vitro for antifungal activity at 28 °C against *Fusarium oxysporum*, *Botrytis cinerea*, *Aspergillus flavus*, and *Alternaria alternata*. The statistical analysis of the antifungal activity of LAB showed significant differences after a seven-day period. The results of the direct confrontation on the PDA (Potato Dextrose Agar) and MRS (de Man, Rogosa, and Sharpe) Agar medium showed better inhibition by the lactic acid strain on MRS Agar medium. *L. mesenteroides* gave the highest inhibition rate of 57.6% for the pathogen *B. cinerea* and 29.1%, 33.3%, and 26.7%, respectively, for the pathogens *A. alternata*, *B. cinerea*, *F. oxysporum*, and *A. flavus* on the solid PDA medium. However, on the MRS Agar medium, inhibition rates of 88.1% and 80.5% were observed for the pathogens *B. cinerea* and *A. flavus* and a total inhibition of 100% on *A. alternata* and *F. oxysporum* in the presence of the strain *L. mesenteroides*. This study led to suggest that food-derived LAB strains could be selected for biotechnological application to control phytopathogenic fungi.

Keywords: agriculture, antifungal activity, lactic bacteria, Fabaceae, phytopathology

1. Introduction

Agriculture is facing many challenges all across the globe, including climate change, loss of biodiversity, failure to control production techniques, diseases and pests that directly affect the already insufficient production in many developing countries [1]. Common bean (*Phaseolus vulgaris* L.) is the most important source of proteins for nearly five hundred million people in Africa, Latin America, and the Caribbean (LAC) [2]. The production of common beans still remains irregular and seems to be closely linked to a certain number of abiotic (poor cultivation practices, climate change, inadequate choice of land, and especially exhaustion of soil in a term of fertility) and biotic (genetic potential, the proliferation of diseases, pests, etc.) factors; hence the persistence of a significant deficit in production [3]. In Algeria, economically accessible food legumes occupy an important place in food security. These biotic and abiotic constraints are also due to the absence of tolerant or resistant varieties to these constraints; hence the need for varietal selection. Diseases are some of the major biotic factors affecting bean production. Most of the organisms causing these diseases are seed-borne arising from external contamination or being carried in the seed. Indeed, the rapid and insidious development of phytopathogenic agents on the bean crop often generates more or less serious yield losses. Sources of fungal contamination are numerous, and fruits can be infected with fungi during the growing season, as well as throughout the harvest process to storage [4].

The most harmful toxigenic fungi in agriculture belong to the genera *Alternaria*, *Botrytis*, *Fusarium*, and *Aspergillus* [5]. Transmission of these fungi on common bean can be as contaminants that attach or stick to the seed coat or infect the seed [6]. Fungal resistance poses great problems in terms of plant protection. Indeed, there are only a few antifungal products that are effective against certain multi-resistant agents [7]. However, the World Health Organization has banned the use of certain chemical fungicides due to their long-term adverse toxicological effects, including carcinogenicity [8]. In Algeria, common bean yields and disease resistance are significantly affected by variable weather and soil conditions. Global climate change is also an important consideration in agricultural production. Suboptimal water supply and temperatures exert adverse effects on common bean growth and yields [9].

On the other hand, pathogenic microorganisms are difficult to control because they can survive in the soil for long periods [10]. Therefore, it would be wise to search for microbial antagonists capable of ensuring the healthy bioprotection of the common bean within microorganisms, which are generally tolerated by

humans and animals. Among these microorganisms, lactic acid bacteria (LAB) have antimicrobial activities frequently used in developing fermented foods, and they could be promising agents [11]. In addition, they are devoid of any toxicity for the consumer, which is why they enjoy a GRAS (Generally Recognized as Safe) status [12]. LAB are, therefore, quite harmless microorganisms and appear to be beneficial for the health of consumers. A number of studies have shown that species belonging to the genus *Leuconostoc*, mainly *L. mesenteroides*, can inhibit the growth of several pathogenic bacteria due to their ability to produce organic acids, diacetyl, and bacteriocins, the best known of which being mesenterocin Y105 produced by *L. mesenteroides* [13], [14]. Our study focused mainly on LAB (*L. mesenteroides*) isolated from goat milk in order to study its effect in vitro against four phytopathogenic fungi. The fungal species chosen in this study were *Fusarium oxysporum*, *Botrytis cinerea*, *Aspergillus flavus*, and *Alternaria alternata.*

2. Materials and methods

2.1. Study area

The region of Relizane (Northwest Algeria) has a hot Mediterranean climate with dry summer, where the average temperature is 19.9 °C, and rainfall is on average 407.6 mm. The region of Relizane is characterized by an arid to semi-arid climate, especially at the level of the plain area. There are cold and rainy winters and hot summers with snowfall in some western regions exceeding 800 meters altitude and in the mountains of Ouarsenis, in the high mountains of Bourokba, and in the mountains of Beni-Chougrane, Mendes, Zemmora, and Dahra. The region of Relizane is characterized by a pronounced summer drought and a rainfall deficit, which makes irrigation compulsory. The low rainfall (279 mm/year) and the irregularity of annual rainfall (45% are recorded during the months of November and December) cause a water deficit estimated at 85 mm/year. The average temperatures vary between 11 °C and 30 °C for the months of January and August. The annual average temperature is 20 °C. The coldest months are January and December (11 °C), while the months of August and July are the hottest months with an average of 30 °C [15].

2.2. Biological material

2.2.1. Lactic acid bacteria (LAB)

The control strains of *Leuconostoc mesenteroides* were obtained from the collection of the Laboratory of Applied Microbiology of the Biology Department (Faculty of Sciences, University of Oran, Algeria).

2.2.2. Phytopathogenic fungi

Isolates of *A. alternata*, *B. cinerea*, *F. oxysporum*, and *A. flavus* were obtained from 70 plants of common bean (*Phaseolus vulgaris* var. *Djedida*). Their stems, pods, and leaves showed typical symptoms of the diseases caused by these pathogens. Samples were collected from commercial farms in Relizane (North-West Algeria) during the spring of 2020. Fragments were excised and placed aseptically on a PDA medium and incubated at 28 °C. The morphological identification of fungi mainly involves the cultural and morphological characteristics of the fungi isolated in the pure state [16]. We use pure cultures isolated from single conidial isolation (monospore cultures).

Identifications of the pathogen were based on a macroscopic study of the morphological characters of the culture. The distinction between pathogenic germs is expected to be made based on specific criteria *viz.* pigmentation, the appearance of mycelium, growth rate, and abundance of pycnidia [17]. Sometimes symptoms can be attributed to certain fungal species, such as discolouration, shrivelling, and cracks on common bean stems, pods, and leaves. In most cases, identification of a pathogen based only on such symptoms is not recommended, as these can be common to several fungi [18]. The predominant major foliage and pod pathogen was identified as *A. alternata*, while the common stem pathogens were identified as *F. oxysporum* and *B. cinerea*. Another important fungus that was isolated from leaves is *A. flavus*.

In a second step, a microscopic study was carried out on the evaluation of the morphological characters of the various organs of asexual reproduction and of the mycelium. Microscopic examination of a fungal culture was done under 40X magnification [19].

2.3. Confrontation method and evaluation of antifungal activity

In this study, we used the method of direct confrontation in vitro, which consists in inoculating the lactic strain on the PDA (Potato Dextrose Agar) or MRS (Man, Rogosa, and Sharpe) Agar medium in a Petri dish in two parallel streaks of 2 cm and incubating it at 30 °C for 48 hours [11]. Isolates on PDA medium were used as a control direct confrontation test. On the same medium and after 48 hours, a 0.5 cm agar disc of the 5-day-old fungal strain was deposited in the centre of the Petri dish (90 mm in diameter) and incubated at 28 °C for seven days. To determine the influence of the lactic acid strain, the radial growth of the fungal strains was measured daily in two perpendicular directions and compared with the controls [20]. The inhibition percentage (I) was calculated according to the method of [21].

$$\textit{Inhibition percentage } (I)(\%) = \frac{C - T}{C} \times 100,$$

where C: Colony diameter in control (cm) and T: Colony diameter in treatment (cm).

2.4. Statistical analysis

The experiments were conducted with five replicates, and the results were expressed as means. All the data were subjected to one-way analysis of variance (ANOVA) and Tukey's HSD multiple comparisons test using SAS Version 9.0 (Statistical Analysis System) (2002) software. A value of $p < 0.05$ was considered as significant.

3. Results and discussions

3.1. Microscopic observation of Leuconostoc mesenteroides

Table 1 showed the characteristics of macroscopic and microscopic observation and physiological and biochemical tests. The study of these characteristics served as a basis for the identification of the genus and the species. The obtained characteristics made it possible to verify and confirm the relationship of the species that were isolated from the goat's milk of the region of Sig (Mascara, Algeria). The macroscopic appearance of the colonies of *L. mesenteroides* isolated and purified on MRS Agar medium showed that the colonies were small, round, and lenticular with a whitish colour (*Figure 1*).

According to *Table 1*, the microscopic appearance carried out after Gram staining revealed that our strains were Gram-positive, ovoid-shaped in pairs and in even, short, and curved chains. These results are similar to those of Ogier et al. [22]. The physiological and biochemical characteristics agree with the criteria specific to the genus *Leuconostoc*, and the results obtained are analogous to those obtained by [23].

Table 1. Microscopic and macroscopic observation and characteristics of some biochemical and physiological tests of the studied strain L. mesenteroides

Gram	**Fermentation type**		**Microscopic observation**			**Macroscopic observation (Colony)**			
+	Hetero		Ovoid in pair/short, curved chains			Round, small, and lenticular shape, whitish in colour			
	Physiological test							**Biochemical test**	
	Growth at different temperatures (°C)					**Growth pH**		**Gas production**	**Catalase test**
4	37	40	45	55	63	4.8	6.5	+	-
-	+	+	-	+	-	+	+		

Notes: (+): growth, (-): no growth.

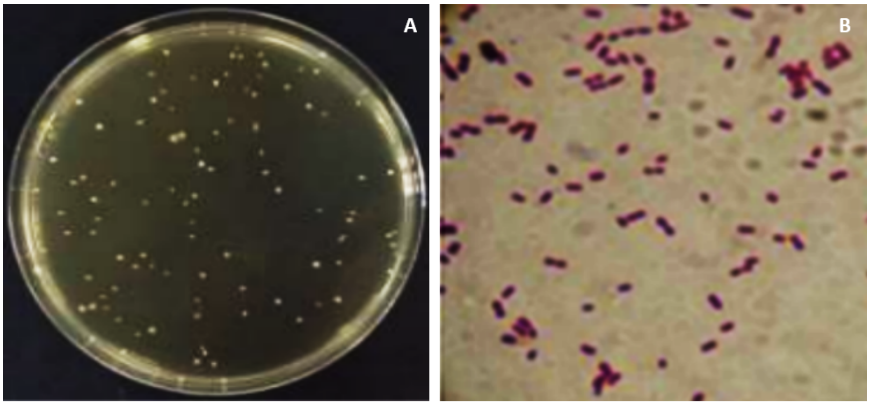

Figure 1. L. mesenteroides observation in a petri dish (A) and microscopic observation under 100X magnification (B).

3.2. Macroscopic and microscopic appearance of fungal pathogens

The predominant major foliage and pod pathogens were identified as *A. alternata*, while the common stem pathogens were identified as *F. oxysporum* and *B. cinerea*. Another important fungus that was isolated from leaves is *A. flavus*.

Botrytis cinerea: Colonies of *B. cinerea* on PDA medium were visually classified into two morphological types, without marked differences in sporulation. The identified mycelium was examined in the study, characterized by the absence of sclerotia formation and by a rather grazing mycelium. The pathogen *B. cinerea* produces a mycelium with articulated filaments, brownish or olive, sometimes cylindrical at the level of the median septum, the diameter of which varies considerably depending on the conditions of hyphal development. When the mycelium is in the fructification stage, it produces clumps of greyish, rounded-branched conidiophores containing clusters of conidia (*Figure 1A*).

Alternaria alternate: The colour of colonies ranged between light to dark olivaceous. The majority of the colonies have a fluffy or cottony appearance, and slight variations in mycelial growth with regular or irregular borders can be observed, with or without concentric zones. These observations are in agreement with those of [24]. Species of the genus *Alternaria* possessed septate conidia with transverse and longitudinal partitions in simple or branched chains, brown, irregular (20–80) x (9–18) µm (*Figure 2B*), more often with a short but well-differentiated apical rostrum [19].

Aspergillus flavus: The macroscopic study of *A. flavus* is performed with the naked eye after seven days of incubation, on PDA at optimal temperature (25 ± 2

°C). Indeed, *A. flavus* develops rapidly (2 to 3 days) on this medium. The shape of the colonies is downy to powdery, first white, then yellow, and then green-yellow. The microscopic examination of the strain under investigation revealed a segmented mycelium. The aspergillus head appeared as a spherical vesicle with phialides forming on metules (biserium head), each phialide producing globular conidia to subglobose, pale green, echinulate. *Aspergillus* heads are borne on hyaline conidiophores (*Figure 2D*). All of these observations – namely: macroscopic and microscopic – corroborate those of Tabuc [25], who indicates that they belong to *A. flavus*.

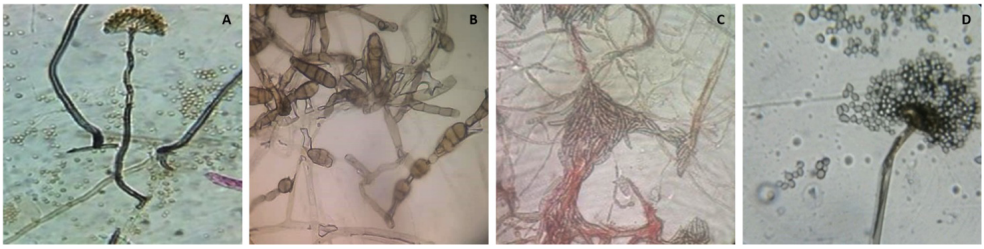

Figure 2. Microscopic observation of pathogens under 40X magnification [*B. cinerea* (A), *A. alternata* (B), *F. oxysporum* (C), *A. flavus* (D)]

Fusarium oxysporum: After purification of the isolates by monospore culture, several major morphological types were observed among the offspring. The aerial mycelium was fluffy in appearance, thick, dense, and relatively sparse. Microscopic observation showed the presence of a septate thallus with short monophialids, on which microconidia were found, as well as the presence of chlamydospores (*Figure 2c*).

3.3. Direct confrontation in vitro

The results of the method of the direct confrontation of the strain *L. mesenteroides* against the four fungal isolates (*A. alternate*, *B. cinerea*, *F. oxysporum*, and *A. flavus*) on PDA medium and MRS Agar are shown in *Table 2* and *figures 3–4*. According to the results of the analysis of variance, the effect of the fungal strain was very significant ($p < 0.001$), just as the effect of the medium and the interaction of these two factors (strain x medium) were also very significant ($p < 0.001$).

Table 2. Inhibition percentage of *B. cinerea*, *A. alternata*, *F. oxysporum*, and *A. flavus* by *L. mesenteroides* after seven days of incubation at 28 °C in two different culture media

Fungi isolates	Inhibition percentage (I) (%)	
	MRS Agar Media	PDA Media
B. cinerea	88.1[b]	57.6[a]
A. alternata	100[a]	29.[1]c
F. oxysporum	100[a]	33.[3]b
A. flavus	80.5[c]	26.[7]d
P-value	< 0.001	< 0.001

Note: Means with similar letters in each column with 5% probability are not statistically different.

3.4. Direct confrontation on the PDA medium

Direct confrontation of the *L. mesenteroides* strain isolated from goat's milk on a PDA medium gave a level of inhibition that varied from one pathogen to another. The highest rate of inhibition for *Botrytis cinerea* (57.6%) was observed on the 7th day of incubation. The inhibition of the 1st day for this isolate did not exceed 12.5%. On the other hand, for *F. oxysporum*, the highest inhibition rate was 33.3% on the 6th day of incubation compared to the 1st day and inhibition of 8.3%. The rate of inhibition of pathogenic strains *A. alternata* with *A. flavus* is very close (29.1% and 26.7% respectively). The highest rate of inhibition of these isolates was achieved on the 4th and 5th day of incubation (*Figure 3*). The results obtained showed that the radial growths of the tested fungal strains are much lower than those of the controls (*Table 2*).

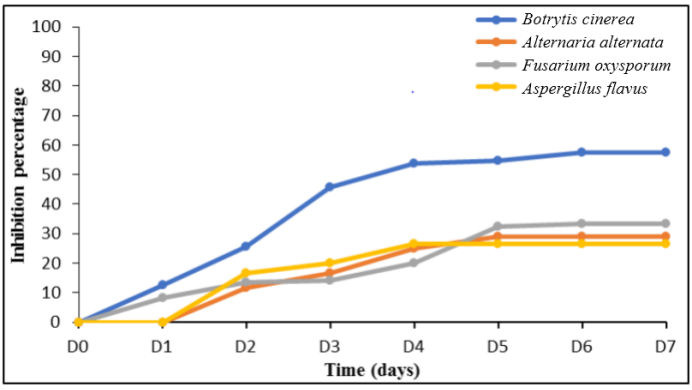

Figure 3. Kinetic of inhibition percentages of *B. cinerea*, *A. alternata*, *F. oxysporum*, and *A. flavus* by *L. mesenteroides* after 7 days of incubation at 28 °C in PDA media

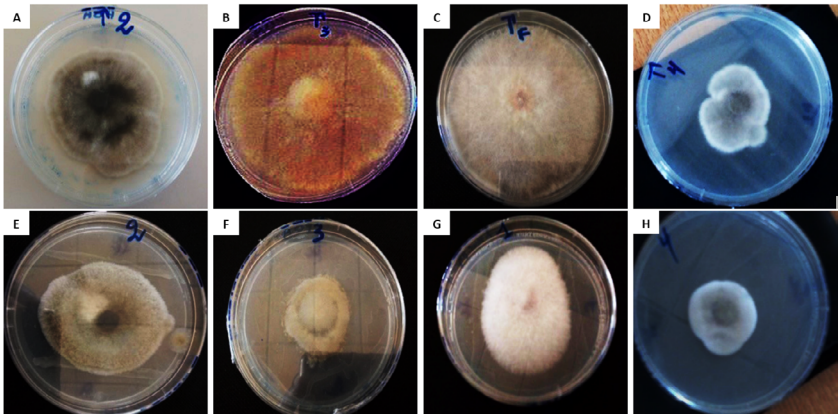

Figure 4. Antifungal activity of selected LAB strain (*L. mesenteroides*) evaluated by confrontation assay against *A. alternata* (A – control, E – confrontation assay), *B. cinerea* (B – control, F – confrontation assay), *F. oxysporum* (C – control, G – confrontation assay), and *A. flavus* (D – control, H – confrontation assay) after incubation at 28 °C for 7 days in PDA medium

3.5. Direct confrontation on the MRS Agar medium

The direct confrontation of the *L. mesenteroides* strain with the four fungal pathogens (*Botrytis*, *Fusarium*, *Aspergillus*, *Alternaria*) on the MRS Agar medium is illustrated in *Figure 5*. The LBA *L. mesenteroides* produced a strong inhibitory effect, which gives a percentage inhibition between 28.5% and 88.1% against *B. cinerea* (*Figure 6B,F*) and a moderate effect for the pathogen *A. flavus* at a percentage of 11.1% up to 80.5% (*Figure 6D,H*). On the other hand, for the pathogens *F. oxysporum* (Figure 6C, G) and *A. alternata* (*Figure 6A,E*), it was observed that there is no fungal growth on the MRS Agar medium in the presence of the *L. mesenteroides* strain, and this amounts to the fact that the lactic strain produces remarkable inhibitory substances on its specific medium (*Figure 6*).

Lactic acid bacteria (LAB), considered safe for human and animal health, are widely used for the fermentation and preservation of food. LAB are capable of producing various antifungal materials [11]. Research on the biological control of phytopathogenic fungi has intensified in recent years. The development of these methods would make it possible to define prevention strategies that are more respectful of the environment. Naturally occurring substances biosynthesised by bacteria, fungi, or higher plants have been shown to be important sources of molecules capable of inhibiting the growth of fungi [26]. Lactic acid bacteria exhibiting antifungal activities have also been isolated from other biological systems such as silages [27], raw milk, and sausages [28]. Antimicrobial compounds by lactic acid bacteria have attracted great attention due to their

application in biological control and are well known for their abilities to exhibit antifungal activity; they are currently used in dairy products for their antifungal properties [29].

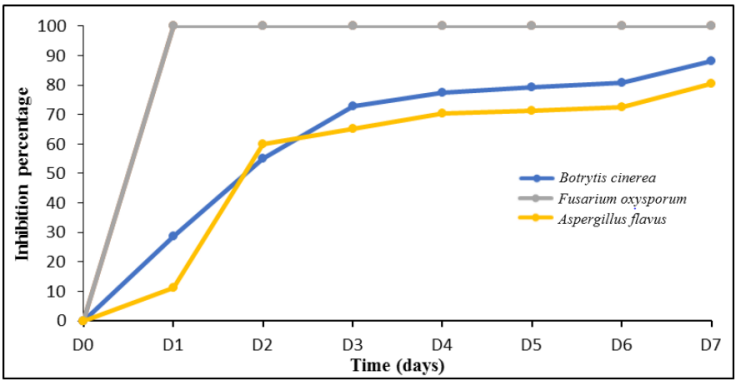

Figure 5. Kinetic of inhibition percentages of *B. cinerea, A. alternata, F. oxysporum*, and *A. flavus* by *L. mesenteroides* after 7 days of incubation at 28 °C in MRS Agar media

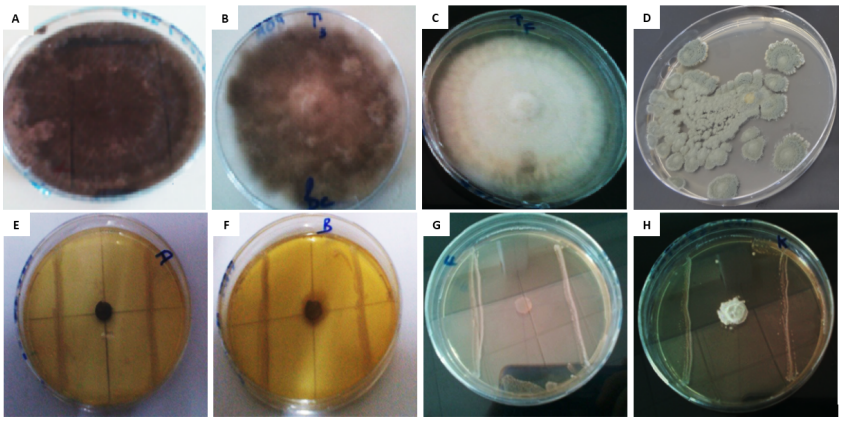

Figure 6. Antifungal activity of selected LAB strain (*L. mesenteroides*) evaluated by confrontation assay against *A. alternata* (A – control, E – confrontation assay), *B. cinerea* (B – control, F – confrontation assay), *F. oxysporum* (C – control, G – confrontation assay), and *A. flavus* (D – control, H – confrontation assay) after incubation at 28 °C for 7 days in MRS Agar medium

The results illustrated in this work present a comparison between the inhibition rates of the *L. mesenteroides* strain on the two culture media used for the comparison (PDA and MRS Agar) to see their antifungal influence on the isolated fungal strains. The inhibition rate of the *L. mesenteroides* strain is greater on the MRS

Agar medium than on the PDA medium since it is the specific medium for these lactic acid bacteria [30]. On the MRS Agar medium, the percentage inhibition is maximum and reaches 100% from the first day for the pathogens *A. alternata* and *F. oxysporum*. On the other hand, the inhibition reached 88% and 80.4% from the 7th day for *B. cinerea* and *A. flavus*. These results are close to those of Bianchini [31], who studied the effect of antifungal compounds produced by *Lactobacillus plantarum* on the growth of *Aspergillus spp*.

According to Dalie [11], the most convincing results are obtained when the bacteria were inoculated into the MRS Agar culture medium 48 hours before inoculation of the fungi. Significant activation of toxin production associated with a reduction in fungal growth is observed suggesting the existence in this medium of compounds, which would activate or act in synergy with the entities produced by the lactic acid strain. These hypotheses are in line with the conclusions of Schillinger and Villarreal (2010) [32], namely that certain compounds of the MRS Agar medium, *viz.* sodium acetate, influence the antifungal activity of lactic acid bacteria. In the light of the results found, we can conclude that the antifungal substances produced by the lactic acid bacteria of the genus *L. mesenteroides* are probably bacteriocins and exert a strong inhibitory effect on the growth of fungal strains isolated from beans.

4. Conclusions

During this work, the study of the antagonist influence of *L. mesenteroides* in vitro was based on the ability to inhibit the mycelial growth of phytopathogenic moulds, which affect common bean (*Phaseolus vulgaris*), their inhibition rate being higher on MRS Agar medium than on PDA medium since it is the specific medium for these lactic acid bacteria. The strain of *L. mesenteroides* is very interesting. The latter draws a conclusion that isolated bacteria are promising natural biocontrol agents and should be further studied and tested for the control of numerous plant diseases. Additional studies are required to definitively determine their mode of antifungal action, safety, and biocompatibility.

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Multifunctional agriculture in the framework of the Sustainable Development Goals (SDGs): Bibliometric review

Nancy Harlet ESQUIVEL-MARÍN,[1] Leticia Myriam SAGARNAGA-VILLEGAS,[1,*] Octavio Tadeo BARRERA-PERALES,[2] Juan Antonio LEOS-RODRÍGUEZ,[1] José María SALAS-GONZÁLEZ[3]

[1] Centro de Investigaciones Económicas, Sociales y Tecnológicas de la Agroindustria y la Agricultura Mundial (CIESTAAM), Universidad Autónoma Chapingo (UACh), Chapingo, Estado de México, México

[2] Centro de Investigaciones en Geografía Ambiental (CIGA), Universidad Nacional Autónoma de México (UNAM), Campus Morelia

[3] Departamento de Sociología Rural, Universidad Autónoma Chapingo (UACh), Estado de México, México

* Corresponding author: msagarnaga@ciestaam.edu.mx

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Abstract: The aim of this work was to analyse the systemic structure of multifunctional agriculture (MFA) and its nexus with sustainability through a bibliometric review of existing literature. By monitoring articles published on the Web of Science platform, a sample of 432 documents was identified. Two software packages, Bibliometrix and VOSviewer, were used to map scientific collaboration networks. The results made it possible to identify the authors, journals, and countries that had given rise to the current structure of knowledge. Four broad thematic clusters were identified: a) MFA and sustainability; b) ecosystem services and biodiversity; c) European public policies; d) governance and urban agriculture. It is concluded that despite an increase in publication rates research is concentrated in Europe, and, furthermore, there are few collaborative networks between different disciplines, suggesting that SDG17 is not being achieved.

Keywords: ecological planning, scientific production, sustainable agriculture, systematic review

1. Introduction

Multifunctional agriculture (MFA) is interpreted as the property of agriculture to create a co-production of marketable and non-marketable goods [1]. Although some argue that agriculture has always been multifunctional, it was not until the 1970s that it was recognized in the literature that agriculture plays multiple roles, where, in addition to producing food and fibre, it contributes to the development of multifunctional landscapes [2].

The multifunctional nature of agriculture allows links to be made between society, the economy, and the environment. As such, it is intrinsically linked to sustainability. These concepts are key in the current debate on agricultural policy reform and rural development in many countries and international platforms [3]. Several authors [3, 4] suggest that sustainable development is a global goal that includes multifunctional agriculture.

However, to date, there are gaps in the knowledge frontier regarding the synergies between the concepts of MFA and sustainability [5, 6]. In addition, researchers come from different disciplines and develop different definitions, approaches, and scales to assess and measure multifunctionality. This leads to fragmentation of information and lack of collaboration between multidisciplinary groups.

Cooperative networks are key to achieving the ambitions of the 2030 Agenda, as partnerships, transnational and interdisciplinary research are essential to achieve progress towards the 17 Sustainable Development Goals (SDGs) [7]. Specifically, SDG17 recognizes the importance of partnerships and collaborative governance to improve coherence between national and international policies and initiatives.

This study is based on the hypothesis that scientific production related to MFA is Eurocentric and lacks integration of the different disciplinary approaches, and therefore Goal17 of creating partnerships and cooperative networks is not being met.

