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EFFECT OF CLIMATE CHANGE ON INCREASING ESTABLISHMENT OF INVASIVE PLANT SPECIES: AN IMPORTANT CHALLENGE FOR BIODIVERSITY OF NATIVE SPECIES IN CHANGING WORLD

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Abstract
Climate change has created many challenges in natural ecosystems around the world. This phenomenon can change the structure of the forests, destroy the biodiversity, alter the distribution of the plant species, make extinct the endemic species, and it may increase some non-native species in different ecosystems. One of the most important effects of climate change on ecosystems is establishment of invasive plant species (IPS) in these areas. Investigation of the impacts of climate change on establishment of IPS is essential to control them in the changing world. This study was performed to investigate the effects of climate change on distribution of IPS, to introduce the most important climatic variables on establishment of IPS, and to evaluate the role of climatic scenarios on prediction of future distribution of IPS in different ecosystems. Results of reviewing several papers in this study showed that climate change has many direct and indirect effects on increasing establishment of IPS in different natural areas. However, direct effects of climate change on distribution of IPS are more than indirect impacts of climate change in this phenomenon. Among all disturbances due to climate change, fire has been one of the most important disturbances increased the establishment of IPS in natural ecosystems. In addition, based on the findings of most studies, temperature and precipitation have been the most important climatic variables in increasing establishment of IPS in natural areas. Although prevention of climate change is inevitable, reduction of effects of this phenomenon on ecosystems is possible using some practical methods and modern technologies. The practical methods presented in this study are simple and applicable to detect the effect of climate change on establishment of IPS in natural areas. In addition, these methods will be helpful for estimating the current distribution of IPS in the ecosystems based on climatic variables. Furthermore, climatic scenarios are very effective tools for prediction of future distribution of IPS in different ecosystems which are helpful for protective management of natural areas against establishment of IPS in the changing world.

Keywords: Temperature, precipitation, invasive plant species (IPS), biodiversity, climatic scenario.

Abbreviations
ANN: Artificial Neural Network
DT: Decision Tree
GBIF: Global Biodiversity Information Facility
GEE: Google Earth Engine

GIS: Geographic Information System
IDW: Inverse Distance Weighting
IPCC: Intergovernmental Panel on Climate Change
IPS: Invasive Plant Species
IUCN: International Union for Conservation of Nature
KNN: K-Nearest Neighbor
MaxEnt: Maximum Entropy
NAISMA: North American Invasive Species Management Association
RF: Random Forest
SVM: Support Vector Machine
UNESCO: United Nations Educational, Scientific, and Cultural Organization

1. Introduction

Climate change and global warming have created many concerns in different natural areas around the world. Based on some reports, most of this global warming is because of increasing greenhouse gases such as CO_2 due to human activities in the earth (Finch et al., 2021). Therefore, human activities can lead to some changes in weather conditions at local, national, and global scales. When the weather conditions change in a specific area over time, the climate of that region will be altered which is defined by "climate change". Climate change can alter the normal conditions of natural biomes, create many disturbances in natural ecosystems, reduce biodiversity, and expose many endemic species to extinction risk (Shrestha et al., 2021). When the climate of a specific area changes, the trend of meteorological parameters also alters; For example, temperature increases (Bogale & Tolossa, 2021), and precipitation and humidity decrease over time, which all of these changes create some disturbances in the natural ecosystems around the world (Bogale & Tolossa, 2021; Burgiel & Muir, 2010; Fettig et al., 2013). The main disturbances due to climate change in the ecosystems are fire, drought, storm, flood, etc. (IPCC, 2007; Bogale & Tolossa, 2021; Finch et al., 2021). Climate change can affect the natural areas and may decrease the ecosystem services. It may change the range, structure, composition, and distribution of plant species, and therefore, climate change can affect the biodiversity in natural ecosystems (Burgiel & Muir, 2010) by threatening the habitat of native species and providing the proper conditions for establishment of invasive plant species (IPS) (Early et al., 2016). Therefore, knowledge of this subject that how distribution patterns of IPS are affected by climate change, is important to control these species, to protect the endemic species, to conserve the biodiversity, and to perform a protective management for natural ecosystems. In fact, if we can predict how distribution of IPS will be changed in a specific ecosystem under changing climate, we can perform a protective plan to control the IPS in these ecosystems (Finch et al., 2021).

In this regard, study about effect of climate change on establishment of IPS in natural ecosystems is very important. So far, some studies have been performed to investigate the effect of climate change on distribution of IPS at different spatial scales (Burgiel & Muir, 2010; Dai et al., 2022; Dyderski et al., 2018; Early et al., 2016; Fettig et al., 2013; Finch et al., 2021; Renteria et al., 2021; Singh et al., 2023; Smith et al., 2012; Tu et al., 2021). Some studies have been carried out at a global scale (Bellard et al., 2018; Dai et al., 2022; Early et al., 2016; Finch et al., 2021; Thuiller et al., 2008; Walther et al., 2009). For example, one study has focused on biotic and abiotic interactions among IPS and global changes (Dai et al., 2022). Another one has reviewed the threats of alien species in the ecosystems and has investigated the national response capacities of ecosystems at global scale (Early et al., 2016). On the other hand, some other studies have been performed at regional scales. One study has investigated the role of climatic factors and edaphic variables on distribution of IPS in understory of the mixed forests in Australia (Singh et al., 2023). Other studies have investigated the effects of climate change on establishment of some IPS in Canada (Smith et al., 2012), California (Renteria et al., 2021), China (Tu et al., 2021),

Eastern Africa (Shackleton et al., 2017), and Eastern Ethiopia (Bogale & Tolossa, 2021). Some studies have focused on climatic scenarios to predict the future distribution of IPS in natural ecosystems under climate change (Bell et al., 2021; Renteria et al., 2021; Tu et al., 2021).
Despite the performed studies, there is no comprehensive study which investigates some important subjects about effect of climate change on distribution of IPS. In fact, no study has been performed to investigate the direct and indirect effects of climate change on establishment of IPS, to detect the most important climatic variables on this establishment, to introduce a simple and practical method to identify the effects of climate change on distribution of IPS, and to describe the role of climatic scenarios on prediction of future distribution of IPS in natural ecosystems. This study has investigated all these subjects by replying to the following questions:
1. How may climate change affect the establishment of IPS in the ecosystems?
2. Which climatic variables are the main effective factors on increasing establishment of IPS?
3. Which method is more usual and practical to investigate the effect of climate change on the current establishment of IPS?
4. What is the role of climatic scenarios to simulate the future distribution of IPS?
Based on the above questions, the main objectives of this study are responses to the mentioned questions. In this paper, some direct and indirect effects of climate change on establishment of IPS will be investigated. In addition, the most important climatic variables which may affect the establishment of IPS in ecosystems will be detected. Furthermore, a simple and applicable method to investigate the effect of climate change on establishment of IPS will be presented. Finally, the role of climatic scenarios on simulation of future distribution of IPS will be investigated. The findings of this study will present the effective views for protective management of natural ecosystems against increasing establishment IPS under climate change.

2. Methodology

2.1. How may climate change affect the establishment of IPS in the ecosystems?

Climate change may mainly affect the forest areas and natural ecosystems around the world. For example, it can decrease the ecosystem services, reduce the biodiversity, and alter the forest structure (Burgiel & Muir, 2010). In addition, this phenomenon can change the composition and distribution of the plant species in the natural forests, make extinct the native species, make dry the endemic trees, increase the non-native species, and establish some IPS in these ecosystems. In addition, climate change may create many disturbances and natural hazards in natural ecosystems (Bogale & Tolossa, 2021; Fettig et al., 2013). In general, climate change has many direct and indirect effects on increasing establishment of IPS and decreasing population of native species (Burgiel & Muir, 2010) which have been described below.

2.1.1. Direct effects

Based on recent reports, change in weather conditions has many direct impacts on increasing establishment of IPS and decreasing of native plant species in the natural ecosystems (NAISMA, 2022); Because climate play an important role on establishment of many plant species in different ecosystems around the world (Dyderski et al., 2018; Li et al., 2020; Xiong et al., 2019). The changes in climatic conditions affect the habitat of native tree species and increase the establishment of IPS in different ecosystems (Walentowitz et al., 2019; Finch et al., 2021). Climate change can directly affect the survival of native species in natural ecosystems (Burgiel & Muir, 2010); Because changes in weather conditions can change the situation of ecological factors which affect the bioclimatic needs of native species (Burgiel & Muir, 2010). For example, change in climatic conditions certainly alters the temperature, precipitation, and other climatic factors in a specific region, while establishment of each plant species (both native and non-native) in a certain area depends on the climatic conditions of that region. In this regard, climate change (especially increasing temperature) may be one of the most important factors of establishment of IPS (e.g. *Acacia Melanoxylon*) in natural habitats of native species (e.g. *Pinus* *pinea*) in some protected areas in Spain which threatens the survival of several native species in these biodiversity hotspots (Figs. 1 and 2).

Fig. 1. Establishment of IPS (*Acacia Melanoxylon*) in natural forests of Cies Islands in northern Spain (Photo by: Saeedeh Eskandari, June of 2024)

Fig. 2. Establishment of IPS (*Acacia Melanoxylon*) in natural forests of Ons Islands in northern Spain (Photo by: Saeedeh Eskandari, July of 2024)

Furthermore, the same situation about increasing establishment of some IPS has been observed in Hyrcanian forests of northern Iran (Amini et al., 2020). In this case, a wide distribution of some evergreen IPS (e.g. *Solanum pseudocapsicum* L.) in understory of Hyrcanian forests has created many concerns about extinction of some valuable native species (e.g. *Gleditsia capsica*, and *Parrotia persica*) in these forests with ancient of 25-50 million years (UNESCO, 2019) (Fig. 3).

Fig. 3. Establishment of IPS (*Solanum pseudocapsicum* L.) in understory of native species (*Gleditsia capsica*, and *Parrotia persica* stands) in Hyrcanian forests of northern Iran (Photo by: Habib Zare, autumn of 2020, (Amini et al., 2020))

In addition, changes in weather conditions can alter the soil characteristics and water accessibility in a specific environment, while growing and surviving of many native plant species directly depends on the edaphic and aquatic conditions of that environment (Singh et al., 2023; Thuiller et al., 2008). This phenomenon is usually observed in the aquatic ecosystems where the high salinity causes the immigration of endemic species and provides the proper conditions for some other non-native species (Burgiel & Muir, 2010). For example, decreasing water and increasing salinity in the soil of riparian forests in southern Iran has increased the establishment of some invasive halophytes (salt-tolerant plants) in these forests around Karkheh River which has decreased the population of native species (*populus euphratica*) in these forests in recent years (Fig. 4). Although, *populus euphratica* is a salt-tolerant species (Rajput et al., 2016), but when the salinity of environment increases to the higher level of ecological threshold of this species, these trees cannot survive, especially where the available water for growing this hydrophilic species decreases (Fig. 4).

Fig. 4. Establishment of invasive halophytes (salt-tolerant plants) in riparian forests around Karkheh River in southern Iran (Photo by: Saeedeh Eskandari, October of 2022)

In general, change in climatic and ecologic factors can change the establishment of native species; especially when these changes are more than bioclimatic resistance of these species. In this regard, native species leave their habitats and distribute in some other areas where have better conditions according to climatic and ecologic needs of these species (Burgiel & Muir, 2010).

On the other hand, the disturbed habitats provide the proper conditions for the establishment of non-native species. In fact, when weather is getting warmer, alien species can rapidly move to the higher or lower elevations, because they have high ability and tolerance for establishing in new habitats, where native species cannot establish anymore because of change in climatic and ecologic conditions (IPCC, 2007; Pauchard et al., 2016; Walther et al., 2009). Based on some reports, alien species can even change the time of flowering under climate change, but it is impossible for native species (Wolkovich et al., 2013). Therefore, IPS can quickly adapt themselves to the new conditions of ecosystems under changing climate (Bogale & Tolossa, 2021). However, some ecosystems like freshwater ecosystems and temperate forests benefit from some specific environmental boundaries (such as thermal borders) which can restrict the invasion of alien species in normal conditions; however, when the climate changes, these ecosystems also become more proper to establish the IPS. Therefore, these ecosystems will be exposed to invasion of IPS when the weather is getting warmer (IPCC, 2007). Based on some reports, the probability of establishment of some IPS in the warmer places of the forests (with higher temperature), is more than other places (with lower temperature) (Paz-Kagan et al., 2019); Because the native species cannot survive in warmer weather, while the IPS which are more resistant to difficult and disturbed conditions can tolerate. Therefore, in the warmer climates, the native species are replaced by IPS over time and the original biodiversity of a specific natural area will be decreased by these changes in climatic conditions.

2.1.1. Indirect effects

In addition to the direct effects of climate change on establishment of IPS, climate change can indirectly provide the suitable conditions for increasing establishment of IPS in different ecosystems. These phenomena especially occur in the environments where expose to natural disturbances because of climate change (Burgiel & Muir, 2010). As a key finding, climate change can create some disturbances in natural ecosystems (Bogale & Tolossa, 2021; Burgiel & Muir, 2010; Fettig et al., 2013). For example, when climatic conditions in a specific area changes, that region will be exposed to some unexpected environmental phenomena such as hurricanes, droughts, and floods (Finch et al., 2021) which transfer IPS to the new places (Bogale & Tolossa, 2021; Burgiel & Muir, 2010). In addition, these disturbances can provide the proper conditions for establishment of some IPS in the specific areas (Bogale & Tolossa, 2021; Burgiel & Muir, 2010), and may decrease the tolerance of that area to invasion of alien species (IPCC, 2007). Furthermore, climate change and global warming may create some other disturbances in natural ecosystems such as fires (Eskandari et al., 2023; Westerling et al., 2006; Bogale & Tolossa, 2021; Fettig et al., 2013). It has been reported that several IPS tend to establish in the natural forests after fire occurrence (Alba et al., 2015; Hellmann et al., 2008; Shackleton et al., 2017), and therefore, fire enhances the establishment of some IPS (Alba et al., 2015) (Fig. 5). Replacement of native species by alien species may occur in different periods after fire (one year, two years, five years, ten years, etc.). However, the important point is that the IPS occupy the habitats of native species after fire occurrence over time (Curatola Fernández et al., 2013) (Fig. 5). Therefore, native plant species will be replaced by IPS after fire occurrence, step by step. This will change the composition of tree species (both native and non-native species) over time which is one of the most important effects of climate change on biodiversity of forest ecosystems (Alba et al., 2015).

Fig. 5. Establishment of invasive herbaceous species in a burned forest of *Juniperus excelsa* after 4 years of fire occurrence, Central Alborz Mountains, Iran (Photo by: Saeedeh Eskandari, September of 2018)

2.2. Which climatic variables are the main effective factors on increasing establishment of IPS?
The most important climatic variables which can affect the distribution of IPS have been reported in different studies. Based on reviewing the studies performed in this subject, the climatic variables in table 1 are the most important climatic variables on establishment of IPS in different areas around the world (Renteria et al., 2021; Shrestha et al., 2021; Singh et al., 2023; Tu et al., 2021).

Table 1. The most important climatic variables on establishment of IPS

Main variable	Sub-variable	Reference
Temperature	Mean temperature	Wolkovich et al., 2013; Truong et al., 2017; Tu et al., 2021; Finch et al., 2021; Bell et al., 2021; Mouta et al., 2021; Singh et al., 2023; Fusco et al., 2023; Huang et al., 2024
	Maximum temperature	Truong et al., 2017; Tu et al., 2021; Renteria et al., 2021; Shrestha et al., 2021; Mouta et al., 2021
	Minimum temperature	Truong et al., 2017; Tu et al., 2021; Renteria et al., 2021; Shrestha et al., 2021; Mouta et al., 2021
Precipitation	Mean precipitation	Wolkovich et al., 2013; Truong et al., 2017; Walentowitz et al., 2019; Shrestha et al., 2021; Mouta et al., 2021; Singh et al., 2023; Fusco et al., 2023; Huang et al., 2024
	Maximum precipitation	Truong et al., 2017; Renteria et al., 2021; Mouta et al., 2021
	Minimum precipitation	Truong et al., 2017; Tu et al., 2021; Renteria et al., 2021; Mouta et al., 2021
Solar radiation	-	Walentowitz et al., 2019
Moisture and humidity	-	Yang et al., 2022; Singh et al., 2023
Aridity	-	Singh et al., 2023

Although the annual data of climatic variables have also been considered in some studies (Boyte et al., 2016; Huang et al., 2024; Mouta et al., 2021; Renteria et al., 2021; Shrestha et al., 2021; Truong et al., 2017; Walentowitz et al., 2019; Yang et al., 2022), the seasonal, monthly, and even daily data of climatic variables have been more important than annual data to get better results in studies about effect of climate change on establishment of IPS (Renteria et al., 2021; Shrestha et al., 2021; Singh et al., 2023; Truong et al., 2017; Tu et al., 2021); Because the important point is that these climatic variables should be considered in the growth season of IPS, so that the effect of climate change on establishment of IPS can be investigated during the growth season of these species. It can lead to more accurate results about effect of climate change on current establishment of IPS in the forest ecosystems.

2.3. Which method is more usual and applicable method to investigate the effect of climate change on the current establishment of IPS?

For detection of effect of climate change on establishment of IPS, the relationships between climatic parameters and distribution of IPS should be investigated over a long time. Investigation of these relationships in time series will be helpful for forest managers to know how the significant changes in climatic conditions have affected the distribution of these species in different periods. In this regard, accessibility to the historical data of climatic variables and locations of IPS over time (in time series) is very important. A general procedure of application of a practical method for investigating these relationships has been described in following sections.

2.3.1. Obtaining data of climatic parameters in time series

In this regard, the data of effective climatic parameters on establishment of IPS in growth season (temperature, precipitation, relative humidity, etc.) can be downloaded from the related websites which have efficient databases of climatic and meteorological variables. For example, if a study is performed at global scale, these websites are recommended:

IPCC: https://www.ipcc-data.org/

Copernicus: https://cds.climate.copernicus.eu/#!/home

WorldClim: https://www.worldclim.org/data/index.html

In addition, there are some other databases of climatic and weather variables as geospatial maps in Google Earth Engine (GEE):

https://earthengine.google.com/

However, if a study is carried out at national or regional scale, it is better if the climatic data are obtained from meteorological stations of national or regional websites. For example, for obtaining the climatic petameters across some provinces in Spain such as Galicia, this website is suggested (https://www.meteogalicia.gal/observacion/rede/redeIndex.action?request_locale=gl_E). On the other hand, for downloading the climatic data of different regions of Iran, this website is recommended (https://data.irimo.ir/).

2.3.2. Data analysis of climatic variables

The climatic data which are downloaded from regional or national websites are usually in "Microsoft Excel" format. Therefore, analysis of climatic data for each meteorological variable in each station is necessary. In addition, data of each meteorological variable should be organized for each year, season, or month of the study. It means that the climatic data would be arranged for certain year (annual mean), season (seasonal mean), or month (monthly mean) based on the objective of the research. This process can be performed in "Microsoft Excel" or "IBM SPSS Statistics" software.

2.3.3. Preparation of digital maps of climatic parameters

If climatic data are downloaded from global websites (Copernicus, WorldClim, IPCC, GEE, etc.), the spatial maps of climatic data will be available. However, if the study is performed at regional or national scales, the climatic data will be in "Microsoft Excel" format which should be converted to climatic maps by some spatial analysis such as interpolation methods (IDW, kriging, co-kriging, etc.) in GIS software. In this regard, maps of all climatic parameters in the time series can be obtained using interpolation methods in GIS.

2.3.4. Provision of spatial maps of distribution of IPS

For preparing the spatial maps of location of IPS, the researchers can use the existing databases or they can create the distribution maps of these species using satellite images, aerial photos, and field data. For the first one, one of the most applicable websites is GBIF (Global Biodiversity information Facility) which presents the spatial distribution (geographical coordinates) and many other data of several plant species over time (https://www.gbif.org/) (GBIF, 2024). In this website, researchers can search for a specific plant species and find the most useful information about that. Data of this website has been applied by some researchers to find the approximate locations of IPS in different ecosystems around the world (Kala et al., 2022; Gallardo et al., 2024; Huang et al., 2024; Rodríguez-Casal & Saavedra-Nieves, 2022; Truong et al., 2017; Tu et al., 2021; Walentowitz et al., 2019).

However, for providing a more accurate dataset of distribution of IPS especially in regional scale, application of field data along with remote sensing data (aerial photos, satellite images, etc.) is recommended (Huang et al., 2024; Royimani et al., 2019; Walentowitz et al., 2019). In this regard, classification of different satellite images for identifying IPS can be performed by some new machine learning algorithms. Finally, the best satellite image or aerial photo and the most proper classification method will be detected to obtain the most accurate map of distribution of IPS in the natural ecosystems.

2.3.5. Determination of the relationship between climatic parameters and spatial distribution of IPS over time (spatio-temporal analysis)

In this step, the spatial relationship between climatic variables and location of IPS should be investigated by some models, algorithms, or methods (Huang et al., 2024; Li et al., 2020; Srivastava et al., 2019). In this regard, application of machine learning methods (RF, SVM, DT, ANN, MaxEnt, KNN, etc.) is recommended. For this purpose, spatial relationships between maps of each climatic parameter and map of distribution of IPS are investigated over time (for example 50 years) using some spatial analyses and machine learning algorithms. Based on these analyses, the relationships between each climatic variable and distribution of IPS will be revealed. In addition, the main climatic variable in the establishment of IPS will be detected.

2.4. What is the role of climatic scenarios to simulate the future distribution of IPS?

Prediction of future distribution of IPS will be very useful for protective management of natural areas under climate change conditions. However, this prediction under climate change is a complex subject, because several variables may affect the establishment of IPS in different ecosystems around the world (Mainali et al., 2015). In addition, in distribution of IPS in different ecosystems, natural factors (such as climate change), and anthropogenic factors (such as land cover change) are related together in a complicated way (Bellard et al., 2013; Finch et al., 2021). However, by exact prediction of future climatic conditions, we can predict the future distribution of some IPS in natural ecosystems and protected areas. In this regard, early detection of IPS is an efficient method to control these species and to prevent further spread of IPS in natural ecosystems (Jarnevich et al., 2010).

In recent years, some climatic scenarios have been designed and developed to simulate and predict the future distribution of IPS in different ecosystems around the world (Bell et al., 2021; Renteria et al., 2021; Tu et al., 2021). For this purpose, the current locations of IPS should be investigated. In this regard, data of current distribution of IPS can be obtained using fieldwork and remote sensing data or available data from online websites (such as GBIF). Furthermore, climatic data should be obtained from a proper dataset such as IPCC, WorldClim, Copernicus, etc. Then, the future climatic scenarios for different periods can be designed for simulating the climatic models (Hausfather et al., 2019). Finally, a proper predictive model should be applied to predict the future distribution of IPS under climate change scenarios (future climatic periods) (Tu et al., 2021). As it mentioned before, for applying these models, some data of current distribution of IPS and climatic variables which affect the distribution of IPS are required (Katz & Zellmer, 2018; Srivastava et al., 2019; Tu et al., 2021).

Based on performing these models, early detection of IPS distribution will be predicted (Burgiel & Muir, 2010). For this purpose, ecological niche models can be applied which are very helpful to detect the potential regions of establishment of IPS (Yan et al., 2020). Among all ecological niche models, MaxEnt, BIOCLIM, and DOMAIN models have been the most efficient models to detect the future distributions of IPS in natural areas (Tu et al., 2021). Finally, the current distribution of IPS and climatic data are entered into a favorite model and the future distribution of IPS will be predicted in different climatic scenarios in temporal periods (e.g. 2025-2050, 2050-2075, 2075-2100).

4. Discussion

In this study, reviewing the papers related to the effect of climate change on distribution of IPS demonstrated that climate change has many direct and indirect effects on increasing establishment of IPS in different ecosystems around the world. In this regard, climate change decreases the population of native plant species, increases the establishment of IPS, and reduces the biodiversity of the natural ecosystems. When the climatic conditions change, the temperature increases, while precipitation and humidity decrease. It can directly affect the normal conditions of ecosystems around the world. For example, by changing the climatic conditions, the original habitat of native species is destroyed, and they cannot survive in their destructed habitats in natural ecosystems. On the other hand, establishment of IPS will be increased in the destroyed habitats of native species, because many IPS have high tolerance to grow in the disturbed ecosystems and they can adapt themselves to the new conditions of environment (Bogale & Tolossa, 2021). Therefore, the native species in the natural ecosystems will be replaced by IPS under climate change over time (Dyderski et al., 2018; Fettig et al., 2013; Pauchard et al., 2016). On the other hand, climate change can cause some natural hazards and disturbances (fire, flood, drought, etc.) in the ecosystems and provide the proper conditions for establishment of some IPS where the habitat of native species has completely been destroyed by these disturbances. It is an indirect effect of climate change on increasing establishment of IPS in the natural areas. However, the direct effects of climate change on increasing establishment of IPS in the specific ecosystems are more than its indirect effects on ecosystems (Li et al., 2020). About indirect effects of climate change on increasing establishment of IPS, fire has been one of the most important disturbances increased the establishment of IPS in some natural ecosystems (Alba et al., 2015; Hellmann et al., 2008; Shackleton et al., 2017). Establishment of IPS in the burned areas may occur one, two, five years after fire or even later. However, the important point is that fire occurrence increases the establishment of IPS in different ecosystems (Alba et al., 2015; Curatola Fernández et al., 2013).

Although prevention of climate change is difficult in our changing world, we can adjust and decrease the effects of climate change on increasing establishment of IPS in natural ecosystems. It is possible by investigating the relationships between distribution of IPS and climatic variables. We can discover the most important climatic variables on increasing establishment of IPS in different areas via these analyses. The findings of most studies have demonstrated that temperature and precipitation have been the most important climatic variables affected the establishment of IPS in natural ecosystems over time (Bell et al., 2021; Finch et al., 2021; Fusco et al., 2023; Huang et al., 2024; Mouta et al., 2021; Singh et al., 2023; Truong et al., 2017; Tu et al., 2021; Wolkovich et al., 2013; Yan et al., 2020). Therefore, protective management of natural ecosystems regarding these factors is nessessary.

In addition, some predictive models can be applied to estimate the current distribution of IPS in natural ecosystems. In this regard, field data, aerial photos, and satellite images along with new methods, algorithms, and models will be very efficient (Huang et al., 2024; Royimani et al., 2019; Walentowitz et al., 2019). It will be helpful for early control of IPS, proper conservation of native species, and effective protection of biodiversity in the natural ecosystems.

Finally, the early detection and prediction of future spread of IPS in natural areas using accurate predictive models and new technologies will be useful for performing the protective measures in natural ecosystems around the world. In this regard, climatic scenarios can be designed and developed for future distribution of IPS in ecosystems. In fact, climatic scenarios can predict the future spread of IPS in temporal periods (Bell et al., 2021; Renteria et al., 2021; Tu et al., 2021).

These scenarios will be efficient for protective management of natural ecosystems against IPS under climate change conditions.

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MAPPING THE LAND USES IN PROTECTED AREAS OF SPAIN USING HIGH-RESOULUTION SATELITE IMAGES AND RANDOM FOREST MODEL: A CASE STUDY IN ONS ISLAND

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Abstract

Mapping land uses in natural areas is an important issue for protective management of these areas. Ons Island is a protected area in northwestern Spain which its natural condition is being altered by many factors. Increasing establishment of some invasive plant species (IPS) such as *Acacia sp.* because of natural or human-made factors has changed the natural conditions of this Island in recent years. In this regard, provision of forest cover and land use maps using advanced methods is a useful way to detect IPS in the forests and to discover the relationships between establishment of invasive plant species and natural and human-made factors in Ons Island. For this purpose, composite PlanetScope satellite images of the study area (imagery date: June of 2024) were ordered and downloaded from Planet website. Then, training areas for each land use (forest, shrubland, rangeland, bare soil, stone and sand, residential area, cropland, road, and water) were selected on Google Earth satellite images. For this purpose, enough and scattered training areas were selected for each land use in the island. Then, supervised classification of satellite images was done using Random Forest (RF) method because of its high efficiency for classifying the pixels in heterogenous areas. Finally, the newest land use map of Ons Island was obtained in 3m spatial resolution for 2024. Accuracy assessment of land use map was done using some square plots on Google Earth images (different from training areas) and accuracy indices. Results showed that rangeland (200.74 ha) is the largest land use and residential area (3.05 ha) is the smallest land use. Results of validation of land use map showed that PlanetScope satellite images and random forest model have high efficiency (*OA*: 92%, kappa index: 0.89) for land use mapping in the study area. The forest map extracted from land use map in this research is a valuable map to identify *Acacia Melanoxylon* inside forest cover in Ons Island. In addition, human-made land uses obtained from land use map in this study can be used for investigating the relationships between location of human activities and distribution of *Acacia Melanoxylon* which will be performed in our future research following this study.

Keywords: Forest, Invasive plant species, Random Forest model, PlanetScope satellite image, Protective management

Abbreviations

$\hat{k}$: Kappa Index
ANN: Artificial Neural Network
CART: Classification And Regression Trees
DEM: Digital Elevation model

DT: Decision Tree
IPS: Invasive Plant Species
KNN: K-Nearest Neighbour
MaxEnt: Maximum Entropy
OA: Overall Accuracy
PA: Producer Accuracy
RF: Random Forest
SVM: Support Vector Machine
UA: User Accuracy

1. Introduction

The protected areas are valuable ecosystems which have many environmental and ecological values (Willis et al., 2012; He and Wei, 2023). One of the most important protected areas in Spain is Ons Island. This area is a biodiversity hotspot in northwestern Spain which provides valuable habitats for many endemic species. Unfortunately, the natural resources of this Island have been destructed by many factors in recent years. One of the destructive factors of native plant species is increasing establishment of invasive plant species (IPS) such as *Acacia Melanoxylon* in Ons Island which threatens the habitat of some native tree species (e.g. *Pinus Pinaster*) in this Island. The first step to know about distribution of *Acacia Melanoxylon* in this protected area is mapping the different land uses (natural and human-made) by high-resolution satellite images, new technologies, and efficient models.