Bibliometrics makes it possible to visualize international networks between countries, authors, and institutions, which is of particular interest for SDG17, which emphasizes the importance of analysing these partnerships to achieve progress towards the other SDGs [7]. Other authors find it useful to use bibliometrics to analyse the intellectual structure and its connection to the SDGs [8, 9].

Bibliometrics is a discipline that analyses bibliographic material from a quantitative perspective. One of its main advantages is that it allows the analysis of a specific research area, considering articles, journals, authors, institutions, and countries, thus providing a general picture of a research area. There are several bibliometric studies in the literature [10]. However, no article has been published that provides an overview of research on MFA and sustainability.

Therefore, the aim of the present work was to perform a quantification, systematization, and mapping of the scientific production published in the field of MFA and its link with sustainability in the framework of the Sustainable Development

Goals; to provide a holistic view of the existing literature, identifying: (a) the main areas of study; (b) the authors, journals, and countries that have conducted most of the research; (c) the main collaborative networks in this area; (d) emerging areas.

Multifunctional agriculture as a tool for understanding sustainability

The relationship between multifunctionality and sustainability is generally considered implicit and is rarely mentioned in research. This often leads to confusion between the two concepts [11]. For example, definitions of multifunctional agriculture and sustainability are similar, including the phrase "social, environmental, and economic development".

The Organization for Economic Cooperation and Development [1] prepared an analytical framework for studying the concept of multifunctionality, which is a reference document for understanding how these concepts are tied. It states that MFA leads to the study of the foundations of positive policy, as opposed to the concept of sustainability, which leads to foundations that lead to normative policy. MFA and sustainable development are related concepts in the sense that the latter provides the framework for describing and evaluating all the economic, social, and environmental objectives associated with MFA since MFA is not an objective but contributes to the understanding and realization of the different aspects of sustainable development in an agricultural and rural context.

These concepts are integrated under the branch of welfare economics, where the integration of both concepts in turn implies a shift from previous perspectives on productivity to the new perspectives introduced by the sustainability discourse [3].

The notion of MFA is thus a way of operationalizing sustainable development and reintroducing a range of different perspectives on agricultural development. However, the question of what types of functions are accepted as components of multifunctional agriculture for sustainable development remains open. Member countries of the World Trade Organization (WTO) do not agree on what constitutes multifunctional agriculture. This issue is territorially intrinsic, as each society wants to create favourable living conditions for its members, and the criteria for quality of life are planned in different ways. Research on multifunctional agriculture must therefore be multidisciplinary [12] since no single discipline can encompass all these different perspectives.

Multifunctional agriculture and the Sustainable Development Goals

Multifunctionality inherently implies a plurality of perspectives that are denoted in the various disciplines that study it such as economics, sociology, political science, geography, ecology, and industrial engineering, among others [13, 14]. Since no single discipline can fully incorporate the different nuances, research into multifunctional

agriculture must be analysed from interdisciplinary [15], transdisciplinary [16], multidisciplinary and interdisciplinary [17], or integrated approaches.

This scientific approach of multidisciplinary, interdisciplinary, or transdisciplinary teams is the way forward to address pressing issues facing our planet [18]. This is relevant when considering the SDGs because there are no simple answers to achieving any of them. After all, interdisciplinary research approaches are required for problems that have significant societal implications, along with diverse opinions and interests of the groups involved [19].

SDG17 – *Strengthen the means of implementation and revitalize the global partnership for sustainable development* is key to realize the aspirations of the other SDGs, as it recognizes stakeholder partnerships as important means to mobilize and share knowledge, experiences, technologies, and financial resources to support the achievement of the Sustainable Development Goals in all countries, especially in developing ones [21, 22].

MFA is a promising topic for territorial development, not only in rural areas and developing countries but also in urban areas and in developed countries. Partnerships are needed to implement an integrated approach to understanding MFA and its contribution to achieving the SDGs [22, 23].

2. Methodological design

Data collection

In this work, as a first step, the metadata were collected, consisting of citation information, bibliographic information, abstract and keywords, as well as funding details, for which the Web of Science (WoS) database of Clarivate Analytics was used since it is one of the most important digital repositories [23].

To compile the metadata, the search string was constructed using the following as part of the title, abstract, or keywords: "multifun* agri*" OR "agri* multifun*" OR "multifun* farm*" AND "sust*". Boolean operators (OR) and (AND) were adopted, as well as different combinations of the selected keywords and both plural and singular forms. The search included papers published before the year 2022. (This restriction is to improve comparability during the bibliometric analysis, as more recent publications had not had time to receive an adequate number of citations [24].)

Bibliometric analysis

Two main techniques were used for bibliometric analysis: performance and scientific mapping. Performance analysis can adopt several indicators, mainly related to the number of publications or citations of the documents within the

dataset and classifying them by authors, journals, countries, and affiliations [23], while mapping gives the researcher the hidden links or patterns in conceptual, social, or intellectual structures, providing an overview of the most important research [25]. In this paper, the number of articles and citations were used as performance indicators, and the type of analysis used for mapping was co-occurrence and co-citation.

To carry out these bibliometric analyses, two software programs with relevance in scientometrics were used: Biblioshiny, an R-based application [26], and VOSviewer [27].

For the realization of bibliometric networks, VOSviewer allowed the execution of different clustering algorithms to position and classify co-citations and pairings in similar groups.

Co-occurrence analysis is a technique based on the number of articles in which two keywords occur together. The size of the keyword node indicates weight, that is, how many documents a keyword appears in. Thicker lines mean more co-occurrences (how many documents a keyword appears in together with another keyword). The smaller the distance between the nodes, the stronger the relationship between them (how many articles these two keywords appear together and compare their relative co-occurrence with other keywords). The same colour of nodes and keywords means that they belong to the same cluster [28]. This allows for a more advanced description of the research, creating a mapping of the relationships between different terms and their association in thematic clusters [29].

Co-citation occurs in two units of analysis (they can be references, sources, or authors), which in turn are cited by other documents published after them; that is, it measures joint citations and assumes that the observed citation patterns reveal how multiple authors typically recognize the documents to promote important concepts [30].

3. Results and discussion

Performance analysis

This section presents the general evolution of the field. Thus, it is possible to know how the literature on MFA and sustainability has evolved and what impact it has had on the scientific community.

A total of 432 documents were found in WoS, of which 126 are case studies. This agrees with Parra-López et al. [31], who point out that in the literature one can find mostly papers on theoretical discussions but few reports that integrate case studies. The most studied subjects are maize, olive trees, and the wine industry.

No bibliometric work analysing MFA was found, while more than a hundred bibliometric studies refer to sustainability [33, 34], but none address sustainability and multifunctionality, which again highlights the relevance of this construct.

Journals

All 180 journals were found to contain at least one article published in the field. This indicates that the contribution is low, representing less than 1% of the universe of journals indexed in WoS [34]. Only 20 journals have published over 5 articles on this topic, with a contribution of 202 out of the 432 articles found in WoS; therefore, 230 are scattered in 160 journals.

The *Journal of Rural Studies* is the most cited one with 1,531 TC (total citations received retrievable in WoS). *Land Use Policy* is the journal that published the highest number of articles of the sample, representing 7.17% of the total published. However, it occupies the second place in terms of number of citations, with 1,409 (*Table 1*).

Table 1. Journals with higher impact

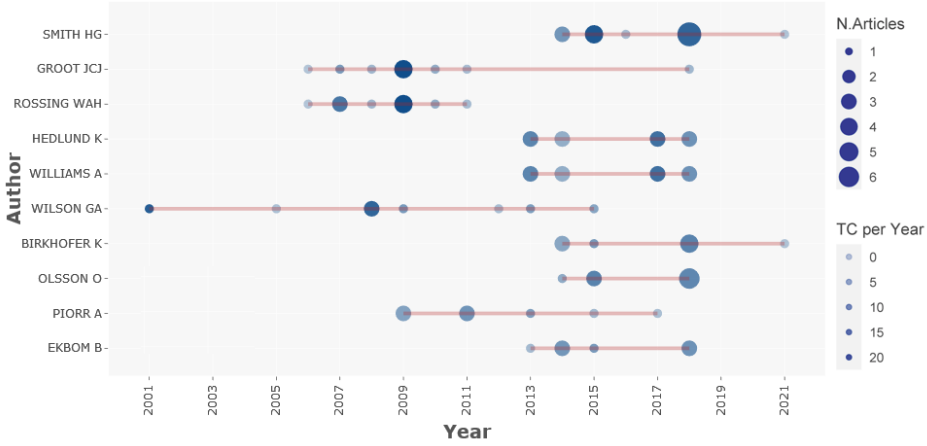
Number	Journals	TC	Articles
1	*Journal of Rural Studies*	1,531	26
2	*Land Use Policy*	1,409	31
3	*Agricultural Systems*	662	8
4	*Transactions of the Institute of B. G.*	626	2
5	*Journal of Environmental Management*	623	12
6	*Agriculture Ecosystems & Environment*	478	11
7	*European Review of Agricultural Economics*	352	6
8	*Proceedings of the National Academy of S. U.*	277	1
9	*Global Environ. Change – Human and Policy D.*	239	1
10	*Journal of Applied Ecology*	224	4

Authors

According to Forliano et al. [23], evaluating an author's relevance in a field should consider two relevant aspects: productivity and impact. In this work, both measures were considered to summarize the 10 most productive authors in this field (*Fig. 1*), where productivity was evaluated by the number of articles published per author, and impact was evaluated by the number of citations received per year.

It was found that Wilson G. A. is a pioneer in this field since from 2001 to 2015 he has had 8 publications as first author. His first publication is the most cited one: *From Productivism to Post-Productivism … and Back Again? Exploring the (Un)changed Natural and Mental Landscapes of European Agriculture*, where the author debates productivist/post-productivist thinking and opens a thread on the concept of agricultural multifunctionality, which encapsulates the heterogeneity

of rural societies. Similarly, in his later contributions, he provides a normative framework where he delimits multifunctional agriculture by productivist and non-productivist actions in a spectrum of weak, moderate, and strong multifunctional agriculture. The strongest form is manifested when the dimensions of social, economic, cultural, moral, and environmental capital are fulfilled.



Note: The larger and darker the circle, the more articles and citations the author had in that year.

Figure 1. Production of the authors with the greatest impact on MFA and sustainability over time

Other important authors are Groot, J. C. J. and Rossing, W. A. H. Their research contributes to decision making on the best alternative land uses while respecting agrobiodiversity.

It was revealed that the authors' disciplinary backgrounds belong to an ecological perspective, which is in line with Nowack et al. [15], who state that the "social functions" of multifunctional agriculture have received little attention, are rarely conceptualized, and are inconsistent compared to studies on ecological functions.

Countries

There were 54 countries represented by at least one paper. The 15 with the highest impact represent almost 92% of the total number of citations (*Table 2*). The remaining 8% of citations are distributed among 39 countries.

Table 2. Top 15 most cited countries based on the WoS 2022 dataset

Number	Country	Times cited	Articles	Average of citations	R&D (%GDP)
1	United Kingdom	2,708	51	84.62	1.71
2	Netherlands	1,847	89	52.77	2.18
3	USA	1,440	140	33.49	3.17
4	Germany	1,087	57	41.81	3.17
5	Sweden	771	86	30.84	3.39
6	France	668	72	41.75	2.19
7	Spain	534	65	17.23	1.25
8	Italy	378	94	8.22	1.46
9	Australia	338	26	30.73	1.83
10	Czech Republic	282	71	7.23	1.93
11	Norway	211	20	30.14	2.15
12	China	184	35	13.14	2.24
13	Austria	179	15	22.38	3.13
14	Switzerland	172	23	21.50	3.15
15	Belgium	146	9	36.50	3.16

Note: An article may represent more than one country. Research and development (R&D) expenses – 2019 value assumed.

Authors from the United Kingdom stand out; not because they are the country that generates the most documents, but because they are the origin of the greatest number of citations. The United States, despite being the country that generated the most documents, ranks third in terms of the impact of its contributions.

Applied research is mainly based in the European Union and China, where specific policies on MFA have been implemented to support rural development and promote sustainable rural communities; in contrast, MFA has rarely appeared explicitly in rural development policies in America or Australasia [2]. In the United States, there are still debates about the use of this policy [37].

Based on data from the World Bank, it can be seen that the countries in the table allocate a higher percentage of GDP to research and development (R&D) than the average expenditure of countries in Latin America and the Caribbean (.67%). This is consistent with Guerrero-Casado [38], who found that the countries with more articles in the agricultural sector are the ones with greater economic resources and not those where agricultural activities are more important.

Bibliometric network analysis

Co-occurrences

Four clusters were created, and each cluster was named according to the keywords of greatest importance. The terms MFA and sustainability belong to the same cluster and are at the centre of the analysis. The clusters were as follows: red (MFA and sustainability), green (ecosystem services and biodiversity), yellow (European public policies), and blue (governance and urban agriculture) (*Fig. 2*).

The number of research approaches coincides with Renting et al. [17], as they classify MFA into four main categories but differ along the lines of market regulation, land use approaches, public regulation, and stakeholder-oriented approaches, as they use the specific governance mechanisms and level of analysis for their classification.

The cluster in red includes the terms: MFA, agriculture, sustainability, landscape, rural development, producers, food security, post-productivism, organic agriculture, diversification, and environment. Because of the words that prevail in this cluster, it can be said that it is the theoretical root that gives rise to the other clusters since the concept of multifunctionality helps to explain the agricultural change concerning the productivist/post-productivist model [14]. On the other hand, Filepné Kovács et al. [39] found that the balanced diversification of the landscape limits the exodus and is a factor of rural development. This cluster is broad, but it basically seeks sustainable agriculture; and although agriculture is only explicitly mentioned in SDG 2 (zero hunger), most of the 17 SDGs can be related to agriculture in some way [40].

The cluster in green is formed of 10 keywords: ecosystem service, biodiversity, management, land use, conservation, economic valuation, diversity, trade-offs, agricultural landscapes, and indicators. The concept of ecosystem service prevails, as this is closely linked to MFA; they are two important concepts in the current debate on sustainable resource use [41]. This group is oriented towards case studies on the multiple options presented by landscapes and the conservation and sustainable use of soil, which have a direct impact on the SDGs of zero hunger (2), health and well-being (3), clean water and sanitation (6), responsible production and consumption (12), climate action (13), and life of terrestrial ecosystems (15). These studies are a priority, as land degradation is a threat to the fulfilment of the SDGs [19].

The cluster in yellow has two keywords: Europe and policies. Cheng et al. [42] highlight that policy studies and their impact on SDG realizations are prevalent in Europe. It should be noted that policy implementation is a critical and leading measure to achieving the SDGs.

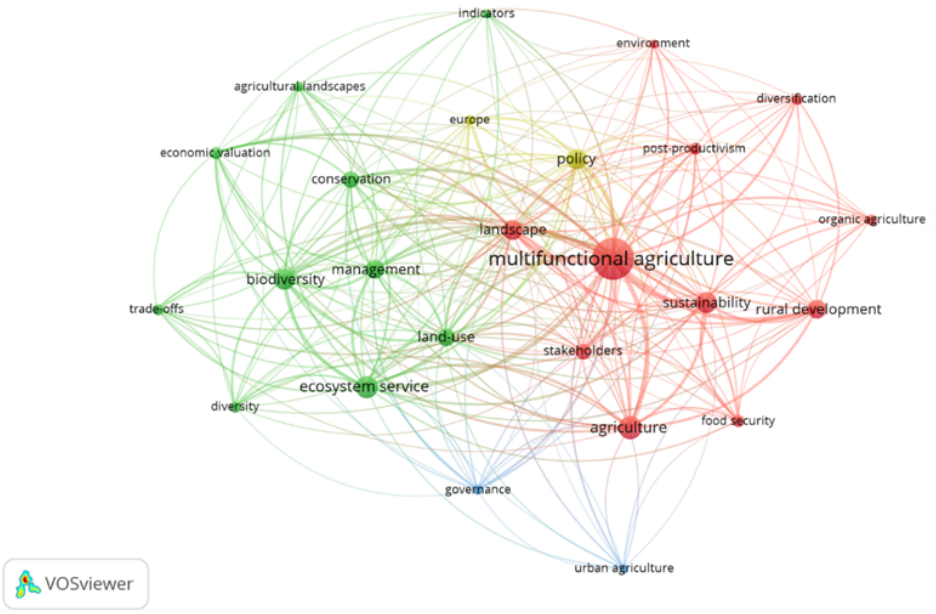


Figure 2. Co-occurrence links with all keywords, considering 12 words as the minimum number of occurrences of a word

The blue cluster consists of two keywords: governance and urban agriculture. The governance mechanism helps to organize the provision of public goods and services produced by agriculture. Governance is key to achieving the 17 SDGs; for while the SDGs have great potential, collective success will depend on several factors such as the extent to which states formalize their commitments, so strengthening global governance helps translate global ambitions into national and local contexts [43].

The five keywords that appeared most often in the search and that define MFA and sustainability are ecosystem service, landscape, biodiversity, rural development and policy.

To know the conceptual trajectory, a temporality network was used from the beginning of 2010 to the end of 2020, where the nodes in purple are the oldest words, and the nodes in yellow the most recent ones (*Fig. 3*). The keywords that appear in the first studies are post-productivism, optimization, and the European Union, among others. This analysis recognizes which are the emerging lines in the topic of MFA and sustainability. Eight emerging words were found: soil organic carbon [44], climate change [45], agroecology [16], case studies [46], Italy and agritourism [47], urban agriculture [48], social agriculture [49], and "trade-offs" [50].

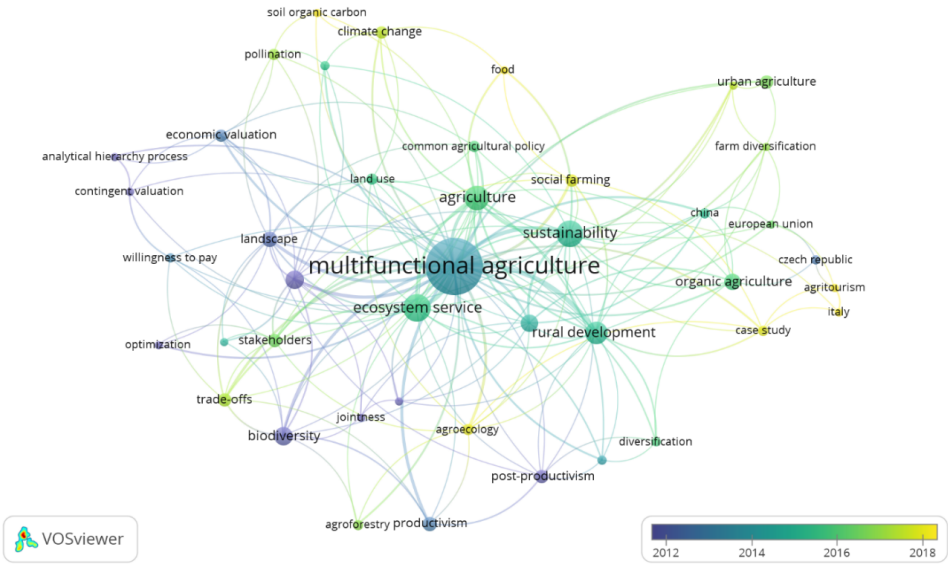


Figure 3. Overlay visualization by year of co-occurrence links with author keywords, considering four words as the minimum number of occurrences of a word

Author co-citation

Four main clusters of author co-citation are observed (*Fig. 4*), as visualized in the keyword co-occurrence map.

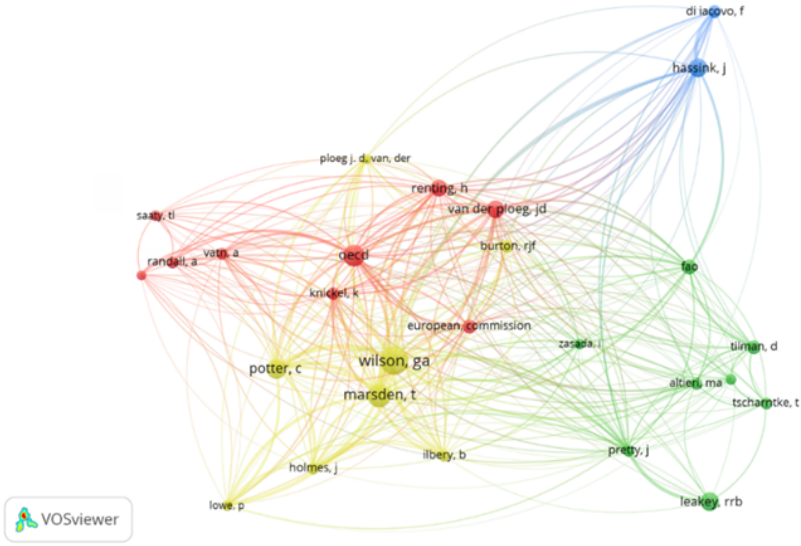


Figure 4. Author co-citation links, considering a minimum of 30 citations per author

The red cluster (MFA and sustainable development) is closely linked to the cluster in yellow (European public policy). At the centre of the map is the OECD paper *Multifunctionality: Developing an Analytical Framework*. This work [1], together with previous works by the European Commission [51, 52], laid the foundations for subsequent studies linked to European public policies, such as the work of Wilson, Potter, and Marsden. In the green group (Ecosystem services and biodiversity), authors such as Leakey stand out, who have works oriented towards agroecology [50], Zasada has works oriented towards agricultural diversification [53], and in the blue group (governance and urban agriculture) two authors, Di Iacovo and Hassink, stand out. These authors talk about social agriculture for global change [54] and "care agriculture" [55].

4. Conclusions

It was observed that primary and secondary studies on MFA in Latin America, Asia, and Africa are scarce, confirming the hypothesis that the scientific production on MFA is Eurocentric, which leads to the following question: Is MFA a reality outside the European context? The importance of generating more studies for the Global South region is highlighted, considering the European Union's experience with MFA.

To move beyond theory, empirical work needs to address the specific needs of each area, considering each country's capacity, budgetary constraints, and agricultural patterns. In addition, studies need to combine multiple disciplinary perspectives as a basis for building new interdisciplinary understandings. Although there is currently no unified conceptualization of sustainable development, most perspectives share the similarity that sustainable development should be committed to the coordinated development of the social economy and the environment, where MFA is increasingly recognized as a key concept in realizing the SDGs. It was found that research on MFA has made significant progress in recent decades; however, most studies have focused on historical changes, and future spatiotemporal changes have not been sufficiently explored. Therefore, it is suggested that future studies address MFA as a means of rural development planning, as well as a framework for assessing existing agricultural activities to determine the extent to which they contribute to the achievement of the 169 global goals or directly to any of the 17 SDGs.

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Effect of salinity on the germination of three species of the *Acacia* genus (*A. karroo*, *A. saligna*, and *A. tortilis*)

Abdenour KHELOUFI,[1,2] Lahouaria Mounia MANSOURI,[1*] Fouad MERADSI[1]

[1] Department of Ecology and Environment, Faculty of Natural and Life Sciences, University of Batna 2, 05078 Batna, Algeria

[2] Laboratory of Biotechnology for Food and Energy Security, University of Oran 1, Oran 31000, Algeria

e-mails: a.kheloufi@univ-batna2.dz,* l.mansouri@univ-batna2.dz, f.meradsi@univ-batna2.dz

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Abstract. This scientific paper explores the impact of soil salinity on plant growth, with a particular focus on the relationship between salt tolerance and seed germination. To investigate this, three species of *Acacia* (Fabaceae), namely *A. karroo*, *A. saligna*, and *A. tortilis*, were selected, and their seeds were collected from Algeria. To overcome seed coat inhibition, seeds were treated with concentrated sulphuric acid, followed by a wash with distilled water before being sown in a culture medium containing varying concentrations of salt, specifically sodium chloride (NaCl) ranging from 0 to 600 mM. The germination tests were conducted over a 21-day period, with measurements taken at intervals of three days, and both the final germination percentage (FGP) and mean germination time (MGT) were calculated. The results showed that at 600 mM NaCl concentration no germination occurred during the experiment. The germination rates exhibited three phases, an initial latency phase, a second exponential phase of accelerated germination, and a third plateau phase. *A. karroo* seeds demonstrated the highest salt tolerance, germinating under high salinity conditions of 400 mM NaCl, with a FGP of 66%. In contrast, *A. tortilis* showed significantly lower salt tolerance, with only 20% germination at the same concentration. *A. saligna* had the lowest salt tolerance, with germination only occurring under 150 mM NaCl concentration and with a FGP of only 18%. Based on these findings, the rank order of the studied species in terms of decreasing tolerance to high salinity conditions, as determined by their respective germination capacities, is as follows: *A. karroo* > *A. tortilis* > *A. saligna*. Although *A. saligna* was the most sensitive species, it was still categorized as a salt-tolerant glycophyte. Overall, this study provides valuable insights into the impact of salt stress on *Acacia* species and could contribute to the development of salt-tolerant crops in the future.

Keywords: Fabaceae, germination, salt tolerance, *Acacia*, NaCl, soil salinity

1. Introduction

The ecosystems of arid and semi-arid regions are characterized by sporadic precipitation and extended periods of drought, which contribute to soil salinization [1]. One strategy to address this issue is to investigate halophyte species that can thrive in saline environments and have the potential to restore damaged soils, thus limiting soil and irrigation water salinization [2]. Halophytes with high growth rates and the ability to store salt in their leaves are particularly suitable for rehabilitating and desalinating arid and semi-arid lands [3].

Seed germination is a critical process for agroforestry and horticulture since our reliance on plants is primarily based on their ability to germinate. However, various environmental factors, including temperature, salinity, light, soil moisture, oxygen concentration, pH, and soil structure, often limit the germination process [4]. Halophyte species that exhibit strong and rapid germination after overcoming unfavourable conditions have a significant ecological advantage in terms of seed dispersal and establishing the next generation of seedlings. This is particularly relevant for annual halophyte species that produce seeds only once during their life cycle and have developed survival mechanisms to ensure seed viability and species persistence [5].

Numerous research teams have concentrated on the detailed and overall characterization of the adaptation of specific *Acacia* species to stressful environmental conditions, whether indigenous or exotic, to incorporate them into sustainable afforestation/reforestation programmes ([6]–[8]). Algeria's soil appears to support an interesting species diversity of the *Acacia* genus with a rustic character, which can survive in extreme pedoclimatic conditions, and it would be beneficial to reintroduce them into reforestation programmes. In Algeria, Kheloufi et al. [9] identified ten *Acacia* species, including *A. albida*, *A. laeta*, *A. ehrenbergiana*, *A. nilotica*, *A. seyal*, and *A. tortilis*, classified as native species [10], as well as introduced species such as *A. farnesiana*, a native of America [11], *A. karroo*, native to South Africa [12], and *A. saligna* and *A. decurrens*, both native to Australia [13]. These species have a significant advantage due to their ability to form a symbiotic relationship with soil microorganisms such as rhizobium and mycorrhizae. This association allows them to survive in soil that is deficient in essential nutrients [14].

Salinity is a major abiotic stress that has a negative impact on plant growth and development globally. In regions with arid and semi-arid climates, the salinization process occurs due to high evaporation rates and insufficient precipitation. The aim of this study is to explore the impact of varying levels of salt stress (NaCl) on *Acacia* seed germination, with nine different levels tested, ranging from the lowest to the most severe. However, only three of the ten Acacia species found in Algeria will be examined in this study, based on their geographic distribution and

density. *A. saligna* represents the northern region, *A. tortilis* the southern region, and *A. karroo* is an overlapping species present in both areas.

2. Materials and methods

Harvest and origin of seeds

Table 1 presents the provenances of the seeds used in this study for the three *Acacia* species, *A. karroo*, *A. saligna*, and *A. tortilis*. Additionally, the table includes biometric data for each species such as the weight of 1,000 seeds and the length and width of seeds. The measurements were taken based on a sample of 100 seeds per species. The experiment took place at the Department of Biotechnology Laboratory at the University of Batna 2 in Algeria.

Table 1. Characteristics and origins of the Acacia seeds studied

	A. karroo	***A. saligna***	***A. tortilis***
1,000 seed weight (g)	44.8	14.7	53.4
Length (cm)	0.54 ± 0.05	0.49 ± 0.02	0.59 ± 0.03
Width (cm)	0.32 ± 0.02	0.28 ± 0.01	0.42 ± 0.01
Region in Algeria	Djelfa	Aïn Témouchent	Tamanrasset
GPS coordinates	34˚40’ N; 3˚09’ E	35°26’ N; 1°13’ W	22°46’ N; 5°34’ E
Altitudes (m)	1,155	2	1,409

The seeds used in the experiment were obtained by manually crushing the naturally dried pods harvested from 10 trees of each *Acacia* species. To minimize inter-genetic variation, the seeds were mixed after harvest. The mixed seeds were then stored in paper bags at a temperature of 4 °C for three months, simulating the vernalization period [15].

Figure 1. Seeds of the different studied Acacia species

Seed germination

The seed coats of *A. karroo*, *A. saligna*, and *A. tortilis* have an anatomical structure typical of the *Acacia* species, resulting in strong seed coat inhibition of germination (*Figure 1*). This implies that natural or artificial scarification of the seed coat is necessary to allow the imbibition and germination of the seeds. To overcome seed coat inhibition, immersion of the seeds in concentrated sulphuric acid (98% H_2SO_4) for 120 minutes was necessary to achieve near 100% germination success under non-saline conditions for *A. saligna* and *A. tortilis* and only 30 minutes for *A. karroo* seeds ([15], [16]). After the acid immersion, the seeds underwent washing with distilled water to eliminate any traces of acid. They were then dried on absorbent paper until immediately sowing them in a suitable culture medium containing different salt concentrations.

Experimental design and application of salt stress

In order to study the effect of salt on germination, we used sodium chloride (NaCl). Seeds (3 species × 5 Petri dishes × 10 seeds × 1 salt × 9 concentrations) were germinated in 10-cm-diameter Petri dishes lined with two layers of Whatman N°1 paper, which were moistened with 20 ml of distilled water for the control (0 mM) and with 20 ml of one of the saline solutions having the following concentrations: 50, 100, 150, 200, 250, 300, 400, and 600 mM. It was important to ensure that the seeds maintained a certain level of humidity throughout the experiment. The counting of germinated seeds with a perforated testa was carried out every 3 days during the 21-day experiment. The Petri dishes were then placed in darkness at room temperature: 25 °C (± 2 °C). The seeds were moistened every three days with 15 ml of appropriate NaCl solution. Also, the papers were replaced every three days to prevent salt accumulation [17].

In the germination tests, the final germination percentage (FGP) and the mean germination time (MGT) for each species and treatment were calculated using the following procedures and formulas:

$$\text{FGP (\%)} = \frac{\sum ni}{N} \times 100,$$

where FGP is the final germination percentage, *ni* is the number of germinated seeds on the last day of the test, and *N* is the total number of seeds incubated per test [18].

$$\text{MGT (days)} = \frac{\sum(ti.ni)}{\sum ni},$$

where MGT is the mean germination time, *ti* is the number of days since the beginning of the test, *ni* is the number of germinated seeds recorded at time *t(i)*, and *Σni* is the total number of germinated seeds [19].

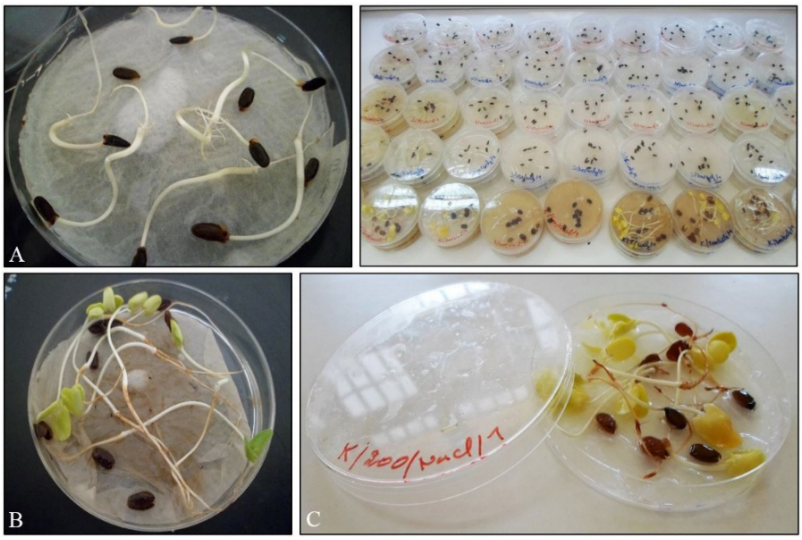

Figure 2. Experimental design and germination in Petri dishes
(A) *A. saligna*; (B) *A. tortilis*; (C) *A. karroo*

Statistical analyses

The effects of different NaCl concentrations on the two variables studied were tested by analysis of variance (ANOVA). Differences between treatments following ANOVA were made by means comparison. Multiple comparisons of means were carried out using Tukey's test ($p \leq 0.05$). Pearson's correlation coefficient was calculated for the three variables studied ($p \leq 0.05$). For germination kinetics, we applied the GLM (General Linear Model) procedure with univariate tests of hypotheses for the between-subject and within-subject effects. All statistical analyses were performed using SAS software Version 9.0 (Statistical Analysis System) (2002).

3. Results

Germination kinetics

The data presented in *Figure 3* displays the overall germination rates for seeds of three different *Acacia* species (*A. karroo*, *A. saligna*, and *A. tortilis*) over a period of 21 days as a function of increasing NaCl concentrations (mM). The figure

highlights three distinct phases: an initial phase of seed imbibition resulting in a latency period, followed by an exponential phase of rapid germination, and, finally, a plateau phase indicating a cessation in germination (stationary phase) (*Figure 3*). Notably, none of the seeds from the three species were able to germinate at a NaCl concentration of 600 mM during the 21-day experimental period.

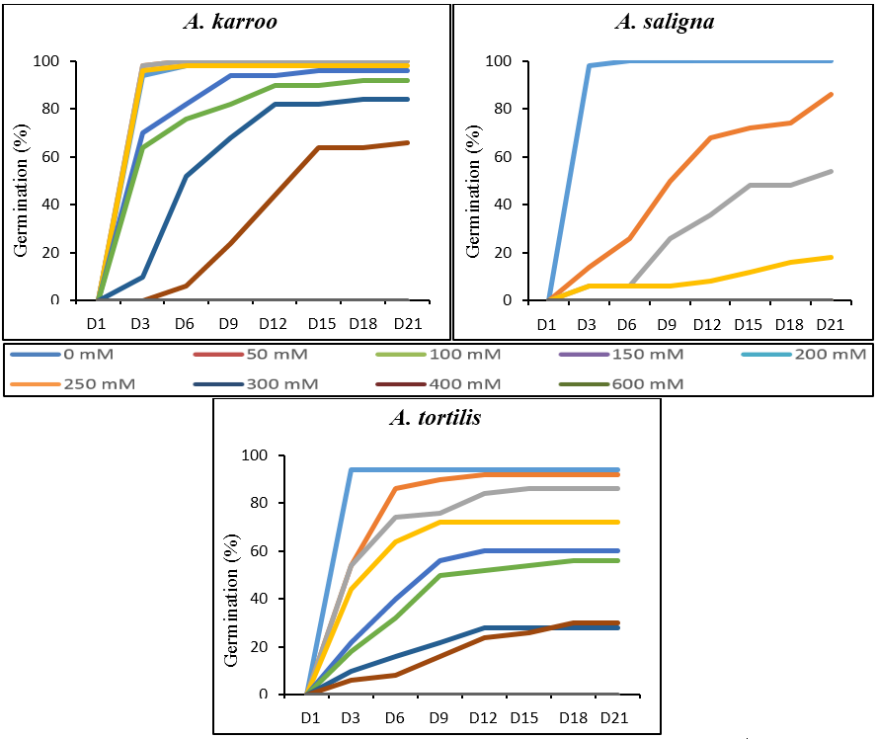

Figure 3. Effects of NaCl concentrations ranging from 0 to 600 mM on the germination kinetics of A. karroo, A. saligna, and A. tortilis seeds over 21 days

With increasing salinity, the germination curve is altered, causing a delay and reduction in the germination rate (*Figure 3*).

For *A. karroo*, the stationary phase begins on the 6th day in the control group, while seeds treated with 50, 100, and 150 mM reach the stationary phase on the 3rd day with almost 100% germination. As salinity levels rise, the stationary phase is further delayed, and under 400 mM NaCl, it exceeds 21 days.

For *A. saligna*, no germination occurs above 200 mM NaCl during the 21-day period, while the control group shows 98% germination by the 3rd day with a stationary phase starting on the 6th day. Seeds treated with 50, 100, and 150 mM have a low initial germination rate, which improves over time.

For *A. tortilis*, the stationary phase begins on the 3rd day in the control group with 94% germination. As salinity increases, the stationary phase starts at around the 15th day, and the germination rate decreases with increasing NaCl concentration. As the NaCl concentration increases by 50 mM, the exponential phase begins with a lower germination rate than the previous level, reaching 6% germination for the 400 mM treatment compared to 54% for the 50 mM treatment.

According to *Figure 3*, the germination rates of all the species examined decrease with increasing salinity stress, and NaCl has a significant impact on this reduction ($p < 0.0001$). In addition, it is evident that the length of the latency period varies among species and increases as the concentration of NaCl increases. A repeated measures analysis of variance (performed over a 21-day period with evaluations every three days) indicates that there is a highly significant effect ($p < 0.0001$) between various factors and variables, such as treatment (TRT), species (SP), and time (T), with both between-subject and within-subject effects and their correlation being observed (*Table 2*).

Table 2. Analysis of variance for the variables: final germination percentage (FGP), mean germination time (MGT) of seeds (A. karroo, A. saligna, and A. tortilis) (SP) in response to saline stress (TRT) induced by NaCl over 21 days (T)

Parameters	Sources of variation	Degree of freedom	F of Fisher	P-value
FGP	TRT	8	197.71	< 0.0001
	SP	2	427.37	< 0.0001
	TRT×SP	16	28.06	< 0.0001
MGT	TRT	7	26.49	< 0.0001
	SP	2	58.95	< 0.0001
	TRT×SP	10	18.25	< 0.0001
Germination kinetics	**GLM procedure**			
	Repeated analyses of variance measures			
	Univariate hypothesis tests for between-subject effect			
	TRT	8	273.93	< 0.0001
	SP	2	612.11	< 0.0001
	TRT×SP	16	31.75	< 0.0001
	Univariate hypothesis tests for within-subject effect			
	T	7	1513.43	< 0.0001
	T×TRT	56	66.46	< 0.0001
	T×SP	14	131.67	< 0.0001

Final germination percentage

The three *Acacia* species had a final germination rate of over 90% when grown in distilled water. However, when grown in the presence of NaCl, a highly significant effect of NaCl on germination rates was observed through analysis of variance ($p < 0.0001$) (*tables 2–3*).

Germination was delayed in most species as the concentration of NaCl increased. To determine the salt stress tolerance ranking of the species, we used 200 mM as a reference concentration. At this concentration, there was a wide range of variation among species. *A. saligna* had no germination power, while *A. tortilis* had half the germination power compared to the distilled water control. In contrast, *A. karroo* had a 96% final germination percentage, indicating a high salt stress tolerance. The ranking of the species based on decreasing tolerance is *A. karroo* > *A. tortilis* > *A. saligna*. This ranking is also supported by Tukey's test (*Table 2*).

Table 3. Effects of NaCl on the final germination percentage (FGP) and mean germination time (MGT) of seeds (A. karroo, A. saligna, and A. tortilis) after 21 days of treatment

Species	TRT [NaCl]	FGP (%)	MGT (days)
A. karroo	0 mM (Control)	100 ± 0.00^{a}	3.24 ± 0.25^{d}
	50 mM	100 ± 0.00^{a}	3.06 ± 0.13^{d}
	100 mM	100 ± 0.00^{a}	3.06 ± 0.13^{d}
	150 mM	98.0 ± 1.47^{a}	3.06 ± 0.13^{d}
	200 mM	96.0 ± 3.47^{a}	4.39 ± 0.52^{cd}
	250 mM	92.0 ± 4.9^{ab}	4.89 ± 0.58^{c}
	300 mM	84.0 ± 3.2^{b}	7.46 ± 1.69^{b}
	400 mM	66.0 ± 2.94^{c}	11.8 ± 2.01^{a}
	600 mM	0.00^{d}	--
A. tortilis	0 mM (Control)	94.0 ± 3.47^{a}	3.00 ± 0.00^{e}
	50 mM	92.0 ± 2.36^{a}	4.47 ± 0.56^{d}
	100 mM	86.0 ± 1.47^{a}	4.96 ± 1.02^{cd}
	150 mM	72.0 ± 3.36^{b}	4.48 ± 0.85^{d}
	200 mM	60.0 ± 3.8^{c}	6.09 ± 0.71^{bc}
	250 mM	56.0 ± 3.47^{c}	6.96 ± 0.96^{b}
	300 mM	30.0 ± 2.36^{d}	6.75 ± 1.27^{b}
	400 mM	20.0 ± 2.07^{d}	9.80 ± 1.48^{a}
	600 mM	0.00^{e}	--

Species	TRT [NaCl]	FGP (%)	MGT (days)
A. saligna	0 mM (Control)	100 ± 0.00^{a}	3.06 ± 0.13^{b}
	50 mM	86.0 ± 2.7^{b}	12.0 ± 2.33^{a}
	100 mM	54.0 ± 3.9^{c}	11.2 ± 1.09^{a}
	150 mM	18.0 ± 1.47^{d}	12.0 ± 2.37^{a}
	200 mM	0.00^{e}	--
	250 mM	0.00^{e}	--
	300 mM	0.00^{e}	--
	400 mM	0.00^{e}	--
	600 mM	0.00^{e}	--

Note: For each species, the different letters in the same column indicate a significant difference at $p \leq 0.05$, as evaluated by Tukey's test.

Mean germination time

Table 2 and *Table 3* demonstrate that salt has a notable impact on the germination of most *Acacia* species. As salt concentration in the culture medium increases, germination rates substantially decline, and a delay in seed germination is evident (as indicated by the mean germination time).

In addition, *Table 4* reveals a significant negative correlation between the variables under investigation (FGP and MGT). This implies that as FGP increases, MGT tends to decrease.

Table 4. Pearson's correlation coefficient between final germination percentage (FGP) and mean germination time (MGT) for different species of Acacia exposed to different NaCl concentrations (DF = 100)

	FGP	MGT
FGP	1.00000	-0,63112
P-value		< 0.0001
MGT	-0,63112	1.00000
P-value	< 0.0001	

4. Discussion

According to Ungar [5], salinity has a negative impact on the ability of both halophytes and glycophytes to germinate and can also cause a delay in the germination process. However, the way in which plants respond to salinity varies depending on the species. It has been observed that excessive salinity in soil, usually over 100 mM of sodium chloride, can lead to reduced growth in glycophytes, including important agronomic species, as reported by Sun et al. [20].

Our findings indicate that saline stress led to a decrease in germination percentage and delay across all species. According to Fernando et al. [21], metabolic imbalances within the seeds were responsible for the reduced germination under stress conditions. The extent of reduction varied for each species. *A. karroo* displayed a higher germination percentage than *A. tortilis* and *A. saligna*. *A. saligna* seeds were only able to germinate under 150 mM or lower saline stress, with germination completely inhibited at higher levels. Genetic and environmental factors both affect seed germination, and different species have developed diverse mechanisms to adapt to sodium-chloride-induced adverse conditions.

Bradford [22] reported that salinity affects seed germination, by either osmotic or toxic effect depending on the species. Osmotic effects are caused by insufficient water absorption, which prevents seeds from reaching their critical hydration level for germination. Toxic effects arise from salt accumulation in cells, which disrupts enzyme activity and prevents dormancy breaking, ultimately leading to reduced germination capacity. In severe cases, excessive ion accumulation can alter metabolic processes and cause embryo death [23]. The findings are in agreement with previous studies conducted on *A. saligna*, *A. tortilis*, and *A. karroo* by Meloni et al. [24], Jaouadi et al. [6], and Kheloufi et al. [25] respectively. These studies showed that the germination rate decreased significantly as the salt concentration in the culture medium increased.

Several researchers have shown that the imbibition and average time of germination are affected by the osmotic potential caused by NaCl, but not the final germination rate. Additionally, Tiryaki and Andrews [26] suggest that it is the germination capacity, rather than the delay caused by salt, that is most important for the final crop yield. Environmental factors, such as water availability and salt content in the soil, also play a role in regulating germination, in addition to genetic characteristics [27]. The low external potential can hinder the enzymatic activity of seeds, causing a delay in the release and growth of the radicle [28].

The toxic effects on seed germination are caused by the absorption of sodium ions, which disturb the movement of Ca^{+2} and Na^{+} ions at the cell wall level and can prevent radicle growth [29]. Sodium chloride has been found to increase the influx of external ions and cytosol efflux solutions, thereby affecting plasma membrane permeability [30]. Moreover, it stiffens the cell wall and reduces the fluidic conductance of the plasma membrane [31]. During seed germination, the degradation of reserves occurs through the action of amylases, phosphorylases, and glucosidases. The products of hydrolysis are then transported to the embryo to support its growth and development [32]. The mobilization of reserves may be slowed down by delayed activation and synthesis of hydrolases or the transfer of inhibition of hydrolysis products from the endosperm to the embryo, which can occur due to the effects of salinity [33].

The order of tolerance of the species under study based on their germination capacity can be arranged in descending order as follows: *A. karroo* > *A. tortilis* > *A. saligna*. Although *A. saligna* is classified as a salt-tolerant glycophyte, it appears to be highly sensitive compared to the other two species. Meanwhile, *A. karroo* and *A. tortilis* can be classified as halophytes at this stage.

In the Mediterranean region, high levels of salinity are reached at the surface of the sand during summer and early autumn [34]. In the case of *Acacia*, it is important to note the strong correlation between salt and temperature: germination occurs more readily at low temperatures in the presence of salt and gradually decreases as temperature increases [24]. This has significant ecological implications, as it highlights the necessity of reducing soil salinity for successful seed germination. Typically, germination in saline environments occurs in the spring when temperatures are cooler, and soil salinity decreases at the end of winter and spring [5]. This pattern is common in most halophyte seeds, which show optimal germination in freshwater ([35], [36]). Despite the differences between halophytes and glycophytes in their adaptation to salinity, both types of plants are affected by high salinity levels when it comes to seed germination [5].

5. Conclusions

The main objective of this study was to examine how NaCl-induced salinity stress affects the germination kinetics of three *Acacia* species: *A. karroo*, *A. saligna*, and *A. tortilis*. The findings indicated that as the salinity stress increased, the germination rates of all three species decreased. The germination curves of each species went through three phases: an initial latency phase due to seed imbibition, a second exponential phase characterized by rapid germination, and a final plateau phase indicating a halt in germination. The latency phase differed among the three species and increased with higher NaCl concentrations. Statistical analysis demonstrated that NaCl significantly impacted the germination rates of these species. These results suggest that all three species are sensitive to salinity stress, with varying degrees of sensitivity. *A. saligna* was the most sensitive, displaying no germination at NaCl concentrations above 150 mM, while *A. karroo* and *A. tortilis* were tolerant to salinity stress, with *A. karroo* displaying greater tolerance than *A. tortilis*.

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Complex evaluation of wild pear

Adrienn GYURKÓ,[1,2] Borbála BAKTAY,[1] Anna VARGA,[3] Zsolt SZANI[4]

[1] National Centre for Biodiversity and Gene Conservation

[2] Hungarian University of Agriculture and Life Sciences, Institute of Genetics and Biotechnology

[3] University of Pécs, Faculty of Humanities and Social Sciences Department of Cultural Anthropology

[4] ECPGR *Malus/Pyrus* Working Group Hungary

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Abstract: The National Centre for Biodiversity and Gene Conservation (NBGK) started the gene bank collection and agrobotanical evaluation of wild pear varieties in 2022. The survey of individuals and the recording of descriptors was carried out in several rounds. Part of the data was recorded in situ on the field, and the other part was analysed after the trip. For the in-situ collection of wild fruit plant species and varieties, it was necessary to develop a method for the collection and description. This applied to the development of the survey methodology as well as to the design of the working method.

Keywords: gene bank, wild pear, collection, biodiversity

1. Introduction

Several scientific fields have processed and evaluated a subset of wild pear genotypes according to their own criteria. [8] has made a botanical description of the wild pear population of the Pannonian region [8]. [5] presents wild pear as rootstocks used in fruit production. Eplényi has shown, using the example of the Kalotaszeg (Rom. *Ţara Călatei*) region, that fruit trees and orchards can be of great visual importance at the landscape level, even despite their small share in the proportion of cultivated species [2][3]. It can be a landscape character and pattern that gives a unique distinctness to the whole area. In the same region, Varga studied the ethno-ecology of pasture wild fruit populations such as wild pears [11]. Szani described the different uses of wild pears based on the ethnopomological traditions of Nógrád County [7].

Both the number and the diversity of wild pears are threatened due to dwindling habitats. We suppose it is necessary to change the paradigm and to consider the diversity of the native wild pear population as an intrinsic value and to describe it as complexly as possible. This requires the development of a complex, species-specific assessment system.

2. Materials and methods

The *Pannonian Seed Bank Project*, which was implemented between 2010 and 2014 to collect and conserve seeds of native wild plant species in the long term, focused mainly on herbaceous plant species/ecotypes. Following the successful implementation of the project, the National Centre for Biodiversity and Gene Conservation considered it timely to continue the activity and extend it to other crop sectors such as wild fruit plants. Wild pear was chosen as the pilot plant species [4].

International guidelines for the description of vegetatively propagated, elder fruit pear cultivars are available, such as IBPGR (1982, 1983), UPOV (2000), or ECPGR (2022), which are well suited for the phenotypic description of the cultivars of fruit varieties and for the distinction of varieties from each other [6], [9], [10]. Brózik tried to make pear variety descriptions as complete as possible from the point of view of commercial fruit production [1]. However, a new, more comprehensive descriptive system needs to be developed for generatively propagated wild fruit species.

The National Centre for Biodiversity and Gene Conservation (NBGK) started the gene bank collection and agrobotanical evaluation of wild pear varieties in 2022 to assess and evaluate the wild pear population. The survey of individuals and the recording of descriptors was carried out in several rounds. Part of the data was recorded in situ on the field, and the other part was analysed after the trip.

For the in-situ collection of wild fruit plant species and varieties, it was necessary to develop a method for collection and description. This applied to the development of the survey methodology as well as to the design of the working method.

Before the ripening of wild pears, we carried out a trial collection, during which we collected 20 lots on one location. We are not yet able to collect fruit samples from the lots. During the on-site data collection, it was important to be able to collect all data relevant to the gene bank. The recording is carried out as efficiently as possible due to the defined time frame, and later the data can be recorded, evaluated, and compared in a uniform database. During the planning of the trial collection route, it was formulated that we need a special observation sheet to document the items.

For this purpose, we wanted to create a questionnaire on which all relevant information can be recorded in the field within a short time and which can be

easily read later, so instead of handwriting, it should be marked. During the design phase of the questionnaire, we took as a basis the experiences of previous gene bank fruit collection trips and surveys in civil community cooperation. It was an important aspect that, in addition to recording the basic data, we could also record as much of the grouping properties of the items as possible, as well as that it should be a maximum of 2 pages so that its use and filling in would not cause technical difficulties during the fieldwork.

3. Results and discussions

On the collection site, we created a collection sheet to record the data of the items to be collected. Specialized for field recording, it consists of a standard A4, 2-sided sheet, so it is easy to use, and there is no need to turn pages during documentation. The first page of the questionnaire details the circumstances of the collection, while the second page describes the collected item.

First, we record the collection location of the item (country, town). If there is a person providing information, their name and contact details are put down. The item to be collected is first marked at the species level, which is selected from the list on the collection sheet and then assigned a unique code. In the case of coding, we use at least one letter and two digits, for example: K01. We record the date of collection, the name of the person(s) collecting, and the collection location of the item using GPS coordinates. In addition to the coordinates, we also prepare a sketch of the lot's surroundings and location. On the sketch, we note the characteristic points of reference in the vicinity, such as roads, buildings, groups of plants, in relation to the position of the lot; also, environmental elements characteristic of the position of the item are noted down, which can be used to identify the location of the item later on. In accordance with the international gene bank guidelines, applying them to the case of fruit-bearing plants, we document the collected lot:

– the source of the collection (unknown / wild species / cultivation / trade / home garden / institution, organization / other);

– the status of the sample (unknown (seedling) / wild / feral / landscape variety / line / variety / other);

– we also include the method of propagation of the lot if it can be established (seeding, grafting / from seed / scion / cuttings, budding / other);

– we assess the condition of the tree, which ranges from the young, non-productive condition to the decaying state (dying / desiccating / old, sick, severely damaged / fertile, sick / fertile, not vigorous / fertile, vigorous / young, not yet productive / other);

– in terms of the use of the item, we indicate whether the fruit, other plant parts, or both can be used (fruit / other plant parts / both);

– the method of using the fruit is also indicated on the collection sheet, where it is important to take into account that in the case of wild pears, this is often multi-purpose; in addition to the human use of the fruit, it can also be used for animal husbandry or game management (fresh consumption / consumption after storage / jam / vinegar / pálinka (distillate) / aszalvány / juice (must) / fruit wine / healing / habit / other);

– we also include the use of other plant parts, which can be rootstock, pollinator, multi-purpose use, wild form, or other.

Regarding the wild pear batch, if there is fruit on the collected batch, we also record some important grouping descriptors for the fruit on site: the size of the fruit, the largest diameter of the fruit, the shape of the fruit, the basic colour of the shell, if there is a cover colour, the extent of its covering. If the collection is done during flowering or the flowering time is known, then the time of flowering is recorded. We also write additional comments on the collection sheet, which can be considered a special identification of the item, as well as the characteristics of the fruit's taste.

We also do photo documentation on site. We photograph the item according to a uniform setting. To illustrate proportionality, we use a measuring stick or a device whose exact dimensions we know. We photo-document the budding, fruiting, leaf colour, and backside of the item. This is done on an A4 white square grid sheet, and a 30-cm ruler is used to illustrate the scale. For subsequent recordings, we collect leaves from the middle third of the shoots, as well as at least 10 pieces of fruit.

The collected samples are stored in a refrigerator until processing. The following quantitative tests are performed on the leaf samples: measurement of the length and width of the leaf blade, measurement of petiole thickness and length. Then we record the visual parameters, for which we apply the ECPGR pear guideline, UPOV recommendations. In the case of fruit samples, we measure the length and width of the fruit at the widest point, the length and thickness of the fruit stalk, and then also record the qualitative parameters. We supplement the recordings with pomological photo documentation. The seeds are extracted from the fruits for long-term preservation in the seed bank. The gene bank method for maintaining generatively propagated wild pear genotypes and populations is yet to be developed.

We work as a team during collection work. Based on our experience, a proper session requires a minimum of three people, optimally four-five people. In the latter case, during the collection, one person fills out the pre-prepared collection sheet, one person collects and labels leaf samples for later recordings, one person collects and labels fruit samples, and two people carry out the photo documentation of the genotype and its surroundings, as well as its plant parts in scale such as its shoot, its leaves, and fruit on the spot.

Figure 1. On-site photo documentation of the genotype

Figure 2. Pomological photo documentation

One of the largest wild pear specimens surveyed during the collection trips in 2022 was examined in Bogyiszló. The height of the tree was 8.49 meters, and the diameter of the crown was 11.21 meters. The floor area of the crown was 98.6 m^2, while the volume of the crown was 538.3 m^3. The collected wild pear specimens can be evaluated from several points of view based on the on-site survey. When evaluating wild pear genotypes as elements of landscape character, in addition to the size of the tree, the general shape of the crown, the habit of the skeletal branches, and the ratio of the height and width of the crown are also decisive. In *Table 1*, we evaluated the wild pear populations in each collection location according to the above three aspects.

Table 1. Evaluation of wild pear genotypes

Place of production	Number of collected lots	Crown shape		
		Hemi-sphere	Sphere	Pyramid
1	7	14%	14%	71%
2	10	40%	20%	40%
3	6	33%	17%	50%
4	11	18%	18%	64%
5	20	65%	20%	15%
All	***54***	***41%***	***19%***	***41%***

Place of production	Number of collected lots	Position of the main branches				
		Disruptive	Half disruptive	Diverging	Stooping	Hanging
1	7	-	57%	29%	14%	-
2	10	-	20%	70%	10%	-
3	6	-	33%	50%	17%	-
4	11	-	45%	36%	18%	-
5	20	-	20%	45%	35%	-
All	***54***	-	***31%***	***46%***	***22%***	**-**

Place of production	Number of collected lots	Ratio of tree height/width				
		Strongly flattened	Flattened	Medium	Elongated	Strongly elongated
1	7	-	57%	43%	-	-
2	10	-	30%	30%	40%	-
3	6	-	33%	50%	17%	-
4	11	27%	64%	9%	-	-
5	20	-	10%	40%	40%	10%
All	***54***	***6%***	***33%***	***33%***	***24%***	4%

Note: Places of production: 1: Abaliget; 2: Bogyiszló; 3: Csokonyavisonta; 4: Nagybajom; 5: Tiszaug.