Regarding importance of land use/ land cover mapping, many studies have been carried out for mapping the land uses and forest area in different regions around the world using various classification algorithms. In recent years, application of machine learning algorithms such as RF (Random Forest), SVM (Support Vector Machine), DT (Decision Tree), MaxEnt (Maximum Entropy), KNN (K-Nearest Neighbour), CART (Classification And Regression Trees), ANN (Artificial Neural Network), etc. has been more common for land use mapping in different natural areas. In this regard, many studies have used RF method to detect the forest cover and classify the various land uses in natural areas around the world (Rodriguez-Galiano et al., 2012; Nguyen et al., 2018; Abdi, 2019; Svoboda et al., 2022; Tikuye et al., 2023). Results of these studies have shown the high efficiency of RF model to classify different land uses especially in the heterogenous landscapes.

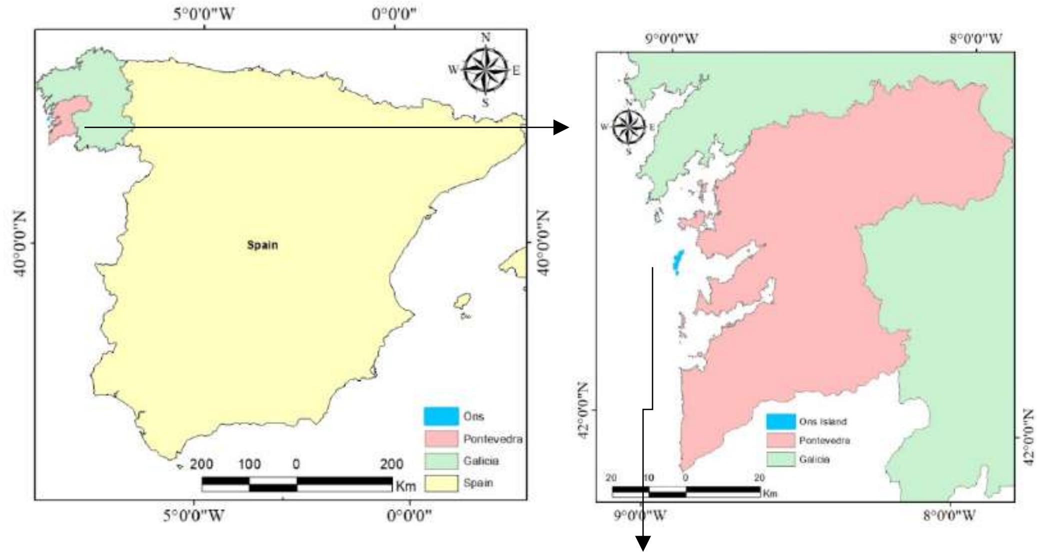
Regarding the destruction of natural habitats in Ons Island as a biodiversity hotspot in northwestern Spain in recent years, knowledge of different natural and human-made land uses in this Island is very important which can be performed via land use mapping using efficient algorithms. This mapping enables us to focus on the forest cover in Island to identify *Acacia Melanoxylon* as an IPS and as a factor that decreases biodiversity in this island. In addition, land use map gives us the useful information of distribution and area of human-made land uses which is important to know about relationships between human activities and destructive factors (e.g. establishment of IPS) in this Island. Therefore, this research aims to map the natural covers (mainly forest, shrubland, and rangeland) and human-made land uses (such as residential areas, croplands, road, etc.) in Ons Island using high resolution satellite images of PlanetScope and RF algorithm. The results of this research will be valuable to identify *Acacia Melanoxylon* in forest cover of Ons Island which will be performed in our future research.

2. Material and Methods

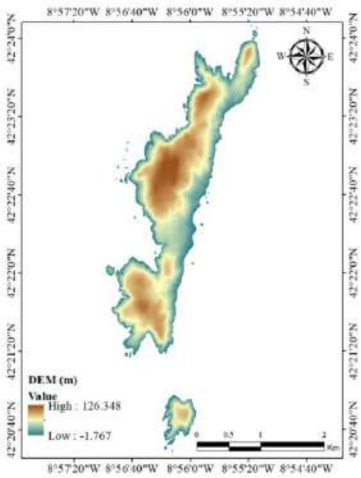
2.1. Study area

The study area of this research is Ons Island in northwestern Spain (Fig. 1a). It has located inside the Atlantic Ocean in Pontevedra, Galicia province, in northwestern Spain which covers an area about 426.1 ha. This island is a part of Atlantic Islands of Galicia National Park. The European Union has considered Ons Island as a specific protected area for the life of several birds in 2001. Some parts of Island have been covered by mixed forests. The main species in the forest cover are *Pinus pinaster*, *Quercus robur*, *Eucalyptus globulus*, *Cupressus macrocarpa*, etc. (Banco de Datos de la Naturaleza, 2021).

About topography, range of elevation from sea level is different from -1.76 m to 126.34 m (based on 2m DEM of study area) (Fig. 1b). About climatic characteristics of the Island, mean annual temperature is different from 10.5 ˚C (January) to 19.5 ˚C (August) in 2023 based on information of meteorological station of Ons Island. Furthermore, mean annual rainfall ranges from 4.1 mm (February) to 320.4 mm (October) in 2023 (Data obtained from: https://www.meteogalicia.gal/observacion/estacions/estacions.action?idEst=10125).



(a)



(b)

Fig. 1. Location of Ons Island in Pontevedra, Galicia province, and Spain (a), and DEM of study area (b)

2.2. Data

2.2.1. PlanetScope satellite images

To perform this research, the composite satellite images of PlanetScope for Ons Island were ordered and downloaded from Planet website (https://www.planet.com/explorer/) (Planet Team, 2022). These images have 3m spatial resolution which composite images including information of 8 spectral bands (Table 1).

The imagery date of these images was 27th of June of 2024, when all land uses are detectable on satellite images. In addition, at this time of the year, the forest cover includes the healthy trees with complete canopy cover which makes the identification of this cover easier on satellite images (Karlson et al., 2015). Therefore, all land uses are more distinguishable at the end of spring and during the summer. These seasons have been recommended for acquiring the satellite images to provide the land use and forest cover maps in the previous studies (Karlson et al., 2015; Eskandari et al., 2020; Svoboda et al., 2022). Thus, we also used PlanetScope satellite images related to the end of spring (June) for mapping different land uses in Ons Island.

Table 1. Characteristics of satellite image applied in this study

Satellite image	Type	Cloud cover	Number of spectral bands	Spatial resolution	Date of imagery
PlanetScope	Composite	<1%	8 bands	3m	June of 2024

2.2.2. Google Earth satellite images

Another dataset used in this study was Google Earth satellite images with better spatial resolution than PlanetScope satellite images. We applied these images to distinguish different land uses and to select training areas for each cover with more accuracy in Ons Islands. In addition, these images were used as reference data in this study to assess the accuracy of the land use map. These satellite images have been used as ground reality data in several studies because of high spatial and horizontal accuracy (Pulighe et al., 2016; Goudarzi and Landry, 2017; Guo et al., 2021). For this purpose, Google Earth satellite images related to June of 2024 were used in this study which were accordant to the date of PlanetScope satellite images (June of 2024).

2.3. Methods

2.3.1. Selection of training areas

Number and distribution of training areas is very important to get the accurate results from supervised classification method (Svoboda et al., 2022).

In this study, the expected land uses in Ons Island were included forest, shrubland, rangeland, bare soil, stone and sand, residential area, cropland, road, and water. The training areas for each land use were selected on Google Earth satellite images (related to June of 2024). Other researchers have also used the orthophotos for verification of training areas on satellite images during the classification process (Svoboda et al., 2022). For this purpose, enough and scattered training areas were selected for each land use on Google Earth satellite images. Totally, we considered 367 polygons of training areas scattered across the Island for all land uses to classify PlanetScope satellite images (Fig. 2).

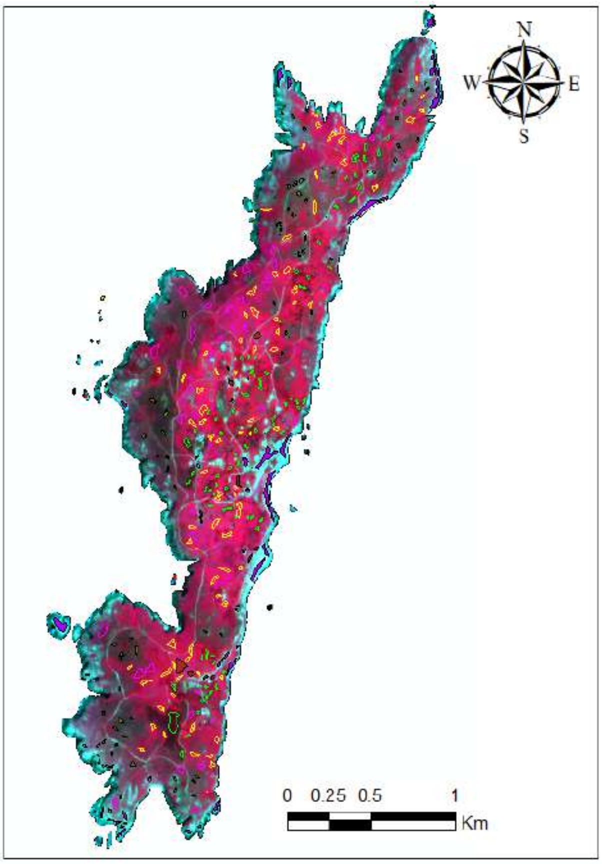


Fig. 2. Distribution of training areas in Ons Island

2.3.2. Supervised classification of PlanetScope satellite images using RF model

After considering proper training areas as several polygons, the supervised classification of PlanetScope satellite images was done using RF model. We applied this method for classifying satellite images in our study, because of the high efficiency of this non-parametric method for classifying the pixels of satellite images in heterogenous areas (Nguyen et al., 2018; Abdi, 2019; Svoboda et al., 2022; Tikuye et al., 2023). After supervised classification of PlanetScope satellite images using training areas and RF model, the land use map of Ons Island was obtained in 3m spatial resolution for 2024.

2.3.3. Accuracy assessment of land use map

Accuracy assessment of land use map was done using 180 square plots on Google Earth images (different from training areas). For this purpose, we considered 20 plots for each land use. Dimension of square plots were 3×3 m which was accordant to the pixel size of PlanetScope satellite images. Land use of each plot on Google Earth, was compared to land use of that plot on land use map. Then, accuracy indices such as overall accuracy (*OA*), kappa index ($\hat{\boldsymbol{k}}$), producer accuracy (*PA*), and user accuracy (*UA*) were applied to validate the land use map of Ons Island. These indices are given in equations 1 to 4 (Congalton and Green, 2008).

$$OA = \frac{\sum_{i=1}^{j} n_{ii}}{n} \times 100 \quad (1)$$

$$\hat{k} = \frac{n\sum_{i=1}^{j} n_{ii} - \sum_{i=1}^{j} n_{i+} n_{+i}}{n^2 - \sum_{i=1}^{j} n_{i+} n_{+i}} \quad (2)$$

$$Producer\ accuracy = \frac{n_{ii}}{n_{+i}} \quad (3)$$

$$User\ accuracy = \frac{n_{ii}}{n_{i+}} \quad (4)$$

Where n is total number of reference plots, n_{i+} is the sum of items on row i, n_{+i} is sum of items on column i, n_{ii} is sum of items on the main diameter of confusion matrix and j is number of land use/cover (Congalton and Green, 2008).

3. Results

3.1. Land use map

The land use map of Ons island including all current land uses has been shown in fig. 3. In addition, area of different land uses (forest, shrubland, rangeland, bare soil, stone and sand, residential area, cropland, road, and water) is given in table 2.

Based on the results, rangeland (200.74 ha) is the largest land use, and residential area (3.05 ha) is the smallest land use in this island.

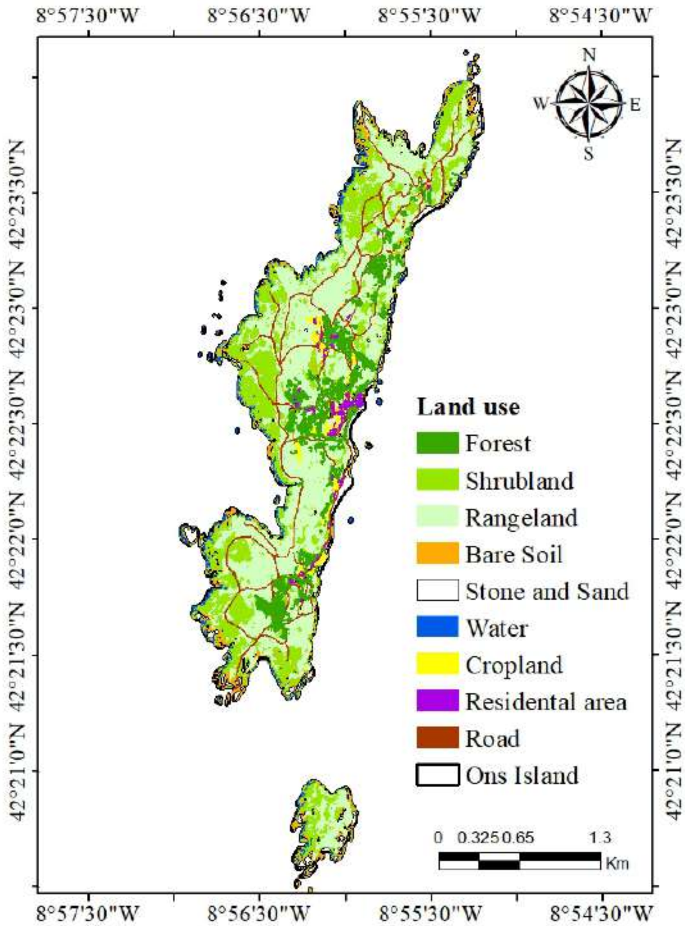


Fig. 3. The land use map in Ons Island

Table 2. Area of different land uses in Ons island

Row	Land use	Area (ha)	Area (%)
1	Forest	27.74	6.51
2	Shrubland	100.26	23.53
3	Rangeland	200.74	47.11
4	Bare soil	41.80	9.81
5	Stone and sand	20.97	4.92
6	Residential area	3.05	0.72
7	Cropland	7.82	1.83
8	Road	11.55	2.71
9	Water	12.17	2.86
10	Total	426.1	100

3.2. Accuracy assessment of land use map

The confusion matrix of land use map in Ons Island based on comparisons of land use of reference data (square plots in Google earth) and land use of pixels (in classified land use map) is given in table 3. The numbers on the main diameter of this matrix demonstrate the number of corrected classified plots. For example, regarding the forest cover, 18 plots of 20 plots have been classified correctly in land use map. In addition, accuracy indices for different land uses in the study area have been shown in table 4. Based on the results, water has shown the highest accuracy (*OA*: 100%) among all land uses, while shrubland and cropland have had the lowest accuracies in land use map (*OA*: 85%).

Table 3. Confusion matrix of land use map in Ons Island

	Number of control square plots in Google Earth (reference data)										
Number of control square plots in land use map (classified data)	Land use	Forest	Shrubland	Rangeland	Bare soil	Stone and sand	Residential area	Cropland	Water	Road	Total
	Forest	18	2	0	0	0	0	0	0	0	20
	Shrubland	2	17	1	0	0	0	0	0	0	20
	Rangeland	0	1	19	1	0	0	3	0	0	24
	Bare soil	0	0	0	18	0	0	0	0	0	18
	Stone and sand	0	0	0	1	19	1	0	0	0	21
	Residential area	0	0	0	0	1	19	0	0	1	21
	Cropland	0	0	0	0	0	0	17	0	0	17
	Water	0	0	0	0	0	0	0	20	0	20
	Road	0	0	0	0	0	0	0	0	19	19
	Total	20	20	20	20	20	20	20	20	20	180

Table 4. Accuracy indices of different land uses in Ons Island

Row	Land use	Number of reference data	Number of corrected classified plots	*OA* (%)	*UA* (%)	*PA* (%)	$\hat{k}$
1	Forest	20	18	90	90	90	0.87
2	Shrubland	20	17	85	85	85	0.82
3	Rangeland	20	19	95	79	95	0.92
4	Bare soil	20	18	90	100	90	0.88
5	Stone and sand	20	19	95	90	95	0.93
6	Residential area	20	19	95	90	95	0.92
7	Cropland	20	17	85	100	85	0.81
8	Water	20	20	100	100	100	1.0
9	Road	20	19	95	100	95	0.92
10	Total	180	166	92.2 %	92.6%	92%	0.89

4. Discussion

4.1. Land use map

Land use mapping is an important subject in natural areas which provides new knowledge of natural and human-made land uses in these areas which is important for ecosystem management especially in protected areas. This study was done to map different land uses in Ons Island in northwestern Spain using PlanetScope satellite images and RF method. For this purpose, nine land uses were considered in this Island.

Results of analysis the land use map showed that rangeland is the largest land use and residential area is the smallest land use in Ons Island. Results of other studies about land cover mapping in natural areas around the world have also shown that rangeland (grassland) is the widest land use in natural ecosystems which is accordant to the results of our study (Eskandari et al., 2020, Tikuye et al., 2023).

4.2. Validation of land use map

Results of validation of land use map showed that RF model has had a high accuracy (*OA*: 92%, kappa index: 0.89) for land use mapping in Ons Island. Investigation of accuracy indices for different land uses show that water has ideally been classified with the highest accuracy (*OA*: 100%). It means that no error pixels of water have not been observed in the land use map. After water, residential area, stone and sand, road, and rangeland have been the most accurate classified land uses in land use map (*OA*: 95%). Then, forest and bare soil have shown the highest accuracy in classified land use map (*OA*: 90%). Finally, shrubland and cropland (*OA*: 85%) demonstrated the lowest accuracy in land use map.

Totally, RF model has shown a high efficiency to provide the land use map in the study area. Results of other studies have also shown the high accuracy of RF model to classify different land uses in heterogenous areas (Rodriguez-Galiano et al., 2012; Nguyen et al., 2018; Abdi, 2019; Svoboda et al., 2022; Tikuye et al., 2023), which is accordant to the results of this study. Therefore, application of this model is suggested for land cover mapping in Ons Island in the future studies.

In addition, results of this study demonstrated that PlanetScope images have good capability to detect different land uses in the study area which is mainly related to high spatial resolution of these images. Furthermore, 8 spectral bands of these images provided this possibility for us to detect 9 land uses on these images in Ons Island. On the other hand, accessibility to these images with high resolution is usually possible for small areas in different time series. Thus, usage of these images is highly recommended to detect the land use change in this island in future studies.

4.3. Land use map and protective management in Ons Island against invasive plant species

In recent years, increasing establishment of IPS such as *Acacia Melanoxylon* is one of the most important challenges for biodiversity of the native species in Ons Island. In this regard, planning for a protective management in the Island is very important. Although, some measures have been begun in this Island to eliminate *Acacia Melanoxylon*, but these actions should be continued in the future. For performing such protective measures, knowledge of current distribution of *Acacia Melanoxylon* in the Island is very important. In this regard, the first required data is knowledge of forest distribution. The results of this study give us the new and useful information of forest area in Ons Island for June of 2024. The forest distribution map extracted from land use map in this research is a valuable map to identify *Acacia Melanoxylon* inside forest cover.

Another challenge regarding *Acacia Melanoxylon* is that which factor has been effective in increasing establishment of it. For this, we need information of all land uses in proper spatial and temporal scales. In this regard, other useful information obtained from the land use map is human-made land uses in the Island. These human-made land uses can be applied to discover the spatial relationships between human activities and distribution of *Acacia Melanoxylon* in Ons Island which will be performed in our future research following this study.

5. Conclusions

Mapping different land uses in protected areas is an important subject for performing protective managements of these biodiversity hotspots. Based on results of this research, PlanetScope satellite images had high capability to detect different land uses in the Island. Furthermore, RF model has been an accurate method to classify different land uses in the study area. Based on the land use map obtained from this research, there are 27.87 ha of forests and 225 ha of rangelands in Ons Island which provide habitats for several fauna and flora. Therefore, protective management of these habitats is necessary. Rangelands should be protected against human-made land uses such as croplands because croplands have mainly located inside the rangelands. Natural forests should be conserved against some threating factors such as invasive plant species like *Acacia Melanoxylon*. As this species quickly establishes in the natural forests, mapping forest cover and *Acacia Melanoxylon* distribution in Ons Island is recommended using PlanetScope satellite images and RF model in certain time series.

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ENHANCING CLIMATE CHANGE GOVERNANCE IN SERBIA: STRATEGIC AND LEGAL FRAMEWORK IN FORESTRY AND RELATED SECTORS

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Introduction

Climate change governance requires a comprehensive understanding of its socio-economic impacts in order to effectively formulate and implement appropriate policies (1). At the same time, strategic and legal frameworks support the implementation and realization of specific policy objectives through numerous documents and procedures as well as the necessary policy interventions (2). Serbia is in the process of harmonizing the national strategic and legal framework with EU requirements and there is also a need to comply with international commitments (3). However, previous research has shown that climate change issues are insufficiently integrated into strategic and legislative documents (4,5) and that there is a need to include them in forest and nature conservation policies in Serbia (6). Addressing climate change governance is a secondary goal of most strategic documents in the field of forestry and nature conservation in Serbia (7). The need to create appropriate links between functionally related sectors and to harmonize the legal framework is emphasized. This approach aims to avoid the overlap of certain legal solutions and to increase the possibilities to respond more efficiently to all challenges in the fight against climate change (7, 8). This paper aims to identify the opportunities for improving the strategic and legal framework for climate change governance in the forestry and related sectors in Serbia. Therefore, the following research questions were formulated:

- How can the strategic framework for climate change governance in Serbia be improved?
- How can the legal framework for climate change governance in Serbia be improved?

Methods

The data was collected in 23 interviews between May and June 2022 (Table 1). A judgmental sample was used to select the interviewees. Decision-makers and experts from the public and civil sectors in forestry and related sectors (nature conservation, environment) as well as "best practice" examples of stakeholder collaboration in climate change governance were selected. Decision-makers are representatives of institutions and organizations involved in the processes of adopting and implementing strategic and binding decisions (9) in forestry and related sectors in the field of climate change governance, while experts are understood as independent professional staff, consultants, project managers, etc. The selection of "best practice" examples is based on: a) sector affiliation (public sector: public enterprise (PE) "Srbijašume", local self-government (LS); civil sector: non-governmental organizations (NGO)); b) lower impact compared to perceived interest in climate change governance (10); c) general impression of the researcher based on the conducted first research phase (5) and prominent „best practice" examples of stakeholder collaboration in climate change governance.

Table 1. List of respondents

Code	Institution/organization	Date (2022)	Code	Institution/organization	Date (2022)
Decision-makers			**Experts**		
MEP1	Ministry of Environmental Protection	18.05	MEP2	Ministry of Environmental Protection	03.06.
DF1	Ministry of Agriculture, Forestry and Water Management–Directorate of Forests	27.05	INC2	Institute for Nature Conservation	18.05.
DF2		24.05	IF2	Institute of Forestry	27.05.
INC1	Institute for Nature Conservation	20.05	SŠD2	PE “Srbijašume” – General Directorate	19.05.
IF	Institute of Forestry	27.05.	SŠD3		19.05.
PENPĐ	PE “NP Đerdap”	25.05.	SŠD4		19.05.
PENPK	PE “NP Kopaonik”	03.06.	VŠD	PE “Vojvodinašume” – General directorate	14.05.
SŠD1	PE “Srbijašume” – General directorate	19.05	NGO1	Standing Conference of Towns and Municipalities (SCTM)	22.05.
			NGO2	WWF program in Serbia	21.05.
“Best practice" examples					
FMUZA	PE “Srbijašume”, FE “Timočke šume”, FMU “Zaječar”	25.05.	LSKU	Local self-government Kučevo	26.05
FEB	PE “Srbijašume”, FE “Timočke šume” (main office)	25.05	NGO3	NGO “Entuzijasti Kučeva”	26.05.
FMUKU	PE “Srbijašume”, FE “Severni Kučaj”, FMU “Kučevo”	26.05.	NGO4	NGO “Timočki omladinski centar”	25.05.

Source: original

Two questionnaires divided into four parts, were used in the research. The questionnaire for decision-makers consisted of 22 questions, including the strategic (questions 3-6) and legal frameworks (questions 7-9) for climate change governance. The questionnaire for experts consisted of 18 questions, including questions on the legal framework (questions 3-5), while the questions on the strategic framework were omitted as the experts were not involved in its adoption. The data was processed using content analysis. The responses were summarized and the respondents were grouped according to their attitude ("supporter", "non-supporter", "neutral"), using the "advocate-opponent" matrix.

Enhancing strategic and legal framework for climate change governance

Regarding the **strategic framework** in the field of climate change in Serbia, insufficient cross-sectoral coordination of climate change governance policies and measures was identified. It should be emphasized that at the time of the research, the Strategy for Low Carbon Development of the Republic of Serbia with the Action Plan was publicly discussed and was not analyzed in this paper. The Nature Protection Program of the Republic of Serbia for the period 2021-2023 (11) highlights the lack of connection between the different stakeholders dealing with climate change issues and the National Environmental Protection Program underlines the lack of effective cross-sectoral collaboration (12).

The need to increase the potential of forests as a renewable energy source is emphasized in the strategic document for the energy sector (13), but the harmonization of goals with other sectors is not mentioned. Strategic frameworks for forestry and related sectors do not recognize the role of the public and civil sector in identifying local problems and defining the necessary policy measures for climate change governance at the regional level (PE for state forest management "Srbijašume" - forest estates (FE), Public Enterprises National Parks (PE NP)) and local level (PE "Srbijašume" - forest management units (FMU), LS, NGOs). Activities related to information exchange and harmonization of activities and support measures for climate change governance at the lower levels are very limited (5).

Certain problems were identified in the analysis of the legal framework (5). The Law on Climate Change (14) establishes the development of a national climate change adaptation plan and the harmonization of various public policy documents, including the planning documents of LS units for sectors vulnerable to changing climate conditions, including forestry. It is not clear from the law whether and in what way the existing program includes the development of local action plans for adaptation (LAPA) to changing climate conditions based on the needs defined at the regional and local governance level. Cross-sectoral, as well as collaboration in climate change governance at regional and local levels is not sufficiently addressed in the legal framework for forestry and related sectors (5, 7, 8).

On the basis of the above-mentioned problems, solutions were proposed for improving the strategic and legal frameworks for climate change management in Serbia, as well as possibilities of application and necessary activities for their implementation. The matrix of qualitative analysis of respondents' attitudes towards the proposed improvements of the frameworks is presented in table 2.

Table 2. Matrix of respondents' attitudes towards the suggested solutions

Suggested solutions		Public sector																			Civil sector			
		PA				LS	PS				PE										NGO			
		DF		MEP		LS	IF[1]		ICN		PENP		SŠD				VŠD	FE	FMU		NGO			
		DF1	DF2	MEP1	MEP2	LSK	IF1	IF2	ICN1	ICN2	PEPNPK	PENPĐ	SŠD1	SŠD2	SŠD3	SŠD4	VŠD	FEB	FMUKU	FMUZA	NGO1	NGO2	NGO3	NGO4
Strategic	Improvement of cross-sectoral collaboration of policies and measures for climate change governance	+	-	+/-	/	/	+	/	+	/	+	+	+	/	/	/	/	/	/	/	/	/	/	/
Strategic	Recognized role of private and civil sector stakeholders from the regional and local level in climate change governance	+	+/-	+	/	/	+	/	+	/	+	+	+	/	/	/	/	/	/	/	/	/	/	/
Legal	Development of LAPA to changing climate conditions	+	-	+/-	+/-	+	+	+	+	+	+	+	+	+	+	+	+	+/-	+	+	+	+	+	+
Legal	Increasing cross-sectoral collaboration in climate change governance on regional and local level	+	+/-	+	+	+	+	+	+/-	+	+	+	+	+	+	+	+	+	+/-	+/-	+	+	+	+

Legend: + support - do not support +/- neutral attitude/ not competent to answer

[1] A part of the activities of the Institute of Forestry (IF) that falls under public service duties was observed

Source: original

Two solutions have been proposed to improve the strategic framework and the activities needed to implement it. To improve the cross-sectoral coordination of policies and measures for climate change governance, it is proposed to set up a working group within the National Climate Change Committee. The working group should include experts from different sectors (representatives of ministries, educational organizations, representatives of the civil and private sectors) as well as representatives of those sectors considered vulnerable to changing climate conditions. Based on information on the necessity and feasibility of certain climate change governance measures, the formed working group would also propose actions to guide and coordinate the improvement of policies, support measures and activities. An example of good practice from Croatia is the establishment of two technical working groups within the Commission for cross-sectoral coordination of climate change mitigation and adaptation policies and measures (15). In Slovenia, the need to establish a national body to coordinate and promote the implementation of measures at local level was also highlighted (16).

The majority of respondents are in favor of the proposed solution, while some representatives of the public administration (PA) take a neutral or negative attitude. They justify this with the fact that there are already many working groups and committees that are often not active enough in solving problems, as well as with a lack of financial resources (DF2, MEP1). However, other respondents who support this solution point out that this enables an "...*operational approach to solving this problem*" (SŠD1), which is necessary to achieve the goals set (DF1).