4. Conclusions

Based on the experience of the collection routes of the first year, it is worth expanding the documentation to document the habitat, to describe the tree as a landscape element. This is also necessary for the development of the method of seed bank maintenance and cultivation for populations of wild, foreign-fertilizing, native fruit species.

In Hungary, there is a high genetic diversity of the wild pear genotypes. From the point of view of biodiversity, the persistence of genotypes has a huge importance.

Our institute aims to continue the examination of the wild fruit species and to assess the population and the genetic diversity.

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Monitoring of the water quality of Lake Blidinje and examination of the prognostic model

Anita IVANKOVIĆ,[1] Marina MARIĆ,[1] Danijela PETROVIĆ,[1] Stanislava TALIĆ,[2] Anita MARTINOVIĆ BEVANDA[2]

[1] University of Mostar, Faculty of Agriculture and Food Technology, Biskupa Čule bb, Mostar, Bosnia and Herzegovina; e-mails: anita.ivankovic@aptf.sum.ba, marina.maric@fpmoz.sum.ba, danijela.petrovic@aptf.sum.ba

[2] University of Mostar, Department of Chemistry, Faculty of Science and Education, Matice hrvatske bb, Mostar, Bosnia and Herzegovina; e-mails: stanislava.talic@fpmoz.sum.ba, anita.martinovic.bevanda@fpmoz.sum.ba

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Abstract: The largest mountainous lake in Bosnia and Herzegovina, Lake Blidinje, is located 1,185 meters above sea level. Polymictic lakes are those in which the entire water column is frequently mixed. Shallow lakes function very differently than deep lakes in many ways because of the strong sediment–water interaction and the possibility for significant water impact on vegetation. In the multiple regression analysis, two models were developed with coefficients of multiple determination (R^2 = 0.720 and R^2 = 0.497) for the independent variable chlorophyll a. The resulting models are compared with water quality monitoring data from 2017 to 2019.

Keywords: physical-chemical indicators, multiple regression analysis

1. Introduction

The largest mountainous lake in Bosnia and Herzegovina, Lake Blidinje, is located 1,185 meters above sea level. According to Directive 2000/60/EC of the European Parliament and the Council [1] of the Western Balkans, Lake Blidinje is categorized as belonging to the fifth ecoregion for rivers and lakes in the Dinaric Alps. When Krasić and Zelenika [2] investigated ways to preserve the waters of Blidinje Nature Park in 1998, they came to the conclusion that the lake loses roughly 24,000 m^3 of water daily, or 4-5 mm during spring. The lake filters sediment and experiences an annual sediment growth of 1 mm. Large amounts of suspended particles in the lake water contribute to its high turbidity. The 15 cm clarity of the lake reflects this in a sizable way. The lake loses water from

evaporation because of its wide surface, particularly in the summer. Most of the animals of this region and the surrounding area use this lake as a watering place.

Polymictic lakes are those that regularly have the entire water column intermingled in them. The functioning of shallow lakes differs significantly from that of deep lakes in many ways because of the intensive sediment–water interaction and the potentially significant influence of aquatic vegetation. Simple criteria, such as the traditional correlation between algal growth and nutrient load, do not apply to shallow lakes [3]. Shallow lakes frequently have "catastrophic" rather than "mild" responses to eutrophication. Many lakes have been seen to change repeatedly from having clear water to having cloudy water without any discernible external influences. This entirely distinct behaviour of shallow lakes has seemingly repelled rather than attracted researchers throughout time, given the relative neglect of shallow lakes in the limnological literature. The few available deep lakes have long been the sole subject of limnological research, even in nations where nearly all lakes are shallow such as Denmark and the Netherlands [4]. Due to a lake's delicate and complicated ecosystem, even a tiny amount of external stress (such as tourist activity) combined with biogenic element inflows might contribute to eutrophication [5]. High mountain lakes are characterized by great diversity and high sensitivity to external influences. Each lake in itself is an expression of interactions within the ecosystem, which in the scientific sense imposes special standards (e.g. water quality) but also special scientific approaches and methodologies. The experiences of researchers highlight the need to use chemical analysis methods with high sensitivity and high precision, that is, the strict application of the quality system, in order to determine, model, and correctly interpret possible changes. Almost every mountain in Bosnia and Herzegovina with peaks above 2,000 m has a "mountain eye" (Vranica, Čvrsnica, Maglić, Zelengora, Lelija, Treskavica) – lakes whose quality status is little known. Where humans are permanently present, there are indications of anthropogenic pollution (Blidinje, Lake Prokoško). In order to achieve or maintain a good ecological status of these waters in line with the EU's *Water Framework Directive*, the identification and assessment of the condition of these lakes serves as the first link in the management of these water resources. Remote mountain lakes are the most sensitive water ecosystems in Europe. They are the focal points of mountain landscapes, habitats for unique plants and animal communities. Such ecosystems, for example, in the areas of the Arctic and the Alps, represent the most preserved ecosystems in Europe. They are vulnerable and impacted by pollution and climate change. Due to their sensitivity, remote highland lakes are excellent environmental change sensors, and the excellent quality of their sediment can be used to calculate the rate, direction, and biological consequences of changes in climate and air quality [6, 7]. Shallow polymictic lakes are more prevalent than

deep lakes in many regions of the world, and even when they are small, they are nevertheless important, especially in densely populated areas. In many aspects, their ecology is different from stratified lakes. The concept of "alternate stable equilibrium" describes how lakes might transition from a clear water condition where macrophytes are dominant to a state where algae are dominant during the eutrophication process [8]. Although the area of Lake Blidinje is not densely populated due to a large number of livestock grazing in the immediate surroundings during the summer months, an influence on the eutrophication process cannot be ruled out. In comparison to deep lakes, shallow basins are often smaller in volume and have a lower capacity for incoming nutrients. This makes shallow basins more susceptible to anthropogenic influences. Additionally, shallow lakes have more frequently strong water–sediment interactions and are more likely to have sediment resuspension, which increases internal nutrient loading and productivity [9].

The model of multiple regression analysis makes it possible to forecast system dynamics and future situations. Multiple regression analysis models are recommended by many advocates of empirical limnology for predictions [10–14]. The prognostic model created in previous studies with the help of the multiple regression analysis of the markers of water quality is looked at in this study. Data collected during water quality monitoring from 2017 to 2019 are compared with the resulting model.

2. Materials and methods

When creating the multiple regression analysis model, data from earlier research was used [15, 16]. In the multiple regression analysis, chlorophyll a was selected as the dependent variable, and the following independent factors were employed as the independent variables:

a) With chlorophyll a, transparency and total phosphorus are factors that characterize the trophic state of the lake.

b) Total phosphorus and electrical conductivity, variables that, according to the literature [12], showed a strong and statistically significant relationship with chlorophyll a.

c) SO_4^{2-}, Fe, NH_4^+, temperature, hardness, PO_4^{3-} of filtered samples, N/P ratio. All abiotic indicators were chosen as independent variables, and then the variables with the least statistical significance were gradually eliminated until these seven variables were found, all of which had an effect on chlorophyll a, which was statistically significant.

d) Temperature, Fe, SiO_2, pH, TN/TP of filtered samples. The variables that have a statistically significant correlation (p 0.05, p 0.01) with chlorophyll a in the first phase were first chosen as independent variables. Following that, the variables that did not show a statistically significant link were gradually removed. There

was no relevant link with chlorophyll a until the multiple regression technique had reached these five parameters, all of which have a statistically significant impact on chlorophyll a. Data sources from earlier investigations were used [15].

The resulting models are compared with water quality monitoring data from 2017 to 2019 [17–19], which is regularly carried out by the Agency for Watershed of the Adriatic Sea. The monitored indicators are: water temperature, air temperature, pH, suspended matter, electrical conductivity (CND), dissolved oxygen, oxygen saturation, amount of oxygen required (BOD), permanganate index, NH_4^+, NO_3^-, total nitrogen (TN), total phosphorus (TP), TN/TP, PO_4^{3-}, Cl^-, SO_4^{2-}, total dissolved carbon (TOC), Cu, Cr, Zn, SiO_2, chlorophyll a [17–19]. For the monitored indicators, the Pearson correlation coefficient was determined. The monitoring was conducted in compliance with European Parliament and Council Directive 2000/60/EC [1].

3. Results and discussions

Multiple regression analysis was used to investigate the relationship between the concentration of chlorophyll a and SO_4^{2-}, Fe, NH_4^+, water temperature, hardness, dissolved reactive phosphorus, and Redfield's N/P ratio. The coefficient of multiple determination (R^2 = 0.720) demonstrated that up to 72% of the variance of the chlorophyll concentration during the observed time was explained by the link between the aforementioned parameters, which was determined to be quite strong (R = 0.849). As a result, this model can be regarded as typical.

Table 1. Multiple regression, dependent variable chlorophyll a, independent variables: SO_4^{2-}, Fe, NH_4^+, temperature, hardness, PO_4^{3-} of filtered samples (dissolved reactive phosphorus), N/P ratio (Redfield's)

Variables Entered/Removed				
Model	Variables entered	Variables removed		Method
1	N/P Fe Temperature Hardness NH_4^+ SO_4^{2-} PO_4^{3-} [a]			Enter
a. All requested variables entered b. Dependent variable: Chlorophyll a				
Model summary				
Model	R	R^2	Adjusted R^2	Std. error of the estimate
1	0.849[a]	0.720	0.670	0.36331
a. Predictors: (Constant), N/P, Fe, Temperature, Hardness, NH_4^+, SO_4^{2-}, PO_4^{3-}				

Anova[b]

Model	Sum of squares	df	Mean square	F	Sig
1					
Regression	13.239	7	1.891	14.329	0.000[a]
Residual	5.148	39	0.132		
Total	18.387	46			

a. Predictors: (Constant), N/P, Fe, Temperature, Hardness, NH_4^+, SO_4^{2-}, PO_4^{3-}
b. Dependent variable: Chlorophyll a

Coefficients[a]

Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		
1					
(Constant)	-0.179	0.466	-0.257	-0.384	0.703
SO_4^{2-}	-0.067	0.030	0.248	-2.224	0.032
Fe	0.418	0.169	0.327	2.470	0.018
NH_4^+	1.779	0.596	0.923	2.987	0.005
Temperature	0.084	0.010	-0.262	8.752	0.000
Hardness	-0.018	0.006	0.237	-2.770	0.009
PO_4^{3-}	23.354	11.486	0.272	2.033	0.049
N/P	0.003	0.001		2.602	0.013

a. Dependent variable: Chlorophyll a

The regression model's individual significance test revealed that each of the independent variables that were chosen had a statistically significant impact on the level of chlorophyll a. The link between sulphate and hardness as independent variables and chlorophyll a as a dependent variable is inversely proportional, but the relationship between the other independent parameters in this model and chlorophyll a is linearly proportional. Following the selection of all abiotic characteristics as independent variables, the variables with the least statistical significance were gradually eliminated until these 7 variables were reached, all of which having had a statistically significant impact on chlorophyll a. Because sulphate concentrations are higher in the spring months following snowmelt, independent of chlorophyll a, the inversely proportional link between sulphate and chlorophyll a does not necessarily imply that sulphates affect the concentration of chlorophyll a. The correlation between chlorophyll a and hardness can also be explained as a coincidence. It is hypothesised that orthophosphate and ammonia will have a direct proportional relationship with chlorophyll a.

Chlorophyll a concentration dependence on water temperature, Fe, SiO_2, pH, and TN/TP limit of filtered water samples found in the multiple regression analysis revealed a strong relationship between the mentioned parameters ($R = 0.705$), while the coefficient of multiple determination ($R^2 = 0.497$) revealed that up to 49.7% of

the aforementioned independent variables contributed to the explanation of the variance of chlorophyll a concentration throughout the observed time. As a result, this model can be regarded as typical. Each of the selected independent variables had a statistically significant impact on the level of chlorophyll a according to the individual significance test of the regression model. The relationship between the TN/TP ratio of filtered samples as an independent variable and chlorophyll a as a dependent variable is inversely proportional, in contrast to the other independent variables in this model, which are all directly proportional to chlorophyll a. This selection of variables was made by first selecting as independent variables all those that have a statistically significant correlation (p 0.05, p 0.01) with chlorophyll a in the first phase. Following that, the variables that did not show a statistically significant link were gradually removed. There was no relevant link with chlorophyll a until the multiple regression technique had reached these five parameters, all of which have a statistically significant impact on chlorophyll a. Since SiO_2 and chlorophyll a have a direct proportional connection, SiO_2 can be a limiting nutrient for the growth of Bacillariophyceae class algae.

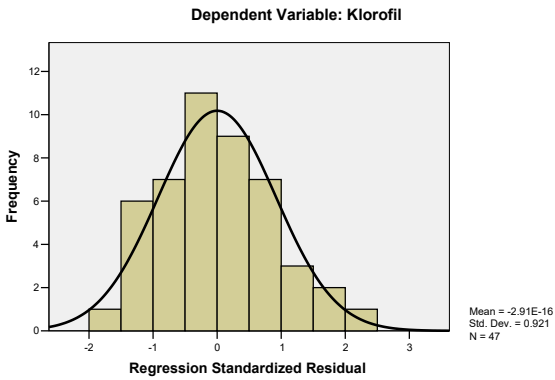

Figure 1. Multiple regression histogram, dependent variable chlorophyll a; independent variables: SO_4^{2-}, Fe, NH_4^+, Temperature, Hardness, PO_4^{3-} of filtered samples (dissolved reactive phosphorus), N/P ratio (Redfield's); dependent variable: Chlorophyll a

Temperature and chlorophyll a were shown to be directly proportional in both typical models of multiple regression analysis, as predicted. Given that iron is required for the synthesis of chlorophyll a as a micronutrient, Fe also shows a directly proportionate connection with chlorophyll a in both representative models of multiple regression.

Future studies can use these two representative multiple regression models to forecast chlorophyll a concentration. Some researchers have already described such models for forecasting the concentration of chlorophyll a [20].

Table 2. Multiple regression, dependent variable chlorophyll a, independent variables: Temperature, Fe, SiO2, pH, TN/TP of filtered samples

Variables Entered/Removed			
Model	Variables entered	Variables removed	Method
1	TN/TP pH Fe SiO_2 Temperature[a]		Enter
c. All requested variables entered d. Dependent variable: Chlorophyll a			

Model summary				
Model	R	R^2	Adjusted R^2	Std. error of the estimate
1	0.705[a]	0.497	0.453	0.47476
a. Predictors: (Constant), TN/TP, pH, Fe, SiO_2, Temperature				

Anova[b]					
Model	Sum of Squares	df	Mean Square	F	Sig
1					
Regression	12.839	5	2.579	11.440	0.000[a]
Residual	13.073	58	0.225		
Total	25.966	63			
a. Predictors: (Constant), TN/TP, pH, Fe, SiO_2, Temperature b. Dependent variable: Chlorophyll a					

Coefficients[a]					
Model	Unstandardized coefficients		Standardized coefficients	t	Sig.
	B	Std. Error	Beta		
1					
(Constant)	-3.744	1.540	0.413	-2.431	0.018
Temperature	0.039	0.009	0.273	4.082	0.000
Fe	0.534	0.186	0.213	2.879	0.006
SiO_2	1.747	0.809	0.263	2.160	0.035
pH	0.527	0.194	-0.226	2.722	0.009
TN/TP	-0.003	0.001		-2.235	0.029
b. Dependent variable: Chlorophyll a					

Pearson's correlation index was calculated to determine the significance of the relationship between certain physico-chemical indicators of water, with an emphasis on their connection with the concentration of chlorophyll a. Observing the water quality monitoring indicators for the period from 2017 to 2019, water temperature, dissolved oxygen, nitrates, total nitrogen, TN/TP ratio, chromium, and SiO_2 show a statistically significant positive correlation with the concentration of chlorophyll a. To some extent, these results confirm the previously created prognostic model.

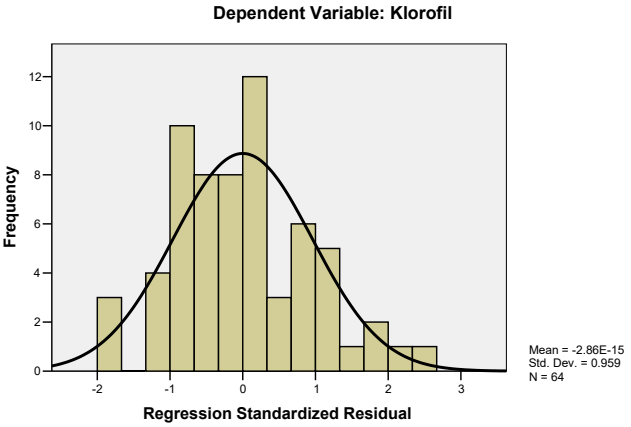

Figure 2. Multiple regression histogram, dependent variable chlorophyll a; independent variables: Temperature, Fe, SiO_2, pH, TN/TP of filtered samples; dependent variable: Chlorophyll a

Table 3. Pearson's correlation coefficient with the concentration of chlorophyll a

Parameter	**Pearson's correlation coefficient**
Temperature water (°C)	-0.79273
Temperature air (°C)	-0.57882
pH (pH unit)	-0.54017
Suspended matter (mg L^{-1})	0.286044
CND (µS cm^{-1})	0.297066
Dissolved oxygen (mg L^{-1})	**0.719949**
Oxygen saturation (%)	-0.35766
BOD (mg O_2 L^{-1})	0.17771
Permanganate index (mg O_2 L^{-1})	-0.14558
NH_4^+ (mg L^{-1})	0.293404
NO_3^-(mg L^{-1})	**0.829257**
Total N (mg L^{-1})	**0.868945**
Total P (mg L^{-1})	0.195754
TN/TP	**0.635985**
Cl^- (mg L^{-1})	-0.03122
SO_4^{2-} (mg L^{-1})	-0.20641
TOC (mg L^{-1})	-0.10359
Cu (µg L^{-1})	0.501051
Cr (µg L^{-1})	**0.822928**
Zn (µg L^{-1})	0.171639
SiO_2 (mg L^{-1})	**0.737193**

4. Conclusions

Analysing the quality of the water can be done for a variety of reasons. In this respect, they represent prospective data and information for prognostic models, ranging in importance from those of general importance to those that are extremely particular, such as warning, monitoring, or projecting the development of the situation. A water quality monitoring model that is gradually developed will allow for the determination of the factual condition, including basic to crucial supplementary data. It is necessary to continue to develop new and improve existing prognostic models in order to be able to predict the concentration of chlorophyll a. In this way, it is possible to prevent the eventual occurrence of eutrophication of the lake through a responsible approach to the sustainable management of the wider area of Blidinje Nature Park.

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Landscape-based design as an instrument for sustainable development in heritage conservation

Raluca Ramona RUSU,[1] Alexandru CIOBOTĂ[2]

[1] West University of Timisoara, Romania; e-mail: ruraluca@gmail.com

[2] West Regional Developement Agency, Timisoara, Romania; e-mail: ciobotalex@gmail.com

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Abstract: The project for restoring and valorizing the "Jumelț" iron furnace historical monument dating from the 19th century and located in Zimbru village, Arad County, has been substantiated by a series of analyses and specialized investigations. The landscape study, based on a hybrid methodology, has led to a specific set of knowledge of the local physical and social conditions and to the highlighting of the relations between the heritage object and its proximity. In conclusion, the knowledge generated by the landscape research has directly influenced the design process, has indicated the legitimacy of reconnecting the heritage monument to its context, as a non-speculative design approach, and has also facilitated the integration of sustainable development measures.

Keywords: interdisciplinarity, research-based design, knowledge, preindustrial heritage, integrated conservation

1. Introduction

Exploiting historical monuments is currently on an upward trend in Romania, but in practice it is limited predominantly to architectural objects and scenarios (such as rendering a historical visual image of a place) and has less to do with the landscape and local communities per se. The conservation and restoration process is focused on protecting the heritage monument, and the valorization programmes indicate a preoccupation with the material culture and especially with prestigious monuments (e.g. archaeological sites, medieval fortresses, etc.), as well as a usage of general knowledge. This approach enhances the loss of authenticity and increases the risk of generating speculative scenarios (pseudohistory, idealization of the past, or utopias), eventually leading to a reverse narration starting from (speculative) fiction and ending with a certain "reality" of the object (e.g. historical/pastiche architecture).

The protection of the monument has been achieved by establishing protection areas [1], but in practice the tendency is more to control proximity and less to use

the vicinity for heritage knowledge and valorization. Nevertheless, it is relevant in the case of the monument to relate on a landscape level, and the participation of the landscape in the formation of local cultures is a commonly accepted fact [2]. Thus, a different perspective on the value of the heritage, as well as on the knowledge process, opens up.

Landscape research involves a complex knowledge process and may be conducted by producing research results, but also by discovering manners in which to transform research knowledge into something useful and practical such as "planning and design guidelines". For example, site-specific features in the professional practice of landscape programmes have sometimes been contrasted to knowledge that can be generalized, and this should not be the case [3]. Design programmes require both general (or scientific) and local (or situational) knowledge, as well as combining or integrating these two types of knowledge [4].

Our study proposes landscape research as a method of acquiring knowledge, in order to reintegrate the monument on a landscape level and to include sustainable development as a solution for the integrated conservation of the heritage.

2. Materials and methods

In the debate concerning the methods of acquiring knowledge of the landscape applied as part of the programmes for protecting and valorizing the heritage, we have chosen classified historical monuments. Of all the heritage categories, research is limited to technical and industrial cultural sights, which have been and still are largely co-dependent on the nature of the neighbouring landscape (particular conditions, resources, accessibility, etc.).

The landscape of preindustrial and industrial monuments (and not only) preserves the essential components of the manufacturing process [5], but also social structures, which makes landscape research more important.

The technical heritage in Romania is largely at risk of disappearing and being devalued [6], as well as at risk of its protection focusing solely on the object and on valorization through speculative scenarios (e.g. utopias or pastiches), this being one of the reasons for the narrow focus of the study. We would like hereby to mention that we have identified this tendency in other categories of monuments as well (e.g. archaeology).

In Romania, technical and industrial monuments were entered under heritage protection quite late (during the 50s), without being allotted a specific category and instead being cumulated with the architectural (bridges, mills, etc.) and the historical ones (foundries, the ruins of the furnace in Moneasa, Arad County). As per the *List of Historical Monuments* of 1992, absorbed by later versions as well, only 2.99% of them are monuments with technical value or associated with industry [7]. The current situation is difficult to estimate in the absence of an

inventory anchored in on-site research, the technical inheritance being in fact much more generous than mentioned. The lack of representation of this type of heritage in the *List of Historical Monuments* is due to the prioritization of great or "classical" cultural monuments, but also to the scant interest and bibliography, correlated with the lack of specific legislation.

The site selected for this study is the "Jumelț" iron furnace located in Zimbru village, Arad County, a historical monument dating back to the 19th century and classified under the code LMI AR-II-m-B-00661 [8]. After selecting the research material, we analysed the landscape located in the monument's proximity.

2.1. Methodology

The research based on landscape knowledge has generated a hybrid methodology and has a vast multidisciplinary coverage, from evolutional territorial analyses based on data from natural, social, and human sciences and arts, to site survey. The various types of knowledge accessed both by the team of landscape architects and by the project collaborators have been managed during the analysis of the landscape on two levels: the macro level of the territory and that of the historical monument site, both being later on integrated into the design process focused on heritage protection and valorization.

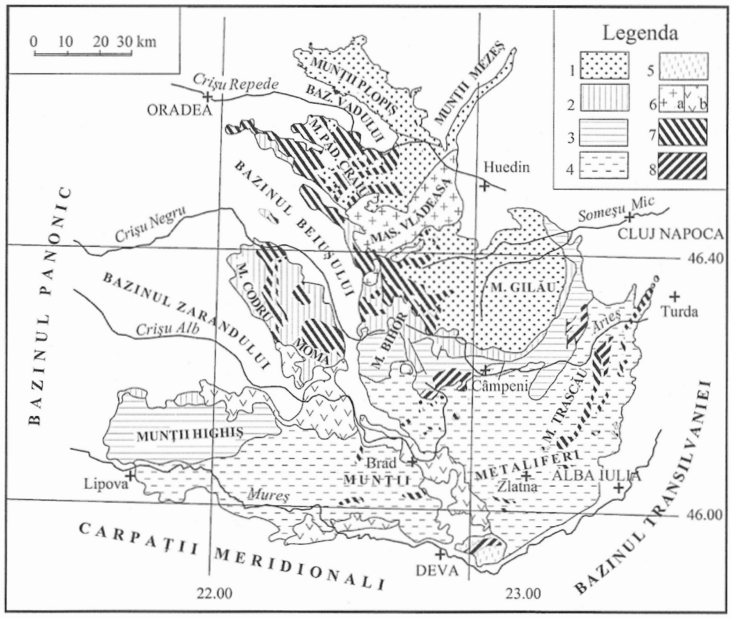
2.1.1. The territorial level

– *The evolutional analysis of the territory* has revealed the points of reference in terms of macro-identity of the local landscape correlated with the historical regions, but also the multifaceted identities delineated by narrower frames belonging to various disciplines (history, geography, ethnology, etc.). Thus, the landscape analysed is part of the historical region of Crişana, the cultural subarea associated with the Crişul Alb River, delineated by the eastern side of Zărandului Hollow and by Codru Moma Mountains.

– *The geographical research has determined the identification of the type of natural landscape* and was based predominantly on geological, hydrological, and forestry data. In addition to the geographical description of the place, we have also identified the resources directly involved in iron exploitation and processing (the useful mineral resource – the iron deposit, the motory resource – the local hydrographical network, the combustion/burning resource – the wood charcoal and the human resource – specialized and non-specialized multiethnic communities) (see *Figure 1*).

– *The analysis of the administrative policies in the context of preindustrial ferrous metallurgy in the region (heritage specificity)* has facilitated the reconstruction of a history researched in a fragmented manner, focusing on specific fields (ferrous

metallurgy, science history, local monographies, etc.). The methods of analysis included researching the metallurgical policy enforced by the Austro-Hungarian Empire in the Partium and Transylvania regions (including e.g. colonizations), historical urbanism research (military maps) and local history (monographies, seals and postcards), as well as the history of noble families. The usage of the analysis lies within the identification of the legibility of the preindustrial patrimonial resource preserved in the heritage objects, meaning the ruins of the furnaces used to melt ores, and is anchored in the (in)visibility of the landscape of the studied area (Moneasa–Dezna/Răschirata–Zimbru) (see *Figure 2*).



Source: Orăşanu, 2020

Figure 1. Structural map of Apuseni Mountains

– *The science history analysis* has facilitated the familiarization with the technical and chemical processes involved in the iron metallurgy, which was useful for understanding the constructive evolution of the furnaces, as well as their impact on the microlandscape in their proximity.

– *The compared analysis* between similar objects from the researched area (Moneasa, Dezna/Răşchirata, Zimbru) and the historical evolution of the plots of land and that of the microlandscape has led to identifying the resources involved in the technological process and the relations between the objects of the furnace.

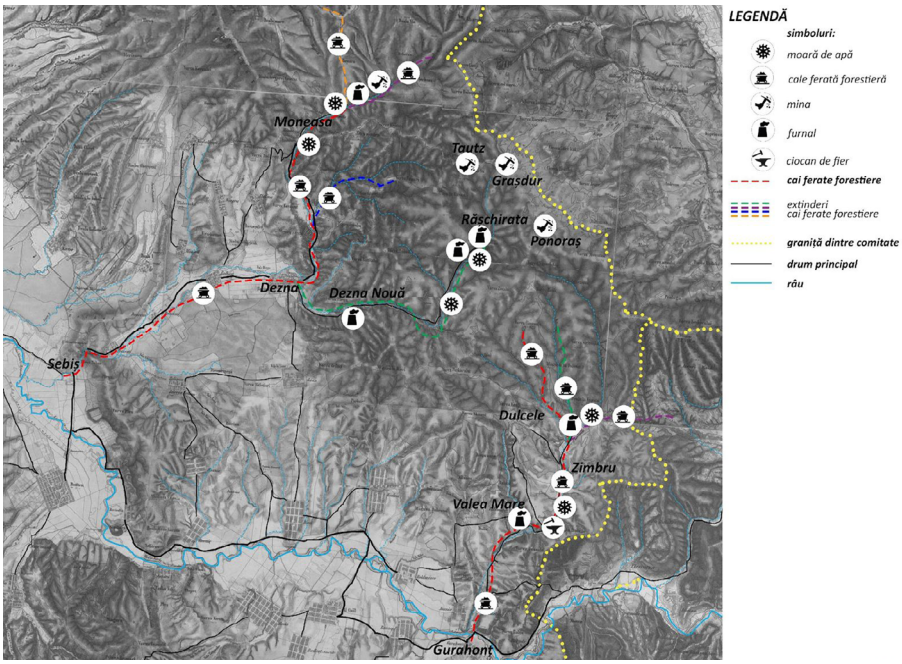


Figure 2. Map of the ferrous metallurgy in the cultural landscape (Moneasa, Dezna/Răşchirata, Zimbru), based on The First and Second Military Survey of the Habsburg Empire

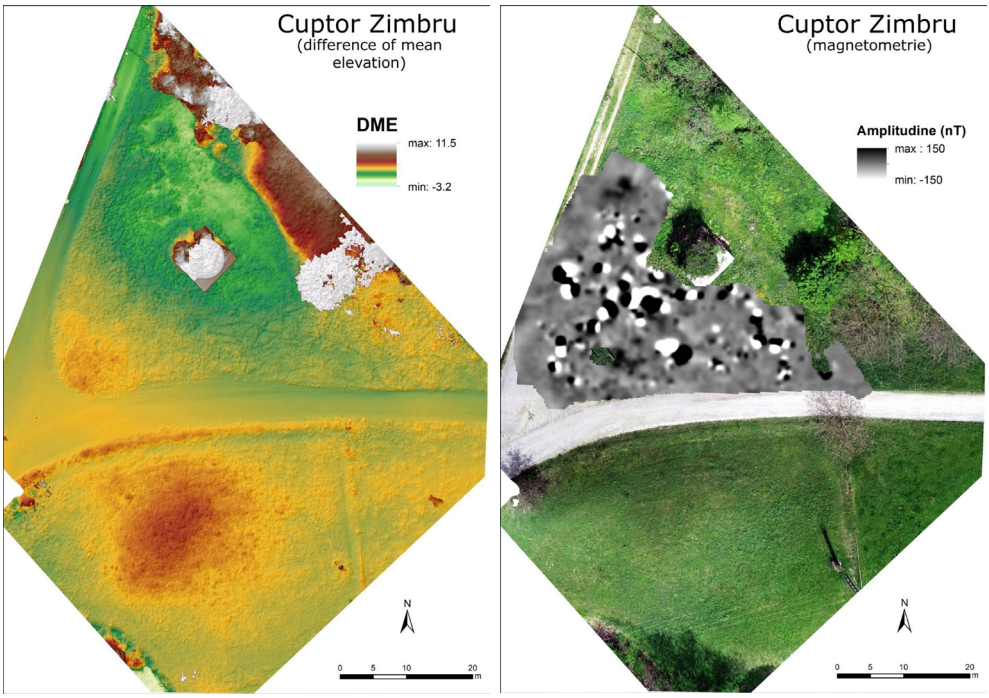
2.1.2. On a parcel level

– *The analysis of the existent situation of the parcel* has been correlated with the specialized investigations conducted by our collaborators and together have determined the identification of the current site conditions and components (topography, geology, archaeology, hydrography, etc.). The detailed land measurements have indicated the microtopography of the historical monument site, together with the new technology surface investigations.

– *The non-invasive investigations used to acquire knowledge about the terrain* (digital surface models – D. S. M., digital elevation models – D. E. M.) have contributed to obtaining the microtopographic details of the site (altitude, elevation of existent elements, vegetation coverage of the surfaces, etc.) (see *Figure 3a*).

– *The magnetometric measurements* have contributed to prospecting the potential archaeological resource existent on the site (see *Figure 3b*).

– *The preventive archaeological investigations* aimed to identify *in situ* the potential building remains of the technological complex from Zimbru, illustrated on historical military maps and identified through magnetometric investigations (see *Figure 4*).



Source: geogr. A. Ardelean, geogr. A. Sărăşan, The National Museum of Banat, 2022

Figure 3. 3a. Difference of mean elevation/DME; 3b. Magnetometric measurement

Source: archg. Victor Sava, 2022 [9]

Figure 4. Archaeological sections

– *The physical and chemical expertise of the type of materials used to build the masonry of the monument* was conducted through a series of microscopic, XRF spectroscopic, petrographic, and chemical analyses. The composition of the furnace components indicated the type of materials used (quarry stone, ferruginous limestone, carbonaceous limestone, burnt bricks, mortars based on loam sand and mortars made of sand and lime, slag), information useful for identifying the local resources, practices, and construction models (part of the diffused knowledge).

– *The chemical analyses conducted on the slag sample taken from the proximity of the furnace* indicated a mineral resource rich in iron between 80% and 81% and manganese between 20% and 25%, which confirms the proximal provenance of the ferrous deposit, the Vaşcău Plateau (the Codru-Moma Mountains), as per the geological data.

– *The ethnological survey* provided information (qualitative data) on the history of preindustrial metallurgy in the area and descriptions of the *Jumelț* iron furnace in Zimbru, as well as local practices, customs and legends, nature management and tourism, obtained through various specific methods: semi-guided interviews, discourse analysis and participatory observation, etc. (see *Figure 5*).

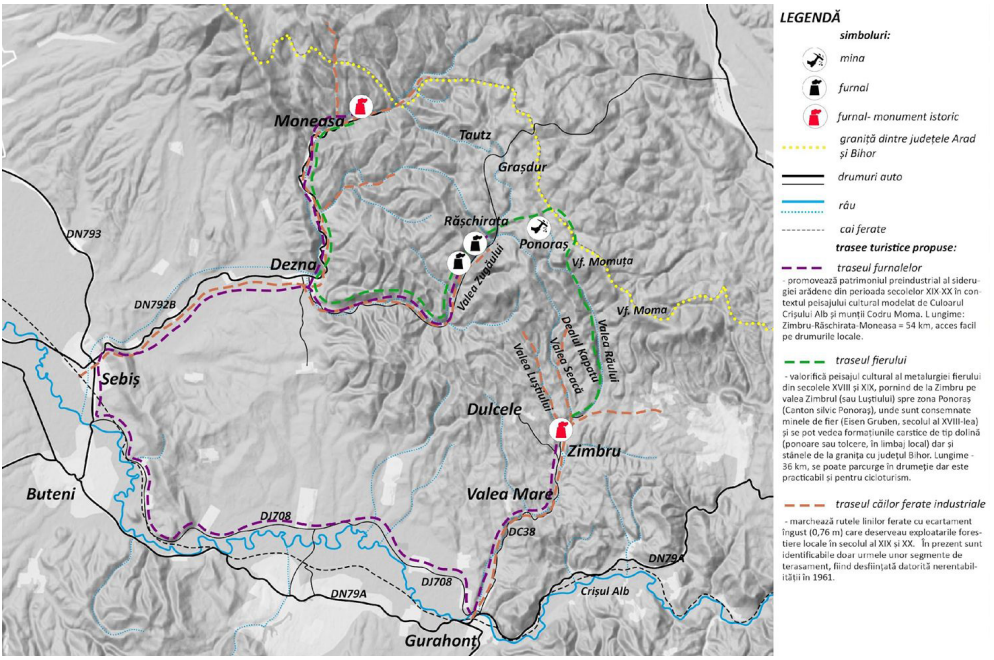
Source: authors' photo, 2022

Figure 5. Ethnological survey expeditions, the Vaşcău Plateau

3. Results and discussions

The multidisciplinary landscape research has generated different complementary types of knowledge. The proximal landscape becomes a (re)source of specialized and local knowledge, especially in the case of historical monuments that have lost their historical function. From the analysis of the topics debated within the landscape study, we can synthetise four directions relevant for the design programme that aims to valorize the monument.

3.1. (Re)connecting the heritage object with the landscape



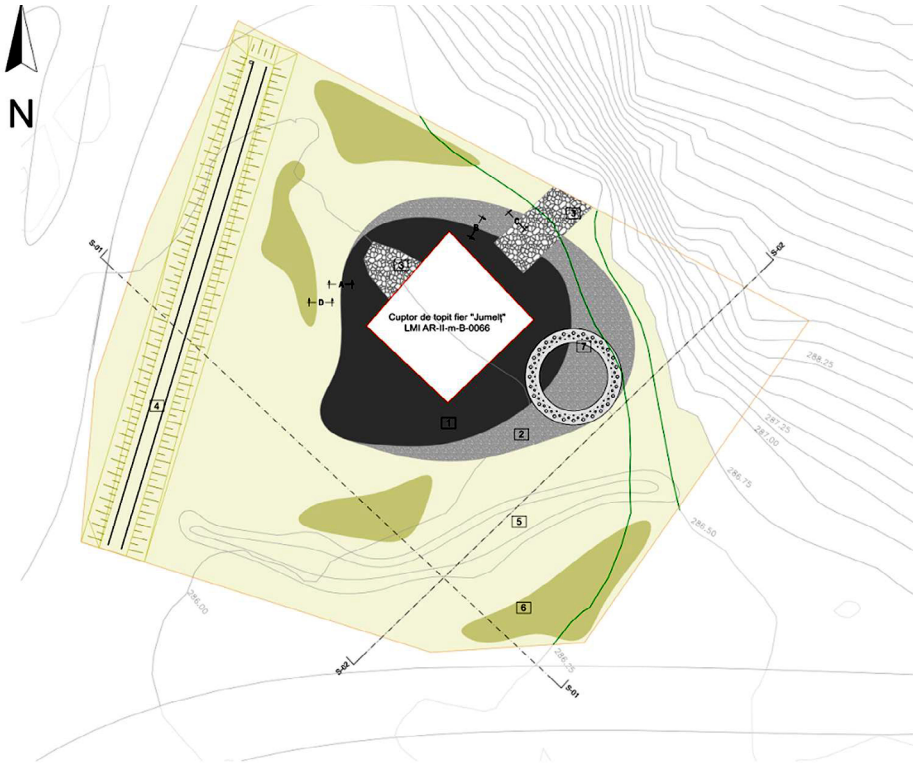
Source: authors' compilation

Figure 6. Map with suggested thematic touristic routes

Lining up the object with its proximity again was achieved through an exercise of territorial memory recovery (memory residing in a diffused knowledge, minimally researched by science, and in the overlapping of historical events with local legends) on the one hand and by means of a horizontal connection with the landscape on the other. Suggesting thematic routes correlated with the technological routes of local preindustrial metallurgy, with natural and historical resources, becomes a sustainable way of heritage and touristic (economic) valorization of the area

(*Figure 6*). Reconnection with the landscape, understood as a passing from the protected object to the subject (approach generated by value) is an integrated conservation method that, by incorporating the local and nature in the heritage experience, becomes a generating factor in the social and economic development of proximal communities. At the same time, connecting the historical monument with the local landscape becomes a catalyst in the promotion of the common asset (both the heritage and the landscape).

3.2. Negotiating identity layers



Source: authors' compilation

Notes: 1), 2), 3) trafficable areas with a draining role; 4) narrow railway bed (reinterpreted); 5) water gutter (reinterpreted); 6) meadow area (flower assortment); 7) steel art installation, an interpretation of wood charcoal production.

Figure 7. Landscape plan proposal

The research conducted on the site level has revealed various cultural layers overlapping on the monument site. In addition to clarifying the composition of

the 19th-century technical complex (the furnace for reducing the iron ore, annexes, mounds for loading the materials, water gutter, traffic routes to the exterior, etc.), we have also identified elements dating from after the preindustrial metallurgical period and a narrow forest railway used for sylvan exploitation in the area. As shown in the historical analysis, these forest tracks were part of the local industrial landscape (Sebiş–Moneasa, Moneasa–Dezna–Răşchirata, Gurahonț–Zimbru), even if in the present day only fragments of the railway bed are still visible. The design programme proposed a vertical connection of the identity layers of the site and a horizontal connection to the landscape (the Codru Moma Mountains forest railway line). Reinterpreting the forest railway on-site (identified in *The Third Military Survey from 1869–1896*) by employing a multifunctional landscape element (limit with the forest road, rest area, signs, and cycloparking) also becomes a way to recover the memory connected to the local forest industry (*Figure 7*).

3.3. Integration in the local ecology

Following the complex landscape analysis, the design programme included a nature-based solution for the valorization of the heritage, but also for the protection and sustainable management of the modified ecosystems (preindustrial site). Part of the solutions were derived from discussions with the locals, in which they described the landscape and the agricultural practices (the ethnographic survey method).

Reinterpreting certain elements of the preindustrial technical complex (watercourse) or other identity layers (the railway bed of the forest railway) has included solutions for a sustainable management of meteoric water (collection and deep infiltration). Another objective of the sustainable design programme was to ensure the draining of the water surrounding the historical monument by employing environment-friendly solutions (dry surfaces made of mineral aggregates that ensure the drainage of the water) and building a drain on the sides located near the high area of the site.

The vegetation works aimed to preserve the meadow habitat existent on the site and proposed interventions meant to facilitate biodiversity (useful assortments of flowers, plantations of irises/*Iris pseudacorus*). The proposals to eliminate the potentially invasive species (*Fallopia sp.*) identified on the site have targeted sustainable methods of intervention (repetitive cuts before fructification in order to exhaust the invasive plant) that are meant to support the health of the ecosystems and the management of biodiversity. The nature-based design solution includes an important educational component, associated with patrimonial protection and valorization.

3.4. Landscape curation

Landscape curation is a concept introduced by the authors in the landscape architecture design programmes and refers to the landscape interpretation of various topics; in this case, of the technical and industrial heritage.

The understanding and legibility of the heritage technical complex is an important objective in valorizing and supporting the educational role of the heritage. In addition to the idea of rendering the missing components of the technical process in a figurative language, we opted in favour of the author resorting to a landscape interpretation and to an artistic representation of certain elements. Due to a multidisciplinary collaboration, we proposed an immersive work that renders in a sculptural language one of the components of the technological process used to melt iron, wood charcoal (*Figure 8a*).

Recovering the historical material identified on site (bricks, stone blocks, pieces of cast iron, etc.) and using it in the design solution becomes a gesture of creative approach towards the integrated heritage conservation programme.

In addition, the knowledge generated by the landscape research has been transferred both to the heritage valorization component and to the signage programme, where various objects and materials display complementary information relevant to the local heritage experience (local landscape and thematic routes, the technical process, the family of local entrepreneurs, etc.) (see *Figure 8b*).

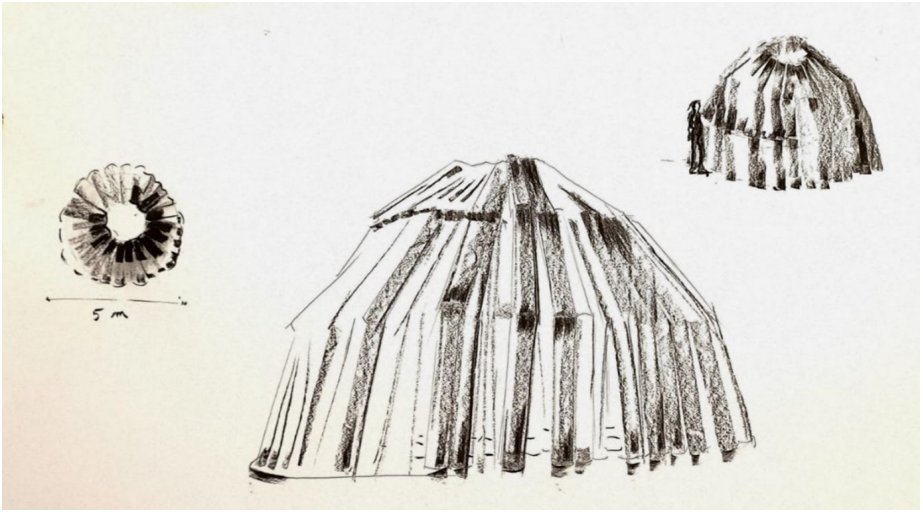


Figure 8a. Steel art installation, an interpretation of wood charcoal production (Rom. *bocşerit*) – sculptor Dan Vişovan

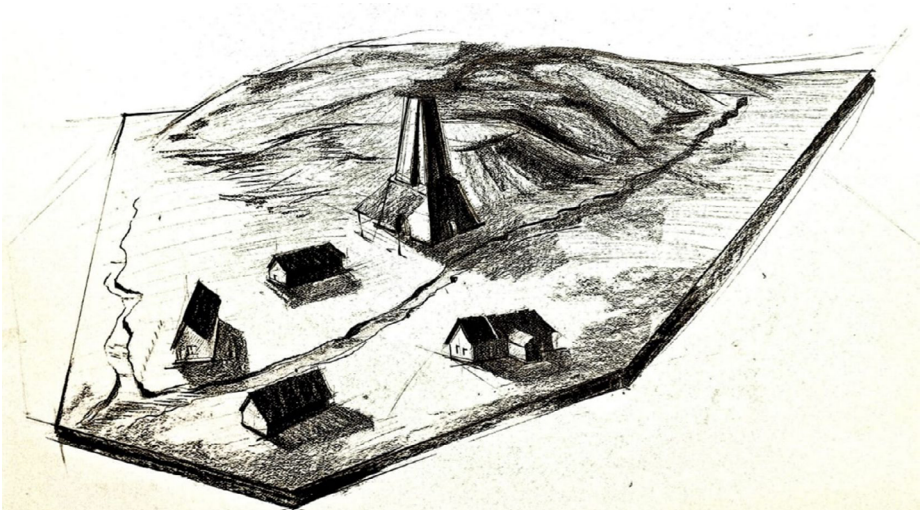


Figure 8b. Cast iron model of the historical site to be integrated into the landscape concept – sculptor Dan Vişovan

4. Conclusions

The multidisciplinary and multi-levelled approach of landscape has revealed visible, diffused, and invisible connections to the historical monument. On a territorial level, identifying the geographical specificity and locating the natural resources used in preindustrial metallurgy and the similar technical monuments situated in the proximity have facilitated the proposal of thematic visitation routes for the area, a horizontal (re)connection. On the other hand, identifying the functional technical complex on a site level (watercourse, constructions, etc.), but also the vertical connection of the overlapping cultural layers, have determined conservation and reinterpretation approaches as part of the design solution. Incorporating landscape research in heritage valorization projects leads to the conclusion that reconnecting the heritage object with its cultural landscape highlights complex relations and valences in the proximity area and facilitates sustainable development measures for the protection of the heritage.

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Propagation of rose varieties by cuttings under the effect of different rooting hormones

Endre KENTELKY, Zsolt SZÉKELY-VARGA,* Géza BALLA

Department of Horticulture, Faculty of Technical and Human Sciences, Sapientia Hungarian University of Transylvania, Calea Sighişoarei 2, 540485, Târgu-Mureş/ Corunca, Romania

* Corresponding author: zsolt.szekely-varga@ms.sapientia.ro

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Abstract: Roses are one of the oldest and perhaps most noble and beautiful plants in the world. The propagation of roses by stem cutting is the simplest and a largely used method to multiply them. For the present experiment, seven varieties of roses were selected as follows: 'Monika', 'Mr. Lincoln', 'Queen Elisabeth', 'The Fairy', 'Peace', 'King's Ransom', and 'Don Juan'. To assess the differences between the varieties, growth, root length, root number, and frost damage were determined. The aim of the experiment is to determine which rose varieties are better suited to propagation by cuttings, which varieties have a higher rooting tendency, and to what extent the rooting could be influenced by rooting hormones [Incit-8 (0.8% of 1-Naphthaleneacetic acid) and Radi-Stim®]. From our results, it could be determined that the Incit-8 positively influenced the number of roots of all varieties. In conclusion, our research suggests that the rooting hormones increase the rooting of the rose varieties; moreover, that proper rooting could also be depending on the appropriate conditions.

Keywords: cuttings, rooting hormones, rose, varieties, vegetative propagation

1. Introduction

Roses have been known worldwide for centuries for their beautiful blooms, are commercially important shrubs [1], and can also have many landscaping uses: they can be placed as accent plants or used to form hedges or ground covers. Roses offer a rainbow of colours and a variety of forms and fragrances, and their sizes range from miniatures to tall climbing plants. Roses may be grown under many climatic and soil conditions, and, with care, they thrive and produce flowers for many years. Roses are one of the most admired ornamental plants worldwide, and, with the growing demand

for ornamental plants, prompt innovations are needed [2]. The nurseries need newer and quicker methods to obtain high-quality plants. Of course, tissue culture could be a successful propagation method; however, when acclimatizing them, only 50% of the seedlings survive [3]. Therefore, most of the nurseries are opting for vegetative propagation, and in order to promote root initiation, rooting hormones are used [4].

Root growth and differentiation in plants are closely related to plant hormones [5]. Furthermore, root formation can also be influenced by environmental factors, nutritional levels of the mother plant, rooting medium, cutting type, and the treatment [6]. Hormones used in the propagation stage could reduce propagation time and improve the percentage of rooting [7]. Plant hormones are substances naturally processed by plants, which can control root growth, plant growth, and even fruit maturation [8, 9]. Adventitious root formation is a crucial process for the successful vegetative propagation of many species [10, 11]. According to [12], adventitious root formation in cuttings is a multiphase development process, as because of the wounding at the cutting site, that part of the plant is isolated from the resources and the signal is transmitted to the whole plant. Furthermore, it is also mentioned that adventitious root formation is a multifactorial response guiding the plant to produce new roots at the base of the cutting, and then a new autonomous plant is developed [13]. In previous studies, it has been mentioned that rooting hormones can be used to increase the rooting capacity of different varieties in a positive way and even to obtain a higher number of rooted cuttings in a shorter period of time [14–17].

The aim of the present study was to test the effect of two different rooting hormones (Incit–8 and Radi-Stim®) on seven rose varieties often used and much liked in Romania to determine which varieties are better suited to propagation by cuttings, which varieties have a higher rooting tendency, and whether the rooting could be influenced by rooting hormones.

2. Materials and methods

The experiment was conducted at Sapientia Hungarian University of Transylvania, Târgu-Mureş (46°31'17" N 24°35'54" E). The cuttings were collected with secateurs from the nursery of the local rose farm on 4 July. The cuttings were immediately transported to the experimental site to prevent desiccation. For each variety, 10 cuttings per replication were used, with three replications. Disease and pest-free propagation material was applied between 9 and 11 cm in length. The leaves from the lower part of the stem were removed. Two types of rooting hormones were used: Incit-8 contains 0.8% of 1-Naphthaleneacetic acid (AMVAC Chemical UK Ltd., Surrey, UK) and Radi-Stim®; unfortunately the product content of the latter is not revealed to the users (CCDB Bios, Cluj, Romania). The cuttings were constantly kept wet with an automatic humidifier in order to provide the 80–90% humidity. After the treatments were applied, the rose cuttings were placed into plastic trays filled

with perlite (granulation: 1–3 mm, density: 0.05 kg/L, and pH: 7–7.5) with a depth of 20 cm; to assess the differences between the varieties' cuttings, length (cm), cutting width (cm), root length (cm), and root number were determined on 22 August, after seven weeks. For each variety, 30 cuttings/treatment were immersed in Incit-8 and Radi-Stim®, and the stem cuttings without any treatment were considered the control.

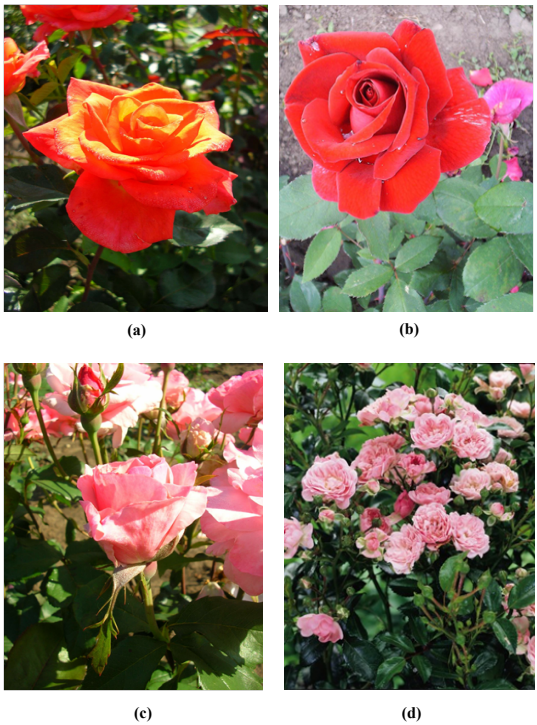


Figure 1. Selected rose varieties: 'Monika' (a), 'Mr. Lincoln' (b), 'Queen Elisabeth' (c), 'The Fairy' (d)

As plant material, the following seven varieties of roses were selected:

The 'Monika' (*Fig. 1a*) variety is characterized by a strong habit, the reddish branches break upwards. It can reach a height of 90 cm; on average, the shrubs have 40 cm in diameter. The branches are covered with a few thorns; the leaves are large, shiny, and dark green.

'Mr. Lincoln' (*Fig. 1b*) has a medium growth rate; the branches are straight and break upwards. It can reach a height of 80 cm. The shrub diameter is 45 cm on average, and the stem has many thorns. The leaves are matte dark green, and the flowers are dark red.

'Queen Elisabeth' (*Fig. 1c*) is a powerful, upright shrub, and the flowers grow high on long stems. It can reach a height of 140–180 cm; the flowers are full pink throughout the summer and the autumn.

'The Fairy' (*Fig. 1d*) is a densely branched variety with semi-double pale pink flowers. The flowers are rosette-shaped, with 25 petals.

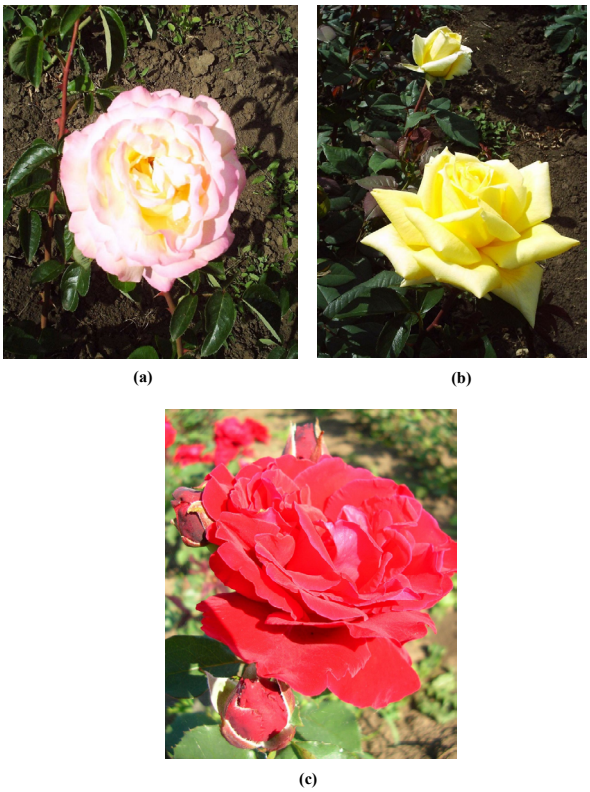


Figure 2. Selected rose varieties: 'Peace' (a), 'King's Ransom' (b), and 'Don Juan' (c)

The 'Peace' (*Fig. 2a*) variety can reach up to a height of 120 cm, with a flower diameter of 14 cm; its colour is a light, bright yellow with a reddish pink petal edge, and it is moderately fragrant. It has brownish-green, large, plate-like thorns and bright green, leathery foliage. It has a good resistance to frost and diseases.

The 'King's Ransom' (*Fig. 2b*) flower's diameter is approximately 13 cm, has a golden yellow colour with a pale-yellow streak, and is moderately fragrant. It blooms from mid-spring to mid-autumn. It has brownish-green, dense, recurved plate thorns. The leaves have a dark green shade, are leathery, oval, with a slightly serrated edge. Most specimens form their flowers on long shoots. It can reach a height of 80–90 cm.

The 'Don Juan' (*Fig. 2c*) variety can reach a height of 250 cm and has large thorns. It is characterized by a strong growth force, develops flowers on long shoots. The flowers' diameter is 10 cm, their colour is velvety, dark red, and it blooms from May until the frosts. The leaves are matte green.

The significance of the differences between the cuttings were tested by applying one-way ANOVA. When the ANOVA null hypothesis was rejected, Tukey's post-hoc test was carried out to establish the statistically significant differences at $p < 0.05$.