To recognize the role of stakeholders from forestry and related sectors at regional and local governance levels, from the public and civil sectors, it is proposed to define the need for their participation in the development of regional and local climate change adaptation action plans. This approach was identified in strategic documents on climate change governance at EU (17) and Croatian (15) level and confirmed by the respondent's attitudes. With the exception of one PA representative, who takes a neutral position, all respondents agree with the proposal to involve stakeholders from different sectors in the development of the LAPA on changing climate conditions. The reason for the neutral position of the PA representative is that "...*some processes of reporting and involving the local community exist, but the local community is inert*" (DF2). PA, PE and Public Service (PS) representatives also pointed out that the current "top-down" system of climate change governance with a centralized decision-making approach should be changed (DF1, PENP1, PENP2, SŠD1, IF, INC1). Furthermore, the possibility of involving local communities is emphasized (MEP1), with additional support measures to encourage their participation (DF2).

In order to improve legal framework, it is proposed to develop LAPA to changing climate conditions. This relates primarily to the identification of organizations involved in data collection and information sharing at regional and local governance levels. Hence, it is proposed to involve PE NP and PE "Srbijašume" - FE at the regional governance level, and PE "Srbijašume" - FMU, LS and NGOs at the local governance level. It is, therefore, proposed to designate an organization responsible for coordinating the exchange of information on local challenges in order to assess the necessary support measures and promote their implementation. This approach has been identified in Croatia, where action plans are defined based on the assessment of local and regional challenges (at county level) and include guidelines, measures and goals for climate change mitigation and adaptation (15). However, the majority of PA representatives are neutral towards this proposal and some respondents have a negative attitude. One of the main obstacles to the development of LAPA to changing climate conditions is the lack of legislation (MEP2) and unawareness of "...*climate change issues based on regional needs*" (DF2). However, the PA representative emphasized that some municipalities in Serbia, with the support of NGOs, have already taken steps to create LAPAs to changed climate conditions due to the "...*perceived need to define support measures at the local governance level*" (MEP2).

The proposed activities to implement this solution include: a) identification of stakeholders responsible for collecting relevant data and information exchange between the public and civil sectors at the regional and local governance level; b) identification of the organization responsible for coordinating the information exchange and defining the necessary support measures at the regional and local governance levels; c) identification of the organizations responsible for implementing the support measures.

The PA representative has a neutral stance on this proposal and points out that "... *a necessary focus is on the development of the regional governance level*" (MEP1). All other representatives of PS, PE (with the exception of one representative with a neutral position), LS and NGOs support the preparation of the LAPA for the changing climate conditions and the proposed activities. At the same time, the representative of PENP points out the need to amend the law "...*with the aim of prioritizing and implementing the proposed solution*" (PENP2). Representatives of NGOs point out that in some cities and municipalities (e.g. Bečej, Zrenjanin) there are already LAPAs on changing climate conditions (NGO1), but that "...*an appropriate methodology and mechanisms for their elaboration need to be defined*" (NGO2). The majority of respondents believe that the responsibility for collecting relevant data and sharing information related to climate change should be assigned to LS, PENP, FE, FMU, NGOs, but also to the city departments and municipal divisions for emergency management. Regarding the responsibility for the implementation of support measures in dealing with climate change at the regional and local levels, the respondents emphasize the role of NGOs from the aspect of information dissemination, but also of LS, FE, FMU and PENP (LSK, IF2, NGO3, FEB, VŠD, SŠD2, IF1, SŠD3, INC2). Furthermore, some respondents emphasized the role of the MEP (IF2, SŠD3, FMUZA) and the LS in *"...coordinating the various stakeholders*" (VŠD, IF2, NGO2).

In order to improve cross-sectoral collaboration in addressing climate change at regional and local levels, it is proposed that the principles of cross-sectoral cooperation between stakeholders be defined and included in the strategies and plans as prescribed by the Law on Climate Change in Serbia, at all three levels of climate change governance (14). This principle would emphasize the need to develop dialog, disseminate knowledge and expand collaboration between the public, civil and private sectors at the different governance levels. This approach can be found in the European Climate Law (18), the Law on Environment (19) of Montenegro and the Law on Nature Conservation (20) in Serbia. Numerous representatives of the public and civil sectors have expressed their support for this proposal. Representatives of PA and PS emphasize the need to define stakeholder collaboration (DF1, IF2) in the relevant laws (DF1) and to harmonize their activities (IF2). Representatives of NGOs point out that in this way the role of the civil sector in addressing climate change is recognized (NGO1, NGO2).

Discussion

The results of previous studies show that in Serbia, as in other neighboring countries (Slovenia, Croatia, Bosnia and Herzegovina), there is a complex strategic framework for climate change governance that recognizes the need to integrate climate change support measures into the strategic documents of all sectoral policies (6, 8). In addition, it was found that cross-sectoral collaboration for climate change governance in Serbia needs to be further developed and improved, as well as the harmonization of national policies of different sectors (8). The results of this study also confirm the need to integrate the issue of climate change into the policies of forestry, nature conservation and related sectors through the application of various support measures. The importance of further developing the strategic framework is emphasized, with a focus on stakeholder collaboration and harmonization of support measures between the different sectors.

The results of previous studies on the legal framework in the EU show that many Member States have harmonized climate change legislation in line with EU regulations and the objectives of various binding and non-binding documents (3,8). Nevertheless, the need for "...*better integration of climate change issues into national policies and their implementation through national legislation*" (21) has been identified. Previous study has shown that in some cases the need to apply LAPA to changing climate conditions is not always clearly defined, nor is the role of stakeholders in the climate change adaptation planning process (22). A similar situation was also found in this study. It was noted that one of the shortcomings of the legal framework related to climate change is the unclear definition of the pathways and mechanisms of collaboration between stakeholders at different levels of governance (23), which was also confirmed in this study.

Conclusions

To improve the strategic framework for climate change governance, based on the attitude of the majority of public sector representatives, it is proposed to recognize the role of public and civil stakeholders at regional and local governance level by involving them in the development of LAPA on changing climate conditions. In this way, a change in the way of governance, i.e. towards the "bottom-up" principle, would be promoted. Although different attitudes were found towards the proposal to improve cross-sectoral coordination of policies and support measures for climate change governance, the majority of respondents believe that the establishment of a working group within the National Climate Change Committee is necessary. This would improve the conditions for achieving the goals set and the harmonization of policies and measures between sectors.

Regarding the improvement of the legal framework, in particular the development of LAPA to changing climate conditions, there were differences in the responses of the PA representatives. However, the majority of public representatives and all from the civil sector believe that the need to develop LAPA to changing climate conditions is recognized by the examples of individual municipalities and that with the establishment of clear working and support mechanisms, the implementation of local action plans is possible. They also believe that it is necessary to define the stakeholders at the regional and local governance level in data collection, coordination and implementation of support measures. The majority of representatives from both sectors are in favor of defining the "principle of cross-sectoral collaboration" as an activity related to strengthening cross-sectoral and multi-level collaboration in climate change governance.

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EXPLOITING HYPERSPECTRAL DATA TO PREDICT PHOTOSYNTHESIS AND WATER STATUS PARAMETERS AND DISCRIMINATE OZONE (O_3) EFFECTS ON GRAPEVINE (*Vitis vinifera* L.)

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Introduction

The development of Infra-Red Gas Analysers (IRGAs) has allowed researchers to measure net carbon dioxide (CO_2) assimilation (A_{Net}), and water vapour released through transpiration (E) *in vivo* [1]. From the response curves of A_{Net} to intercellular CO_2 concentration (C_i), known as A_{Net}/C_i curves, several eco-physiological parameters can be derived by parameterization methods, providing a more comprehensive understanding of the impact of abiotic stresses on plant [2]. Despite improvements in IRGAs performance, the need to assess crop *status* across multiple phenological stages has driven researchers to develop new and more time- efficient techniques. Vegetation spectroscopy is a high-throughput sensor technology, enabling the simultaneous assessment of various plant traits over extended periods [3]. Ozone (O_3) is a phytotoxic air pollutant that can impair grapevine photosynthetic capacity and bud fertility, posing risks to wine production [4]. The main goals of this study are to (*i*) develop partial least squares regression (PLSR) models to estimate from spectra photosynthetic process and hydric dynamics-related parameters commonly impaired by O_3 exposure, (*ii*) evaluate the potential of hyperspectral phenotyping to accurately disentangle the plant responses to a gradient of O_3 concentrations, and (*iii*) investigate the role of Chlorophyll Index (CI) and Normalized Difference Water Index (NDWI) in O_3-grapevine interaction.

Material and Methods

Grapevine plants (*Vitis vinifera* L. cv Cabernet sauvignon) were exposed to three O_3 levels, i.e., ambient air (AA), moderate O_3 (MO: 1.5 × AA), and elevated O_3 (EO: 2.0 × AA), in an Free-Air O_3 eXposure (FO_3X) facility (Sesto Fiorentino, central Italy) [4]. Leaf gas exchange was measured using a portable IRGA (LI6800, Li-Cor, Lincoln, USA) on 4-8 July and 5-7

September 2022. A_{Net}/C_i curves were obtained at 9 CO_2 levels (50, 100, 200, 410, 600, 800, 1200, 1600, 2000 µmol mol^{-1}). A_{Net} and E were determined at CO_2 concentration of 410 µmol mol^{-1}. The Variable J method was used to infer the mesophyll conductance (g_m) [5] and the carboxylation capacity of ribulose-1,5-biphosphate carboxylase/oxigenase (Rubisco) (V_{cmax}) was derived according to the A_{Net}/C_i curves [1,2]. Full-range (350-2,500 nm) leaf reflectance profiles were collected at bunch closure (BBCH-77) and berry ripening (BBCH-83), using a spectroradiometer. A_{Net}, E, g_m, and V_{cmax} were predicted from reflectance profiles using a PLSR approach. The effects of O_3 exposure (O), phenological stage (PS) and their interaction (O × PS) on the leaf profiles were determined by permutational multivariate analysis of variance (PERMANOVA). Spectral responses were visualized using principal coordinates analysis (PCoA) on the same spectral data tested by PERMANOVA. Partial least squares discriminant analysis (PLS-DA) was used to determine the ability of hyperspectral data to classify experimental groups showing statistical significance from PERMANOVA [3]. CI and NDWI were computed from the grapevine leaf profiles collected at BBCH-77 and BBCH-83 [3].

Results

A. Prediction of leaf traits

Different wavelength ranges and number of components were initially tested to optimize PLSR statistical outputs. Final models utilized the following wavelength ranges: 400-2,400 nm for A_{Net}, g_m and V_{cmax}, while 1,300-2,400 nm were used for E. Predictive models accurately characterized g_m and V_{cmax} (for cross-validation, R^2: 0.52 and 0.40; %RMSE: 13 and 18), and even more A_{Net} and E (for cross-validation, R^2: 0.65 and 0.54; %RMSE: 15 and 16) (Table 1; Figure 1).

Table 1: Range of wavelengths, number of components (Comp), model goodness-of-fit (R^2), root mean square error (RMSE), and percent RMSE of the data range (%RMSE) for cross-validation data for the PLSR models predicting leaf grapevine traits. Data are shown as mean ± standard deviation. Traits: A_{Net}: net CO_2 assimilation (µmol CO_2 m^{-2} s^{-1}), E: transpiration (mol H_2O m^{-2} s^{-1}), g_m: mesophyll conductance (mol CO_2 m^{-2} s^{-1}), and V_{cmax}: carboxylation capacity of ribulose-1,5-biphosphate carboxylase/oxigenase (Rubisco) (µmol CO_2 m^{-2} s^{-1}).

Trait	Range (nm)	Comp	R^2	RMSE	%RMSE
A_{Net}	400-2400	16	0.65 ± 0.11	2.49 ± 0.66	15
E	1300-2400	14	0.54 ± 0.15	0.00 ± 0.00	16
g_m	400-2400	9	0.52 ± 0.14	0.04 ± 0.01	13
V_{cmax}	400-2400	12	0.40 ± 0.17	12 ± 2.10	18

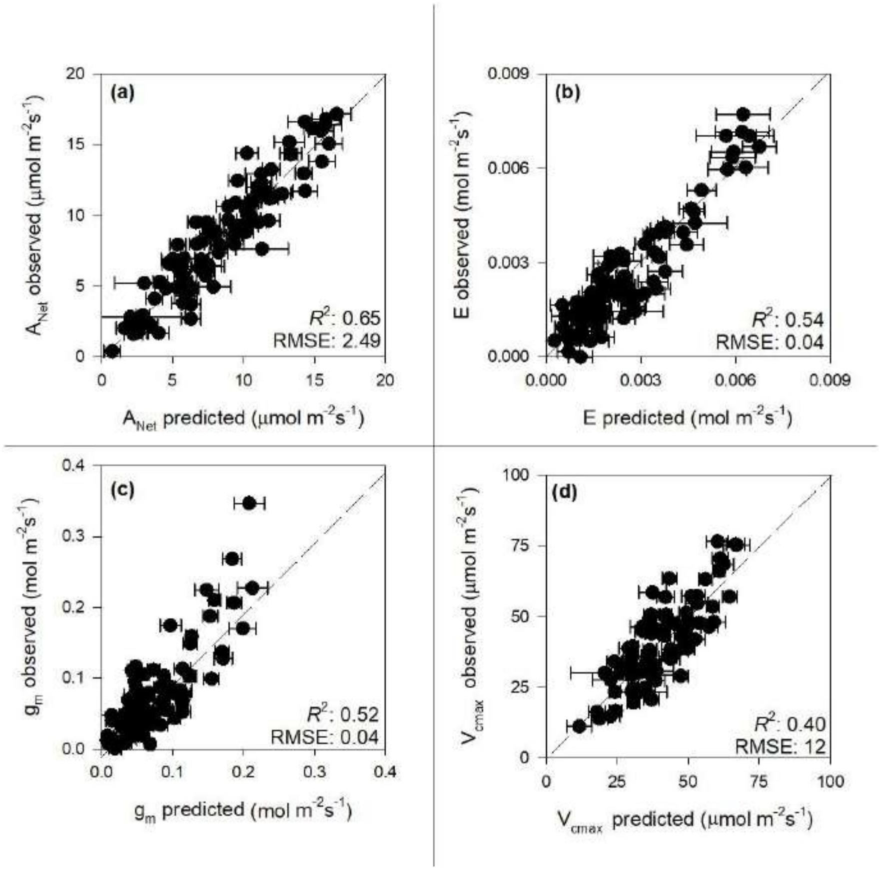

Figure 1. Observed and PLSR-predicted values of leaf grapevine traits; error bars for predicted values represent the standard deviation generated from 500 simulated models; dashed line is 1:1 relationship; model goodness-fit (R^2), and root mean square error (RMSE) for validation data generated using 80% of the data for calibration and 20% for validation are reported. Bias outputs are not shown as they were always lower than 0.01. (**a**) A_{Net}; (**b**) E; (**c**) g_m; (**d**) V_{cmax}.

B. Analysis of spectral signatures

Recorded using the full range (i.e., 400-2,400 nm), PERMANOVA showed a significant effect of O (*df*: 2), PS (*df*: 1), and O × PS (*df*: 2), on grapevine spectral profiles (Table 2).

Table 2. *P*-levels of PERMANOVA for the effects of O, PS, and their combination on the full-range (400-2400 nm) reflectance profiles of grapevine. *df* represents the degrees of freedom. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$; ns: $P > 0.05$.

Effect	*df*	*P*
O	2	***
PS	1	**
O × PS	2	*

Average spectral profiles were visualized in Figure 2 (**a**) and (**c**). *Kappa* and Accuracy from PLS-DA were 0.36 ± 0.11 and 0.54 ± 0.21 (mean ± standard deviation) at BBCH-77, and 0.32 ±

0.13 and 0.66 ± 0.18 (mean ± standard deviation) at BBCH-83, highlighting the capability of spectroscopy to discriminate plants exposed to AA (white circle) from plants exposed to MO (gray circle) and EO (black circle) (Figure 2 (**b**) and (**d**)).

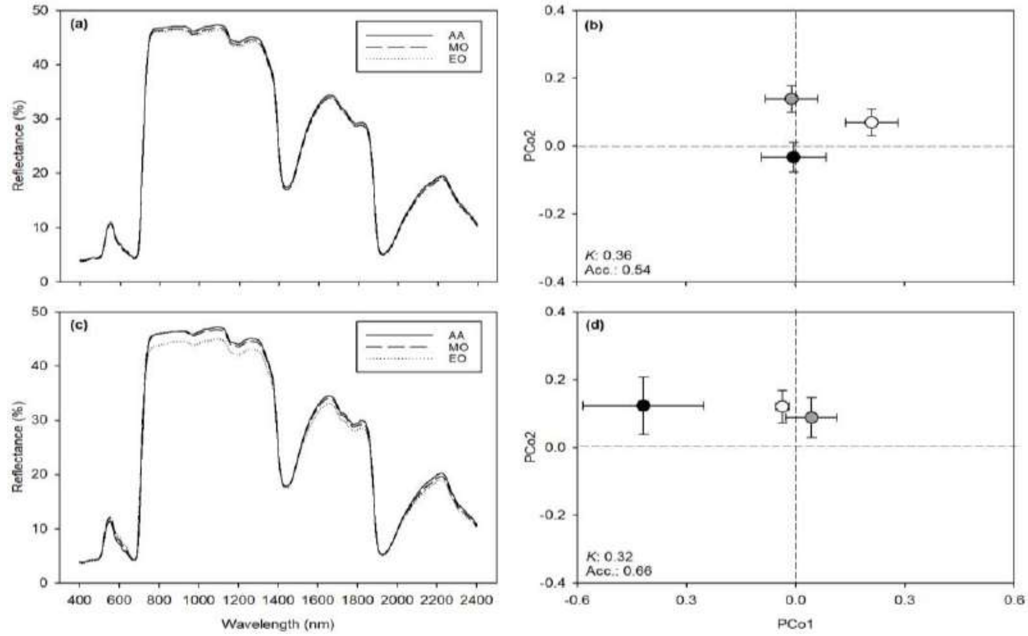

Figure 2. Average leaf reflectance profiles of grapevine exposed to AA, MO, and EO at BBCH-77 (**a**) and BBCH-83 (**c**). Scores (mean ± standard error) for the first and second principal components from PCoA of reflectance data (400–2400 nm) collected from grapevine leaves at BBCH-77 (**b**) and BBCH-83 (**d**); average accuracy (Acc.) and *Kappa* (K) values from PLS-DA are reported on the bottom-left corner of each panel.

C. Variation of VIs computed from spectra

Two-way ANOVA showed a significant effect of O, PS, and their combination on CI and NDWI computed by the spectra collected at BBCH-77 and BBCH-83 (Table 4; Figure 4).

Table 4. *P*-levels of two-way ANOVA for the effects of O, PS, and their combination on CI and NDWI. *df* represents the degrees of freedom. *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$; ns: $P > 0.05$.

Effect	CI		NDWI	
	df	*P*	*df*	*P*
O	2	***	2	***
PS	1	***	1	***
O × PS	2	**	2	***

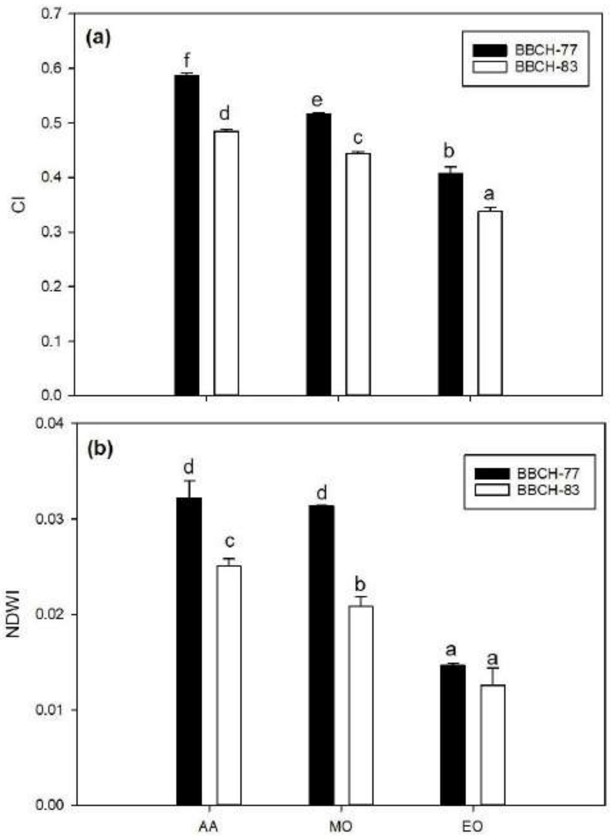

Figure 4. Variations of CI (a) and NDWI (b) derived from grapevine leaf spectra under AA, MO, and EO at BBCH-77 (black) and BBCH-83 (white). Data are shown as mean ± standard deviation. According to Tukey's post-hoc test, different letters indicate significant differences among means ($P \leq 0.05$).

Conclusions

The present study shows the potentials of vegetation spectroscopy as a rapid, non-destructive, and relatively inexpensive tool to accurately monitor grapevine-O_3 interaction, even before the onset of O_3 visible injuries.

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THE POTENTIAL OF USING GRAIN BY-PRODUCT IN THE DEVELOPMENT OF 3D-PRINTED SNACKS

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Introduction

The development of 3D-printed food has emerged as a promising innovation within the food industry, offering new possibilities for personalized nutrition, sustainable production, and creative culinary designs. This technology is linked to sustainability in the food sector by enabling the use of alternative ingredients, such as grain by-products and plant-based proteins [1, 2]. Grain by-products like bran, germ, husk and oil-seed press cake, which are often discarded or underutilized in traditional food production, are rich in dietary fiber, vitamins, minerals, proteins, and bioactive compounds, making them valuable for creating nutritious and eco-friendly food products. Incorporating these grain by-products into 3D-printed snacks not only supports sustainability goals but also helps reduce food waste while enhancing the nutritional profile of these snacks [3, 4]. This study, therefore, aimed to explore the potential of using various grain by-products (wheat bran, defatted flaxseed flour, and pumpkin seed cake) in 3D savory and sweet snacks through evaluation of their effect on dough rheology, 3D printing precision, physical properties and dimensional stability.

Material and Methods

Wheat bran was sourced from an Farina mill (Granolio Inc., Zagreb, Croatia), fresh pumpkin seed press cake from Grbić (Požega, Croatia), deffated flaxseed flour from SME Siladi (Croatia), and glucose oxidase (GOX, EC 1.1.3.4., 500 U/g) from BIO-CAT (Troy, VA, USA). Oat flour (Garden Ltd., Zagreb, Croatia), pea and rice protein (both from Biovega Ltd., Zagreb, Croatia), and other dough ingredients were purchased at the local market. Particle size distribution of ingredients was measured using the Mastersizer 2000 instrument with Scirocco dry dispersion unit (Malvern Instruments, Worchestershire, UK) [5]. Formulations for savory snacks with pumpkin seed cake (SSP) or flaxseed flour (SSF), and sweet snacks with sugar (SWSS) or honey (SWSH) are shown in Figure 1. Dough was hand mixed (M350LBW, Gorenje, Slovenia) in three steps (Figure 1) and immediately used for rheology analysis or 3D printing. Oscillatory measurements were performed with a parallel plate geometry using an MCR 92 rheometer (Anton Paar, Graz, Austria) as previously described [3], and the storage modulus (G'), loss modulus (G"), and complex viscosity were calculated with Anton Paar RheoCompass software. Dough was extruded (heart or quadratic shape, Figure 1) using the 3D Food Printer-Multi-Ingredient Support (Ningbo Createbot Electronic Technology Co., Ltd, Ningbo, China) with a 1 mm nozzle and printing speed of 25 mm/s at 20 °C, and then baked in a deck oven (EBO 64–320 IS 600, Wiesheu GmbH, Germany) at 180°C for 18 min (savory) or 10 min (sweet snacks). The printing precision and shape deformation were determined with digital image analysis and calculated as previously demonstrated [3]. The hardness of baked snacks was analysed with a cutting test using a texture analyser (Ametek Lloyd Instruments Ltd., West Sussex, UK) [6]. The lightness (L^*), redness (a^*), and yellowness (b^*) of baked snacks was measured with a colourimeter (Konica Minolta CM-700d, Tokyo, Japan) and the total colour change (ΔE^*) of the dough 1h after printing and browning index (BI) of snacks after baking were calculated [3]. Peroxide value (PV) was monitored under ambient conditions for 30 days in snacks with and without added pumpkin seed cake, stored in PPmet/PE bags [7].

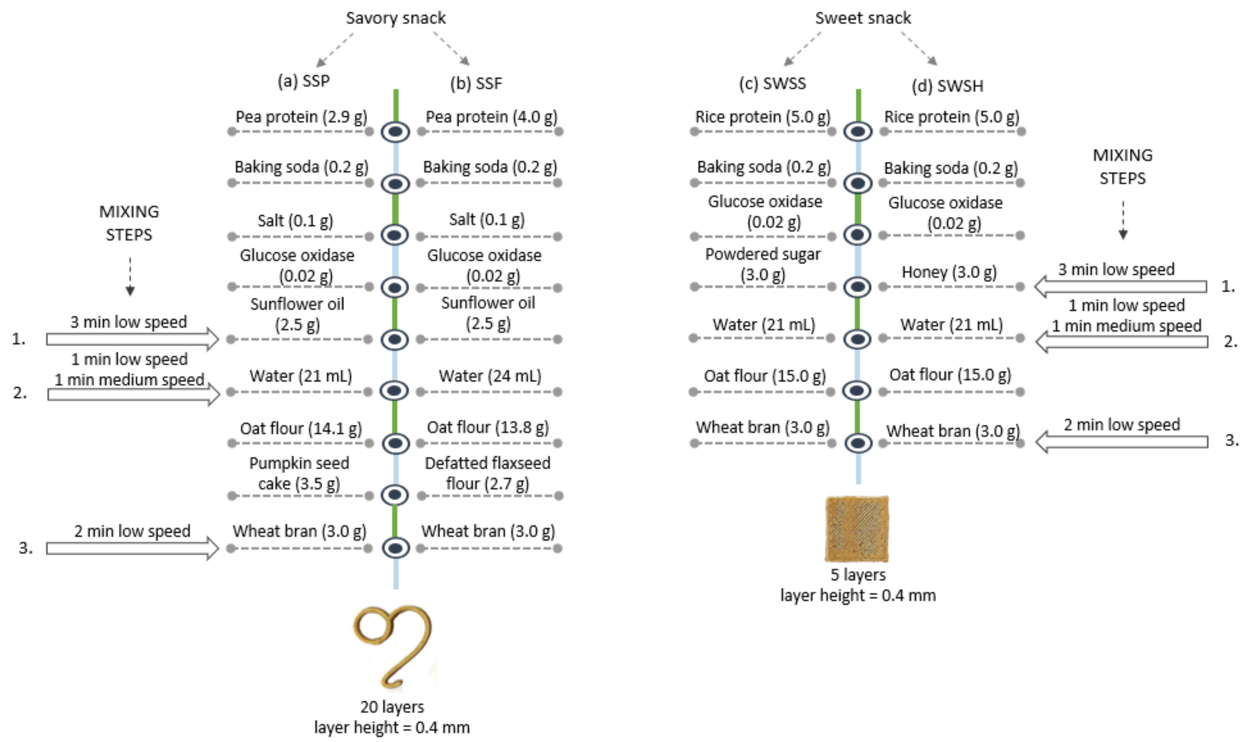

Figure 1. Snack formulations, steps of dough preparation, and 3D printed shapes used in this study

ANOVA and Tukey's post hoc test ($p < 0.05$) were performed to evaluate snack formulation effects on rheology and printability of the dough, and physical parameters of the snacks.

Results and Discussion

The main particle size distribution parameters are shown in Table 1. Wheat bran has previously exhibited a median diameter at the 50th percentile of 177 ± 2.3, at the 90th percentile of 401.6 ± 0.9, surface weighted mean of 63.0 ± 0.7, and a span of 2.1 ± 0.0 [7]. Although results for the pumpkin seed cake and defatted flaxseed flour indicated a potential risk of nozzle clogging, this issue did not arise during the extrusion of the SSP or SSF dough.

Table 1. Particle size distribution of ingredients

	Oat flour	Pea protein	Rice protein	Pumpkin seed cake	Flaxseed flour
d (0.5) (µm)	35.8 ± 4.9	51.7 ± 0.5	17.8 ± 0.2	497.4 ± 79.5	993.2 ± 1.8

d (0.9) (µm)	376.8 ± 8.2	101.4 ± 0.6	41.3 ± 0.3	1087.0 ± 134.5	1529.5 ± 1.6
D[3,2] (µm)	20.9 ± 1.9	38.0 ± 1.1	10.9 ± 0.3	142.1 ± 21.6	922.8 ± 1.4
Span	10.5 ± 1.7	1.54 ± 0.0	2.0 ± 0.0	2.1 ± 0.1	0.92 ± 0.0

d (0.5), median diameter of 50th percentile; d (0.9), median diameter of 90th percentile; D[3,2], surface weighted mean.

The rheological properties of the doughs were significantly influenced by the type of the dough mixture ($p < 0.05$). The solid-like behaviour of all dough samples was confirmed, with G' values higher than G'' (Table 2), which is essential for successful 3D printing and ensures dimensional stability of shapes after extrusion-based 3D printing [3]. The largest G' and G'' values were noticed in SWSH dough. Reducing oil content generally increases the dough's complex viscosity [8]. Consequently, sweet doughs had higher complex viscosity than the savory ones, most likely due to the absence of oil and the addition of sugar [9]. Further, the complex viscosity of SWSH mixture was higher than that of SWSS due to the presence of honey [10].

Table 2. Effect of the dough mixture on dough rheology

	SSP	SSF	SWSS	SWSH
G' (Pa)	42817 ± 1284 ^{a}	23689 ± 2799 ^{b}	46997 ± 1842 ^{a}	56948 ± 1788 ^{a}
G'' (Pa)	8808 ± 282 ^{a}	6033 ± 111 ^{a}	11328 ± 169 ^{b}	13104 ± 277 ^{b}
Complex viscosity (Pa s)	6970 ± 11 ^{a}	3891 ± 157 ^{b}	7694 ± 201 ^{a}	8834 ± 240 ^{a}

Different letters within the same row indicate significant differences ($p < 0.05$).