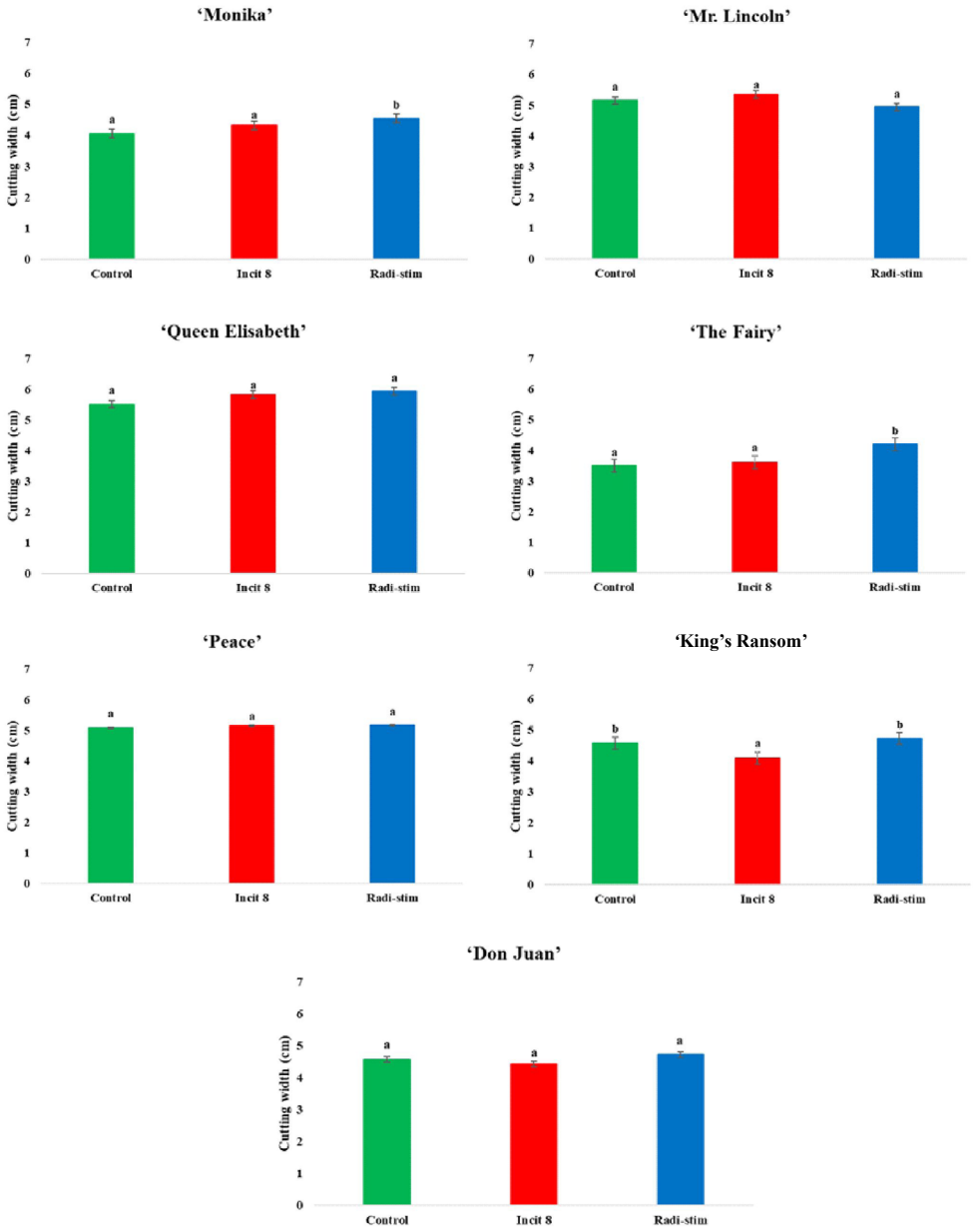
3. Results and discussions

Considering cutting width, the rooting hormone treatments influenced in different ways (*Fig. 3*). For the 'Monika' variety, a significant increase was determined at the cuttings treated with Radi-Stim® compared to the other two. Furthermore, with 'The Fairy' and 'King's Ransom', the same rooting hormone (Radi-Stim®) significantly influenced the cutting width; however, with the second variety mentioned above, the control and Radi-Stim® cutting width were similar, in this case decrease being reported at Incit-8 compared to the other two treatments. Regarding the other rose varieties ('Mr. Lincoln', 'Queen Elisabeth', 'Peace', and 'Don Juan'), the rooting hormones did not influence statistically the cutting width.

Under our experimental conditions, the cutting length was statistically significantly influenced in almost all cases (*Fig. 4*). In the case of 'Monika' Radi-Stim®, cutting length significantly increased. No significant differences were observed on 'Mr. Lincoln'. For 'Queen Elisabeth' and 'The Fairy', Incit-8 significantly increased cutting length. Further, for the 'Peace' variety, cutting length was increased under the Incit-8 treatment, however, not significantly compared to control, and a significant decrease was observed upon the Radi-Stim® treatment. Regarding 'King's Ransom' and 'Don Juan', a significant decrease was recorded on the cuttings treated with Incit-8.

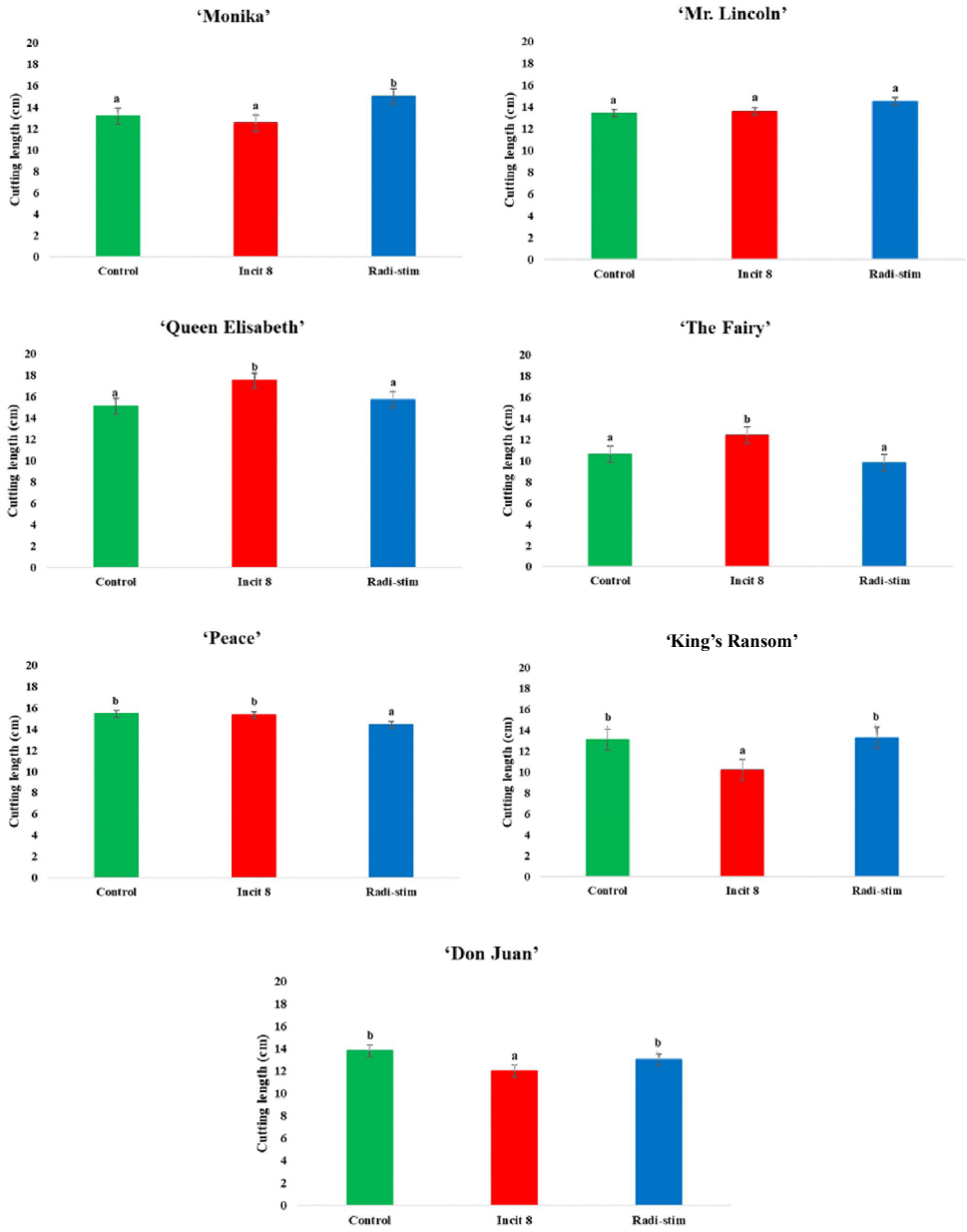
From our results, it could be clearly observed that the rooting hormones have had different effects on the rose varieties' root length (*Fig. 5*). No statistically significant differences were observed between 'Monika' and 'Don Juan'. A significant decrease was reported on the 'Mr. Lincoln' variety when the cuttings were treated with Radi-Stim®. In the case of 'Queen Elisabeth', 'The Fairy', and 'King's Ransom', Incit-8 positively influenced the root length, yielding statistically significant differences compared to the other two treatments. Furthermore, for the 'Peace' variety, both rooting hormones significantly increased the root length of the rose cuttings.

As expected from the previous results, the rooting hormones resulted in different influences on the rose varieties' root number (*Fig. 6*). In the case of 'Monika', 'Queen Elisabeth', and 'Don Juan', no statistically significant differences were determined when comparing the treatments. Additionally, for the 'Mr. Lincoln' variety, a significant increase was observed with Incit-8 compared to the other two treatments and a significant decrease when cuttings were treated with Radi-Stim®. In the case of the 'The Fairy', 'King's Ransom', and 'Peace', Incit-8 significantly increased the root number; moreover, for the 'Peace' variety, a significant increase was achieved with the Radi-Stim® treatment.



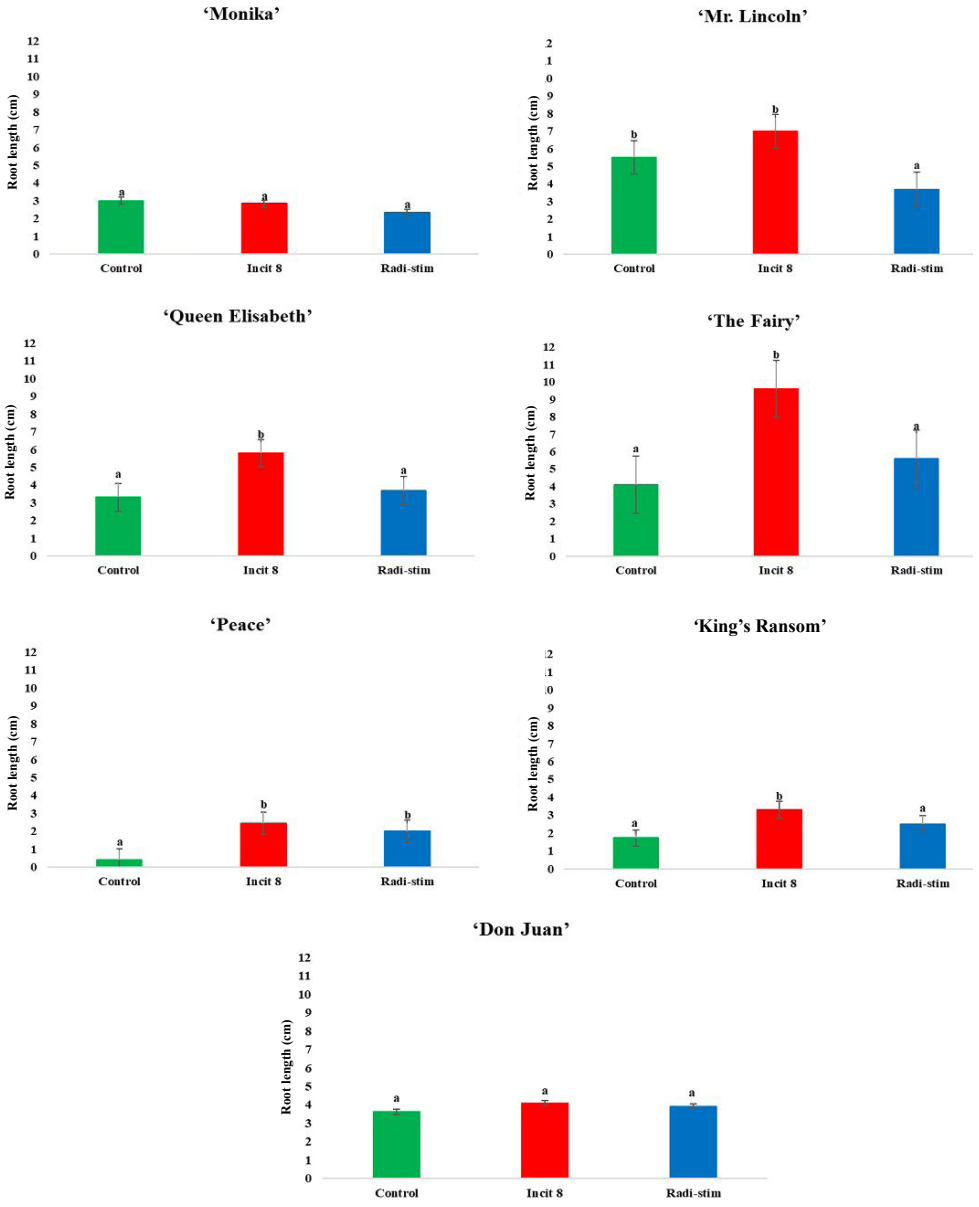
Note: Different letters above the bars indicate significant differences between the treatments.

Figure 3. The effect of the rooting hormones (Incit-8 and Radi-Stim®) on the selected rose varieties' cutting width (cm)



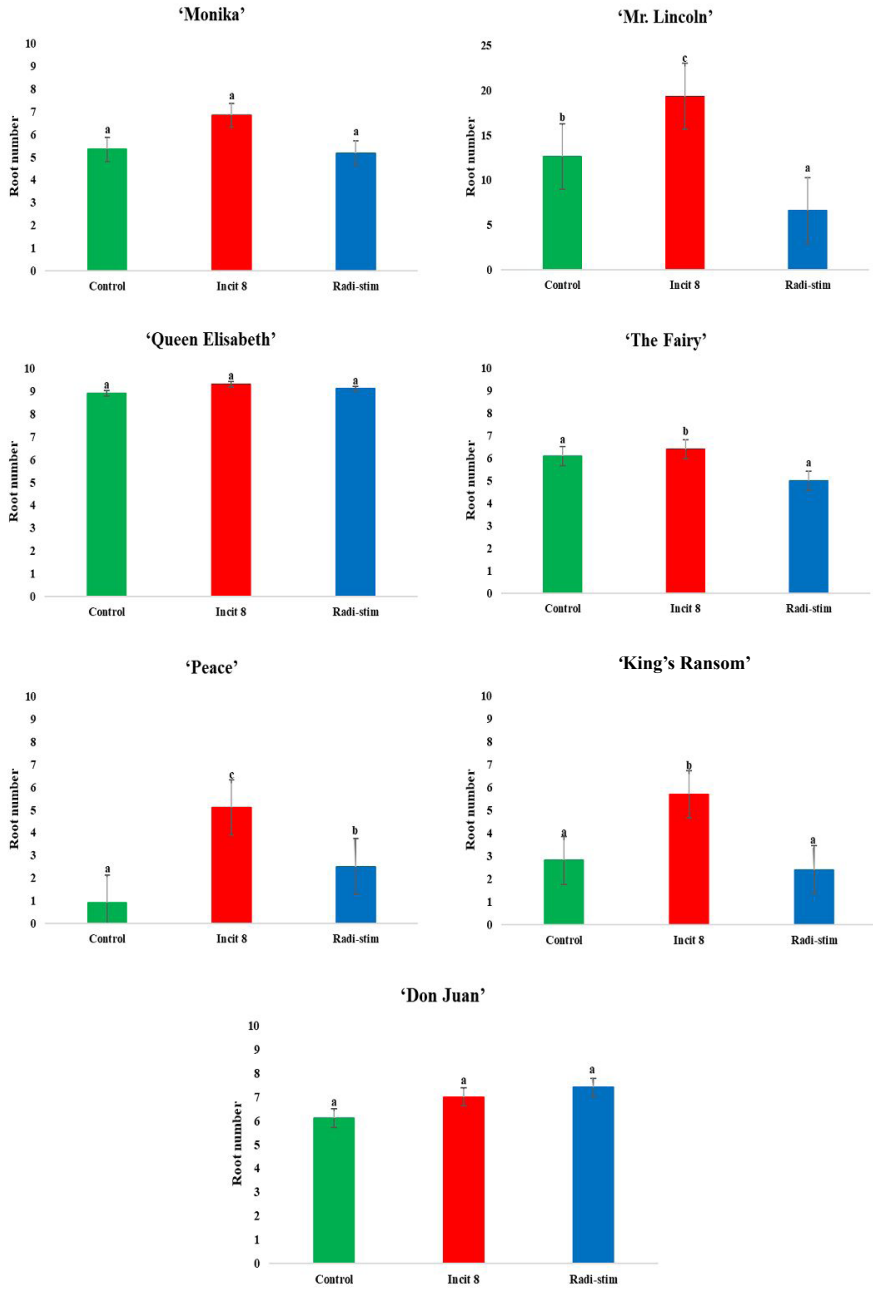
Note: Different letters above the bars indicate significant differences between the treatments.

Figure 4. The effect of the rooting hormones (Incit-8 and Radi-Stim®) on the selected rose varieties' cutting length (cm)



Note: Different letters above the bars indicate significant differences between the treatments.

Figure 5. The effect of the rooting hormones (Incit-8 and Radi-Stim®) on the selected rose varieties' root length (cm)



Note: Different letters above the bars indicate significant differences between the treatments.

Figure 6. The effect of the rooting hormones (Incit-8 and Radi-Stim®) on the selected rose varieties' root number

In a study, it was found that 75 ppm of indole-3-butyric acid recorded the greatest results in the number of leaves, shoot fresh weight, root length, and number of roots in the case of rose cuttings compared to the naphthaleneacetic acid [18]; however, the concentrations of the naphthaleneacetic acid used are lower than in our experiment. In another study, the highest percentage of rooted cuttings were reported upon propagating *Nerium odorum* with indole-3-butyric acid rooting hormone. [19] reported that when combining blue light with naphthaleneacetic acid treatment, rooting percentage was higher for *Chrysanthemum* cuttings. The highest rooting percentage of *Jasminum parkeri* was obtained at a concentration of 0.3% of naphthaleneacetic acid [17]. However, when *Hydrangea* 0.01% was combined with 0.01% of GA_3 [20] and in the case of *Ficus benjamina*, only 0.001% of naphthaleneacetic acid [21] improved rooting percentage.

4. Conclusions

From the present study, it could be concluded that rooting hormones can have a positive effect on the rooting of rose cuttings. However, it is important to mention that root formation can also be influenced by climatic conditions and can act as a variety-dependent factor. As a conclusion, the Incit-8 rooting hormone yielded better results than the Radi-Stim®.

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Evaluation of the aphicidal activity of *Salvia microphylla* (Lamiaceae) aqueous extracts against *Aphis pomi* (Aphididae)

Toufik BENHIZIA,[1] Salim LEBBAL,[1*] Azzeddine ZERAIB,[1,2] Bilal HASROURI,[1] Youcef Alamine MESSAOUDIA,[1] Ammar ZIANI[1]

[1] Department of Agricultural Sciences, University of Khenchela, Algeria

[2] GBVBR Laboratory, University of Biskra, Algeria

* e-mail salim-leb@hotmail.com

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Abstract: The aphid *Aphis pomi* represents an important pest of apples. The use of botanical extracts may be a safe and effective way to control this pest. In this context, we studied the aphicidal and repellent effects of aqueous extracts of *Salvia microphylla* against *A. pomi*. Five concentrations were tested on two main aphid parameters: mortality rate and percentage of repellency. The results showed that concentrations of 10% recorded the highest mortality rate (73.33%) and percentage of repellency (62.59%). After further field investigations, the use of *S. microphylla* may constitute a component of the integrated management of aphids.

Keywords: repulsive effect, apple aphid, corrected mortality rate, extract concentrations, bioinsecticides

1. Introduction

There will be an estimated 9 billion people on the planet by the year 2050, which will increase the demands on agriculture [1]. However, many biotic factors, including insect pests, cause significant losses to crops each year. Among these insects, aphids influence plant production either directly, by sap sucking, or indirectly, through virus transmission. Aphids have a very high rate of multiplication due to parthenogenesis and viviparity, and therefore their attacks frequently result in total crop damage [2]. During the last decades, pesticides constitute the main tool to control aphid populations. Among the most commonly used chemicals, pesticides have greatly benefited humankind by increasing food production and promoting nutrition [3]. Without the use of pesticides, an important portion of agricultural

production in developing countries would be obliterated by pests, according to the Food and Agricultural Organization [4]. However, in certain cases, they pose serious threats to the environment, animals, and humans [5].

In recent years, there has been a growing apprehension among both producers and consumers due to the excessive and unscientific utilization of pesticides [6]. This concern centres on their residues and the potential health consequences such as nerve disorders, skin and eye irritation, and the emergence of long-term chronic ailments such as cancer [7]. Additionally, the persistent application of synthetic pesticides available in the market has led to the emergence of significant levels of resistance in numerous insect pests [8]. This resistance phenomenon now affects over 550 species, rendering most existing insecticide categories ineffective and creating a pressing need for innovative insecticide targets [9].

Thus, the growing concerns and regulations surrounding the utilization of various pesticides because of their detrimental impacts and toxicity have increasingly encouraged the exploration of botanical insecticides as an alternative means of managing insect pests [8]. Consequently, numerous researchers from both the industrial and academic sectors are actively engaged in the quest for valuable compounds derived from plants to serve as novel, eco-friendly insecticides [9].

Biopesticides encompass a category of naturally existing protective agents, typically characterized by their gradual onset of action, and they are generally considered safer for humans and have fewer long-lasting effects on the environment when compared to traditional pesticides [10]. In addition, plant-derived insecticides are associated with a range of merits such as sustainability [11, 12]. Encouraging networking and strong connections within the farmer community, small and medium-sized enterprises, and the industry will further stimulate the biopesticide market [13].

It is clear that plants are highly desirable for the development of novel botanical pesticides for the control of insect pest infestations; however, only a small fraction of the over 250,000 species of plants found on Earth have been adequately studied for this purpose [11].

Thus, the main objective of the present study is to assess the aphicidal and repellent effects of aqueous extracts of *Salvia microphylla* (Kunth) against *Aphis pomi* (DeGeer) under laboratory conditions, as the insecticidal activities of this plant have not been reported against aphid species to date.

2. Materials and methods

2.1 Used insect and plant solutions

The plant material used in this experiment consists of uninfected leaves taken from apple (*Malus domestica* Borkh., cv. Golden Delicious) in addition to the aerial part of *S. microphylla*. *Salvia* is the major genus of Lamiaceae and includes

members whose secondary metabolites, such as terpenoids and flavonoids, have therapeutic activities [14]. *S. microphylla*, native to Central America, is a scarlet-flowered ornamental plant [15].

To obtain aqueous extracts, the infusion method was used. Then five concentrations (1, 3, 5, 7, and 10 %) were prepared.

The animal material, however, was made up of the apple aphid *A. pomi* individuals. This pest, commonly referred to as the "green apple aphid", is a major infestation of *Malus spp.* trees grown in and around the Pacific Northwest [16].

2.2 Toxicity test

A total of 18 Petri dishes were prepared in triplicates for each solution.

Apple leaves were dipped in each concentration for a few seconds. Then the treated leaves were kept in the Petri dish with ten apple aphids in each replicate. Mortality was observed after 24 hours. The percentage mortality was corrected using Abbott's formula [17]:

$$\text{Corrected mortality rate} = [(Tmp - Cmp)/(100 - Cmp)] \times 100,$$

where: *Tmp*: mortality percentage on the treated leaf; *Cmp*: mortality percentage on the control (dipped in distilled water).

2.3 Repellency test

For each replicate (Petri dish), two leaves free from aphids were used. Then a leaf was introduced for a few seconds in the treatment solution. Leaves introduced in the treatment were placed on one side and the control on the other side. Ten apple aphids were placed in the middle of each dish. The procedure was replicated three times for each of the five examined solutions. After 24 hours, the number of aphids in each side was recorded.

Percentage of repellency was calculated according to the following equation:

$$PR = [(NC - NT) / (NC + NT)] \times 100 \text{ [18]},$$

knowing that NC is the number of apple aphids attracted to the control, and NT is the number of apple aphids attracted to the treated leave.

2.4 Statistical analysis

The comparison of aphid mortality averages on each treatment was carried out by ANOVA one-way analysis and Student–Newman–Keuls test, using SPSS

software. In addition, a *Probit* analysis was performed using the same software to determine Lethal Concentration (LC) 50 of the plant extract.

3. Results and discussions

3.1 Toxicity test

The statistical analysis indicated significant differences in insect mortality rates between the different solutions. The mortality rate of aphids was low for treatments 1, 3, and 5% (≤ 30%), while the mortality exceeded 50% for treatments 7 and 10% (*Table 1*). The LC 50 value of the studied extract was 7.09%.

Table 1. Corrected mortality percentages of apple aphids on different aqueous extracts of *S. microphylla*

Extract concentrations	Corrected mortality percentages after 24 hrs (Mean ± Standard error)
1%	3.33 ± 3.33 a
3%	20.00 ± 5.77 b
5%	30.00 ± 7.77 c
7%	50.83 ± 5.77 c
10%	73.33 ± 3.33 d
Significance	0.000 *

Note: * significant at $P < 0.05$.

Similarly, Balog et al. [19] revealed that all extracts from five tested plant species with 6% concentrations had significant effects on apple aphid, and the mortality was high compared with control. Moreover, other investigations showed potential insecticidal activities of aqueous extracts against different aphid pests, including damas (*Conocarpus lancifolius*) against sorghum aphid, *Rhopalosiphum maidis* [20], *Aloe zebrina*, *Melia azedarach*, and *Capsicum annum* against cotton aphid, *A. gossypii* [21], *Thymus algeriensis* [22], and *Artemisia campestris* against black bean aphid, *A. fabae* [23] and common bean (*Phaseolus vulgaris*) against cabbage aphid, and *Brevicoryne brassicae* [24] and neem (*Azadirachta indica*) was found to reduce aphid infestations similarly to the tested chemical insecticide [25].

On the other hand, some previous studies confirmed the insecticidal potentialities of many plant species belonging to *Salvia* such as *S. microphylla* against the fall armyworm *Spodoptera frugiperda* [26], *S. officinalis* against the woolly apple aphid *Eriosoma lanigerum* [27], and the methanolic extracts of 21 *Salvia* species and/or their cultivars against the moth *Spodoptera littoralis* [28].

In the present study, it was noticed that the mortality rates increased with the dose. Additionally, Benoufella-Kitous et al. [29] found that the insecticidal effect of sage *S. officinalis* against *A. fabae* seems to depend on the dose.

It is suggested that the aphicidal activity expressed in our case is due to the presence of some secondary metabolites in plant extracts. Secondary metabolites seriously affect aphid behaviour, physiology, and metabolism [30]. For instance, a prior study investigating the insecticidal properties of Amaryllidaceae alkaloids confirmed that N-Allylnorgalanthamine effectively inhibited acetylcholinesterase in *A. citricola* [31]. Besides, saponins are known for their toxicity to detrimental insects, which includes actions such as deterring feeding, disrupting the molting process, regulating growth, and causing mortality [32]. Furthermore, *in vitro* bioassays demonstrated that among the native flavonoids, quercetin and isorhamnetin exhibited significant inhibitory effects on aphid reproduction [33].

3.2 Repellency test

Results presented in *Figure 1* revealed that the extract concentration of 10% of *S. microphylla* was the most repellent to apple aphids (PR = 62.59%), followed by the concentration of 7% (PR = 60.48%).