Printing precision, which refers to the reproducibility and consistency of both the process and final product quality, can be affected by various factors, particularly the rheological properties of the material [1, 2]. Figure 2 shows that printing precision of savory doughs was significantly higher (98% for SSP and 96% for SSF) ($p < 0.01$) and they experienced less deformation during baking compared to sweetened dough.

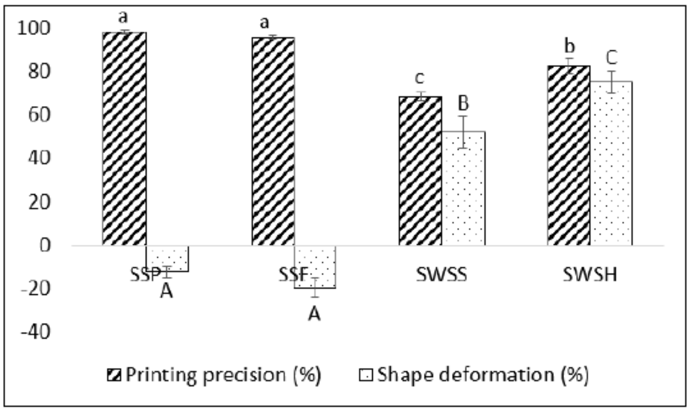

Figure 2. Printing quality and stability of investigated dough mixtures. Different letters within the same parameter indicate significant differences ($p < 0.05$).

Pea protein used in the preparation of savory doughs has previously been shown to improve structural stability and enhance the texture of starch-based printed food products [11]. Indeed, SSP and SSF expressed significantly less deformation during and significantly greater hardness after baking (Figure 2, Table 3). Savory snacks shrank (by -12 and -19%), while sweet snacks spread (by 52 and 70%). Shrinkage is likely due to dehydration, protein denaturation, and starch gelatinization [3]**,** whereas the spread of sweet snacks could be attributed to the presence of sugar and higher oil content [9]. Compared to our previous study [8] the adition of sugar and honey disrupted the stability of the dough during baking, while the partial replacement of oat flour with pumpkin seed cake or defatted flaxseed flour had no significant impact (Figure 2). Snack deformation was positively correlated with hardness (r=0.99), with baked savory snacks being significantly harder (2.47 to 2.84 g) compared to sweet snacks (0.63 to 0.78 g) ($p < 0.01$) (Figure 2, Table 3).

The total colour change of the dough 1h after printing was minimal (Table 3), likely due to glucose oxidase's ability to degrade *β*-carotene, which helps minimize dough browning [12]. Baked sweet snacks were significantly lighter and more yellow than savory snacks ($p < 0.05$). The browning index, which is influenced by the Maillard reaction, starch dextrinization, and sugar caramelization during baking [13], was significantly higher in sweet snacks ($p < 0.05$) (Table 3).

Table 3. Physical properties of investigated dough mixtures and snacks

	SSP	SSF	SWSS	SWSH
3D printed dough				
ΔE^*	1.43 ± 0.33 ^{a}	1.86 ± 0.36 ^{b}	1.83 ± 0.38 ^{b}	1.86 ± 0.25 ^{b}
Baked snack				
Lightness $L*$	53.61 ± 0.68 ^{b}	53.65 ± 1.68 ^{b}	58.59 ± 2.33 ^{a}	57.65 ± 1.06 ^{a}
Redness $a*$	3.63 ± 0.14 ^{b}	4.21 ± 0.39 ^{a}	4.22 ± 0.41 ^{a}	4.28 ± 0.27 ^{a}
Yellowness $b*$	13.06 ± 0.69 ^{b}	12.36 ± 1.12 ^{b}	15.70 ± 1.57 ^{a}	15.72 ± 0.64 ^{a}
Browning index	32.44 ± 1.97 ^{b}	31.44 ± 2.16 ^{b}	35.88 ± 2.71 ^{a}	36.74 ± 1.34 ^{a}
Hardness (kg)	2.47 ± 0.18 ^{a}	2.84 ± 0.22 ^{a}	0.78 ± 0.06 ^{b}	0.63 ± 0.04 ^{b}

Different letters within the same row indicate significant differences ($p < 0.05$). ΔE^*, total colour change.

The primary products of lipid oxidation in food are measured by peroxide value (PV), which can further degrade into secondary oxidation products through the action of peroxidase [14]. Partial replacement of oat flour with pumpkin seed cake resulted in PV of 10.32 ± 0.26 in savory snacks just after one month of storage. Products with a PV level greater than 10 are considered rancid and toxic [7].

Conclusions

Various grain by-products (such as wheat bran, defatted flaxseed flour, and pumpkin seed cake) were utilized in this study to create savory and sweet 3D-printed snacks formulations. The savory and sweet snacks displayed notable differences in their rheological profiles, printing quality, hardness, and colour. Savory snacks outperformed in terms of printing precision and maintaining shape integrity, while sweet snacks excelled in colour properties. Although dough containing grain by-products can be accurately 3D-printed into diverse shapes, attention is required to prevent undesirable shape deformation after processing and to mitigate rapid oxidative spoilage of snacks.

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PHENOLIC CONTENT AND PHYSICOCHEMICAL PROPERTIES OF SOME ALBANIAN HONEY SAMPLES FOR THERAPEUTIC USES

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Abstract

The aim of this study is to evaluate the physicochemical properties and total phenolic contents of various multifloral and monofloral honeys collected from different regions of Albania. The majority of the samples were classified as multifloral, with the remainder being monofloral. Multifloral and *Arbutus* honeys exhibited the highest acidity, while *Salvia officinalis* honey showed the highest electrical conductivity (0,949 mS/cm) among all samples. *Salvia officinalis* honey also had the highest diastase activity and the lowest HMF content. Moreover, the highest total phenolic content (TPC) was found in *Salvia officinalis* honey, followed by *Multifloral*, *Castanea Sativa*, *Arbutus*, and *Erica* honeys. A significant correlation was observed between total phenolic content and diastase activity (DN) based on the data analysis. Consequently, *Salvia officinalis* honey is recommended for health purposes due to its high phenolic content, relatively low HMF levels, and higher diastase activity. Considering that northern Albania is the largest producer of *Salvia officinalis* honey, this study provides valuable recommendations to increase the production of this honey type, which possesses a high TPC level and could be utilized for therapeutic and wound-healing applications.

Keywords: *diastase number, HMF, physicochemical, TPC.*

Introduction

Honey is the most widely consumed bee product globally, valued both for its nutritional content and healing properties. It contains about 200 known compounds, with a typical composition of 38% fructose, 31% glucose, 10% other sugars, 18% water, and 3% other substances. Despite its small proportion, this 3% includes crucial components such as carotenoids and phenolic compounds [1]. The phenolic composition of honey, alongside key physicochemical parameters such as Hydroxymethylfurfural (HMF) content and diastase activity, plays a crucial role in its potential application for wound healing. This study explores these properties in honey collected from various regions, with a focus on understanding how environmental and beekeeping factors influence honey's therapeutic qualities. The geographical location and exposure of the regions, the diversity of plants present, and their phenology significantly affect the phenolic profile and overall quality of honey.

Additionally, the time of honey collection, bee migration patterns, and the methods used for honey harvesting—centrifuge extraction in all stations examined—are important factors in determining its physicochemical properties. Honey rich in phenolic compounds, with low HMF content and high diastase activity, has shown great promise in wound healing applications. This research aims to provide insights into the complex interplay of these variables to better understand how they enhance the medicinal properties of honey for therapeutic use.

Owing to its therapeutic qualities, numerous studies have explored honey's positive effects on human biological functions, such as its antioxidant, antimicrobial, wound healing, anti-inflammatory, and anticancer properties. Many of these health benefits are attributed to honey's unique phenolic profile, which is largely influenced by environmental and seasonal factors [2, 3]. Studies by Kivrak et al. [4] and Can et al. [5] have identified the phenolic profiles of various honey types. Albania's favorable climate and geographic location provide excellent conditions for honey production. However, there is limited information regarding the essential physical, chemical, and biological characteristics of honey produced in the country, Research has shown that these characteristics vary depending on the nectar source and the region [6, 7]. Thus, the aim of this study is to assess the physicochemical and biochemical properties of different honey types, including chestnut, salvia, thymus, and multifloral honey, collected from hives in various regions of Albania.

Materials and methods

Honey samples

Honey samples were provided by various local beekeepers (47 samples in total) from the regions indicated by numbers on the map: 27 multi-floral honey samples, 12 *Castanea* honey samples, 3 *Arbutus unedo* samples, 2 *Salvia officinalis* samples, 2 *Erica* honey samples, and 1 special *Rosacea*-type honey sample (see Figure 1). To avoid temperature effects, the samples were stored at room temperature and protected from sunlight until analysis.

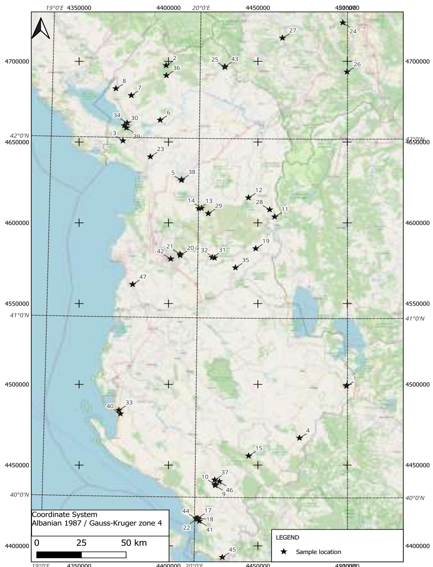

Figure 1. Location of honey samples

Physicochemical analysis for moisture

The moisture content was determined using the refractometric method suggested by Bogdanov. A homogenized honey sample was put in a closed container and placed in a water bath set to 50± 0,5°C to allow all the sugar crystals to be dissolved prior to the analysis. The sample was then cooled to room temperature and stirred again. The refractive indices of honey samples were read using a refractometer (Auto Digital Refractometer A760) at 20°C and corresponding moisture contents were recorded [International Honey Commission].

Free acidity

Titrimetric analysis was performed to determine free acidity following the method given by International Honey Commission. Basically. the acidity was calculated based on the volume of consumed 0.1 M NaOH for titration of the diluted honey sample (10 g:75 mL distilled water) to reach a pH of 8,3 and the results were expressed as milliequivalents per kg of honey.

Electrical conductivity (EC)

A conductivity meter (pH.mv.Cond.TD5. DOO. ISOLAB) was used for EC measurements of honey samples. All measurements were expressed as mS/cm.

Diastase activity

The determination of diastase activity using a standard solution of starch, capable of developing an iodine blue color within a defined range of intensity, is conducted by allowing the enzyme in the sample to act under standard conditions. The decrease in blue color is measured at intervals. A plot of absorbance against time, or a regression equation, is used to determine the time (tx) required to reach the specified absorbance of 0,235. This method is based on the original work of Schade, as presented by Codex Alimentarius. One challenge with methods for diastase activity is achieving accurate and precise analysis. For this reason, the certified reference material FAPAS T2848QC was used to calculate the precision, accuracy, and uncertainty of the proposed methods. The instrumental limits of detection (LOD (mg/L)) and quantification (LOQ) were calculated with respect to the maximum level specified in Regulation (EC) 2001/110/EC [8].

Hydroxymethylfurfural (HMF) content

The determination of HMF content is based on the determination of UV absorbance at 284 nm. In order to avoid the interference of other components at this wavelength. the difference between the absorbance of the clear aqueous honey solution and the same solution after the addition of bisulfite is measured. The HMF level is calculated after subtracting the background absorbance at 336 nm. A developed and fully validated method was applied for the determination of HMF content and diastase activity in honey samples. For the determination of HMF content, quantification was performed using the certified reference material FAPAS T2848QC with a certified value of 31,9$\pm$6,01 in order to avoid matrix interferences.

Total phenolic content (TPC)

The total phenolic content of the honey samples was determined with the Folin–Ciocalteu method as described by Gülçin et al. A 0,2 mL aliquot of honey stock solution was diluted with 3,16 distilled waters, then 0.2 mL of Folin-Ciocalteu reagent was added and mixed thoroughly. Three minutes later, 0,8 mL of Na_2CO_3 (7,5 %) was added and the mixture is followed by incubation at room temperature in the dark for 2 h with intermittent shaking. The absorbance readings at 760 nm were recorded. A standard curve was plotted using gallic acid to calculate the concentration of total phenolic compounds in the honey (mg gallic acid equivalent (GAE)/g honey).

Statistical analysis

All analyses were performed in triplicate. Statistical analyses were conducted using Minitab 17. A one-way analysis of variance (ANOVA) through the general linear model was applied ($\alpha = 0{,}05$). For comparison, significant differences ($p < 0.05$) between the means were further analyzed. All values were normally distributed ($p > 0.05$) according to the normality test, and thus, Pearson's correlation coefficient was applied to understand the relationship between TPC, HMF, and DN results.

Results and discussion

Palynological analysis is essential for differentiating honey produced in distinct geographical and climatic regions. Table 1 presents the frequency distribution of pollen from various plant species identified in the honey samples. The melissopalynological characteristics confirmed the floral origin of the majority of the samples. Out of 47 samples, 27 were classified as multifloral, containing a mixture of pollen from different plant species, with frequencies categorized as (S), (r), and (i). Twelve samples were identified as typical monofloral honey, with a dominant frequency (D) of *Castanea sativa* pollen, reaching approximately 77%. Three samples exhibited a significant presence of *Arbutus unedo* pollen, up to 72%, while two samples had a high frequency of *Erica* pollen (84%), and two contained *Salvia officinalis* pollen. In this study, monofloral honey was confirmed by the high pollen frequency (> 45%), which facilitated the identification of the specific plant species.

Table 1. Pollen analysis of honey samples

Nr. samples	Honey type	Taxon	Pollen frequency
27	Polyfloral	*Quercus(s), Castanea (r), Trifolium(r)*	(S), (s), (r)
12	Monofloral	*Castanea Sativa (D),* Allium (s), Arbutus (r)	77%
3	Monofloral	*Arbutus Unedo (D), Helianthus Galega*	72%
2	Monofloral	*Erica (D), Arbutus (s) Crataegus (r)*	84%
2	Monofloral	*Salvia Officinalis (D, Robinia pseudoacacia (r)*	57%
1	Monofloral	*Rosaceae (D), Medicaago (r), Allium (r)*	55%
D: > 45% pollen; S: 16-45% pollen; s: 3-15% pollen; r: 1-3% pollen; i: >1% pollen			

Moisture

Honey with lower moisture content is more resistant to fermentation and spoilage by microorganisms, as it provides less favorable conditions for their growth. In the honey samples analyzed, moisture levels ranged from 15,86% to 22,05%, with an average of 17,57 ± 2,82% (Table 1). This complies with EU regulations [9], which stipulate a maximum moisture content of 20%. *Arbutus* honey exhibited the highest moisture average content (22,13 ± 2,4%), while chestnut honey had the lowest (15,86 ± 1,91%). These findings are in line with moisture content values reported by Kivrak et al. [4], Šarić et al. [10], and Zappala et al. [11] for multifloral honeys.

Free acidity

Free acidity plays an important role in determining the sensory and physical characteristics of honey, such as its color and conductivity. The acidity levels of the honey samples, detailed in Table 2, averaged 22,65±6,42 meq/kg. Among the varieties, multifloral and *Arbutus* honeys showed the highest levels of acidity. All the acidity values recorded were within the EU's maximum permissible limit of 50 meq/kg for honey [9].

Table 2. Moisture, Free acidity, RI and Electrical Conductivity of honey samples

		MIN	MAX	Mean value
Humidity %	Polifloral	13.06	19.1	16.11±1.94
	Chestnut	13.1	21.8	15.8±2.64
	Arbutus	19.7	24.5	22.13±2.4
	Salvia	17.4	17.6	17.5±0.14
	Erica	13.33	17.3	15.32±2.81
	Mean ± STD	13.1	24.5	17.57±2.78
Free Acidity	Polifloral	27.33	28.29	27.81±0.68
	Chestnut	8.11	37.15	21.06±9.38
	Arbutus	16.22	29.94	24.23±7.14
	Salvia	9.54	15.12	12.33±3.95
	Erica	27.33	28.29	27.81±0.68
	Mean ± STD	4.55	11.33	22.65±6.42
EC mS/cm	Polifloral	0.325	1.433	0.775±0.372
	Chestnut	0.26	1.349	0.736±0.375
	Arbutus	0.268	1.511	0.770±0.064
	Salvia	0.329	1.57	0.949±0.878
	Erica	0.347	0.593	0.47±0.174
	Mean ± STD	0.13	1.57	0.740±0.17

Electrical conductivity (EC)

The electrical conductivity (EC) values of the honey samples are presented in Table 2. The type of honey significantly influences its EC value. Among the samples, *Trifolium* honey exhibited the lowest EC value (0,168 ± 53,74 mS/cm), while the other honey types showed similar EC values, ranging from 0,582 mS/cm for *Thymus* honey to 0,949 mS/cm for *Salvia* honey.

EC values are directly related to the mineral and acid content of honey and are commonly used to determine its botanical origin. For example, although there are exceptions for certain honey types, chestnut and honeydew honeys are generally expected to have EC values above 0,8 mS/cm, according to EU regulations [9]. Thrasyvoulou and Manikis [12] and Šarić et al. [10] reported average EC values of 1,54 and 1,27 mS/cm for Greek and Croatian chestnut honey, respectively. The average EC value for our chestnut honey samples (0,736 mS/cm) does not fully comply with these EU standards [9], though some samples reached values as high as 1,349 mS/cm.

Diastase activity

Together with HMF content, diastase activity is an indicator of honey freshness [40]. As shown in Table 3, the honey samples exhibited a wide range of diastase numbers (3,00–43,6). All our samples had diastase numbers higher than the minimum limit of 8 Shade units, with an average value of 21,66 ± 4,802. The highest diastase activity was observed in *Salvia officinalis* monofloral honey (25,80 ± 18,81). This result is reasonable, considering that *Salvia officinalis* is a potent plant, and the honey is harvested from hives located at high altitudes, where temperatures are relatively lower. Chestnut honey had significantly lower diastase activity (15,16 ± 12,62) compared to *Salvia officinalis* and multifloral honey (20,90 ± 16,73). However, *Salvia* honey showed the highest diastase activity, followed by multifloral and *Thymus* honey. These differences could be attributed to various factors, but we believe the most significant are the species of bees and the type of plants in the foraging area.

Table 3. Diastase activity. HMF aand TPC content of honey samples

		MIN	MAX	Mean value±SD
HMF	Polifloral	0.06	101.8	36.21±30.61
	Chestnut	0.3	169.46	38.63±49.21
	Arbutus	21.56	73.46	44.79±26.38
	Salvia	32.62	36.38	34.51±2.66
	Erica	33.46	51.52	42.49±12.77
	Mean ± STD	0.10	169.46	42.4±8.08
DN	Polifloral	3.50	43.6	20.90±16.00
	Chestnut	3.30	36.8	15.16±12.62
	Arbutus	3.00	42.1	19.87±16.73
	Salvia	12.5	39.1	25.80±18.81
	Erica	14.4	15.6	15.00±0.85
	Mean ± STD	3.00	43.6	19.35±4.49
TPC	Polifloral	0.122	0.269	0.183±0.038
mgGAE/g	Chestnut	0.071	0.258	0.148±0.058
	Arbutus	0.012	0.174	0.073±0.089

	Salvia	0.164	0.254	0.214±70.92
	Erica	0.034	0.114	0.074±0.056
	Mean ± STD	0.012	0.269	0.168±0.037

Hydroxymethylfurfural (HMF) content

The HMF content of the honey samples is presented in Table 3. Since fresh honey contains no HMF, the presence of HMF is commonly used as an indicator of freshness [45, 46]. The HMF content is significantly influenced by the type of honey. As shown in the table, the average HMF level across all honey types (42,4 ± 8,01 mg/kg) is close to the maximum limit set by EU standards [9] for HMF, which is 40 mg/kg. Out of the 47 samples, 30 had HMF values within the EU standard. However, the remaining 17 samples, all of which were multifloral honey, exhibited HMF levels above the permitted limit, starting from 43,6 mg/kg. The lowest average HMF value was found in *Salvia officinalis* mono-floral honey (34,5 ± 2,66 mg/kg), while the highest average was observed in mono-floral chestnut honey (44,79 ± 26,38 mg/kg). Interestingly, despite chestnut honey generally having lower HMF values, one chestnut honey sample from northern Albania, which is highly regarded in the country, showed the highest recorded HMF level (169,46 mg/kg). This region is a major producer of chestnut honey in Albania. The elevated HMF value in this case could be attributed to the rapid increase in honey production in recent years, potentially leading to improper storage at temperatures exceeding 20°C.

In a study by Chakir et al. [13], 17 Moroccan multifloral honey samples had an average HMF level of 12,91 mg/kg. Similarly, Can et al. [5] reported that the HMF content of acacia honey (12,56 mg/kg) was higher than that of chestnut (9,28 mg/kg) and rhododendron (3,20 mg/kg) honey. Compared to these studies, the HMF values in our 47 honey samples are significantly higher, even though the honey samples were sourced from trusted producers. As observed, HMF content in honey exhibits considerable variability, and it is difficult to draw definitive conclusions unless all honey samples are collected and stored under the same conditions.

Total Phenolic Content

The total phenolic content (TPC) varied significantly among the six types of honey analyzed (Table 3). In the 47 honey samples, TPC ranged between 0,012 and 0,214 mg GAE/g (Table 3). Based on their phenolic content, the honey samples were ranked as follows: *Salvia* > *Polyfloral*> *Chestnut Ericacea* > *Arbutus.* The average TPC for all types (0,168 ± 0,037 mg GAE/g) was similar to averages reported in other countries. For example, Czipa et al. [41] recorded an average TPC of 0,164 mg/g in Hungarian acacia honey. In a related study, Bertoncelj et al. [54] found an average TPC of 0,05 mg/g, while Can et al. [5] and Kivrak et al. [4] reported averages of 0,16 mg/g and 0,19 mg/g for acacia honey. The TPC levels in our honey samples are consistent with these findings. Among the types, *Arbutus* honey had the lowest TPC (0,073 ± 0,089 mg GAE/g), followed by Erica (0,073 ± 0,056 mg GAE/g), while *Salvia officinalis* honey exhibited the highest value (0,214 ± 0,070 mg GAE/g) [18, 38]. These differences are likely due to the seasonal and regional variability in the honey samples.

Conclusions

The aim of this study was to assess the quality of 47 monofloral and multifloral honey samples for potential use in wound healing, based on their physicochemical properties and total phenolic content, while highlighting some specific factors that need to be considered. Monofloral honey, such as that from *Salvia officinalis, Chestnut and Arbutus unedo,* is often regarded as higher quality. This research outlines the essential properties that distinguish honey samples with high phenolic content and diastase activity, which are critical for therapeutic applications, particularly in wound care. Based on previous studies, it can be concluded that while seasonal and regional variations impact honey quality, each type of honey possesses unique characteristics that support its potential in therapeutic treatments. Notably, monofloral *Salvia officinalis* honey fully satisfied the required standards for low HMF values (34,5$\pm$2,66 mg/kg). high diastase activity (25,8$\pm$ 8,81). and elevated phenolic content (0,213$\pm$0,071 mg/100g GAE/g). Meeting these criteria suggests that the honey is of high quality and holds significant potential for use as a therapeutic product.

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PHYSICOCHEMICAL AND MICROSTRUCTURAL CHARACTERIZATION OF BACTERIAL NANOCELLULOSE OBTAINED BY *KOMBUCHA* WATERMELON RIND FERMENTATION

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Introduction

In recent years, more research has focused on developing sustainable biomaterials that use renewable resources, including incorporating living biological systems. One of the best biomaterials is bacterial nanocellulose (BNC). Research has shown that agricultural waste can be a cheap and sustainable way of synthesizing BNC, which simultaneously meets the ecological requirements for the disposal and reuse of vegetable residues. BNC is a multifunctional biopolymer with high crystallinity, specific high degree of porosity, purity, relatively high permeability for liquids and gases, extremely high water absorption capacity, structural strength, ultrafine network structure, as well as biodegradability [1].

This study investigated two media based on three different carbon sources for BNC synthesis. The sugars included glucose, fructose, and sucrose in standard HS (Hestrin-Schramm) chemically defined media, each in the concentration of 30 g/L and watermelon rinds complex medium (WMR) with 25 g/L glucose, 21 g/L fructose, and 6 g/L sucrose. During the 12 days of fermentation with *Komagateibacter xylinus* DSM 2004, the production of organic acids (acetic and gluconic) was measured. BNC yield, pH changes, and residual sugar concentrations were monitored. The scanning electron microscopy (SEM) analysis covered microstructural BNC pellicle morphology and Fourier-transform infrared spectroscopy (FT-IR) chemical composition and crystallinity. The present study showed that the WMR medium provides all nutrients required for *K. xylinus* growth, supporting the synthesis of BNC with high yield and properties similar to those produced by BNCs with commercially available nutrients.

Materials and Methods

Chemicals: Glucose, fructose, saccharose, and mannitol were purchased from (GRAM MOL, Zagreb, Croatia), yeast extract and peptone (Biolife, Milano, Italy), Na_2HPO_4, citric acid and agar (Merck, Darmstadt, Germany), acetic acid and $CaCO_3$ (Kemika, Zagreb, Croatia).

Media: The HS [2] chemically defined media for growth and BNC production consisted of (g/L): glucose (HS-Glc), fructose (HS-Fru), and sucrose (HS-Suc) 30; peptone 5.0; yeast extract 5.0; Na_2HPO_4 2.7 and citric acid 1.15. The pH of the media was adjusted to 5.0 with acetic acid. The YPM medium for the growth of *K. xylinus* consisted of (g/L): mannitol 25; yeast extract 5.0; peptone 3.0, and the GYC medium used for the growth and maintenance of *K. xylinus* consisted of (g/L): glucose 20; yeast extract 10, and $CaCO_3$ 20 [3]. Complex medium with watermelon rind (WMR) consisted of (g/L): glucose 25; fructose 21; sucrose 6; N and P sources were from the watermelon rind.

Bacterial strain: Frreze-dried culture of *Komagataeibacter xylinus* DSM 2004 was initially obtained from the Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig, Germany).

Maintenance of *K. xylinus*: According to the manufacturer's instructions, a commercial pure freeze-dried culture of *K. xylinus* was activated in a 5 mL liquid YPM culture on a rotary shaker at 25 °C and 140 rpm for seven days of cultivation in aseptic conditions. 1 mL of the grown culture was inoculated onto Petri plates with GYC agar using the hair method, and these plates were placed in a thermostat for eight days at 25 °C. After that, 10^8 CFU/mL were inoculated from the grown plates into 100 mL of liquid YPM culture and grown again on a rotary shaker at 25 °C for five days. The culture grown in this way was used in further research.

Preparation of complex medium (WMR): The watermelons were grown in the Neretva Valley, harvested at the end of May 2024, and purchased at a local store in Zagreb. Watermelons were thoroughly washed under running water to remove soil and impurities and rinsed twice. After they were cut into slices, the pulp with the stones was removed entirely with a knife, down to the white part of the pulp and the rind. To prepare the WMR medium, 200 g of WMR was suspended in 500 mL of water and ground with a blender. 480 mL of a homogeneous mixture was thus prepared, and this procedure was repeated three more times. Four unique WMR homogenates were combined and then mixed. The resulting homogenate was passed through three layers of cheesecloth to remove coarse particles. The filtrate was filtered through filter paper, separating the clear solution from the remaining solid particles. The clear solution was sterilized by autoclaving for 20 minutes at 121 °C and was then used as a nutrient medium in further research.

***Kombucha* fermentation and BNC synthesis:** The pH of each media was adjusted to 5.0 using 0.1 M NaOH or 0.1 M HCl. All media were sterilized and inoculated with 10 mL of pre-grown pure *K. xylinus* culture and 10% (v/v) pre-fermented media. Erlenmeyer flasks of 300 mL containing 100 mL of medium were covered with sterile gauze to ensure aerobic conditions. Fermentation was statically carried out at 28 °C for 12 days in aseptic conditions. Sintetized pellicles floated on an air-liquid surface were harvested every 48 h to determine the mass of BNC (g). The BNC produced (g/L) in media with the associated carbon source concentration was selected for further characterization. Overheated substrates were centrifuged at 2500 rpm for 10 minutes, and the supernatants were used for further determinations. Experiments were performed in triplicate.

Total acidity and pH determination: Samples were titrated with 0.1 M sodium hydroxide until the pH reached 8.0 [3]. pH of samples was measured using a calibrated pH meter (Hanna Industrial HI 98103).

Determination of sugar and acid concentrations: The concentrations of residual sugars (glucose, fructose, and sucrose) and occurred acids (acetic acid and gluconic acid) were determined by HPLC (Shimadzu, Japan) using a Supelcogel C610H column (300 mm × 7.8 mm; Supelco Analytical, Bellefonte, PA, USA) and guard column (Supelcogel H Guard Column, 50 mm × 4.6 mm). Analytes were eluted isocratically with 0.1% phosphoric acid at a flow rate of 0.5 mL min^{-1} and 55 °C. A RID detected and quantified residual sugars (Shimadzu RID-10). Organic acids were analyzed using a UV/Vis detector (Shimadzu SPD-M10Avp Diode Array Detector, Shimadzu, Kyoto, Japan) [4].

Microstructural characterization: BNC pellicle samples were air-dried on glass Petri dishes in a thin film and cut into square slices (1 x 1 cm). The analysis of crystallinity was carried out by Fourier-transformed infrared spectroscopy (FT-IR; Bomem MB 100 mid FT). A total of 400 scans were taken with a resolution of 2 cm^{-1} in order to produce the spectra. The BNC network and distribution of AAB and yeasts were observed by scanning electron microscope (SEM; TescanVega 3 Easyprobe). Each sample was coated with gold and palladium, and a microscope operating at 5 kV was used to examine the samples.

Results and discussion

Fig. 1A shows changes in pH in *kombucha* using different carbon sources as substrates during fermentation. The initial pH value of all tested samples was 5.0, with a noticeable drop to pH 3.6 and 3.75 in media with glucose and sucrose. In contrast, after four days of fermentation in media with fructose, the pH value dropped slightly to 4.6. In the media with watermelon rind (WMR), the pH value decreased slightly until the end of fermentation (12 days), when the measured pH value was 3.55. After the eighth day, the drop in pH value in all three chemically defined HS substrates became significantly slower. In Fig. 1A, pH stagnation is visible until the end of the fermentation. The acidity of the nutrient medium is reduced when the pre-fermented mother liquor from the previous fermentation is added during the BNC inoculation, which prevents the appearance of mold and enables protection against undesirable microorganisms [5]. In the WMR medium, total acidity (%) increased with time from 3.1% to 9.64% at the end of fermentation (Fig. 1B). It was previously reported that a rapid decrease in pH during fermentations does not increase BNC production but is mainly due to increased concentrations of organic acids as primary metabolites [6]. During the fermentation, the concentration of acetic acid process in the WMR medium increased with the glucose consumption by AAB. Despite the constant but slow growth from the beginning of cultivation (0.21 g/100 mL), the final concentration of acetic acid was 1.55 g/100 mL at the end of the process. Gluconic acid was the second major organic acid found in kombucha metabolism, but it was formed in lower concentrations than acetic acid (0.85 g/100 mL).

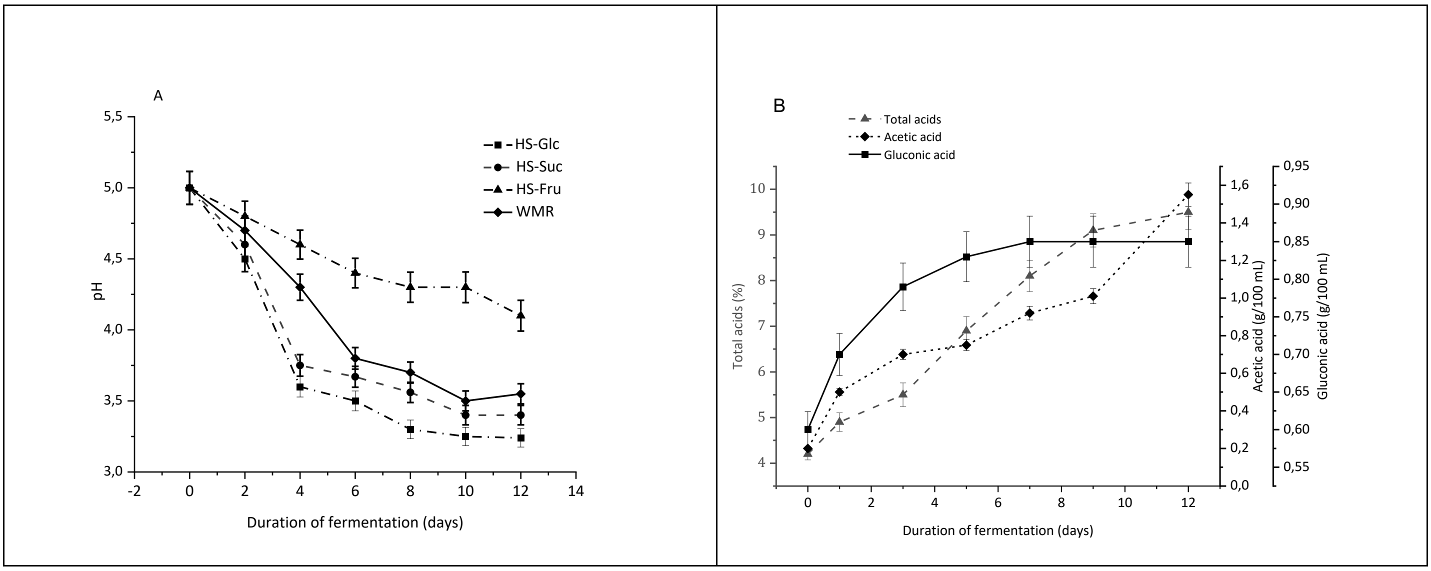

Figure 1. A) Changes during 12 days of *Kombucha* fermentation in A) pH of media with different carbon sources (HS and WMR) and B) acetic and gluconic acids contents during in WMR medium

Exopolysaccharide (BNC) secretion is usually most noticeable when the producer bacteria have abundant carbon and minimal nitrogen sources [7]. The effect of different carbon sources from a chemically defined (HS) nutrient medium, mono-saccharides (3% (wt/vol) glucose and 3% (wt/vol) fructose) and disaccharides (3% wt/vol) sucrose), on the yield of produced BNC is shown in Fig. 2A. Because different bacteria have different enzymatic and metabolic systems, they can use different carbon sources for growth and BNC production with different efficiency. High BNC yields were achieved in HS media with sucrose (11.56 g/L) and glucose (10.34 g/l). In comparison, fructose as a C-source yielded a much lower yield (6.9 g/L) (Fig. 2A). The results of research on a complex (WMR) medium with 6 g/L sucrose, 25 g/L glucose, and 21 g/L fructose as the main carbon sources showed that the content of fructose during fermentation was lower than that of glucose suggesting that yeast cells preferred fructose as a carbon source (Fig. 2B). As a result, the WMR-BNC yield was 9.5 g/L, which presents a higher yield than BNC-Fru from HS medium but lower than HS-Suc and HS-Glc BNC yields. Research by Neffe-Skocinska et al. [8] also showed that after a 23-hour *kombucha* tea fermentation, a significant part of fructose (88%) and only 40% of glucose were metabolized into ethanol. AAB and yeast cells use substrates for fermentation in a WMR medium in complementary but different ways. Until today, the details of the precise, complex mechanism of biochemical changes during the *kombucha* fermentation of sugary nutrient media with the yeast-AAB symbiotic system have not been fully elucidated.

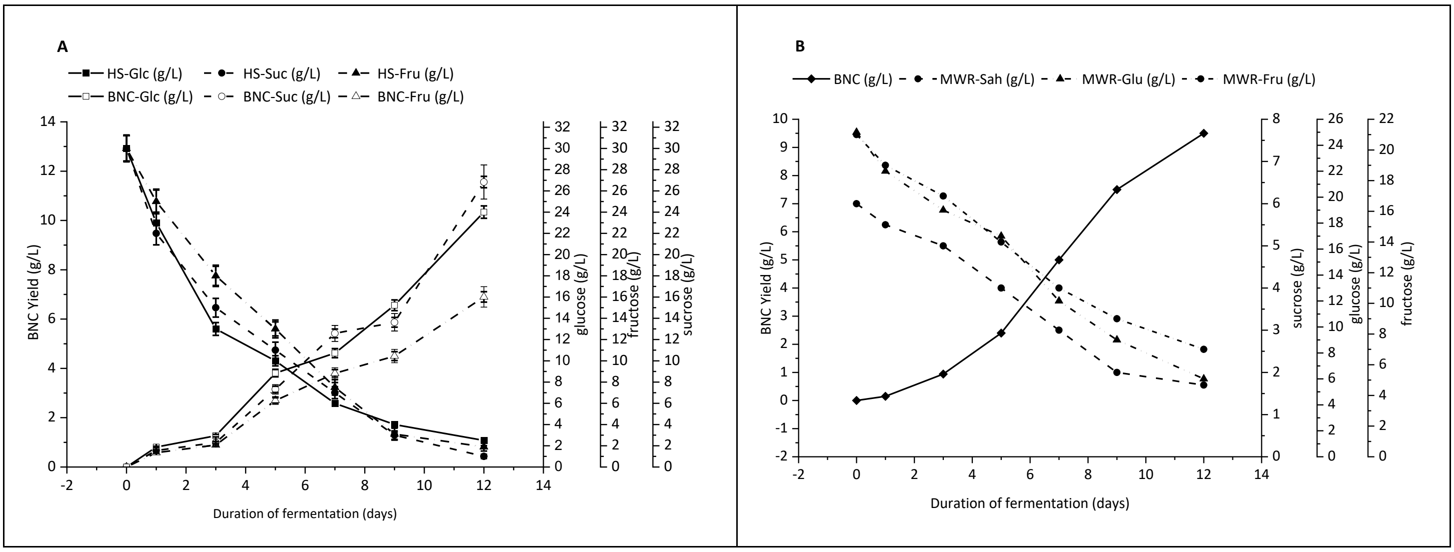

Figure 2. Dynamics of sugar consumption and BNC synthesis in modified HS nutrient medium A) and WMR medium (B)

Fig. 3 illustrates the thickness of the synthesized BNC pellicle (mm) according to the added carbon source. The first thin visible layer of synthesized pellicle was observed already on the first day of cultivation, with a tendency to constantly increase the height of the BNC layer until the end of cultivation. The BNC pellicle's highest synthesis rate and thickness were measured in the HS-Suc medium, where the thickness after 12 days of cultivation was 25 mm. BNC synthesized in HS-Glc medium has a 22 mm measured pellicle. In comparison, the HS-Fru had the most negligible pellicle thickness at the end of cultivation (18 mm), with a slight stagnation in growth on the 11th day of cultivation. The thickness of the WMR-BNC pellicle increased linearly until the end of cultivation when the measured thickness was 21 mm.

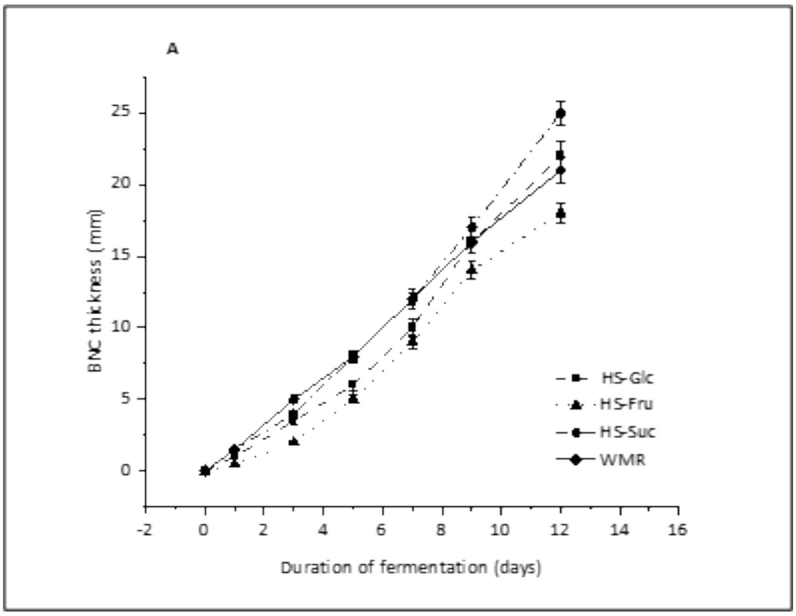

Figure 3. Kinetics of BNC pellicle synthesis in HS and WMR nutrient media

The macroscopic appearance of BNC pellicles synthesized on WMR and HS substrates was similar, visible in the form of gelatinous pellicles formed layer by layer on the surface of the liquid phase of the culture medium. BNC produced by static cultivation is a hydrogel membrane with a three-dimensional (3D) mesh structure that can be observed under an electron microscope [9]. Morphological structures of the produced WMR-BNC pellicles were studied using scanning electron microscopy (SEM) (Fig. 4). Fig. 4-left micrograph shows spherical yeast cells and rod-shaped AAB cells. Bundles of nanocellulose microfibrils were observed directly from the cell surface, forming a 3D- envelope at the air-liquid interface (Fig. 4-right).

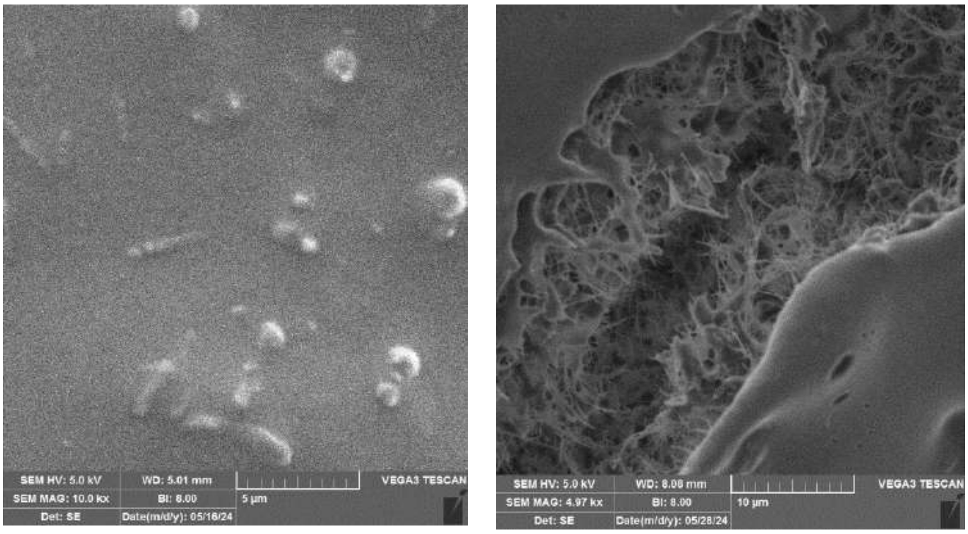

Figure 4. SEM micrographs of a BNC synthesized with visible microbiota embedded within the nanocellulose network of pellicle (left) and 3D nanocellulose network of BNC synthesized after 12 days of fermentation in WMR nutrient medium (right)

FT-IR spectroscopy of WMR-BNC is presented in Fig. 5. A broad absorbing band observed around 3424 cm^{-1} corresponds to the O-H stretching vibrations of the BNC cellulose I. At 2924

cm^{-1}, C-H stretching was observed. The two bands in all studied C-source in WMR medium at 1625 and 1751 cm^{-1} are due to the H-OH bending vibration of absorbed water molecules in BNC. The most complex region of the BNC FT-IR spectrum is the region that ranges from 1430 cm^{-1} to about 850 cm^{-1}. The characteristic signal at 1427 cm^{-1} is attributed to the symmetric stretching of CH_2. According to literature data, the absorption band at 1427 cm^{-1} is attributed to highly ordered (crystalline) areas of the BNC pellicle [10]. The band at 1054 cm^{-1} corresponds to the primary alcohols' C-OH stretching vibration [4].

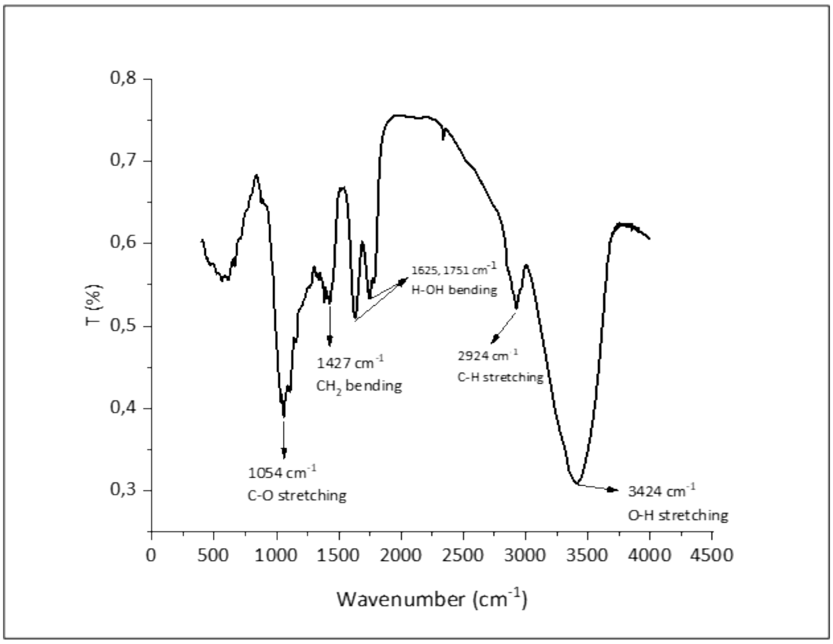

Figure 5. FT-IR spectra of BNC synthesized after 12 days of fermentation in a WMR medium

Conclusions

The investigation elucidated the impact of the carbon source in the nutrient media on BNC yield, structure, and properties. Changes in pH result from the symbiotic metabolic activities of yeasts and AAB, and the formation of organic acids decreases it. The thickness and yield of BNC increased with fermentation time (12 days). Using *K. xylinus* DSM 2004, the sugar mixture from WMR complex nutrient medium gave a relatively high BNC yield (9.5 g/L) harvested on the 12th day of incubation time. As expected, analyzed samples by FT-IR and SEM showed a higher percentage of crystalline regions than amorphous ones. Watermelon rinds proved to be an excellent nutrient substitution for BNC production compared to the standard HS medium. The results obtained in this study showed that some agro-industrial wastes, such as watermelon rinds, can serve as low-cost carbon sources for BNC production.

Acknowledgements

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INFLUENCE OF *Melissa officinalis* L. EXTRACT CONCENTRATION ON BIOSYNTHESIS OF IRON NANOPARTICLES

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Introduction

Green synthesis of nanoparticles uses a precursor and a certain type of biological material, whereby biological materials play the role of a catalyst [1]. In green synthesis, the most commonly used materials are plant extracts, various bacteria, yeasts, algae or fungi, which are rich in bioactive components needed for synthesis, such as polyphenols. In general, plant extracts are often used due to several benefits: (i) plants are cheap and readily available; (ii) synthesis using plant extracts does not require harsh reaction conditions or excessive use of harmful chemicals; and (iii) the reaction is quick and simple [2]. Lemon balm (*Melissa officinalis* L.) belongs to the Lamiaceae family, and it is known as a very adaptable wide spread plant with numerous beneficial health effects. Furthermore, phenolic substances such as flavonoids, tannins and phenolic acids present in lemon balm have the capability to reduce metallic ions to nanoparticles [3, 4]. Due to the above stated, *Mellisa officinalis* L. aqueous extract was chosen for green synthesis of iron nanoparticles in this research. Due to their magnetic properties, high stability, good adsorption and low toxicity, iron nanoparticles have found their application in various fields such as agronomy, water treatment, biomedicine and environmental protection [5]. In the food industry, they are used in food analysis, protein purification and enzyme immobilization, while in the pharmaceutical industry they are used in the diagnosis and treatment of diseases [6,7]. The reaction parameters that affect the quantity and properties of the synthesized nanoparticles are the concentration of the precursor, the concentration of the extract (bio-catalyst), the temperature and the duration of the extraction [1,8]. Furthermore, the pH value affects the stability of the synthesized nanoparticles and their size. Research has shown that lower pH values favor the creation of more stable and smaller nanoparticles, while organic acids additionally stabilize the particles [4]. The reaction duration is also a very important factor since there is possibility of nano particle aggregation into larger clusters [9,10]. The influence of temperature is more pronounced on the quality of bioactive compounds due to their degradation at elevated temperatures [8,11], while the optimal temperature and pH depend on the type of plant or other biocatalyst and its chemical composition [1]. The concentration of the extract also directly affects the synthesis process, mostly in the area of particle size - the higher the concentration of the extract, the larger the nanoparticles and the particles become more susceptible to forming large clusters due to strong connective forces [8].

This study investigated the influence of lemon balm extract concentration (ranging from 2.5 to 100 %) on the biosynthesis of iron nanoparticles from a 0.1 M $FeCl_3$ precursor solution. Different properties of the reaction mixtures as well as the resulting powders were determined: pH, conductivity, color, FTIR, UV-Vis spectroscopy, and the hydrodynamic diameter of particles. Furthermore, the total amount of polyphenols in the extract was determined using the Folin-Ciocalteu reagent and the antioxidant capacity using the DPPH method.

Materials and methods

4.1. Extract and precursor solution preparation

To prepare the aqueous extract, 12 g of dried *Melissa officinalis* L. leaves (Suban, Croatia) were mixed with 360 mL of distilled water at a temperature of 80 °C for 30 minutes, with a stirring speed of 350 rpm in a water bath (IKA HBR 4 digital, IKA-Werk, Staufen, Germany). The extract was filtered using a 13 µm pore size cellulose filter paper and the ChromafilXtra H-PTFE membrane filter with a pore size of 0.2 µm. The filtered extract was then diluted to the following concentrations: 2.5, 5, 10, 25, 50, 75 and 100 %. The precursor solution was prepared by dissolving iron (III) chloride 6-hydrate in distilled water to obtain 0.1 M solution.

4.2. Iron nanoparticle synthesis

The synthesis of iron nanoparticles was conducted by mixing solutions of precursors and extracts in a ratio of 1:4 at room temperature, with uniform stirring at 100 rpm. The color change of the reaction mixture from light yellow to dark blue/gray was considered to be an indicator of nanoparticle synthesis. The supernatant was then separated from the reaction mixture, while the precipitate containing nanoparticles was dried in a laboratory dryer (InkoLab ST60T, Croatia) at 105 °C until constant mass.

4.3. Physical properties of the solutions, reaction mixture and nano powders

The color was measured using a colorimeter (PCE-CSM 3, PCE Instruments, Germany) according to Hunter's scale. The pH value and conductivity were determined using a pH/conductivity meter (914 pH/Conductometer, Metrohm, Switzerland), in triplicate. The hydrodynamic diameter was determined using a Litesizer 500 device (Anton Paar, Graz, Austria). Reaction solutions containing nanoparticles were diluted 100 times with distilled water prior to measurements. Measurements were performed in triplicate.

4.4. Spectral analysis

To assess optical characteristics of the nanoparticles, continuous UV-Vis spectrum was recorded on an UV1601 spectrophotometer (Shimadzu, Japan) in a wavelength range 190 – 800 nm. Solutions were diluted 100 times before measurement. FTIR spectrum was recorded on a Spectrum one spectrophotometer (PerkinElmer, USA), in the range of 4000 cm^{-1}- 450 cm^{-1}. Samples used for FTIR analysis were powdered after drying, mixed with KBr in a ratio 1:100 in favor of KBr and measured using the ATR module. Measurements were performed in triplicate.

4.5. Chemical properties of the extracts and the reaction solutions (supernatants)

Chemical properties were measured for the extract prior to synthesis and for the supernatant separated from the reaction mixture after synthesis. Analyses were done in duplicate. Total polyphenol content (TPC) was determined by the spectrophotometric method using the Folin-Ciocalteau reagent as described by [12], while the antioxidant capacity (AOC) was determined by the DPPH scavenging method described by [13]. Measurements were performed in duplicate.

Results and discussion

To investigate the influence of extract concentration on the biosynthesis of iron nanoparticles, pH and conductivity of the extracts and the reaction mixtures was measured and the results are shown in Table 1.

Table 1. pH and conductivity of the extract before synthesis and the reaction mixtures after synthesis. Results are shown as mean ± standard deviation.

Sample	pH		Conductivity (mS cm^{-1})	
	Extract before synthesis	Reaction mixture after synthesis	Extract before synthesis	Reaction mixture after synthesis
2.5 %	5.73 ± 0.29	2.28 ± 0.11	0.25 ± 0.01	7.54 ± 0.38
5 %	5.79 ± 0.29	2.29 ± 0.12	0.32 ± 0.02	7.70 ± 0.38
10 %	5.77 ± 0.29	2.22 ± 0.11	0.49 ± 0.03	8.14 ± 0.41
25 %	5.87 ± 0.29	2.06 ± 0.10	0.96 ± 0.05	9.41 ± 0.47
50 %	5.85 ± 0.29	2.05 ± 0.10	1.62 ± 0.08	10.18 ± 0.51
75 %	5.80 ± 0.29	2.14 ± 0.11	2.28 ± 0.11	9.65 ± 0.48
100 %	5.83 ± 0.29	2.30 ± 0.12	2.92 ± 0.15	9.17 ± 0.46

The pH of the plant extract is slightly acidic, showing a range of values from 5.73 ± 0.29 (2.5 % extract) to 5.87 ± 0.29 (25 % extract) (Table 1). The pH after synthesis decreases sharply because a very acidic precursor solution with a pH value of 2 was added to the plant extract solutions, and the values range from 2.05 ± 0.10 (50 % extract) to 2.30 ± 0.12 (100 % extract). According to the conductivity values, with range from 0.25 ± 0.01 mS cm^{-1} (2.5 % extract) to 2.92 ± 0.15 mS cm^{-1} (100 % extract), it is evident that the conductivity of the extract solution before synthesis increases with increasing concentration. After mixing the extract solution with the precursor solution, conductivity increased compared to the extract itself, which is a result of conductive properties of the synthesized iron nanoparticles. The values range from 7.54 ± 0.38 mS cm^{-1} (2.5 % extract) to 10.18 ± 0.51 mS cm^{-1} (50 % extract). Conductivity increases by increasing the concentration of the plant extract up to 50 % where it reaches a maximum and after that the value drops slightly.

Table 2. Colour, mass and diameter of the powders

Sample	*L**	*a**	*b**	Chroma	Hue	Mass (g)	Hydrodynamic diameter (nm)
2.5 %	30.38±1.52	1.73±0.09	1.95±0.10	2.60±0.13	48.39±2.42	0.018±0.001	1120.47±43.28
5 %	30.65±1.53	1.50±0.07	2.00±0.10	2.50±0.12	53.14±2.66	0.025±0.001	1515.87±58.73
10 %	28.49±1.42	0.51±0.03	1.24±0.06	1.34±0.07	67.52±3.38	0.034±0.002	1889.85±69.55
25 %	26.41±1.32	-0.13±0.01	-1.02±0.05	1.03±0.05	262.76±13.20	0.050±0.002	5722.91±62.15
50 %	29.17±1.46	0.09±0.00	-0.32±0.02	0.34±0.02	285.97±14.30	0.093±0.005	2105.07±19.36

75 %	26.91±1.35	0.22±0.01	-0.07±0.00	0.23±0.01	343.47±17.17	0.137±0.007	2177.01±37.24
100 %	27.18±1.36	0.40±0.02	-0.05±0.01	0.41±0.02	352.62±17.63	0.166±0.008	1905.12±34.78

After synthesis, the powders were filtered, dried and their color, mass and hydrodynamic diameters were analyzed. *L** values ranged from 26.41 ± 1.32 (powders synthesized from 25 % extract) to 30.65 ± 1.533 (from 5 % extract). As a rule, the *L** value in all measurements decreases with increasing concentration, and the values do not exceed 50, which indicates a dark color of all the samples. The higher the concentration of the plant extract, the more nanoparticles were formed and the darker colored particles were detected. *a** values ranged from -0.13 ± 0.01 (powder synthesized from 25 % extract) to 1.73 ± 0.09 (from 2.5 % extract). All *a** values are positive, which indicates a presence of a red pigment, with the exception of dried nanoparticles obtained with a 25% extract, whose value is negative and indicates a transition from red to green color. The *b** values ranged from -1.02 ± 0.05 (powder obtained from 25 % extract) to 2.00 ± 0.10 (from 5 % extract). In the case of dried nanoparticles obtained from 25, 50, 75 and 100 %, the measured value is negative, which shows a transition from yellow to blue color. Chroma values were in the range from 0.23 ± 0.01 (75 % extract) to 2.60 ± 0.13 (from 2.5 % extract). Chroma values decreased with the increase of plant extract concentration. Hue values ranged from 48.39 ± 2.42 (powder from 2.5% extract) to 352.62 ± 17.63 (powder from 100 % extract). The shade of color (Hue) of dry nanoparticles increases at concentrations of 25, 50, 75 and 100%.

By increasing the concentration, the mass of the synthesized powder also increases (Table 2). This can be explained by the fact that a more concentrated extract contains a larger amount of bioactive compounds that are used in the synthesis of nanoparticles. The influence of extract concentration on the amount of synthesized nanoparticles was investigated by [14], who established that by increasing the concentration of bioactive compounds at a constant concentration of the precursor in the solution, the intensity of the spectral absorption increases, which is directly related to the amount of synthesized nanoparticles. The hydrodynamic diameter was in the range from 1120.47 ± 43.28 nm (particles from 2.5 % extract) to 5722.91 ± 61.6 nm (from 25 % extract). Particles from the most diluted plant extract had the smallest diameter, while the other concentrations ranged from 1500 nm to 2180 nm. Hydrodynamic diameter reaches the highest value at 25% extract and decreases with higher extract concentrations.

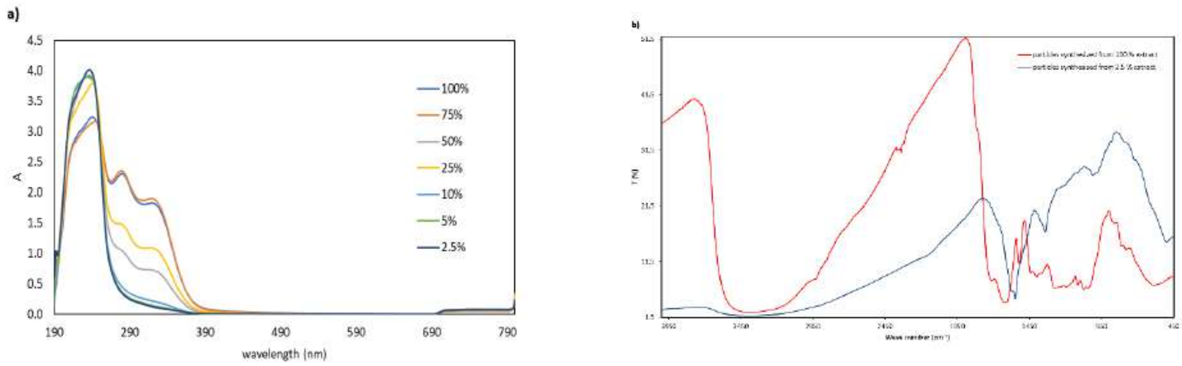

Figure 1. a) UV-Vis and b) FTIR spectra of the synthesized particles

At a wavelength of 280 nm (Fig. 1a), a peak characteristic of rosmarinic acid and a specific peak of iron at 325 nm are visible, which proves the formation of nanoparticles. In the wavelength range from 220 nm to 240 nm, there is a peak specific for the benzoyl group present in flavonoids [15]. Also, from the continuous UV spectrum, a reduction of the peak at a wavelength of 340-350 nm is visible, which indicates the consumption of bioactive compounds for the synthesis of nanoparticles. The mentioned peak is characteristic of flavonoids that act as phytoreducers in nanoparticle synthesis reactions [16, 17].