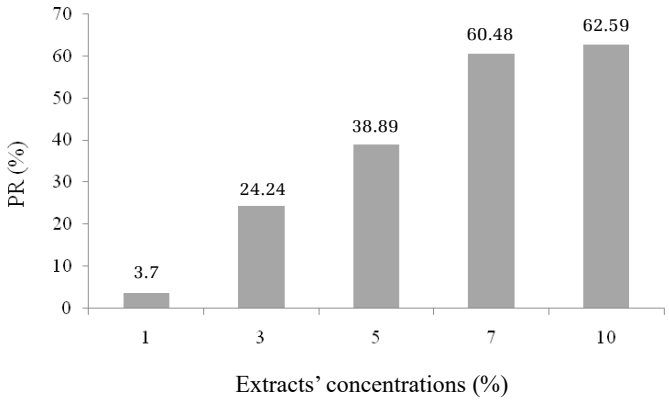

Figure 1. Repellency of the aqueous extracts of *S. microphylla* against the apple aphids

Likewise, Assis et al. [34] found that the garlic extract presented a repellent effect on aphids. Furthermore, the results of Mina [35] indicated that the aqueous extract of marigold (*Tagetes erecta*) flower showed effective repellency against *A. gossypii* and *Myzus persicae* populations. Moreover, watery extracts of five medicinal species demonstrated significant repellency against wingless rose aphid

Macrosiphum rosae in comparison to control [36]. Further, the neem seed aqueous extract recorded a medium repellence index for the concentration of 10% [37].

The selection of host plants by aphids is influenced by a range of physiological and chemical cues but is primarily influenced by gustatory signals observed during the stylet's penetration into the plant's peripheral tissues [38]. It seems that the tested plants deploy some of their components as repulsive to aphids, and their effects change according to the concentrations in the solutions. Thus, triticale hybrids with higher phenolic concentrations were less appealing to cereal aphids compared to non-transgenic varieties [39].

4. Conclusions

The potential insecticidal and repulsive effects of aqueous extracts of *S. microphylla* on *A. pomi* were screened *in vitro*. The findings demonstrated that extracts at higher concentrations had significant aphicidal and repellent properties. As a result, using these extracts could offer suitable alternatives to chemical pesticides for aphid management. It is strongly advised to carry out field bioassays to confirm the results obtained.

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Dendrometric study of stands of *Pistacia atlantica* in southwestern Algeria

Abdelkrim BENARADJ,[1*] Hafidha BOUCHERIT,[1] Anteur DJAMEL,[2] Adda ABABOU[3]

[1] Laboratory of Sustainable Management of Natural Resources in Arid and Semi-Arid Areas, Salhi Ahmed University Center of Naama (Algeria)

e-mail: kbenaradj@yahoo.fr

ORCID: https://orcid.org/ 0000-0001-6555-6008

[1] Laboratory of Sustainable Management of Natural Resources in Arid and Semi-Arid Areas, Salhi Ahmed University Center of Naama (Algeria)

e-mail: h.boucherit@yahoo.fr

ORCID: https://orcid.org/ 0000-0001-6618-0727

[2] Research Laboratory of Water resources and Environment, Dr Moulay Tahar University of Saida (Algeria)

e-mail: anteurdjamel12@gmail.com

[3] Department of Biology, Faculty of Nature and Life Sciences, Hassiaba Ben Bouali University of Chlef (Algeria)

e-mail: ab_adda@yahoo.fr

*Corresponding author

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Abstract: In the southwestern part of Algeria, the stands of Atlas pistachio (*Pistacia atlantica* Desf.) constitute a special natural heritage. They are usually scattered between the steppe plains and the Saharan Atlas. This study was conducted with the aim of identifying the dendrometric characteristics of Atlas pistachio that could be indicators of good productivity. The aim is to investigate the influence of stationary factors (slope, exposure, geology) on the dendrometric characteristics of *Pistacia atlantica*. The results obtained on the dendrometric parameters of this species are very interesting. From a dendrometric approach, *Pistacia atlantica* is a large tree that can reach impressive dendrometric dimensions: 8 to 10 meters in height and a diameter often exceeding 100 cm. This dendrometric analysis allowed us to make a structural characterization of stands of *Pistacia atlantica*, which is an essential step in the management plan and the valuation in the medium or long term. This species of *Pistacia atlantica* deserves adequate measures of protection, especially in its natural environment. Therefore, a better knowledge of their potentialities, problems, and factors of the decline

of this hardy species would contribute to the protection and the preservation to favour their regeneration, which would allow a better extension of this species.

Keywords: stands, *Pistacia atlantica*, southwestern, Algeria, dendrometric

Introduction

The Atlas pistachio tree (*Pistacia atlantica* Desf.) is extremely widespread in the eastern Mediterranean area (Greece, Cyprus, Turkey, Syria, Palestine, Crimea, Iran, Afghanistan, and as far as India) [1]. But it can also be found in the south of North Africa, scattered in the arid and semi-arid regions [2]. It is quite common throughout Algeria; its habitat can extend from the Tell Atlas with a humid soil to the arid and even Saharan regions, where it is scattered (isolated), or in the depressions (*dayas*) on the high steppe plains, the northern Sahara, at the foot of the Saharan Atlas in the best watered parts, and even in the Hoggar in a "relic" state [3–7].

In the southwestern part of Algeria, *Pistacia atlantica* stands have a very extensive botanical area, covering relatively large areas per thousand hectares between the steppe plains and the pre-Saharan region of the Saharan Atlas. These pistachio trees are generally dispersed (rocky outcrops, thalwegs, ravines, and wadi beds) or grouped in the form of a grove, mainly in the alluvial depressions [8–13].

The *Pistacia atlantica* is a sclerophyllous deciduous species, typical of the Mediterranean region. It belongs to the family Anacardiaceae, which once formed dense stands. Due to its poor regeneration, the pistachio tree is becoming increasingly rare because its seeds do not germinate well and are often grazed by both wild and domestic herbivores. *Pistacia atlantica* is one of the endemic (autochthonous) species widespread in the southwestern part of Algeria; it shows a perfect adaptation to extreme conditions.

The aim of the study is to identify the dendrometric characteristics of the *Pistacia atlantica* stands in the southwestern part of Algeria, which could serve as indicators of the potential of a forest management process.

Materials and methods

Study area

On a biogeographic level, the study area belongs to the Mediterranean zone, the highlands sector, and the Saharan Atlas sector, according to Quézel and Santa's subdivisions [14].

A field survey aims to delimit the areas potentially favourable to the development of *Pistacia atlantica*. It was carried out in order to identify and discover the general distribution of *Pistacia atlantica* trees according to the selected criteria (biotope,

climate, edaphic, etc.) on 12 well-distributed populations of the study area (*Tab. 1* and *Fig. 1*).

Table 1. Location of study stations

Station no.	Region	Geographic coordinates		
		X	Y	Z (m)
Station 1	El Bayadh	N 32°33'21.6"	E 0°01'29.8"	835
Station 2		N 33°02'40.3"	E 1°02'20.7"	955
Station 3		N 33°15'32.9"	E 1°36'57.7"	970
Station 4		N 33°19'38.7"	E 1°37'27.5"	990
Station 5	Naama	N 32°44'20.0"	W 0°27'14.4"	1,040
Station 6		N 32°49'06.2"	W 0°13'54.1"	1,145
Station 7		N 32°59'24.7"	W 0°41'51.6"	1,345
Station 8		N 33°23'01.0"	W 0°57'46.6"	1,122
Station 9	Bechar	N 31°58'17.06"	W 1°32'17.80"	840
Station 10		N 32°00'21.00"	W 1°38'05.13"	780
Station 11		N 31°49'03.39"	W 1°45'41.69"	724
Station 12		N 32°04'36.16"	W 2°17'45.31"	960

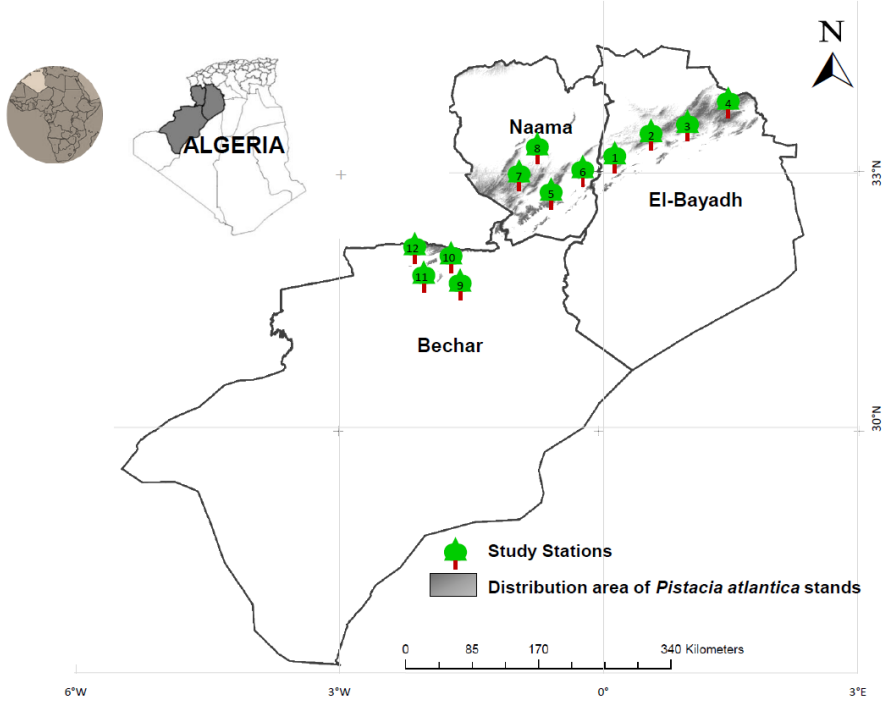

Figure 1. Geographical location of the study area

In terms of climate, the study area is characterized by low and irregular rainfall (between 100 and 250 mm/year) and a fairly long dry period of 6 to 7 months. This explains its belonging to the arid bioclimatic stage [11, 15, 16].

The table below provides the climatic characteristics of the most representative weather stations in the area of *Pistacia atlantica*.

Table 2. Climatic characteristics of study stations (1990–2014)

Region	**Bechar**	**Naama**	**El- Bayadh**
Altitude (m)	807	1166	1341
Rainfall (mm)	112	219	284
Maximum temperature M (°C)	40.7	36.8	34.88
Minimum temperature m (°C)	3.15	0.32	-0.35
Thermal amplitude (M – m (°C)	37.55	36.48	35.23

Materials used (biological and technical)

The study focuses specifically on groups of *Pistacia atlantica* spontaneously occurring in the southwestern part of Algeria.

The following equipment was used:

– Blume-Leiss dendrometer for measuring the heights of trees,
– GPS (Global Positioning System) device for the positioning of trees,
– compass to establish the direction,
– camera to take the pictures,
– tape for the measurement of the circumference at 1.30 m from the ground.

Methodological approach

In the field of forestry, if qualitative criteria are very often used to describe and compare stands, it is sometimes necessary to use numerical data to refine the description and better understand the evolution of a plot [17].

The dendrometric appreciation of a forest stand is based on the study of height, basal area, and density [18].

Various dendrometric measurements were made of the trunk of the trees for *Pistacia atlantica* in the field and to investigate the relationship between the diameter and height of the tree.

The dendrometric analysis included 239 sample trees (individuals) of *Pistacia atlantica* taken at random from the study area. It was based on the determination of the stand dendrometric variables: classes of diameters and classes of heights.

The comparison of the results obtained from the field data was made on the following dendrometric parameters:

Tree height

Table 3. Distribution of trees by height classes

	Class	Height (m)
1	Class 1	> 3 m
2	Class 2	3–6 m
3	Class 3	6–9 m
4	Class 4	9–12 m
5	Class 5	12–15 m
6	Class 6	> 15 m

Tree size

The size of a tree is the most commonly used and measured dendrometric characteristic. According to Lombardini et al. [19], the four broad categories of diameter to differentiate trees are defined as shown in the table below.

Table 4. Tree diameter categories

	Trees categories	Diameter Φ (cm)
1	Small trees	7.5–22.5
2	Medium-sized trees	22.5–42.5
3	Large trees	42.5–62.5
4	Very large trees	> 62.5

Density of trees

Density refers to the number of trees on a given surface area [17]. This was estimated by counting the number of subjects of *Pistacia atlantica* per hectare.

Results and discussions

Determination of the main dendrometric parameters

a) Height

The results obtained from the distribution of trees by height classes are given in the form of histograms (*Fig. 2*). This figure shows the vertical structure of the woody *Pistacia atlantica* stand in the study area.

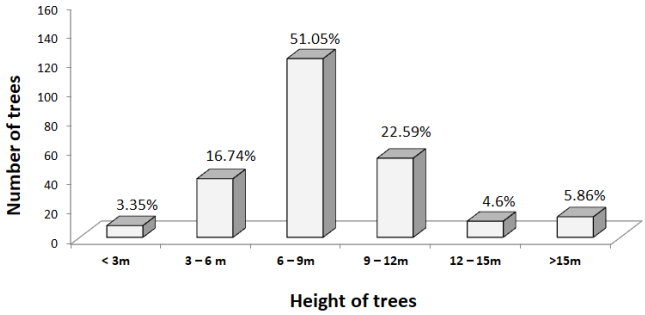

Figure 2. Distribution of *Pistacia atlantica* trees by height class (m)

Results obtained from the distribution of trees by height classes show that the structure is rather heterogeneous because of the high variability of the stems (trunk).

The interpretation of *Figure 2* confirms the following:

– The dominance of heights from 6 to 12 m in the sampling plots at Bechar.
– The first, fifth, and sixth classes are poorly represented in these same plots.
– At the level of the stand sampled at Naâma, there is a dominance of heights of 4 to 10 m and the total absence of trees from 12 to 16 m.
– For the plots studied in the El Bayadh region, we note that the heights mostly range between 6 and 12 m, with the total absence of the subjects in the range of 14–16 m.

The comparison between the results obtained in the different study areas reveals a divergence between the studied Bechar stand and that of Naama and El Bayadh. In fact, the growth in height of *Pistacia atlantica* in Bechar is greater than those of Naâma and Bayadh. This can be justified by the grazing, which is frequent in the areas of El Bayadh and Naama.

Most of the woody species are best represented and grouped in class 6–9 m; this is the class of the most dominant stratum, and it represents 51% of the total number of tree individuals recorded in the study area. The individuals of the classes 9–12 m represent 23%, or 54 subjects of *Pistacia atlantica*. Individuals with a height greater than 12 m are less represented (10%) than the two higher classes (12–15 m and > 15 m) of *Pistacia atlantica* tree. Class 3–6 m individuals take the 3rd place with 17% of total trees measured. The class of < 3 m represents only a small percentage, not exceeding 3.35% of all individuals, which indicates the importance of individuals less than 3 m tall and also testifies to an increased natural regeneration.

All these results and the observations made allow us to deduce that the percentages of height classes differ from one station to another. This is mainly due to several indicators related to the diversity of geomorphological, topographic, climatic, and

hydrologic units, biotope, pedo-climatic characteristics, anthropization (logging, overgrazing), etc.

b) Diameter (Φ)

All mean values of the size (circumference or diameter) of a stand are calculated from the frequency distribution of the numbers of trees by size category. *Figure 3* below shows the distribution of the *Pistacia atlantica* trees by diameter classes given in the form of a histogram.

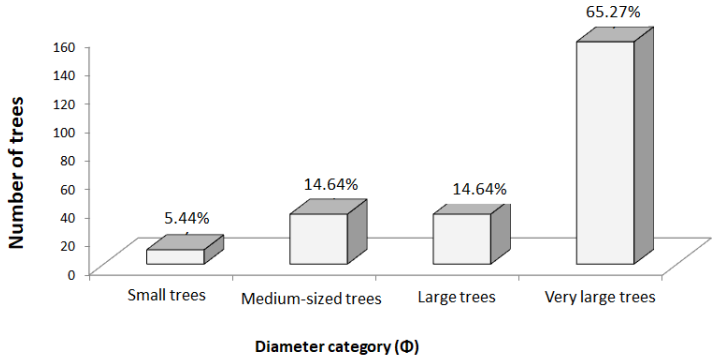

Figure 3. Distribution of trees by diameter classes of *Pistacia atlantica*

The analysis in *Figure 3* shows that the diameter class distribution is quite heterogeneous in the study area. In fact, the majority of the individuals are in the category of very large trees (> 62.5 cm). This category represents 65% of the trees measured in the study area. It is generally characterized by depressions (the case of stations no. 2, 4, 8, and 12); this is explained by favourable humidity and deep soil.

Figure 4. Example of vigorous trees (very large trees) in the study area

For the other categories, individuals of diameters in the ranges of 22.5–42.5 cm and 42.5–62.5 cm represent 14.6% each. Individuals in the small trees category (7.5–22.5 cm) account for only 5.44% of the total trees measured. The latter

category of very large trees is somewhat remarkable, especially under the shelter of the clumps of jujube (*Ziziphus lotus*), which is indicative of the regeneration possibilities of the pistachio tree in the study area.

These results are consistent with those obtained by Terras [20], Benaissa [21], and Mansour [22] and suggest the potential of natural regeneration. This regeneration is generally carried out within the clumps of *Ziziphus lotus* (Rhamnaceae), which protect the young shoots of the *Pistacia atlantica* against pasture and promote the germination of their seeds as well as the growth of their young shoots by enriching the soil with organic matter [5, 23, 24, 25]. The regeneration of the *Pistacia atlantica* was also observed inside a tuft of *Retama retam* [10].

When the young regenerated foot reaches a certain height by adapting to the medium, the jujube is found in a lower layer and gradually disappears from its immediate vicinity [24]. In our stations, we have noticed some cases of regeneration of the Atlas pistachio tree at the level of inaccessible mountain cliffs, also acting as a shelter for the animals feeding on it and retaining water precipitation that promotes seed germination.

c) Density

The dendrometric study will allow the estimation of the density and determination of tree structure in the study area.

Density is defined as the total number of stems per unit area [26]. *Figure 5* summarizes the results of the study regarding the tree density of *Pistacia atlantica* stands in southwestern Algeria.

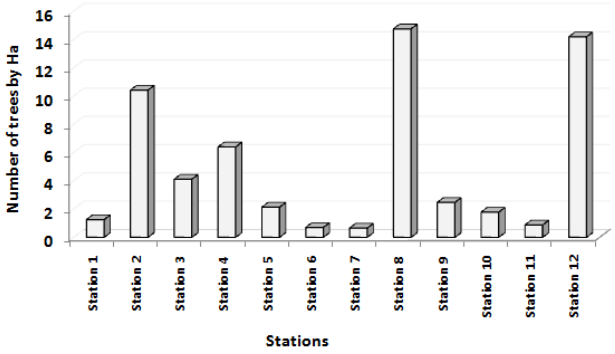

Figure 5. Density of *Pistacia atlantica* trees per hectare

The analysis of the above figure indicates that the highest density of trees is found in the depressions (the case of stations 2, 4, 8, and 12) with 14 subjects, in which case *Pistacia atlantica* is a rather large tree, very massive and spectacular. *Pistacia atlantica* is found in denser stands, i.e. 10 trees/ha in dayas. The regeneration of some young plants has been observed in more or less protected areas. Indeed,

this regeneration is always carried out inside the jujube (*Ziziphus lotus*), which would constitute a good protection to the young shoots against winds and herds.

On the other hand, the lowest density is found at Station 7: with only 0.65, it is well dispersed. Density values for stations 1, 5, and 6 are 2.13, 1.25, and 0.70 respectively. The average density in relation to the total area is 8 subjects/ha, where the trees of *Pistacia atlantica* and *Ziziphus lotus* are scattered in isolated spaces because of the drought conditions that afflict this species in the Saharan region. According to Boudy [27], the drier (arid and semi-arid) a forest region, the lower the density of its mature stands because the roots need a considerable living space to draw water from the soil.

Despite this low density, tree growth is relatively good, which may be due to the location of trees in unfavourable sites: the presence of the species in isolation and on riverbanks (depression, wadi beds). A small difference, which is insignificant, is the influence of certain environmental factors.

Pistacia atlantica trees are generally found in well-ventilated and sunny sites, requiring a large spacing between trees to avoid competition. The distance to be applied is more than 20 m, or 10 to 20 trees per hectare in depressions. Often, however, this spacing is not sufficient and must be given more than 100 m, or 1 to 8 trees per hectare.

Correlations between the diameter and height of trees

The study of correlations between the diameter and the height of the trees of a stand makes it possible to highlight the relationships between the dendrometric characteristics of the subjects studied. It also allows learning about the behaviour of *Pistacia atlantica* trees towards the biotic and abiotic factors of the environment.

The linear trend curve and the equations connecting the diameter and the height of the different subjects of *Pistacia atlantica* studied are represented in *Figure 6*. The linear regression lines obtained made it possible to highlight certain correlations.

Results show that the correlation coefficients obtained are all greater than zero ($r > 0.44$) in all the study stations. Almost all established correlations are in close relationship, which translates into the response of *Pistacia atlantica* to the edapho-climatic conditions of the environment. The correlation coefficient characterizing the relationship between the height and the diameter of the Atlas pistachio trees is 0.445 for Naâma, 0.564 for El Bayadh, and 0.681 for Bechar. This confirms that these coefficients are well linked and correlated.

Based on the value of the correlation coefficient, the tree height of *Pistacia atlantica* is closely related to its diameter. Nevertheless, there is a weak correlation between the height and diameter of young *Pistacia atlantica* shoots of a small diameter. This is probably due to the juvenile age of the stand and overgrazing.

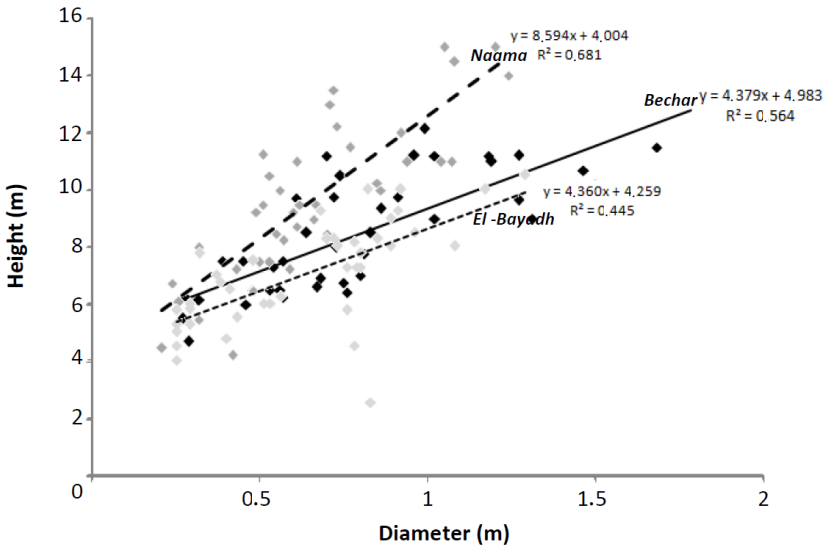

Figure 6. Correlation between the height and diameter of trees

The relationships we found between the measured parameters can be explained by the influence of the stationary, microclimatic, and edaphic factors on the morphology of *Pistacia atlantica* trees.

Diameter growth is generally related to edaphic and climatic conditions and the impact of anthropogenic factors. As an example for low-density stations, the growth of the tree is relatively good; this may be due to the location of the trees in very favourable sites: the presence of the species in an isolated state in the hydrographic network. Therefore, Pistachio trees are generally found in well-ventilated and sunny locations, requiring a large spacing between trees to avoid competition [8, 9, 11, 12, 28].

Pistacia atlantica is one of the tree species inhabiting the arid and semi-arid lands of northern Africa [14]. It is a perennial, hardy, and endemic taxon that can live for several centuries by adapting perfectly to the edaphic and climatic conditions of its habitat [29].

Conclusions

These stands of *Pistacia atlantica* cover vast expanses of the Saharan Atlas and the high steppe plains of Algeria. They constitute an important off-forest heritage of South-West Algeria.

Pistacia atlantica is a large tree that can grow to a large dendrometric size of 8 to 10 meters in height and often more than 100 cm in diameter. Its crown is voluminous and rounded. It is more abundant in the Saharan region (altitude from

700 to 1,400 m). The analysis of the effect of the distribution of *Pistacia atlantica* groups on dendrometric parameters (diameter and tree height) shows a significant difference between the averages of height classes and those of diameters. This indicates that the heterogeneity of the structure of these Atlas pistachio trees between stations depends on the environmental factors of the study environment (geomorphology, lithology, geology, anthropic impacts).

These stands of *Pistacia atlantica* can be an excellent barrier between the high steppe plains and the Saharan space against the advance of the desert. Its rehabilitation and conservation are therefore necessary to safeguard and enhance it so as to contribute to the sustainable development of these fragile arid zones.

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Evaluation of the 'Leányka' ('Fetească Albă') white wine grape variety's qualitative and quantitative parameters in the context of different bud loads

Géza BALLA, Tekla Amália LUNKA,* Zsolt SZEKELY-VARGA, Csaba MOLDOVÁN,* Csongor KENDI, Endre KENTELKY

Department of Horticulture, Faculty of Technical and Human Sciences, Sapientia Hungarian University of Transylvania, Calea Sighişoarei 2, 540485, Târgu-Mureş/ Corunca, Romania

*Corresponding authors: tekla.lunka@gmail.com, csaba.moldovan@ms.sapientia.ro

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Abstract: Wine is a product of human creativity, culture, and nature, and therefore it is an intriguing subject for scientific research and admiration. Among its many kinds, white wine stands out oenologically because of its diverse flavours, aromatic attributes, and capacity to mature. In this experiment, we established 3 different bud load levels (25%, 50%, and 75%) on the well-known white wine grape variety 'Fetească Albă'. The sugar content significantly increased in the 50% and 76% groups, whereas there were no significant changes regarding titratable acid content, must quantity, and cluster weight.

Keywords: bud load, sugar content, titratable acid content, 'Leányka', 'Fetească Albă'

1. Introduction

Wine is a fascinating topic for scientific studies because it is a product of nature, culture, and workmanship. White wine is one of the many varieties, and it stands out oenologically thanks to its complex flavours, aromatic qualities, and ability to age [1].

White wine is a beverage with significant scientific interest because of its diverse sensory qualities and complex winemaking processes. The key to appreciating the complexity of this libation is to understand the complicated biochemistry that underlies the emergence of aromas and flavours, the impact of terroir on grape development, and the fermentation processes that give rise to white wine's distinctive finesse [2, 3].

Romania is situated in Southeastern Europe and has a long history of viticulture extending back for millennia. Its varied areas, which range from low plateaus to rolling hills, provide a variety of microclimates and soil compositions. These characteristics, along with the regional grape types, have produced a specific winemaking culture that is still open to scientific study [1, 4].

Making thoughtful decisions about how many buds to leave on a grapevine is called "bud load management", a vital component of viticulture. This strategy has a direct effect on grape yield, cluster formation, and canopy density. Viticulturists can control the amount of grape must that can be harvested by altering the bud load, which allocates the resources of the vine to either vegetative growth or reproductive development [5].

The relationship between bud load and sugar build-up is closely related to the physiological functions of the grapevine. During photosynthesis, grapevines take in carbon dioxide from the atmosphere and use several complex metabolic processes to convert it into glucose. The number of buds on the vine will determine how these sugars are distributed [6, 7].

More clusters and grape berries are produced as a result of a larger bud load, which raises the competition for limited resources, including water, minerals, and carbohydrates. If resources are distributed among larger berries, the sugar content of each berry can drop. Contrarily, fewer buds enable a more efficient allocation of resources, which can enhance the sugar content of the berries.

Grapevine has numerous internal regulatory mechanisms that further control how bud load affects sugar build-up. Particularly important in the hormonal signalling, which regulates the allocation of resources between vegetative and reproductive organs, are auxins and cytokinins. A greater bud load may encourage vegetative development at the expense of the developing grape berries due to increased auxin production [8].

The production of grape must, a crucial step in the production of wine, requires the presence of titratable acids, including tartaric, malic, and citric acids. These acids have an impact on the final wine's flavour, harmony, and stability. The concentration of titratable acids in grape must has a significant impact on acidity levels, pH, and taste perception. These three parameters also have a significant impact on the sensory profile of the finished wine [6, 9].

The metabolic activities of the grapevine constitute the basis for the interaction between bud load and titratable acid content, which affects acid production. The glycolytic pathway and the citric acid cycle are just two of the metabolic processes that produce titratable acids. The availability of resources, such as minerals and carbohydrates, has a significant impact on the grapevine's capacity to synthesise these acids [10, 11].

More grape clusters produced by a larger bud load will likely deplete the resources needed to produce titratable acids. If more resources are allocated to

vegetative development and more grape clusters are produced, the generation of titratable acids may be impeded. The ability of the grapevine to allocate resources more efficiently to fruit development if there are fewer buds present may boost the synthesis of titratable acids in grape berries.