FTIR spectra (Fig. 1b) show a broad peak characteristic for water (3500 cm^{-1} to 3100 cm^{-1}), the peaks at 1610 cm^{-1} and 1437 cm^{-1} represent the carboxyl groups of phenols, the peak at 1020 cm^{-1} may represent the C-O-C bond or C-O stretching present in flavonoids, and the peak at 1260 cm^{-1} is specific for the carbonyl group. The peaks at 1608 cm^{-1} and 1550 cm^{-1} can be attributed to the vibrations of the aromatic C=C group. Also, a peak in the wavelength range of 500-580 cm^{-1} is characteristic of iron and iron oxides present in the synthesized particles, similar as in the research by [15] and [18].

Table 3. TPC and AOC values of the extracts and reaction mixtures, and the amount of bioactives used for synthesis and stabilization of nanoparticles

Sample	Extract TPC (before synthesis) (mg GAE L^{-1})	Reaction mixture TPC (after synthesis) (mg GAE L^{-1})	TPC used for synthesis (mg GAE L^{-1})	Extract AOC (before synthesis) ($mmol_{Trolox}$ L^{-1})	Reaction mixture AOC (after synthesis) ($mmol_{Trolox}$ L^{-1})	AOC used for synthesis ($mmol_{Trolox}$ L^{-1})
2.5%	75.88±2.28	52.35±1.57	23.53±0.71	0.47±0.02	0.27±0.01	0.20±0.01
5%	118.82±3.36	73.53±2.20	45.29±1.36	1.03±0.05	0.40±0.02	0.63±0.03
10%	205.88±6.18	74.70±2.24	131.17±3.93	1.45±0.07	0.58±0.03	0.87±0.04
25%	407.94±12.24	159.70±4.79	248.23±7.45	5.44±0.27	1.33±0.07	4.11±0.20
50%	1441.18±43.23	505.88±15.18	935.29±28.06	6.69±0.48	3.64±0.18	6.05±0.30
75%	1635.29±49.06	517.65±15.53	1117.65±33.53	16.85±0.84	4.35±0.22	12.50±0.62
100%	2129.41±63.88	658.82±19.76	1470.59±44.12	19.33±0.97	5.36±0.27	13.97±0.70

The TPC in the pure plant extract of Melissa officinalis L. (Table 3), is very high, which shows that lemon balm is an excellent source of reducing substances for the synthesis of nanoparticles [19]. The TPC for the pure extract ranges from 75.88 ± 2.28 mg GAE L^{-1} (2.5 % extract) to 2129.41 ± 63.88 mg GAE L^{-1} (100 % extract), and for the reaction mixture from 52.35 ± 1.57 mg GAE L^{-1} to 658.82 ± 19.76 mg GAE L^{-1}. After mixing the precursor solution with the plant extract, the highest utilization of polyphenols for biosynthesis can be seen for 100% extract (1470.59 ± 44.12 mg GAE L^{-1}), where, consequently, the highest amount of nanoparticles was formed. It can be seen that, at constant precursor solution concentration, the utilization of polyphenols rises with the rise in extract concentration. The AOC of the extract ranges from 0.47 ± 0.02 mmol L^{-1} (for 2.5 % extract) to 19.33 ± 0.97 mmol L^{-1} (for 100 % extract), while the values for the reaction mixture ranged from 0.27 ± 0.01 mmol L^{-1} (for 2.5 % mixture) to 5.36 ± 0.27 mmol L^{-1} (for 100 % mixture). The change in AOC during synthesis ranges from 0.20 ± 0.01 mmol L^{-1} (for 2.5 % extract) to 13.97 ± 0.70 mmol L^{-1} (for 100 %

extract). Similar as with the TPC, higher change in AOC is seen at higher concentration of the extracts used for synthesis.

Conclusions

The consumption of bioactive compounds with antioxidant activity and polyphenols for the synthesis of iron nanoparticles increases with the increase in the concentration of the extract. At a higher concentration of the plant extract, a larger mass of nanoparticles was formed, with the mass of the synthesized powder being proportional to the concentration of the plant extract.

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INHIBITION OF STEEL CORROSION IN HYDROCHLORIC ACID SOLUTION USING CHITOSAN AND CAFFEINE

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Introduction

Corrosion of metals can occur in various forms and in various conditions. The final result of the corrosion process may be the inapplicability of the metal and alloy product or construction, so it is necessary to apply suitable methods for protection the metal from corrosion. It is well known that the most stainless steels in the environment of a hydrochloric acid solution will corrode, because their chromium content is not sufficient to form a protective passive layer [1]. One of the effective ways to protect steel in acid solutions is the application of substances that slow down the corrosion process, i.e., corrosion inhibitors. Nowadays, pollution caused by the use of toxic corrosion inhibitors has led to the search for alternative, more environmentally friendly substances which can be used as corrosion inhibitors, so called green corrosion inhibitors [2]. Today, as corrosion inhibitors, substances that are environmentally acceptable and which, due to their chemical structure, possess good inhibition properties are the most tested, and some of these substances are chitosan and caffeine. Chitosan as a natural polymer has a wide range of applications due to its properties of biocompatibility, biodegradability and nontoxicity [3]. Caffeine is a naturally occurring molecule found in several foods, such as coffee and tea. Therefore, in this work, the inhibition efficiencies of chitosan and caffeine solution, on steel corrosion in a 10% hydrochloric acid was tested. Corrosion rates of steel in 10% hydrochloric acid without and with different concentration of tested inhibitors were determined by the gravimetric method at 25°C, 40°C and 60°C. Surface roughness values were determined on the all tested steel samples, while their surfaces were recorded with an optical metallographic microscope.

Material and Methods

The tests were carried out on alloy steel C. 4732 samples with diameter 18 mm and average thickness 5 mm. The chemical composition (wt.%) of the alloy steel is: C (0.38-0.45), Si (0.15-0.40), Mn (0.50-0.80), P (max. 0.035), S (max. 0.035), Cr (0.90-1.20) and balanced Fe. All samples were polished using SiC paper grit 1200 and diamond paste. After processing, all samples were degreased in 96% ethanol and dried. Corrosion tests were performed in a 10% hydrochloric acid solution, with the addition of different concentrations of chitosan and caffeine. Chitosan solutions were prepared by dissolving a certain mass of chitosan in 10% hydrochloric acid to obtain 0.2%, 0.5% and 1% chitosan solutions. Each chitosan solution was prepared by mixing on a magnetic stirrer and heated to 40°C for 3 hours, or until complete homogenization. Caffeine was added directly to the acid solution in concentrations of 0.2 g/L, 0.5 g/L and 1 g/L. Chitosan (low molecular weight) and caffeine (98%) were purchased by Sigma-Aldrich, Darmstadt, Germany. The corrosion rates of steel in a 10% hydrochloric acid solution without and with the addition of the mentioned different concentrations of tested inhibitors were determined by the gravimetric method at 25°C, 40°C and 60°C. Gravimetric measurement procedure is described previously [4]. The surface roughness of the steel samples was tested on a MITUTOYO SURFTEST SJ-210 portable surface roughness measuring device. The surfaces of all steel samples were recorded with a metallographic optical microscope Olympus Tokio, Epityp II, Type MO21 at a magnification of 250×.

Results and discussion

Table 1. shows the results of gravimetric tests, which are expressed as corrosion rates of steel (mg cm^{-2} h^{-1}) in hydrochloric acid solution without and with the addition of different concentrations of chitosan and caffeine.

Table 1. Corrosion rate data obtained from weight loss measurements for steel C.4732 in 10% HCl solution in absence and with addition of inhibitors at different temperatures, after 2 h immersion time.

Inhibitor	Inhibitor conc.	Corrosion rate, w (mg cm^{-2} h^{-1})		
		25°C	40°C	60°C
Chitosan (%)	0	1.158	3.502	8.483
	0.2	0.658	1.783	3.806
	0.5	0.501	1.267	2.125
	1.0	0.366	0.946	1.855
Caffeine (g/L)	0.2	0.875	3.055	8.252
	0.5	0.516	2.741	6.208
	1.0	0.275	2.150	4.922
Chitosan 0.5% + Caffeine (g/L)	0.2	1.066	2.732	5.855
	0.5	0.458	2.012	4.624
	1.0	0.401	1.670	3.183

The results shown in Table 3 indicate that the corrosion rate of steel in a 10% HCl solution decreases with an increase in the concentration of chitosan, and the lowest values were obtained at the highest concentration of 1% at all tested temperatures. As expected, corrosion rates increase significantly with increasing solution temperature [5]. Similar results were observed with caffeine and the mixture of chitosan and caffeine, although the values of the corrosion rate in the case of chitosan do not show a significant increase at elevated temperatures. In order to better determine the inhibition properties of the investigated compounds, the values of their inhibition efficiency (η) were calculated and expressed in (%). The mentioned results are shown in Figure 1. In Figure 1, the values of inhibition efficiencies are given for inhibitor concentrations of 1% for chitosan, 1 g/L for caffeine and the mixture of 0.5% chitosan and 1 g/L caffeine. From Table 1 and Figure 1, in the case of chitosan, it can be observed that the degree of protection increased with increasing temperature, which means that chitosan shows a better degree of protection at higher temperatures. This behavior of chitosan can be attributed to the fact that chitosan is more soluble in acids at elevated temperatures, where cross-linking of the structure occurs [6].

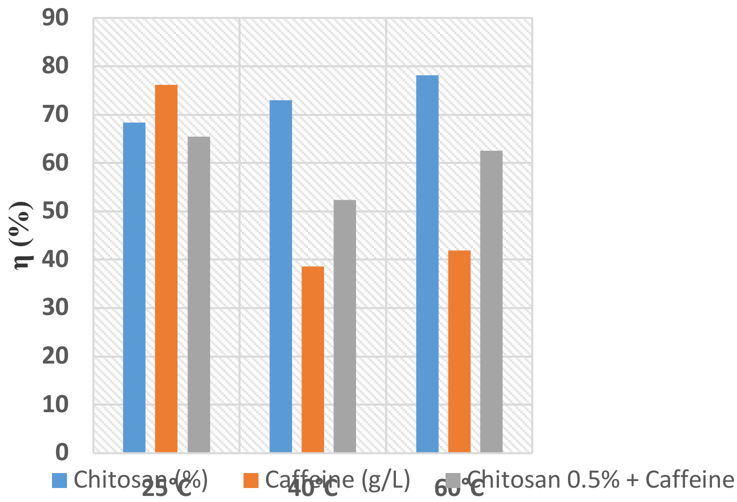


Figure 1. Inhibition efficiencies values (*η*) obtained after 2 h immersion in 10% HCl solution in absence and with addition of tested inhibitors in concentration of 1% and 1 g/L, respectively at different temperatures.

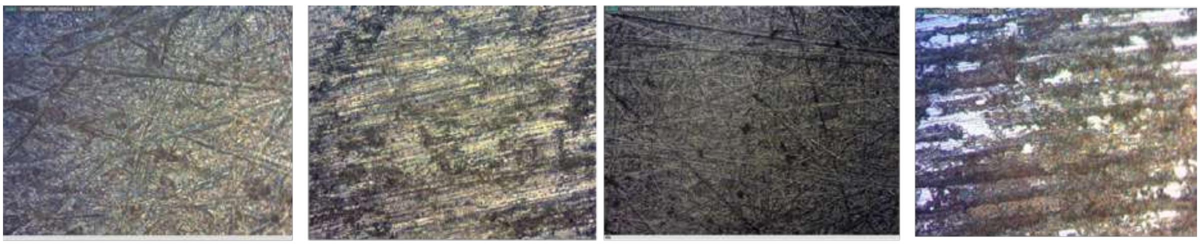
On the other hand, when testing caffeine as an inhibitor for steel corrosion in HCl solution, it is evident that the corrosion rate decreases with an increase in caffeine concentration, but at elevated temperature, caffeine showed that its inhibition effect, i.e. the degree of protection, does not increase as is the case with chitosan. The above indicates that the caffeine as a corrosion inhibitor is more efficient at room temperature with a maximum inhibition efficiency of 76%. The combination of caffeine and chitosan in a concentrations of 1 g/L and 1%, results in levels of effectiveness that are between the values obtained for pure inhibitors. Moreover, from Figure 1., it is clearly visible that the addition of chitosan to a solution with caffeine can reduce the corrosion rate of steel at elevated temperatures compared to caffeine alone. These results show that caffeine has a better protective effect on steel corrosion than chitosan, but only at room temperature, while the inhibition effect of chitosan is significantly better at elevated temperatures.

Table 3. Maximum values of surface roughness obtained as R_a parameter on steel samples after exposure in 10% hydrochloric acid in absence and with the addition of tested inhibitors at different temperatures.

Solution		**R_a (μm) maximum value**		
		25°C	40°C	60°C
10% HCl		6.732	8.765	11.50
Chitosan (%)	0.2	4.235	7.102	9.870
	1	3.841	4.815	6.452
Caffeine (g/L)	0.2	5.825	7.223	10.98
	1	4.003	6.455	8.818

Chitosan 0.5% + Caffeine (g/L)	0.2	5.076	4.865	7.226
	1	4.525	4.021	5.822

Table 3. show the maximum values of surface roughness obtained as R_a parameter on steel samples. The surface roughness of the steel samples was measured before exposure to the 10% hydrochloric acid solution, and after 2 hours of exposure to the acid without and with the addition of tested inhibitors in the lowest and highest concentrations. The results are given as the R_a value (µm), the vertical roughness parameter that represents the mean arithmetic deviation from the profile. The mean value is calculated on the unit length of the surface from the total amounts of the roughness amplitudes [7]. Obtained values of surface roughness clearly indicate a significant corrosion effect of 10% hydrochloric acid solution on the steel surface, and these values increase significantly with increasing temperature of HCl solution. The addition of inhibitors, especially chitosan at a concentration of 1% in the hydrochloric acid solution, has a significant effect on the reduction of the surface roughness value not only at room temperature, but also at elevated temperature. Also, these measurements proved that caffeine shows better inhibition properties at room temperature compared to chitosan.



a) b) c) d)

Figure 2. Steel surfaces recorded by optical metallographic microscope (250X) after exposure to 10% HCl solution in absence (a) and with the addition of 1% chitosane (b), 1 g/L caffeine (c) and their mixture (d) at 25°C.

Figure 2 shows the surfaces of the steel alloy samples exposed to the action of hydrochloric acid without and with the addition of the tested inhibitors at 25°C, obtained by an optical metallographic microscope at magnification of 250X. As was expected, the hydrochloric acid solution creates damage in the form of small holes on the steel surface, indicating the occurrence of pitting corrosion [8]. By adding chitosan and caffeine to the HCl solution, this effect is reduced, and there is significantly less damage to the surface of the steel, although it is evident that at room temperature the protective effect of caffeine is much better compared to chitosan.

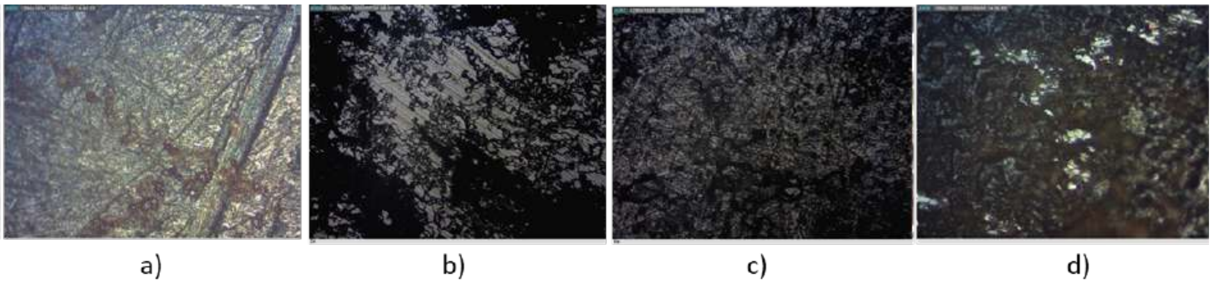


Figure 3. Steel surfaces recorded by optical metallographic microscope (250X) after exposure to 10% HCl solution in absence (a) and with the addition of 1% chitosane (b), 1 g/L caffeine (c) and their mixture (d) at 60°C.

Figure 3 shows the surfaces of the steel after exposure to a 10% hydrochloric acid solution without and with the addition of the tested inhibitors at 60°C. A clear difference between Figure 2 and Figure 3 is visible, because the surface of the steel that was exposed to acid without an inhibitor is significantly rougher, and it can be seen a change in color and the formation of small dark spots, which are actually damage in the form of small holes. It is significant that Figure 3. clearly shows that at 60°C chitosan creates something like a thin film on the surface of the steel and thus protects the steel from pitting corrosion. This is not the case with caffeine, and at 60°C significant surface damage occurs, confirming that caffeine has a better inhibition effect on steel corrosion only at room temperature.

Conclusion

The tested environmentally acceptable inhibitors, chitosan and caffeine proved to be effective inhibitors for steel corrosion in HCl solution. Chitosan showed very good inhibition properties, reducing the corrosion rate of steel in hydrochloric acid at room temperature and especially at elevated temperature. At room temperature, caffeine was proved as better corrosion inhibitor than chitosan with maximum inhibition efficiency of 76%. On the other hand, at elevated temperatures, the inhibition effect of caffeine decreases significantly. The addition of chitosan to the hydrochloric acid solution leads to a significant reduction in the corrosion rate of steel not only at room temperature but also at elevated temperatures. Moreover, adding chitosan to the caffeine solution can improve the inhibition effect of caffeine at elevated temperatures.

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A SUSTAINABLE APPROACH TO THE DISPOSAL OF MERCURY-SATURATED FeS-DOPED NATURAL ZEOLITE BY SOLIDIFICATION/STABILIZATION IN A CEMENT MATRIX

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Introduction

The remediation of a mercury-contaminated environment is quite a challenging task since it is known that mercury is extremely toxic, persistent in the environment and prone to methylation processes. Nowadays, sorption remediation processes require the use of environmentally friendly highly selective sorbents for mercury. Since mercury has a strong affinity for sulphur species, natural sulphur-containing sorbents could have promising properties [1]. Therefore, natural zeolite doped with iron sulphide particles will be used in this paper. As iron sulphides minerals are natural constituents of the geological layer, natural zeolite doped with iron sulphides particles represents a green sorbent. After the application of zeolite for *ex situ* remediation, proper handling of the mercury-saturated zeolite is important, since it represents a hazardous waste. According to the Minamata Convention, the application of mercury is almost completely prohibited, so it seems reasonable to solidify/stabilize (S/S) the mercury-saturated zeolite in the cement matrix [2]. Solidification with Portland cement achieves physical encapsulation of the waste material within the structure of the solidified matrix, while stabilization involves chemical fixation with hydration products of the cement. Leaching of encapsulated heavy metals from the cementitious matrix is quite a complex process, and depends on the type of S/S agent, leachant pH, the characteristics of the heavy metal and the cementitious matrix, as well as the leaching protocol [3]. A chemical evaluation of the mobility of pollutants solidified/stabilized in cement composite, is usually determined by short-term leaching tests such as Toxicity Characteristic Leaching Procedure, while long term leaching is performed by dynamic Dutch leaching test (NEN 7345) over 64 days.

Therefore, the purpose of this paper is to examine the chemical characteristics of FeS-doped mercury-saturated natural zeolite before and after the S/S process via two mentioned leaching tests. The ultimate aim of this research is to gain insight into the possibility of safe disposal or reuse of stabilized/solidified hazardous waste.

Material and Methods

Sample preparation

Natural zeolite (NZ) originating from the Zlatokop deposit (Serbia) of 0.6 − 0.8 mm particle size is used as a starting material to obtain iron sulphides dopped natural zeolite (FeSZ). The 1 g of NZ was refluxed for 2 h at 100 °C with 50 mL of 1 mol/L $Fe(NO)_3 \cdot 9H_2O$, and then for 4 h at 150 °C with 10 mL of 1 mol/L $Na_2S\cdot9H_2O$. FeSZ is completely saturated with 10.220 mmol/L Hg(II) solution in batch mode under optimal conditions (pH = 2, solid/liquid ratio = 10 g/L, t = 24 h and 550 rpm) and labeled as FeSZHg. The initial and equilibrium Hg(II) concentration was determined on a Flame Atomic Absorption Spectrophotometer, AAS.

Preparation of solidified/stabilized zeolite specimens in cement matrix

Ordinary Portland cement purchased from CEMEX Croatia cement plant is homogenized with different proportions (10%, 20%, 30% and 40%) of FeSZHg with the addition of 5 mL of ultrapure water. The total mass of all specimens was 10 g. The specimens were then poured into cylindrical moulds (2 cm × 2.12 cm), isolated from air and cured in a thermostat for 7 days at 20°C.

Leaching experiments

Short-term leaching test from FeSZHg and crushed FeSZHg-Cem specimens was performed with two extraction solutions of pH_o = 2.88 and pH_o = 4.93 according to the Toxicity Characteristic Leaching Procedure, TCLP (USEPA, 1311) [4]. The solid/liquid ratio was 20:1, the leaching time was 18 h at 30 rpm and at ambient temperature. The long-term dynamic leaching test with monolithic FeSZHg-Cem specimens was performed in accordance with the standard Dutch protocol (NEN 7345) [5]. S/S monoliths are immersed in ultrapure water with a pH value of 6.18 (liquid/solid volume ratio = 5:1). Eight leaching cycles were carried out at intervals of 8 h and 1, 2, 4, 9, 16, 36 and 64 days. After each cycle, the leachate was collected and replaced with fresh leachant. The concentration of leached Hg(II) was determined in all leachates using a Mercury Analyzer AMA 254.

Calculation of characteristic parameters

Cumulative leachability of Hg, M_t (mg/m^2) taking into account all eight cycles, is calculated according to the Eq. (1):

$$M_t = \sum_{n=1}^{8} M_i = \frac{c_i \cdot V_i}{A} \qquad (1)$$

where: M_i denotes the interval amount of leached Hg per unit of external surface area of the sample for each leaching cycles (mg/m^2), c_i is the leached Hg concentration in each leaching cycle (mg/L), V_i is the volume of leachant (L) and A is the external geometric surface area of sample (m^2).

The effective diffusion coefficient of Hg(II) during cumulative leaching according to NEN 7345 [3,5] can be calculated according to the analytical expression derived from Fick's second law for a cylindrical sample i.e. from its logarithmic form as follow:

$$M_t = 2 \cdot \rho \cdot M_o \cdot \left(\frac{D_e \cdot t}{\pi}\right)^{\frac{1}{2}} \qquad \log M_t = \log\left[2 \cdot \rho \cdot M_o \cdot \left(\frac{D_e}{\pi}\right)^{\frac{1}{2}}\right] + \log t^{\frac{1}{2}} \qquad (2)$$

where: M_o is the available amount of Hg in the sample (mg/m^2), ρ is the sample bulk density (kg/m^3), D_e is the effective diffusion coefficient (m^2/s) and t is the elapsed leaching time (s). According to the NEN 7375 protocol, a slope value in the range 0.35 − 0.65 indicates diffusion-controlled leaching, a slope > 0.65 is attributed to dissolution, and a slope < 0.35 is accompanied by surface wash off or depletion. The leachability index (pD_e) is calculated as the negative logarithm of the effective diffusion coefficient (D_e) as follows [3]:

$$pD_e = -\log(D_e) \qquad (3)$$

For a value of $pD_e > 12.5$ the pollutant has low mobility, for $11.0 < pD_e < 12.5$ medium mobility and for $pD_e < 11$ it has high mobility [3].

Results and discussion

The results of the leached Hg concentration according to the TCLP test from FeSZHg and FeSZHg-Cem specimens are shown in Figure 1a and b.

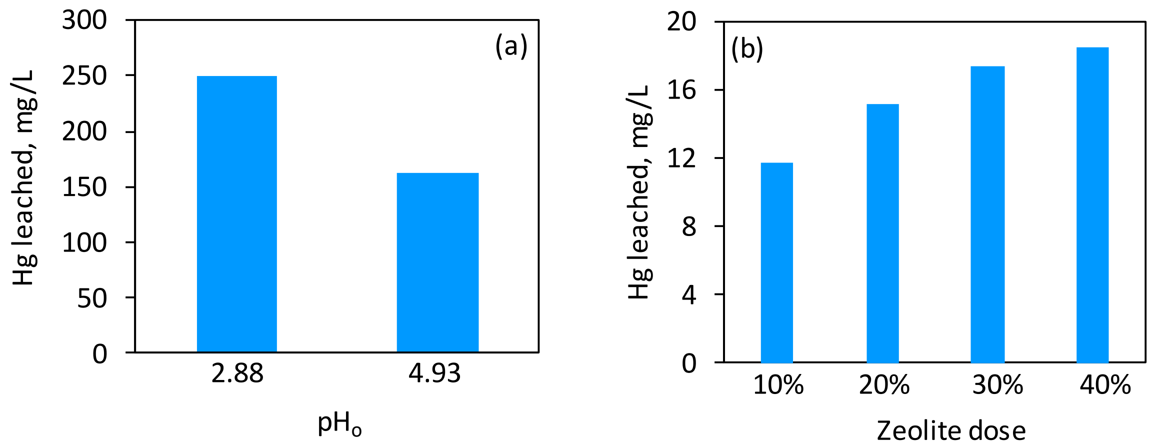

Figure 1. Leached Hg concentrations according to the TCLP test at (a) $pH_o = 2.88$ and $pH_o = 4.93$ from FeSZHg and (b) at $pH_o = 2.88$ from FeSZHg-Cem specimens.

The concentration of leached Hg (Fig. 1a) from FeSZHg is higher at a lower pH value of 2.88 as a result of higher concentration of H^+ ions, which leads to a partial degradation of the zeolite structure, causing more significant leaching of Hg. The concentration of leached Hg at both pH values of the extraction solution is higher than the prescribed TCLP criterion of 0.2 mg/L, therefore FeSZHg is classified as hazardous waste and must be adequately treated before disposal. For this purpose, different doses of FeSZHg were treated, i.e. solidified/stabilized in the cement matrix, and the crushed S/S samples were also subjected to the TCLP test. The obtained results are shown in Figure 1b. The concentration of leached Hg increases with the increase in FeSZHg dose in the cement matrix. The increase in the concentration of leached Hg is not proportional to the increase in the dose of FeSZHg in the cement matrix, indicating that surface wash off and diffusion of Hg from the FeSZHg-Cem specimens is most likely occurring. The concentrations of leached Hg from S/S specimens (Fig. 1b) are significantly lower than from FeSZHg (Fig. 1a), which proves that the cement effectively reduces the mobility of Hg. However, the concentrations of leached Hg from all S/S specimens are above the prescribed TCLP criterion of 0.2 mg/L, whereby S/S specimens should neither be used nor disposed of in crushed form.

For this reason, a Dutch dynamic leaching test (NEN 7345) was performed to determine the leaching characteristics of Hg from monolithic S/S specimens. The results of cumulative (M_t) leaching of Hg expressed as release per unit of external surface from monolithic FeSZHg-Cem specimens during 64 days are shown in Figure 2a.

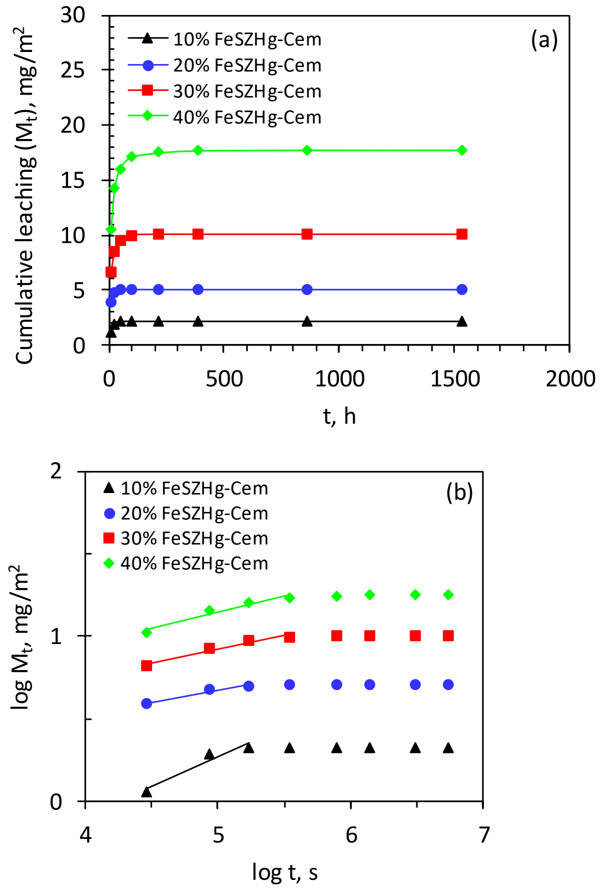

Figure 2. (a) Cumulative Hg leaching(M_t) from FeZHg-Cem monoliths, and (b) log-log plots of cumulative Hg leaching (M_t) vs. time from FeSZHg-Cem monoliths in a period of 64 days.