The relationship between bud load and grape must yield is based on the fundamental principle of resource distribution inside the grapevine. A higher bud load, which leads to more grape clusters, increases the potential yield of grape must. However, the availability of essential resources, such as water, minerals, and carbohydrates, has a direct impact on this increase in output. The grapevine's capacity to supply these components to the developing grape clusters has a significant impact on the eventual volume of grape must [12, 13].

On the other hand, a reduced bud load concentrates the vine's energy on a fewer number of grape clusters. The number of grapes produced may be decreased overall, but they may use resources more efficiently, producing grapes with higher concentrations of sugars, acids, and other ingredients [2].

Source–sink dynamics is a concept that examines the balance between tissues that create resources (like leaves) and those that consume them (like fruit), and it is also crucial. Increased bud load may tip the source–sink balance in favour of vegetative tissues, limiting the resources available for the growth of grape clusters and, as a result, lowering grape must output [2, 14].

The effect of bud load on cluster weight demonstrates the delicate balance between vegetative growth and reproductive processes.

The distribution of resources like nutrients, water, and other resources throughout the grapevine has a substantial impact on cluster formation and weight. A higher bud load causes more potential clusters to develop, competing for few resources. While having more clusters could seem advantageous, it could lead to resource shortages for some clusters, which might limit their ability to grow and gain weight [6].

2. Materials and methods

At the heart of Romania's viticultural heritage lies 'Leányka' ('Fetească Albă'), a grape variety that embodies the mystique of indigenous viticulture. It is known for its resilience in the face of various climatic challenges, adaptability to different terroirs, and the production of wines ranging from dry to sweet. Yet, the genetic and oenological intricacies of 'Leányka' ('Fetească Albă') remain a captivating enigma.

When completely ripe, 'Leányka' grapes are small to medium size and have a greenish-yellow to golden skin. Due to the grapes' high acidity, a well-known feature, a variety of white wines – from dry to sweet – can be made from them. The adaptability of this cultivar in winemaking makes it highly regarded. The variety of white wines it may produce includes dry, semi-sweet, sweet, and sparkling wines.

Grape is excellent for a variety of winemaking techniques due to its inherent acidity and aromatic flavour. The characteristics of the wine could substantially change depending on the particular vineyard and area where it is cultivated because it is very responsive to terroir. It is a fascinating grape for examining the subtleties of various wine-producing regions because of its terroir responsiveness [1, 2].

Three different loads and a control during the experiment were used for the current experiment. There were six vines for each bud load, amounting to a total of 72 vines. Following the start of sap circulation on 8 April, the amount of bud load was determined. Every vine underwent the Guyot training method. A cane with 12 buds and a 2-bud short spur (12 + 2) served as the control. The bud load was determined by this. Only a short cane with 4 buds remained after the first load was 25% higher than the control (12 + 2 + 4). Compared to the control (12 + 2 + 7), the second one had a 50% load and a 7-bud cane left. For the final 75% load compared to the control, one more cane with 10 buds was added.

The samples were titrated to ascertain the titratable acid content, and the sugar concentration was measured using a refractometer.

3. Results and discussions

Considering the sugar content measurements, an increase was detected between the control (217.3 g/L) and the other bud loads. The value of the 25% load is almost the same as the control (218.6 g/L), but there is a significant increase at the 50% (245.8 g/L) and 75% load (243.2 g/L) compared to the control. It can be concluded that the highest sugar content was achieved at the 50% load, with an outstanding value compared to the control (245.8 g/L) (*Fig. 1*).

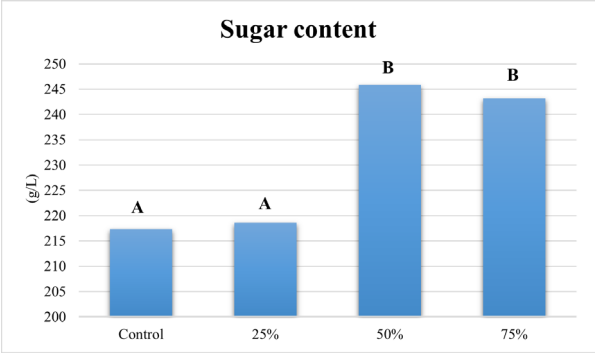

Figure 1. Differences in sugar content with different load levels ($p < 0.05$)

The acid content of the control was 7.7 g/L. The samples of vines with a 25% load were 8.3 g/L, the samples with a 50% load were 8.1 g/L, and the acid content

of individuals with a 75% load was 7.6 g/L. The results show that the load mostly had a positive effect on the formation of acidity, which could be observed at 25% and 50%. In the case of the 75% load, a decrease was recorded compared to the control (*Fig. 2*).

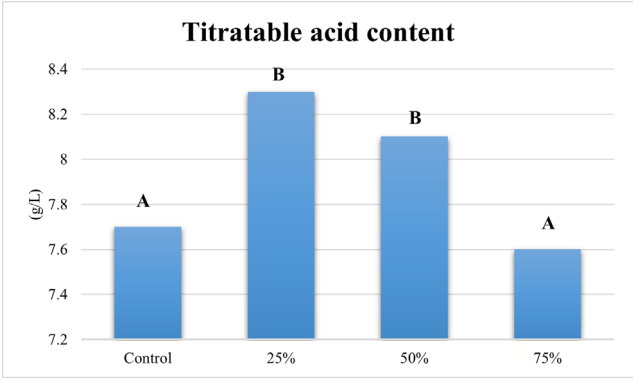

Figure 2. Differences in titratable acid content with different load levels ($p < 0.05$)

The loaded groups mostly show an increase compared to the control (1.78 kg) although there are no particularly large differences. The largest juice quantity was pressed out of the 25% load group (1.98 kg), leaving the 50% load group (1.9 kg) behind by just 0.1 kg. The smallest value was given by the 75% load (1.3 kg), which is almost 0.4 kg less than the control. Based on the results, we drew the conclusion that in the case of 'Leányka', increasing the bud load mostly had a positive effect on the must yield up to a certain level since too much load was already associated with a decrease (*Fig. 3*).

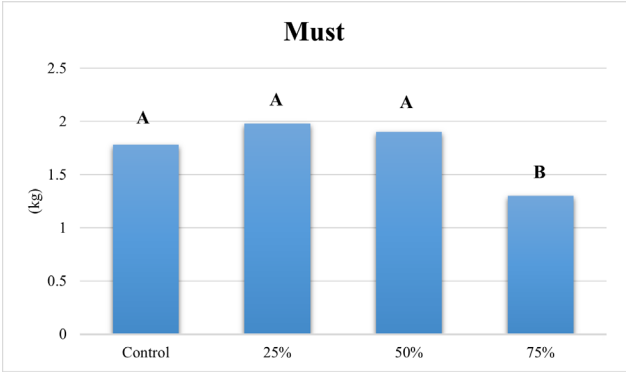

Figure 3. Differences in must quantity with different load levels ($p < 0.05$)

The clusters measured in the control had an average weight of 43.7 g. Among the bud loads, the 25% load level (36.6 g) resulted in a decrease in cluster weight, the average cluster weight of the 50% load (35 g) gave an even smaller value, and, finally, the 75% bud load (30 g) had the smallest average cluster mass. It can be concluded that in the case of this variety, the load was inversely proportional to the weight of the curls (*Fig. 4*).

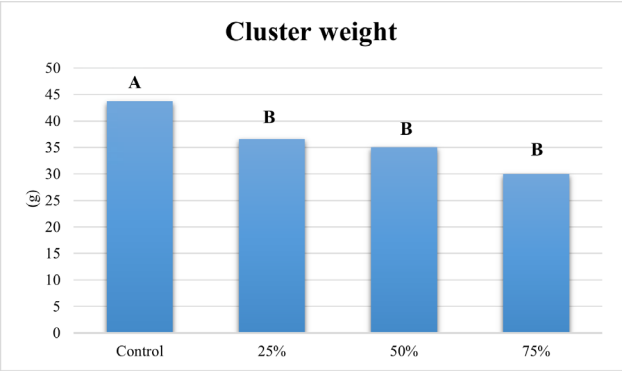

Figure 4. Differences in cluster weight with different load levels ($p < 0.05$)

4. Conclusions

From the present study, it can be concluded that in terms of sugar content, there was a significant increase in the 50% and 75% bud load. With the exception of sugar formation, no significant differences were reported between the control and the loads. It was observed concerning the 'Leányka' ('Fetească Albă') variety – since it is also a grape variety with a strong growth power – that too much bud load results in a too dense foliage, which hinders the performance of phytotechnical operations.

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Injuries in the landscape: The Suseni quarry – A case study from Transylvania, Romania

Ildikó LIHĂT,[1,2] Endre VÁNYOLOS,[1,*] Anna Imola HENNING,[1] Noémi Melitta HEGEDÜS,[1] Zsolt SZEKELY-VARGA,[1] Endre KENTELKY,[1,*]

[1] Department of Horticulture, Faculty of Technical and Human Sciences, Sapientia Hungarian University of Transylvania, Calea Sighişoarei 2, 540485, Târgu-Mureş/ Corunca, Romania

[2] "Ion Mincu" University of Architecture and Urban Planning, Bucharest, Romania

[*] Corresponding authors: kentelky@ms.sapientia.ro; v.endre@ms.sapientia.ro

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Abstract: Mining and quarrying operations exert a substantial influence on both the environment and nearby communities. Plenty of them can be found in mountainous areas of Transylvania. These activities, which involve the excavation of expansive open pits and the disruption of soil and vegetation, can lead to adverse consequences such as soil erosion, deforestation, habitat degradation, and water contamination. Mining and quarrying operations carry notable consequences for both the environment and the nearby communities. The interventions of contemporary humans have changed the landscape on an unprecedented scale. The signs of human involvement are apparent in numerous locations in the Giurgeului Depression; the Suseni quarry site is an example of a place that necessitates a comprehensive rehabilitation plan.

Keywords: mining site, rehabilitation, footprints, NBS (nature-based solutions)

1. Introduction

In his book titled *Urban Space*, architect and urban planner Rob Krier writes that: "I have yet to see a tree which looked aesthetically wrong or defective. The same is true for landscape" [1]. While Krier's statement may hold true in natural environments, in human-influenced territories, there are many landscapes that can appear visually unappealing or flawed. Human interventions in contemporary times have significantly altered landscapes, resulting in many "wrong or defective" landscapes that require remedial action. The formation of landscape wounds or scars

resulting from mining or quarrying leaves enduring impacts on the environment and nearby communities. These effects give rise to a variety of ecological issues, including soil erosion, deforestation, habitat loss for both plant and animal species, and potentially leading to water and air pollution.

According to Professor Mihály Mőcsényi, landscape planning is a discipline that seeks to shape the human environment by applying contemporary ecological and technical knowledge as well as aesthetic principles. Its objective is to enhance the productivity, visual appeal, and overall well-being of the landscape. This is achieved by improving the living conditions of human residents, extending their physiological and aesthetic enjoyment of life. [2].

For centuries, humanity has engaged in the manipulation of the Earth's terrain. Well before the introduction of modern construction materials like drywall and plywood, natural caves, pits, abode, and turf were fashioned to provide shelter. Mountainsides were shaped to support agricultural terraces, roads, canals, and mines, enabling people to sustain their livelihoods. These altered landscapes often bear an artificially shaped character, a design form that becomes an indelible part of the surrounding environment. Interestingly, modern mining activities continue to face criticism despite the presence of similar geometric patterns created by other industries and even by nature itself [3]. These patterns etched on and within the landscape are a testament to the ongoing human impact on the Earth's surface, footprints.

Despite the role of mining in providing an economic foundation and a source of natural resources to enhance the quality of human life [3], quarrying operations have generated a wide range of physical and environmental consequences on a global scale over the course of recent decades. In the past 20 years, there has been an increasing recognition of the importance of addressing the rehabilitation and requalification of quarry sites. To be truly effective, such efforts should be closely aligned with the available local resources and context, as evidenced by diverse rehabilitation strategies implemented in various regions around the world [4].

The industrial landscape stands as a distinctive category, broadening our understanding of landscape to encompass locations like production sites, originally devoid of aesthetic intent. Abandoned extraction sites, in particular, form a distinctive landscape where technological heritage and the natural environment intertwine, creating a multifaceted heritage that necessitates innovative methods of preservation and appreciation. The significance of the industrial landscape is linked not only to the emerging aesthetics connected to workplaces but also to the preservation of the place's cultural value [5]. This can be called the "genius loci", saving the atmosphere of the place while the reclamation of the quarry zone is taking place by allocating a different and original function, multiple functions, compatible with current human activities, adequate to our society and its needs, based on the aims of a sustainable development.

The UN World Commission on Environment and Development defined the concept of sustainable development as "development that meets the needs of the present without compromising the ability of future generations to meet their own needs" [6]. This definition emphasizes the importance of balancing current socio-economic needs with the preservation and responsible use of natural resources to ensure that future generations can also fulfil their own needs.

Nature-based tourism is widely recognized as a rapidly expanding sector within the world's largest industry, and it plays a vital role in supporting conservation efforts. While visitation rates may be declining in certain affluent countries, as evaluated through two different methodologies, data from around three-quarters of nations with available information indicate an overall increase in visits to protected natural areas [7]. This suggests that the trend of visiting protected natural areas is on the rise in many parts of the world, highlighting the significance of nature-based tourism as a contributing factor to conservation.

2. Methodology

The literature review component of the research methodology aims to provide a comprehensive understanding of the subject matter, specifically focusing on mine reclamations, nature preservation, and related aspects. This contextualization helps establishing a foundation for the subsequent analysis. The analysis itself involves a detailed examination of the territory by utilizing map documentation techniques and culminates in the development of a recovery proposal. This proposal aims to maximize the ecological, economic, and social benefits of the territory, ensuring a holistic approach to its restoration and preservation.

The territory requires a comprehensive reclamation concept and landscape rehabilitation plan. The objective of this paper is to analyse the territory and to identify optimal strategies for promoting a more sustainable relationship between humans and nature in the quarry's anthropic environment, with a focus on providing ecological, economic, and social benefits. Nature-based solutions and nature-based tourism must constitute the base of the concept.

3. Case Study

Location

Transylvania, a region in Romania, boasts a rich mining history that traces its origins back to the Roman Empire. Throughout its historical timeline, this area gained renown for its plentiful reservoirs of precious metals such as gold, silver, copper, and various other metallic resources [8]. Today, mining operations primarily focus on extracting salt, coal, and other minerals, with quarries being

a common sight. These open-pit mines, which are used for extracting various types of rock, stone, sand, and gravel, are situated in the mountainous regions of Transylvania.

The Suseni quarry is located in the Giurgeului Depression in Harghita County, Romania, near the settlement of Suseni (*Fig. 1*). It is situated in its administrative area, next to a valuable natural environment [9].

Figure 1. Location of the studied quarry

Historical development

Mining operations in the Suseni quarry began in the late 1930s. Nowadays, after a few changes of ownership, Lafarge, a French mining company operates the Suseni quarry, which is one of the largest and most modern quarries in Romania. In a few years' time, the extraction will end because the area is running out of good-quality stone [9].

Similar quarry areas in the Giurgeului Depression and near-by

Analysing the territory on a larger scale can unveil some important aspects. Traces of human intervention are conspicuous in numerous locations within Transylvania, including the Giurgeului Depression, and the Suseni quarry area is no exception. The map (*Fig. 2*) shows several quarry and mining areas in the Giurgeului Depression and its vicinity.

Such mining areas are the Voslobeni quarry and the Sândominic quarry, which extract dolomite. The Bălani mine was an iron and copper mine until 2006. The Topliţa site is an andesite quarry, and the Gura Haitii quarry was a sulphide exploitation. The Borsec quarry is known for its travertine extraction, and the Bicaz-Chei quarry for limestone. This reveals a diversity of mining material in the mountainous areas of Transylvania.

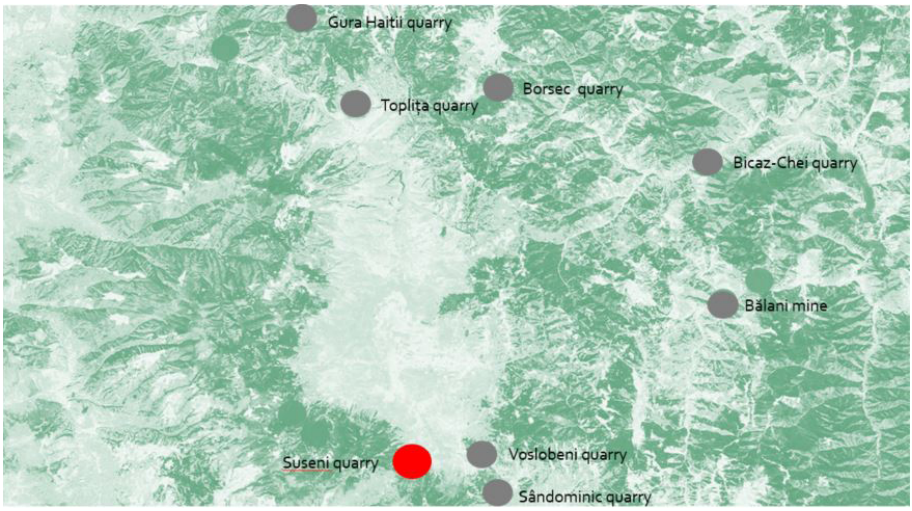

Figure 2. Similar quarry zones near-by

Ecological networks

The protected areas of Natura 2000 [10], as parts of green corridors, constitute the backbone of the European green ecological infrastructure and are located near the studied quarry zone (*Fig. 3*).

Figure 3. Natura 2000 infrastructure in the study area

The territory surrounding the quarry is home to a diverse range of protected and endangered species, underscoring its significant ecological importance. The conservation efforts within this territory are dedicated to preserving the habitats and ensuring the long-term survival of these vulnerable species.

Accessibility

Regarding the quarry's current situation, it can be accessed via County Road 138 and secondary dirt roads. Additionally, there is a railway line that connects in Voşlobeni to the main Ciceu–Deda line. The location of the quarry is thus favourable in many ways. It is situated in a very spectacular location and can be reached via multiple routes, making it a potential tourist destination (*Fig. 4*).

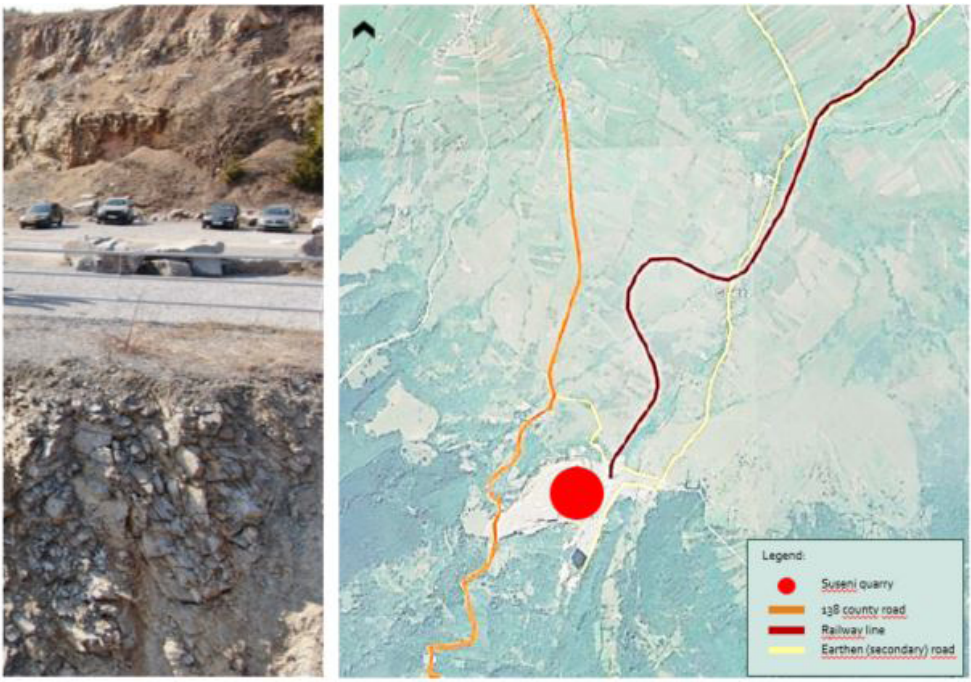

Figure 4. Accessibility analyses

Shape and size

The quarry has an amorphous shape, with approximately 100 hectares of land. Two thirds of the area are occupied by the extraction zone, while the remaining third is used for processing, including loading, transport, and storage (*Fig. 5*). This large area provides ample space for a multitude of activities following rehabilitation.

When studying mining patterns, one can discern elemental shapes and identify potential design possibilities. Barren and harsh landscapes are typically perceived as unappealing unless a distinct and prominent pattern is present [3, 11]. The landscape's character, determined by irregular topography, steep vertical stone

walls, the potential for water features, and a range of microclimates, offers advantages for design and after-use proposals.

Figure 5. Visualization of the shape of the quarry

Material

The material extracted here is hard rock, specifically andesite, which is a volcanic rock formed about 6.5 million years ago (*Fig. 6*) [9]. This is processed using crushing and screening installations. Mining operations have led to surface water loss and soil erosion, exposing a substantial amount of bedrock and greatly impeding the establishment of vegetation.

Figure 6. Extracted material

Pollution analyses

Through the extraction process of the stone, massive air and sound pollution is caused along with significant visual pollution and land pollution in stone quarries. The visual pollution stems from the alteration of natural landscapes, as quarries often result in vast excavations, creating unsightly scars on the surface of the earth. These enormous pits can disrupt the visual harmony of the surrounding environment, replacing scenic vistas with barren, industrialized landscapes.

Additionally, the mining and processing of stone often involve the use of heavy machinery, which can further damage the land. The constant movement of vehicles and equipment contributes to soil compaction and fragmentation, reducing the ability of the land to support vegetation growth. The release of chemicals and contaminants from machinery and processing facilities can also seep into the soil, potentially polluting groundwater and further degrading the quality of the land.

Terrain dynamics

The walls of the pit are almost vertical. Examining the terrain dynamics of the quarry, this has six production levels, and it can be noticed that the area ranges from 800 m above sea level to 920 m, resulting in a difference in elevation of around 120 m, creating a unique landscape (*Fig. 7*).

Figure 7. View of the terrain dynamic

In order to address safety concerns arising from high walls, it is needed to restrict surface reclamation grades from exceeding a 35-degree slope, which is typically the angle of repose for spoil material. Stone quarries possess specific physical characteristics that may necessitate blasting or backfilling to adhere to such regulations, consequently resulting in higher reclamation costs. Moreover, fill material must meet engineering standards to ensure minimal settlings and suitability for future applications [3].

Water elements

There are two water elements on the quarry's territory, a rainwater-accumulating pond (*Fig. 8*) in the middle of the extraction zone and a lake (*Fig. 9*) in the lower part of the territory, which was once used for washing the extracted stone. Those water elements can be integrated in the concept and can be an additional argument for the after-use proposals.

Figure 8. Rainwater accumulation

Figure 9. View from the lake

Flora and fauna

The original soil surface in the mining area has been entirely removed, resulting in the complete destruction of the local ecosystem.

Figure 10. Ruderal plants on the territory of the quarry

Over the past 90 years, quarrying operations have decimated the flora and fauna while giving rise to a range of environmental issues. However, there are some patches of natural elements, such as ruderal plants, attempting to recover the territory following the closure of the mine (*Fig. 10*).

Given enough time, nature has an inherent ability to regenerate and restore itself regardless of the disturbances it may have faced, whether human intervention is involved or not. This gives rise to the saying "time heals all wounds" [3]. In adopting a conscious natural design approach, it may be prudent to adopt a hands-off approach, allowing natural processes to unfold and guide the regeneration process.

4. Proposal

In the rehabilitation proposal, it is firstly important to establish the context for rehabilitation. Clear achievable objectives and goals need to be established. Every site is different, so it is advisable to start on a small scale. Regular monitoring of revegetation is necessary.

Planning applications for quarries in modern times must include detailed proposals for the after-use of the quarry, which must be achievable. Sustainability policy for quarry areas involves several steps in the after-use proposal, including natural regeneration, nature conservation, landfill, leisure and recreation, agriculture, residential and other buildings, and other industrial uses.

The life cycle and impact of a quarry on both society and the environment are intricately connected to the effective planning, operation, and rehabilitation of the site [12]. Efficient planning involves considering factors such as location, extraction methods, and potential environmental and social impacts. During the operational phase, responsible management practices are crucial to minimize the negative effects on the environment, including measures to control dust, noise, and water pollution. Additionally, community engagement and addressing social concerns are essential for fostering positive relationships between the quarry and residents. Rehabilitation plays a significant role in mitigating the long-term impacts of the quarry [13].

This phase involves restoring the site to a condition that is environmentally sustainable and socially beneficial. It may include activities such as reclamation of disturbed areas, landscaping, reforestation, and creating habitats for wildlife. By prioritizing proper planning, responsible operation, and effective rehabilitation, the quarry industry can strive for sustainable practices that minimize adverse impacts and maximize positive contributions to both society and the environment.

Reviving abandoned quarries cannot be accomplished by merely concealing the excavation walls. It is crucial to establish a multifaceted cultural framework for reintegrating these disused areas into a fresh network of shared purposes.

This approach offers a chance to redefine the human-made landscape and its connection with the natural environment. By repurposing former extraction sites, the region gains new prospects in both the economic and ecological domains. In this context, quarries transition from being dormant "grey elements" into versatile "green elements" within an infrastructure system [5].

In the table below (*Table 1*), the possibilities of reclamations for the quarry are presented. This table combines the approaches for reclaiming mine sites from Engler's work, which focused on landfill and sewage treatment, exploring eight different design approaches for waste landscapes. It also incorporates insights from the Arbogast team's work, which emphasizes the human factors in mining reclamation. These two works share similarities and form a basis to a reclamation proposal that can be adopted to the Suseni quarry as well.

Table 1. Quarry reclamation based on Engler's and the Arbogast team's work [3, 15]

Approach	
Natura	Allow nature to reclaim the site with minimal human influence
Camouflage	Conceal the mining facility using visual screens and buffers
Restoration	Restore the land to its approximate original contour
Rehabilitation	Utilize the site for public amenities
Mitigation	Repair a mined-out site from extensive damage
Recycling	Recycle man-made or natural resources on site
Education	Communicate mining- or resource-related information through outreach
Art Integration	Treat the site as a work of beauty and a unique experience
Celebrative Integrative	Combination of approaches integrating art and science

Additionally, it is important to recognize that physical rehabilitation and revegetation are separate tasks. Preparing the site for native vegetation planting can take up to a year or two. It is essential to approach each site individually, as plants will behave differently in different environments. Embracing a progressive rehabilitation approach allows for learning and adapting strategies based on site-specific conditions, as what may work successfully in one place may not yield the same results in another.

In the context of hard rock environments, certain features within the quarry can serve as valuable biodiversity assets. Additionally, cliffs present in the area can serve as rare habitats that are beneficial to certain species.

In order to establish the initial vegetation structure, it is advisable to utilize colonizing species. For example, wattles can contribute by fixing nitrogen and aiding in soil structure establishment, while grasses can offer erosion control.

It is important not to rush the process of revegetation and approach it gradually instead. This approach allows for careful evaluation and adjustment, ensuring that resources are not wasted on ineffective strategies.

5. Conclusions

In modern times, it is important to recognize that protecting a specific area, even a quarry zone, is not sufficient. Instead, we need to consider a larger area or region and think about the surrounding environment and its relationships from the perspective of the multiple interconnected habitats, flora, fauna, and human communities. This approach requires an understanding of the larger ecological context in which the area exists. Therefore, a holistic approach is necessary to ensure the long-term sustainability of the environment and its inhabitants.

While the extraction of rocks has a history as old as humanity itself, the issue of revitalizing abandoned production sites has become a central topic in contemporary discussions about land regeneration.

When engaging in rehabilitation efforts, it is crucial not to commit to unrealistic expectations for obtaining approval. Regulators should be approached with a sense of realism. It is important to understand that the establishment of vegetation communities can be a lengthy process, potentially spanning decades and involving multiple stages. Proper rehabilitation necessitates meticulous planning and preparation.

In conclusion, it can be underlined that the location of the Suseni quarry is appropriate for tourist after-use or recreational zone, but being in the middle of an extended natural area, restoration of the natural environment must be taken into account in the proposal. Quarry restoration requires addressing abiotic and biotic constraints holistically to encourage the self-sustainability of the system. Restorations need not mimic pristine conditions, as alternative approaches can uphold valuable natural assets. Analyses of local plant community composition and structure allow for the assessment of restoration effectiveness, while alternative approaches relying on NBS (nature-based solutions) can avoid expensive and inadequate restoration practices.

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