The results of cumulative leaching of Hg from all monoliths reveal the highest concentration of leached Hg after the 1st leaching cycle (Figure 2a). Then, a slight increase in M_t is observed until the 3rd leaching cycle, after which a steady state is established where leaching is almost negligible. With an increase in the dose of FeSZHg in the monoliths, the maximum cumulative concentration of leached Hg also increases due to the higher available amount of Hg.

The leaching mechanism can be determined from the slope of the linear dependence of the experimental data of cumulative leaching as a function of time by plotting in a log M_t – log t diagram as shown in Figure 2b. Two linear regions can be observed for all monoliths, which indicates a change in the leaching mechanism. With the increase in the proportion of available Hg in the monoliths, the time increment of the first linear part increases, indicating a longer time period of Hg leaching from the monoliths. In the second linear region, there is no leaching, which is attributed to the establishment of a steady state. The slope values for the first linear part, the dominant leaching mechanism for the specified time increment and the corresponding effective diffusion coefficients calculated from Eq. (2) are shown in Table 1.

Table 4. Dominant leaching mechanism for linear time increment, slope of log-log plots, effective diffusion coefficient (D_e) and leachability index (pD_e).

FeSZHg dosage	**Time increment**	**Slope**	**Leaching mechanism**	**D_e, m^2/s**	**pD_e**

10%	1-3	0.3513	diffusion	$1.70\cdot10^{-20}$	19.77
20%	1-3	0.1477	surface wash off	$2.14\cdot10^{-20}$	19.67
30%	1-4	0.1661	surface wash off	$2.86\cdot10^{-20}$	19.54
40%	1-4	0.1969	surface wash off	$4.10\cdot10^{-20}$	19.39

According to the slope values, the dominant Hg leaching mechanism for the S/S specimen with the addition of 10% FeSZHg is diffusion, whereas for the other S/S specimens it is surface wash off in the initial stages followed by delay diffusion. The wash off leaching mechanism for specimens containing FeSZHg > 20% can be attributed to the higher amount of zeolite grains located on the external surface of the specimens. The values of calculated leachability index values > 19 (Table 1) indicates low Hg mobility from monolithic specimen, confirming that Hg is chemically bound to FeSZ and hydration cement products, as well as physically encapsulated in the cement matrix. This indicates the possibility of controlled disposal of S/S FeSZHg-Cem specimens in monolithic form.

Since Croatia, like most EU member states, does not have landfills for S/S hazardous waste and thus no waste acceptance criteria for landfilling, the UK prescribes a leaching limit value for monolithic hazardous waste determined according to the NEN 7345 protocol in the leachate up to 0.4 mg Hg/m^2 [6]. Only the S/S sample with the addition of 10% FeSZHg meets this requirement, indicating its harmless long-term environmental risk and can be disposed of in a hazardous waste landfill.

Conclusion

This study investigated Hg leachability from mercury-saturated FeS-doped natural zeolite (FeSZHg) and its solidified/stabilized form in cement matrix (FeSZHg-Cem). The results of TCLP test showed that both, FeSZHg and crushed S/S FeSZHg-Cem specimens represents a hazardous waste and should neither be disposed of nor reused. The dominant Hg leaching mechanism for the S/S specimen with the addition of 10% FeSZHg is diffusion, whereas for the other S/S specimens it is surface wash off in the initial stages followed by delay diffusion. S/S treatment with cement provides a satisfactory Hg fixation effect in monoliths, significantly reducing its mobility, pD_e > 12.5. The utilization of FeSZHg for special purposes as fillers in concrete structures such as road-based materials seems to be a reasonable method of their safe reuse, which is imperative to meet the principles of sustainable development.

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ISOLATION OF BIOACTIVE COMPOUNDS FROM VEGETABLE BY-PRODUCTS USING A PULSED ELECTRIC FIELD AS PRE-TREATMENT

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Introduction

Every year, millions of tons of tomatoes are processed to produce commercial products such as ketchup, juice and sauce [1]. The main problem with tomato processing is waste, which is generated in the range of 5.4-9.0 tons. By-products consist of nutrient-rich and bioactive components, recycling of which reduces economic and energy costs while increasing product yield. Pulsed electric field (PEF) is a non-thermal technique that increases electroporation and improves extraction without compromising nutrient quality [2]. Tomato by-products, especially seeds and peels, are rich in lycopene. Lycopene is a natural pigment with a characteristic red color that is known as a powerful antioxidant [3]. The stability of the lycopene depends on temperature, exposure to light and processing technology. The development of new non-thermal technologies can enable better lycopene extraction with the higher yield and efficiency [4]. The aim of this work was to investigate the effects of PEF treatment on the extraction of carotenoids and polyphenols from tomato peels.

Materials and methods

Tomato peels from locally grown tomatoes were peeled using the standard hot-cold water breakout method, dried at 60°C for 75 minutes, ground, and stored in desiccator for analysis. Drying and grinding were performed using an Elektrokovina dryer and Imetec Tenacta Group mill, respectively. Pulsed electric field (PEF) treatment was applied using the HVG60/1 PEF system at 2, 5, and 7.5 kV cm^{-1} for 1, 3, and 5 minutes. Samples were sonicated at 100% amplitude, filtered, and separated into supernatant (for polyphenolic content and antioxidant activity) and pellet (for carotenoid content).

Table 5. Experimental parameters of PEF treatment

Sample	R0	R1	R2	R3	R4	R5	R6	R7	R8	R9
Voltage (kV/cm)	0	5	7.5	2	2	7.5	5	7.5	2	5
Time (min)	0	5	3	1	3	5	1	1	5	3

Carotenoid Content Determination

1 g of pellet was extracted with an acetone-hexane solution (4:6 v/v), vortexed for 15 minutes, centrifugated, and the supernatant analysed. Carotenoids were analysed using HPLC with a non-polar C30 column on an Agilent Technologies LC 1200 system. Gradient chromatography was performed with mobile phases A (methanol: MTBE: water) and B (methanol: MTBE), and carotenoid detection was done using DAD at 280 nm. Lycopene and β-carotene standards were used for identification.

Total Polyphenolic Content (TPC) and Antioxidant Activity

TPC was measured using the Folin-Ciocalteu method. Filtered extracts were mixed with FC reagent, sodium carbonate, and water, and absorbance was recorded at 765 nm after 2 hours. Polyphenolic compounds were also identified using HPLC with gradient chromatography and DAD detection. Antioxidant activity was measured via the DPPH assay at 518 nm, calculating the radical scavenging activity (%) based on absorbance values.

Results and discussion

The aim of this work was to evaluate method for extraction of polyphenols and carotenoids from tomato peels using pre-treatment pulsed electric field (PEF).

pH and electrical conductivity

The pH results for all samples were in the range of 5.53 ± 0.04, untreated and treated, which is in correlation with Panthee et al. [5]. The duration of the PEF treatment and the electric strength had no significant impact on the pH of the samples, but a significant effect on the electrical conductivity. Samples with higher electrical conductivity (747 µS cm^{-1}) had a higher total polyphenolic content (2.437 mg g^{-1} d.m.). The sample with the lowest total polyphenolic content (1.955 mg g^{-1} d.m) had the lowest electrical conductivity (622 µS cm^{-1}).

Total carotenoid content UV/VIS

Isomerisation and oxidation are the main reasons for the degradation of lycopene during tomato processing [6]. According to Pataro et al. [7], PEF treatment (5kV cm^{-1}, 5 kJ kg^{-1}) increased the lycopene content in tomato peel by 12-18%. The results show the total carotenoid degradation after treatment. The change in temperature and pH can reduce the concentration of nutritive and bioactive compounds during food processing. For example, a pH of 2-4 leads to a 96-99%, and a pH of 5-8 to a 47-54% reduction in total lycopene content [8]. R0 had the highest lycopene concentration (0.135 mg g^{-1} d.m.) while R1 and R9 samples had the lowest lycopene concentration, while both were treated at 5 kV $cm^{-1}$and also had the same pH (5.54). The concentration of β-carotene in the samples also decreased after treatment. The concentration of chlorophyll A and B are approximately the same in the treated and untreated samples, with the exception of sample B3, which was found to be deficient in chlorophyll B. The decrease in the value of extracted carotenoids due to the increase in duration and voltage can be attributed to the irreversible electroporation of the cell membranes [9].

Total carotenoid content at HPLC

Lycopene is the most represented carotenoid in tomato peel [10], [11]. Luengo et al. [12] recorded a significant increase in lycopene content after PEF induced extraction from tomato peels. The treatment was carried out with voltages of 3, 5 and 7 kV cm^{-1} and the best results were obtained with field strengths of 3 and 5 kVcm^{-1}. After treatment, the total lycopene content was increased by 13-39% in Luengo et al. [12] and 45% in Varvara et al. [13]. The results obtained in this experiment did not confirm the previously made observation (Figure 1). The treatment duration and applied voltages did not significantly improve the extraction of lycopene and β-carotene ($p > 0.05$). Untreated sample R0 contained the highest content of lycopene (4.886 mg g^{-1} d.m.) and β-carotene (5.508 mg g^{-1} d.m.). These unexpected results can be explained by the biodiversity of tomato plants, which causes a reverse PEF effect when carotenoids are extracted. The extraction method also depends on the type of solvent [14]. The aim of this work was to extract both non-polar and polar carotenoids from tomato peels, which proved acetone-hexane to be a suitable solvent for this experiment. The results of the HPLC analysis confirmed the results of the UV/VIS analysis.

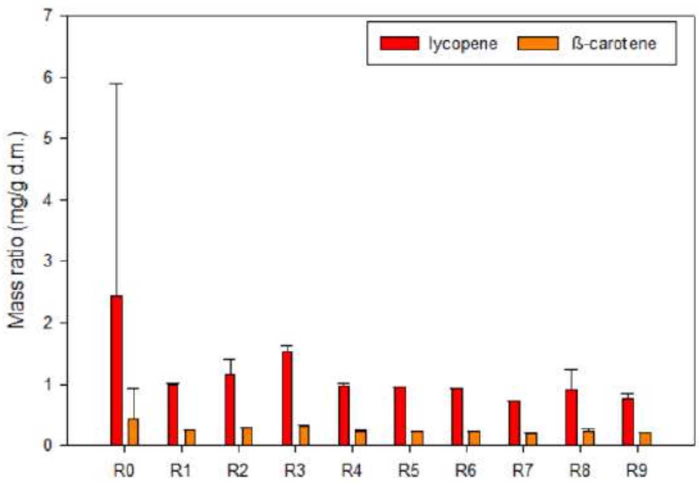

Figure 1. Results of the total carotenoid content in tomato peels analysed with HPLC

Total polyphenolic content UV/VIS and HPLC

In the study by Varvara et al [13], the total polyphenolic content doubled after treatment with 2 kV cm^{-1} and 7000 pulses. UV/VIS and HPLC analyses were used to measure the total polyphenolic content. Both results confirmed the positive influence of PEF on polyphenolic extraction, which is consistent with the previously mentioned studies. It can be concluded that the total number of polyphenols was increased after PEF treatment. Among the numerus different phenolic acids, gallic acid and quercetin-3-glucoside dominate in the tomato peel (Figure 2). According to Elbadrawy and Sello [15], the most important phenols in tomato peel are caffeic, catechin, gallic and vanillic acid. The luteolin content in tomato peels was the lowest in all samples examined.

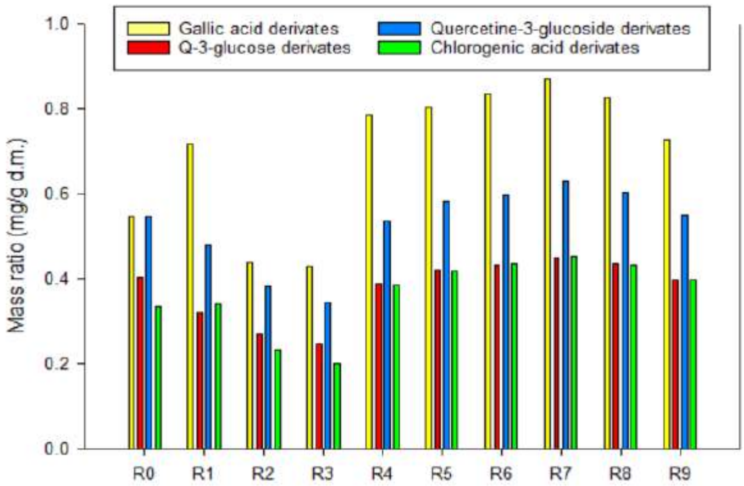

Figure 2. Total polyphenolic content in tomato peels analysed with HPLC

Antioxidant activity

The antioxidant activity was improved after increasing the field strength [15]. In Elkbadrawy and Sello [15], the antioxidant activity of tomato peels was increased by 97.23% and 97.07% for treated samples. The increase in TPC and lycopene content leads to an improvement in antioxidant activity [12]. PEF treatment improved the extraction of lycopene and polyphenols [14], resulting in better antioxidant activity of the samples. The treatment duration and the applied voltages had a significant influence on the antioxidant activity. Sample R7 was treated with the highest field strength (7.5 kV cm^{-1}) and consequently showed the highest antioxidant activity compared to the other samples (Table 2).

Table 6. Antioxidant activity of tomato peels samples

Sample	% reduction of DPPH
R0	75.11 ± 0.29
R1	88.70 ± 1.48
R2	83.89 ± 0.00
R3	77.41 ± 0.88
R4	91.11 ± 0.73
R5	92.79 ± 0.15
R6	92.47 ± 0.00
R7	93.10 ± 0.00
R8	92.05 ± 0.89
R9	90.48 ± 0.43

Conclusion

The results obtained in this work shows advantages of PEF-assisted extraction compared to conventional extraction methods. It can be concluded that the total number of polyphenols was increased after PEF treatment. The treatment duration and the applied voltages had a significant influence on the antioxidant activity. The PEF treatment is a non-thermal, environmentally friendly and food-safe method, which makes it desirable for the extraction of nutrient-rich and bioactive components from vegetable by-products.

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CORROSION INHIBITION PERFORMANCE OF *ARUNDO DONAX* L. LEAVES ALCOHOLIC EXTRACT ON FE B500B STEEL IN ACIDIC SOLUTION

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Introduction

It has been reported that corrosion related costs in several developed countries constitute between 3–4% of their gross domestic product (GDP) [1]. Several industrially relevant metals, including different types of steel, aluminum and copper are highly susceptible to corrosion in acidic environments. Acid cleaning (pickling) is commonly used to remove oxides from the metal's surface [2, 3]**.** Finding new environmentally friendly alternatives to mitigate corrosion has been a continuous challenge for the scientific community [4]. A wide range of environmentally friendly compounds and natural products have been employed as an alternative to organic corrosion inhibitors for steel in acidic solutions. Amino acids, biopolymers, drugs, ionic liquids, natural gums, vitamins and food supplements are among the tested green corrosion inhibitors [5, 6]. However, plant extracts are by far the most used green inhibitors. A wide variation of different extraction techniques, solvents as well as plant's parts (i.e. roots, stem, bark, leaves, flowers, and fruits) have been employed to obtain mostly aqueous or alcoholic extracts [7, 8]. The corrosion inhibition efficiency of the plant extracts is due to the adsorption of one or several of their constituents on the surface of the metals, isolating it from the corrosion environment [7, 8].
Arundo Donax L. (also known as giant reed) is an invasive grass species that belongs to the Poaceae family [9-11]. Different parts of this plant (including stem, leaves, roots and the rhizome) have been found ethnomedicinal applications treating among others circulatory, dermatological, digestive and urinary disorders [11]. The main classes of phytochemicals include alkaloids, phenols, lignin, flavonoids and flavone C-glycoside [10, 11]. These compounds contain several heteroatoms (mainly oxygen and nitrogen), benzoic rings, double and triple bonds, which can act as centers of adsorption.
In this study, the corrosion susceptibility of the Fe B500B steel samples in 1 M HCl solution was studied in the presence of *Arundo Donax L*. leaves alcoholic extract, using weight loss and potentiodynamic polarization measurements. The effect of extract's concentration, temperature and potassium iodide as a possible synergist on the corrosion rate of the steel samples immersed in the acidic solution is also discussed.

Materials and methods

The locally collected *Arundo Donax L.* leaves were first cleaned and then dried at 323 K for 3 days. The ground dried leaves were then extracted with reflux in 80% ethanol as previously described [12, 13]. The obtained alcoholic extract was added in different concentrations (i.e. 5, 10, 25 and 50 mg L^{-1}) to the 1 M HCl solution.

The samples used for the weight loss (cylinders with a 5 cm height and 0.8 cm diameter) and electrochemical measurements (disks with a 1.5 cm diameter and 0.5 cm thickness) were cut from Fe B500B bars with the chemical composition presented in Table 7, provided by Diler Demir Celik Endustri Ve Ticaret A.S. (Gebze, Turkey) [14].

Table 7: Chemical composition of the Fe B500B steel as specified by the provider.

Element	C	Mn	Si	P	S	Cu	N	Ni	Cr	Mo	V	B	Fe
Content (wt.%)	0.20	0.84	0.22	0.014	0.023	0.30	0.0095	0.11	0.11	0.017	0.0012	0.0002	balance

The Fe B500B samples were pretreated as previously described [12-14], including ground with different grinding papers, washed with bidistilled water and degreasing with acetone in an ultrasonic bath. The average weight loss (*W*) of the Fe B500B samples before and after 100 h immersion in 1 M HCl solutions with and without different additions of the alcoholic extract, was used to calculate the corrosion rate (CR) of these samples, the surface coverage (ϴ), and the corrosion inhibition efficiency (IE_{WL}) of the alcoholic extract. Prior to weighing the samples after immersion, they were treated as previously described [14].

$$CR_{(g\ m^{-2}\ h^{-1})} = \frac{W}{S \cdot t} \quad (1)$$

$$IE_{WL}(\%) = \frac{CR^0 - CR}{CR^0}\ 100 \quad (2)$$

$$\theta = \frac{CR^0 - CR}{CR^0} \quad (3)$$

where *S* is the surface of the samples, *t* is the immersion time, and CR and CR^0 are the corrosion rates of the Fe B500B samples with and without additions of the alcoholic extract, respectively. The obtained results will also be used to determine the best fitting adsorption isotherm. The weight loss measurements have been performed mainly at 298 K, while additional measurements at 308 and 318 K were performed only for the samples immersed in solutions containing the optimum concentration of the alcoholic extract.

The electrochemical measurements were performed in an IS-CCS Corrosion Cell Set from PalmSens using a platinum electrode (as counter electrode), a saturated calomel electrode (SCE, as reference electrode), and the Fe B500B steel samples (as working electrode). First the open circuit potential (E_{OC}) of the Fe B500B steel samples immersed for 30 min in 1 M HCl solutions with and without additions of the alcoholic extract was measured. Then, a 0.1 mV s^{-1} scan rate was employed to perform the potentiodynamic polarization (PD) measurements in the E_{OC} ± 120 mV potential range. The corrosion current densities obtained from the Tafel extrapolation of the potentiodynamic curves of the samples immersed in the 1 M HCl solution with (i_{corr}) and without (i^0_{corr}) additions of the alcoholic extract were used to calculate the CR (in mm y^{-1}) and the IE of the alcoholic extract.

$$CR_{(mm\ y^{-1})} = \frac{Kai_{corr}}{nd_{Fe}} \quad (4)$$

$$IE_{PD}(\%) = \frac{i^0_{corr} - i_{corr}}{i^0_{corr}}\ 100 \quad (5)$$

$$R_p = \frac{b_c\, b_a}{2.303\ i_{corr}(b_c + b_a)} \quad (6)$$

where *a*, d_{Fe} and *n*, are the atomic mass, density and valence of Fe (n = 2), respectively. *K* (0.00327) is the conversion factor. The polarization resistance (R_p) is also calculated from the cathodic (b_c) and anodic (b_a) Tafel slopes (Equation 6). In addition to the above-described solutions, 50 mg L^{-1} potassium iodide (KI) was added only to the solution containing the optimum concentration of the alcoholic extract, and the respective PD curve was obtained.

Results and discussions

Weight loss measurements

Table 8 shows a significant decrease in the CR of the Fe B500B steel samples upon addition of 5–50 mg L^{-1} alcoholic extract, from 2.3611 to 0.3048 mm y^{-1}. The IE of the alcoholic extract increased with increasing its concentration, reaching the highest values of 87.09% upon addition of 50 mg L^{-1} alcoholic extract. Further additions of the alcoholic extract did not improve its corrosion inhibition performance, indicating that 50 mg L^{-1} is the optimum concentration.

Table 8: CR and Θ, of the Fe B500B steel samples immersed at 298 K for 100 h in 1 M HCl solution with and without additions of 5–50 mg L^{-1} alcoholic extract, as well as the respective IE_{WL} values, obtained from weight loss measurements.

Solutions	CR ($g\ m^{-2}\ h^{-1}$)	CR ($mm\ y^{-1}$)	θ	IE_{WL} (%)
Blank	2.1212	2.3611	–	–
5 mg L^{-1}	1.0499	1.1687	0.5050	50.50
10 mg L^{-1}	0.5992	0.6670	0.7175	71.75
25 mg L^{-1}	0.3689	0.4106	0.8261	82.61
50 mg L^{-1}	0.2739	0.3048	0.8709	87.09

In order to understand the adsorption behaviour of the alcoholic extract on the surface of the Fe B500B steel samples, which is responsible for mitigating corrosion, the data in Table 8 were fitted to the most common adsorption isotherms, i.e. Temkin, Freundlich, Langmuir, Frumkin, El-Awady, and Flory-Huggins.

$$\frac{C_{inh}}{\theta} = \frac{1}{K_{ads}} + C_{inh} \quad (7)$$

Langmuir (Equation 7), presented in Figure 2 as (C_{inh} θ^{-1}) *vs.* C_{inh}, was found to be the best fitting isotherm, indicating monolayer adsorption of the alcoholic extract's components. The adsorption constant (K_{ads}), determined from the intercept (K_{ads}^{-1}) of the Langmuir isotherm was found to be 0.26 L mg^{-1}.

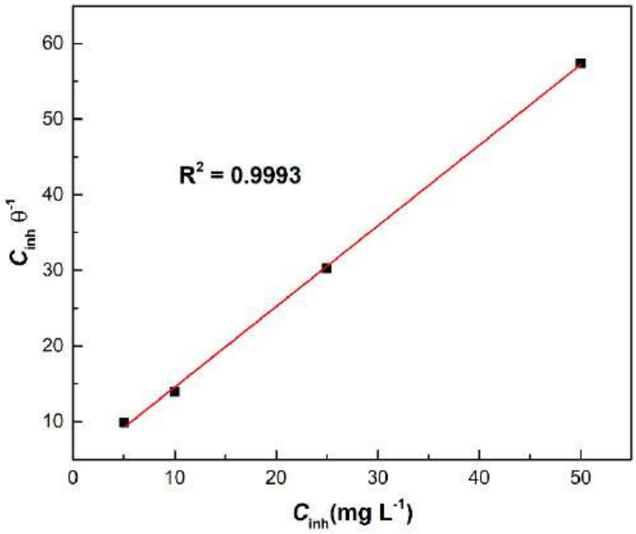

Figure 2: Langmuir adsorption isotherm of the Arundo Donax L. leaves alcoholic extract on the Fe B500B steel samples after 100 h immersion.

The effect of temperature on the corrosion inhibition performance of the alcoholic extract was also studied with the weight loss techniques. Table 9 shows that the CR of the Fe B500B steel samples immersed in 1 M HCl solution containing the optimum concentration of the alcoholic extract, increased with increasing the temperature from 298 to 318 K.

Table 9: CR of the Fe B500B steel samples immersed for 100 h in 1 M HCl solution with and without additions of 50 mg L^{-1} alcoholic extract in the 298–318 K temperature range, and the respective IE_{WL} values, obtained from weight loss measurements.

Solutions	298 K		308 K		318 K	
	CR (g m^{-2} h^{-1})	IE_{WL} (%)	CR (g m^{-2} h^{-1})	IE_{WL} (%)	CR (g m^{-2} h^{-1})	IE_{WL} (%)
Blank	2.1212	–	6.9363	–	19.6297	–
50 mg L^{-1} alcoholic extract	0.2739	87.09	1.5640	77.45	7.7887	60.32

Meanwhile, the IE of the alcoholic extract decreased with increasing temperature, indicating moderate corrosion protection at 318 K. The decrease in the corrosion inhibition efficiency can be attributed to the desorption of the constituents of the alcoholic extract from the surface of the Fe B500B steel.

Potentiodynamic polarization

Figure 3 presents the potentiodynamic polarization curves of the Fe B500 B samples after 30 min immersion in 1 M HCl solutions containing 5–50 mg L^{-1} alcoholic extract. The E_{corr} determined by the PD curve measurements shifted to less negative values with the addition of the alcoholic extract (from –502.53 to –476.74 mV), as shown in Table 10.

Both anodic and cathodic branches of the polarization curves obtained from the samples immersed in 1 M HCl solutions containing alcoholic extract are transferred to lower current densities compared with the samples immersed in the blank solution. In addition, the shift in

E_{corr} is smaller than 85 mV, which would indicate that the *Arundo Donax L.* leaves alcoholic extract behaves as a mixed-type inhibitor [15].

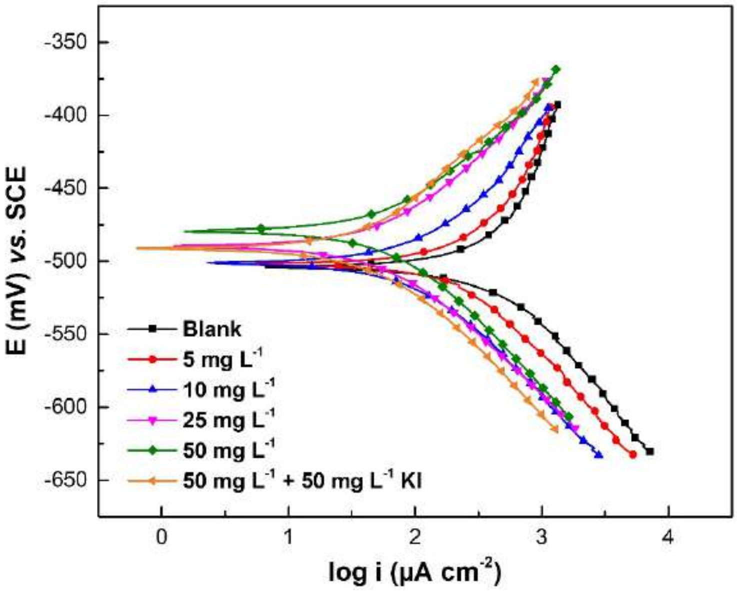

Figure 3: PD curves of the Fe B500B steel samples after 30 min immersion in 1 M HCl solution with and without addition of 5–50 mg L^{-1} alcoholic extract, as well as 50 mg L^{-1} KI added to the optimum alcoholic extracts concentration (i.e. 50 mg L^{-1}).

Table 10 shows that the i_{corr} of the Fe B500B steel samples immersed in inhibited solutions decreased significantly from 424.6 to 53.64 μA cm^{-2} while the CR rate decreased from 4.9169 to 0.6212 mm y^{-1} for the samples in 1 M HCl solution containing 50 mg L^{-1} alcoholic extract.

Table 10: Kinetic parameters obtained from the potentiodynamic polarization curves of the Fe B500B steel samples immersed for 30 min in 1 HCl solutions containing different concentrations of alcoholic extract in addition to 50 mg L^{-1} KI to the optimum inhibitior concentration.

Solutions	**E_{corr} (mV)**	**b_c (mV dec^{-1})**	**b_a (mV dec^{-1})**	**i_{corr} (μA cm^{-2})**	***CR* (g m^{-2} h^{-1})**	**CR (mm y^{-1})**	**R_p (Ω cm^2)**	**IE_{PD} (%)**
Blank	–502.53	–102.97	204.13	424.6	4.4192	4.9169	69.99	–
5 mg L^{-1}	–501.48	–91.4	98.06	198.3	2.0639	2.2963	103.59	53.30
10 mg L^{-1}	–498.63	–90.91	78.29	91.07	0.9479	1.0546	200.56	78.55
25 mg L^{-1}	–488.61	–86.07	85.38	66.20	0.6890	0.7666	281.14	84.41
50 mg L^{-1}	–476.74	–82.00	69.21	53.64	0.5583	0.6212	303.82	87.37
50 mg L^{-1} + 50 mg L^{-1} KI	–488.08	–79.99	80.64	40.67	0.4223	0.4710	428.74	90.42

The possible synergistic effect of 50 mg L^{-1} KI was also studied for the samples immersed in 1 M HCl solution containing the optimum inhibition concentration of the alcoholic extract (i.e. 50 mg L^{-1}). A slight decrease in the CR of the samples immersed in the solution containing 50 mg L^{-1} KI was observed, which was reflected in a slight increase of the IE_{PD} of the alcoholic extract. Moreover, the R_p values increased upon addition of the alcoholic extract, indicating possible adsorption of the components of the alcoholic extract on the surface of the Fe B500B steel samples. It has been previously shown that luteolin-6-C-glucoside, isorhamnetin-3-O-rutinoside, kaempferol 3-O-neohesperidoside, and hesperitin are the main components in the alcoholic extract of *Arundo Donax L*. leaves [10]. These compounds adsorb on the surface of the Fe B500B steel samples through heteroatoms (mainly oxygen), benzoic rings, or double bonds, isolating the surface from the corrosion environment.

Conclusions

In this study the inhibition performance of *Arundo Donax L*. leaves alcoholic extract on the corrosion of Fe B500B steel samples in 1 M HCl solution was studied using weight loss and potentiodynamic polarization techniques. The corrosion rate of the Fe B500B steel samples decreased significantly upon addition of 5–50 mg L^{-1} alcoholic extract. The highest corrosion inhibition efficiency (i.e. 87.37%) was achieved upon addition of 50 mg L^{-1} alcoholic extract, which was found to be the optimum concentration. The corrosion inhibition efficiency of the alcoholic extract decreased with increasing temperature in the 298–318 K range. The alcoholic extract acted as a mixed-type inhibitor suppressing both the anodic and cathodic corrosion reactions. The components of the *Arundo Donax L*. leaves alcoholic extract adsorbed on the Fe B500B steel surface following the Langmuir adsorption isotherm.

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ASSESSMENT OF THE ENERGY POTENTIAL OF FLAX AND HEMP BY-PRODUCTS

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Abstract

The study investigated the potential for energy recovery from food waste, focussing on hemp and flax cake. The selected samples were subjected to characterization before and after their thermal treatment. The aim of this study was to determine whether a given food waste has the potential for energy recovery when the sample is subjected to low temperature pyrolysis in an inert nitrogen atmosphere at a temperature of 300 °C. The results showed that flax has the highest potential for energy recovery. Its calorific value and stability after thermal treatment make it one of the most promising materials for converting food waste into energy. This approach can make a significant contribution to waste reduction and sustainable resource utilisation, which is key to protecting the environment and reducing our ecological footprint.

Keywords: flax, hemp, energy potential, torrefaction, higher heating value

Introduction

Rapid urbanization and population growth contribute significantly to the increase in food waste. In economic terms, food waste causes considerable losses in all segments of the food supply chain [1]. From an environmental perspective, this leads to an increase in greenhouse gas emissions both during decomposition and production. In the food industry, efforts are being made to minimize waste by optimizing production processes and recycling the inedible parts of food waste [2].

Low temperature pyrolysis is a promising solution for improving the storage and energy potential of these by-products. In this thermochemical process, the material is heated in an inert atmosphere at moderate temperatures, usually around 300 °C, which leads to significant changes in the chemical composition and properties of the material. By fine-tuning the temperature and exposure time, the process can improve the energy value, stability, carbon content of the material, and reduce its volatile components. There are three main categories of pyrolysis: slow pyrolysis, fast pyrolysis and flash pyrolysis. The difference between these processes lies in the residence time, at which the material is held at a specific temperature. In slow pyrolysis, the material is subjected to a gradual increase in temperature, up to 400 °C, with a longer dwell time. In fast pyrolysis, the material is subjected to a short dwell time and quickly heated to a high temperature of up to 600 °C. In contrast, flash pyrolysis combines a short dwell time of just a few milliseconds with a rapid heating process that reaches temperatures of over 1000 °C [3].

The aim of this study was to determine whether a given food waste has the potential for energy recovery when the sample is subjected to low temperature pyrolysis in an inert nitrogen atmosphere at a temperature of 300 °C.

Material and methods

Hemp cake is a by-product of the cold pressing of industrial hemp to produce seed oil at a processing temperature between 45 and 48 °C. Hemp cake is a rich source of protein and fibre, with 90 % of the fibre being insoluble and 10 % soluble. Insoluble fibre plays an important role in maintaining a healthy digestive system, while soluble fibre has been shown to regulate blood sugar and cholesterol levels [4]. Hemp cake is a high energy density substance that has significant potential for a range of industrial and energy-related applications. The fat contained in hemp cake has a high energy value, while the protein content also contributes to the total energy value of the biomass [5]. The high fibre and residual oil content of hemp cake increases its calorific value, which is a crucial factor for energy use [4].

Common or Eurasian flax is mainly used for the production of fabrics and linseed oil [6]. Flax fibres consist of 70 - 85 % cellulose, 11 - 20 % hemicellulose, 2 - 12 % pectin and about 2 % lignin, as well as several minor extractives, including fats rich in linolenic and linoleic acid (30 - 40 %). In addition, proteins (25 %) and water (8 %) make up a significant part of the flax fibre composition. Flaxseed cake has a considerable energy value and can be used as a source of energy. In addition, flaxseed is a valuable raw material for the health, cosmetics and other industries. The cake can also be used as animal feed [7].

A sample of hemp and flax oil cake was obtained from Slovenian mills. Before starting all analytical procedures, the samples were ground to ensure homogeneity of the samples. The pyrolysis of the samples was carried out in a laboratory Carbolite tube furnace under a nitrogen atmosphere at 300 °C for a period of 30 minutes. The final product is a dry, stable and energy-rich biomass (biochar), that is easier to store and has better combustion properties. Considering the chemical and physical properties of the selected samples, it was determined that a low temperature pyrolysis process would be most suitable. The pyrolysis yield (MY) was calculated according to Eq. 1.

$$MY(\%) = \frac{\text{mass (treated)}}{\text{mass (untreated)}} \cdot 100 \qquad (1)$$

The dry matter content of the samples was determined for the raw and pyrolyzed samples. Each sample was weighed on an analytical balance and dried at 105 °C until a constant weight was reached. The volatile matter and ash content were determined according to a modified protocol for proximate analysis using thermogravimetric analysis (TGA) [8]. The first four steps of the protocol were performed under a nitrogen atmosphere, while the fifth step took place under an air atmosphere. The procedure began with each sample being thermostatted at 25 °C for four minutes (step 1). This was followed by heating from 25 °C to 110 °C at a rate of 85 °C/min (step 2) and leave the sample at that temperature for six minutes (step 3), then heating from 110 to 900 °C at a rate of 80 °C/min (step 4) and finally burning each sample at

900 °C for five minutes (step 5). The higher heating values (HHVs) of the samples were determined using an IKA Isoperibol C6000 calorimeter. The thermogravimetric analyses were carried out with a TGA/DSC^{3+} (Mettler Toledo) device. The measurements were carried out in a temperature range of 50 to 900°C in an inert nitrogen atmosphere (10 mL /min) at a heating rate of 25°C/min.

Results and discussion

Table 1 shows the low-temperature pyrolysis efficiencies for both samples. The hemp cake showed the lowest mass loss, which corresponds to the highest pyrolysis mass yield (78.03 wt%). The low mass loss indicates that a smaller proportion of the material is converted into gaseous and liquid products during pyrolysis, leaving a larger proportion as solid residue (biochar). This indicates that the hemp cake contains components that are more resistant to thermal degradation, which leads to a higher biochar yield. The low mass loss of the hemp cake sample is advantageous as the goal is to obtain a larger amount of biochar that can be used as fuel or energy-rich waste. The mass loss of the flax cake during pyrolysis is greater than that of the hemp cake, yet the resulting biochar yield remains high (71.53 wt%), classifying the flax cake as a high-energy material.

Table 1: The pyrolysis mass yield

Sample	Pyrolysis mass yield (wt%)
Hemp cake	78.03
Flax cake	71.53

The moisture, volatile matter and ash content of the air-dried samples and the solid products resulting from pyrolysis were determined. The calorific value is a fundamental parameter for assessing the energy potential of food waste, as a higher calorific value indicates a higher energy density per unit mass or volume. The higher the energy density, the less material is required to achieve a certain amount of energy, which is therefore of interest regarding the transportation and storage of food waste. The results are shown in Table 2. After pyrolysis, the moisture content was found to have decreased, with the flax cake still containing 3.81 wt% moisture. The results of the analysis showed that the hemp cake had the highest volatile matter (hydrocarbons, hydrogen, carbon dioxide, carbon monoxide and other gases) after pyrolysis (84.23 wt%). Similar results were observed for the raw samples, with the exception that the volatile content was around 70 wt%. This indicates that these samples burn more slowly or are more flammable.

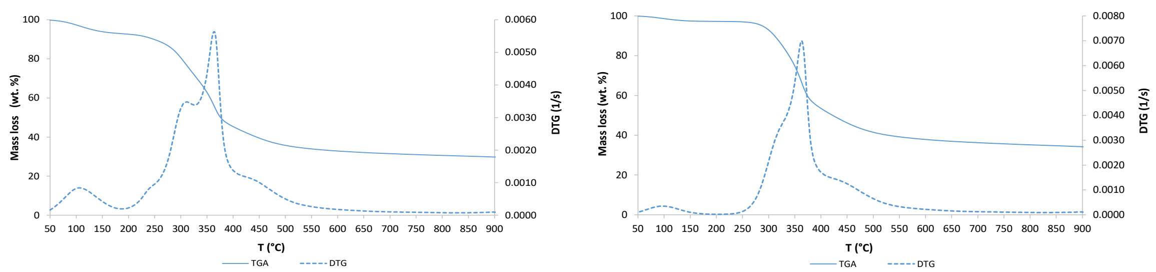
Table 2: Results of moisture, volatile matter and ash determinations of raw samples

Sample	Moisture (wt%)	Volatile matter (wt%)	Ash (wt%)	HHV (MJ/kg)
Hemp cake - raw	4.88	70.53	17.93	19.054
Flax cake - raw	5.63	72.77	13.85	11.067
Hemp cake - pyrolyzed	0.40	84.23	11.29	22.862

Flax cake - pyrolyzed	3.81	79.07	7.35	24.504

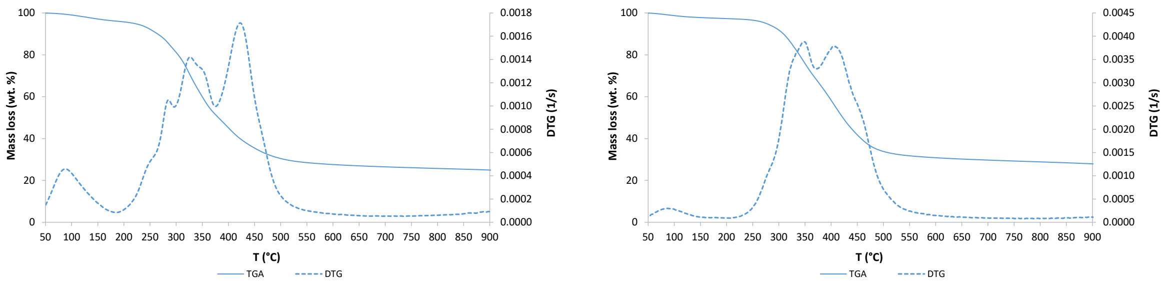
The flax cake had the highest ash content at around 7 wt%. The hemp cake had the highest ash content after pyrolysis at 11.29 wt%. This indicates that the sample is less efficiently transferred to other components as the amount converted to energy of the hemp cake sample is the lowest. As a result of the change in the composition of the sample due to the thermochemical process, the calorific value of the pyrolyzed samples also changes accordingly. It is assumed that the higher carbon concentration will lead to an increase in the higher heating value (HHV) of the pyrolyzed samples [9]. The results confirm the hypothesis that the calorific values of the samples increase after treatment. Biochars from various biomass sources, including invasive plants and wood residues, were shown to have a higher calorific value after pyrolysis. To illustrate: the pyrolysis of materials such as mistletoe and juniper at higher temperatures and longer residence times leads to a considerable improvement in the energetic properties, which is reflected in a higher calorific value of the finished biochar [10]. The largest discrepancy in HHV is observed with flax cake, which is therefore the most energy-efficient material after thermal treatment.

The analysis of TGA and differential thermogravimetric analysis (DTG) results is the key to understanding the thermal stability and degradation of materials. The results of the TGA analyses for selected food waste samples before and after pyrolysis are shown in Figures 1 and 2.



a) b)

Figure 1: TGA of hemp cake sample a) before pyrolysis and b) after pyrolysis



a) b)

Figure 2: TGA of a flax cake sample a) before pyrolysis and b) after pyrolysis

Conclusion

The agri-food industry generates a significant amount of waste, accounting for over 90 % of total waste biomass. This represents a potential source of energy and raw materials that could help reduce dependence on fossil fuels. The thermal treatment of hemp and flax cake samples was carried out by pyrolysis in a laboratory tube furnace under a nitrogen atmosphere. The properties of the resulting solid products were then compared with those of the raw materials.

Hemp was found to have the highest ash content and the lowest volatile matter content of all the samples. The samples were then analysed for weight loss during the pyrolysis process. The results showed that the hemp cake had a lower weight loss compared to the flax cake. Before processing, it was found that the raw hemp cake sample had the highest calorific value. After pyrolysis, the HHV values of the treated samples were measured and a significant increase in calorific value was observed in all samples. The flax cake sample had the highest calorific value after treatment. The TGA/DTG measurements showed that the flax cake sample had the most complex chemical structure. It is a rich source of dietary fibre, proteins and essential fatty acids. A comprehensive analysis of the results shows that flax cake, with its optimal chemical structure and energy value, is a suitable candidate for energy utilisation. In contrast, the hemp cake sample has a comparatively lower energy density.

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INFORMED, AWARE AND COMMUNICATIVE YOUNG CITIZENS: A STRATEGIC APPROACH TO ADDRESS URBAN GREENING CHALLENGES

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Introduction

In the context of the United Nations' 2030 Agenda, public and green spaces play a key role in promoting urban sustainability and citizens' well-being, fostering the connection between humans and nature, as well as providing multiple benefits for both human and environmental health (United Nations, 2015). Urban green spaces are crucial for the wellbeing of citizens, but nowadays are facing social and environmental challenges that compromise its sustainability (Vidal et al., 2020). As cities grow and expand, the green areas are compromised, leading to a host of ecological and social issues, including loss of biodiversity, increased pollution, and reduced public access to natural environments (Aronson et Al. 2017). Considering that "by 2050 the population of the world is projected to be 68 % urban, with urban dwellers numbering 6.7 billion" (United Nations, 2019), the challenges and investments required for effective planning are likely to become even more pronounced. Urban green spaces not only enhance the aesthetic appeal of cities but also play a vital role in improving air quality, regulating temperature, and promoting public health. The ability of urban green spaces, such as street trees, parks, and green roofs, to mitigate some negative impacts of the built environment on human health is well documented (e.g., Roy et al., 2012). For instance, trees play a significant role in reducing air pollution primarily through the passive deposition of pollutants and particulate matter onto their leaves (Xing & Brimblecombe, 2020). Additionally, the shade provided by tree canopies, along with evapotranspiration, helps lower both surface and atmospheric temperatures, creating thermally comfortable environments for residents during hot summer days (Sun et al., 2021). Moreover, the presence of urban green spaces also has significant social benefits, as they serve as gathering places for people, for instance, through physical or recreational activities (Finlay et al., 2015), providing contact with nature, which is linked to reduced stress and increased concentration (Carrus et al., 2017). Considering these considerations, it is essential that local authorities pay particular attention to the planning and development of urban green spaces. For this, a well-designed green space provides a number of benefits related to resident health, climate change adaptation and biodiversity, elements that significantly benefit also the economy of municipalities, taxpayers and landowners (Kruize et Al. 2019). Urban green spaces are generally planned and managed by public authorities and municipalities but in recent decades, citizen participation is a frequent part of green space planning and management. However, participation has often failed to reach its full potential for different reasons; for instance, dedicated resources are not fully activated, are not taken suggestion for improving urban liveability, capacities for collaboration are diluted (Fors et Al. 2021). At the heart of addressing these urban greening challenges is the need for informed, aware, and communicative young citizens. The youth, key stakeholders in urban development and potential future leaders, must be actively engaged in the discourse and decision-making processes, their awareness of environmental issues, coupled with the ability to communicate and advocate for sustainable practices, is crucial for the long-term success of urban greening initiatives. To address the challenges related to the planning of urban green areas outlined at the European level, the project "Future Citizens Involved through European Green Deal Challenge - A Great Fabric of Our Citizenship" (FUCIN) has been developed and funded under the ERASMUS+ Programme, KA220-SCH Cooperation Partnerships in School Education action. It adopts a collaborative and participatory methodology to engage and develop a constructive dialogue between young citizens and institutions to empower students to take a leading role in decision-making regarding environmental and sustainability policies. Through dialogue, lifelong non-formal exchanges, and sharing between students and municipalities, a dynamic community is built to support solid, sustainable, and concrete urban green growth. The present paper focuses on the objectives and methodologies adopted by the project, as well as presenting the preliminary results achieved in the first year.

FUCIN project and the methodology

The FUCIN project (https://fucin-erasmusplus.eu), which involves seven different partners from three countries (Italy, Turkey, and Slovakia), was launched in October 2023 and has duration of 36 months. The project was developed with the aim of promoting active citizenship through collaborations between students and local policy makers with the common objective of the improvement, and communication of sustainable and green local policies in the framework of the European Green Deal. Given that the emerging technology is becoming prevalent in students' lives, it is essential to promote and consolidate strategies of social and active relationships, using as a lever themes and issues that they consider important and promoting languages and communication strategies appealing and appropriate to them. FUCIN aims to address three main challenges identified at the European level: (1) Enhance young citizenship and participation in democratic life; (2) Increase students' skills to make them ready to face environmental challenges; (3) support students to acquire the digital knowledge for addressing the 21st century digital skills. Therefore, it aims to achieve the following key objectives through key activities (Table 1): (1) promote active citizenship with the common objective of the improvement and communication of sustainable and green local policies in the framework of the European Green Deal; (2) Sustain emerging technology, becoming prevalent in students' lives, as essential and strategic tools in promoting and in consolidating social and active citizenship; (3) GD themes, considered by the young citizens very important and pressing, can prove strategic in promoting STEAM (science, technology, engineering, art and math) issues especially if we use a stimulating and engaging language.

Table 1. FUCIN project activities and related goals.

Participative community development between students and municipality.	**Green New Deal and STEAM challenge.**	**Digital immersive tools for future citizens.**
This activity aims to develop a participative community between students and municipality raising the awareness of local and EU	It aims to improve STEAM skills in relation to environmental and European Green Deal challenges to make young citizens aware and	This activity aims to enhance students' digital skills for implementing digital tools to disseminate green practices at EU

green and environmental practices. It is designed with the aim of reducing the distance between students, that are the young citizens, and municipality through their involvement in a process which encourages a constant social dialogue. This participatory process will drive students to discover the municipal administration and to know the policies that are implemented at territorial level, with reference to green practices.	increase their civic engagement. This activity is designed with the aim of increasing students' knowledge that is the key to preparing new generations for a greener future. The lessons that will be organized during the project will provide input to young citizens to face environmental challenges with more awareness. The civic sense will be nourished by the continuous debate with experts and researchers and the exchange with students at European level (students in Italy and Turkey).	level co-designed between students and municipalities. It is designed to increase the digital skills that are not deepened in the school curriculum with the aim of increasing the digital literacy of the students. Laboratories and lessons will be carried out by a team of experts to increase the awareness of young people about their own digital and communication skills on important issues. Furthermore, the aim of the activity is to increase the sense of belonging of the citizens to the municipality, increasing the awareness of the challenges and the green policies that are implemented.

The project is being developed and implemented simultaneously in Italy and Turkey at two schools (ITT Marco Polo in Italy and Vakif K12 School in Turkey) and in two municipalities (Calenzano Municipality in Italy and Marmara Municipalities Union in Turkey). The following partners are providing scientific and educational support to enhance students' skills in areas such as STEAM (Science, Technology, Engineering, Art, Mathematics), the Green New Deal, and innovation: the Institute of BioEconomy of the National Research Council (Italy), the NGO INAK (Slovakia), and the Bilimce Association (Turkey).

Results

The FUCIN project, one year after its launch, has initiated the planned activities between students and municipality. A total of approximately 70 students from both Italy and Turkey have been actively involved through an ongoing participatory process, which has included face-to-face meetings, seminars and the administration of surveys. Specifically, the project began with the distribution of an initial survey to students and municipal staff to explore key topics aimed at addressing environmental, social and economic challenges related to the principles of the Green New Deal (e.g. biodiversity preservation, creating a sustainable food chain, sustainable agriculture and climate action) and to assess the level of knowledge acquired in both school and extracurricular settings. The main objective of this activity was to collect useful data to identify potential areas of intervention. The results will guide the design of educational initiatives, with the aim of fostering the development of innovative and participatory solutions to tackle global challenges from a local perspective.

Following this step, five face-to-face meetings between students and the municipality were organized. The first meetings took place at the school, a familiar setting for the students, where the participatory process began in both Italy and Turkey. During this dialogue, the students gained a deeper understanding of the municipality's functioning by interacting with public employees and analyzing ongoing projects, the implementation processes, and the challenges and opportunities these initiatives offer to citizens. This allowed them to better

comprehend the process of implementing local policies, fostering a constructive dialogue aimed at improving civic participation and raising awareness about public policies.
Through the activation of this participatory process, students have had the opportunity to contribute their ideas using clear language and innovative technological tools, offering their vision for the future. Specifically, the debate on public green projects and environmental policies implemented by the municipality is now officially open, with students taking a leading role. Their attention and curiosity have focused primarily on issues such as biodiversity and climate change, topics to which they have shown a strong sensitivity and a wealth of knowledge. The challenge that students pose to institutions, and to the adult world in general, is to identify and implement concrete projects that can effectively combat habitat loss, mitigate the increasingly evident risks posed by climate change, and address the growing detachment and indifference of some citizens in the face of these global emergencies. Students are calling for serious commitment and a swift, coordinated response from institutions, so that the future can truly be sustainable and inclusive for all.
This first year of activities marked the beginning of a participatory process aimed at bridging the gap between institutions and students. To reinforce this process, the "Students and Municipality Desk" was established and activated as a tangible virtual tool that allows students to interact with the municipality easily and efficiently. At the same time, the municipality now has a direct channel to gather feedback, ideas, and perspectives from young people, fostering closer and more dynamic collaboration between the younger generations and local administrations.
Building on the strengthened relationship between institutions and students, the second year began with a series of six seminars organized by scientific partners, aimed at enhancing students' skills in sustainable development topics and, more specifically, the principles of the Green New Deal. These workshops will provide students with a deeper understanding of the complex interactions between urban and rural ecosystems, climate change, and biodiversity loss. Furthermore, the workshops will offer insights into the principles, challenges, and participatory processes involved in urban green space design, highlighting the importance of innovative and sustainable solutions for the cities of the future.

Conclusions

This work focuses on the first-year results of an Erasmus+ project aimed at fostering active and responsible citizenship among students in Italy and Turkey through a participatory process involving local administrators. One of the key findings highlighted the mutual recognition by both students and the municipalities of the need to strengthen the connection between institutions and citizens. Local governments emphasized the importance of engaging with young people as future citizens, acknowledging that few initiatives had been implemented to bridge this gap. At the same time, it became clear that many young people had limited understanding of how municipal administrations operate, the projects they undertake, and the motivations, challenges, and constraints they face. The necessity of testing these concepts against the realities of the two specific contexts was underscored, moving away from generic participation processes. This approach helps students appreciate the value of dialogue and in-depth discussions on specific topics, aligning the quality objectives for green spaces with practical needs and constraints.

Throughout this first phase of project activities, a key factor emerged as a vital need among students: the importance of acquiring a comprehensive understanding of the scientific and design variables associated with urban green projects. This includes focusing on each operational intervention and correlating it with the broader framework of international policies. Ultimately, the opportunity to enhance their skills, engage with potentially unfamiliar environments, and apply their theoretical knowledge in practical contexts elicited an immediate and collaborative response from the students.

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OVERVIEW OF PLANT TYPES FOR EXTENSIVE GREEN ROOFS

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Introduction

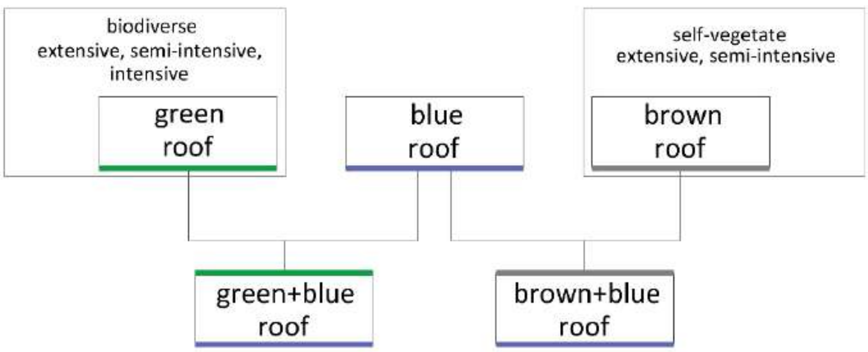
This paper reviews the theoretical principles and applied systems of green roofs, focusing on the methods used to implement different components. With increasing urban interest in sustainable solutions, communities are turning to green roofs to mitigate climate change effects. As part of the Nature-Based Solutions (NBS) initiative, green roofs offer adaptable, sustainable urban infrastructure solutions. Implementing a green roof system is complex and site-specific, requiring clear guidelines to ensure optimal conditions for plant species selection. An overview of plant species suitable for extensive green roofs would facilitate the use of native species, enhancing sustainability. Several factors influence the success of green roof systems, including location, vegetation choice, substrate depth, and the roof's purpose. Green roofs effectively address urban problems like the urban heat island effect, sustainable drainage, and biodiversity enhancement. Their impact on blue and green infrastructure is crucial for climate change mitigation. Local climatic parameters—such as sun exposure, wind, temperature fluctuations, and precipitation—are essential for selecting the appropriate green roof system, substrate thickness, and plant species. Properly selected vegetation is crucial for a successful green roof, especially native species that are better adapted to local climates, reducing the need for maintenance, irrigation, and feeding. Native vegetation also enhances biodiversity and ecological resilience. The depth and composition of the substrate must be compatible with plant species, influencing the vegetation coverage of the green roof. Sustainability can be integrated into green roofs by using recycled materials for substrates, supporting ecological goals and improving biodiversity. Green roofs offer multiple functions beyond biodiversity, including stormwater management, thermal insulation, energy efficiency, and visual enhancement. These functions help determine the specific design, suitable vegetation, and other components like irrigation and substrates. Extensive green roof systems are gaining popularity within NBS initiatives due to their versatility and adaptability. The trend is towards increasing the self-sufficiency of green roofs by integrating green and blue roofs within the same system. This involves creating biodiverse green roofs that accumulate and utilize rainwater and/or grey water on-site. These advancements support the ecological significance of green roofs and enhance their role in urban sustainability efforts.

Material and Methods

Methodology used in this research is based on analysis of scientific and professional literature covering green roofs background and development of the fundamental principles and systematization. Throught analysis and interpretation of practical nature-based concepts and other applied principles, the potentials and limitations of the guidelines were proposed for the implementation of plant types for extensive green roofs in the context of urban areas.

Green roof system as a proposal of the NBS program for urban areas

The development of green infrastructure is upgraded every day by numerous directives and laws as part of European policies. Accordingly, today, the approach and potential for the implementation of NBS solutions should be observed through; The Nature Restoration Law, Energy Performance of Buildings Directive, The Urban Waste Water Treatment Directive, Energy Efficiency Directive and Directive on Soil Monitoring and Resilience. The greening of roofs is a functional example of the application of NBS as a way of reducing temperatures in cities, collecting rainwater, fighting pollution, integrating natural solutions that encourage the construction of a healthier environment and increasing biodiversity. What sets the roof garden system apart as a significantly valuable part of the NBS solution in urban areas is its ability to mitigate rainwater during intensive infiltration. The advantage is that they use the already existing space and do not reduce the functionality of the city existing infrastructure. The undefined guidelines at the state and regional levels prevent the adequate implementation of green and blue infrastructure and biodiversity at the local level. It is necessary to select autochthonous species that will have a positive effect on existing habitats in order to prevent the introduction of foreign plant species, monocultures, harmful insects and diseases, etc. (Eggermont, 2015.) Wider application of roof gardens can serve as a bridge between other NBS and help create a more environmentally conscious urban community. In the literature and in practice, the division according to the typology of the cover is mentioned, so we can divide them into; a) green roofs, b) 'brown roof' and c) blue roof, but also possible combination between them (Picture 1). For green roofs, the established division is defined by the FLL guidelines (German: *Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau* e.V.) and we divide them into: a) extensive, b) semi-intensive and c) intensive. (FLL, 2018)



Picture 1. Typology depending on the dominant character of the system and the type of vegetation with possible combinations of systems

Characteristics of the substrate

The type of substrate affects the selection of the type of plant material, since its characteristics dictate the success of growth in extreme conditions. The components of the green roof vary significantly depending on the climatic conditions of the location, the purpose and the thickness of the system, therefore it is not possible to apply one universal substrate or system. Especially the regional specificities of the conditions for growth, numerous research questions the physical and chemical properties of the substrate and the definition of specific characteristics through a thoughtful adaptation of the FLL guidelines. (Kazemi & Mohorko, 2017) They question: depth, weight, pH, settling factor, particle size ratio, water retention and drainage possibilities, and the ratio of organic and mineral substances. The substrate must also be optimized to be structurally stable, that is, meet certain requirements (Table 1). (FLL, 2018; Van Woert et al., 2005., Sutton et al., 2012) The physical properties of the substrate mostly affect the movement and retention of water in the substrate layer itself, as well as resistance to erosion, chemical properties affect the ability to absorb nutrients, i.e. factors such as: pH, availability of nutrients, capacity for cation exchange, buffering capacity and stabilization of the substrate from chemical changes. (Ampim & Peter et al., 2010)

Table 1. Requirements for vegetation engineering properties of growing media for single-layer intensive and extensive greening (except turf greening) (FLL, 2018)

No.	Properties	Requirements	
		Unit	Value
1	**Particle distribution** [1]		
	Proportion of silting components (d $\leq$ 0.063 mm)	% mass	$\leq$ 10
	Proportion of fine medium gravel (d > 4 mm)	% mass	$\leq$ 75
2	**Bulk density (volume weight)** [2]		
	In dry condition	g/cm³	-
	At max. water capacity	g/cm³	-
3	**Water/air balance**		
	Total pore volume [2]	vol. %	-
	Maximum water capacity – intensive greening	vol. %	30-65
	Maximum water capacity – extensive greening	vol. %	20-65
	Air capacity at maximum water capacity	vol. %	$\geq$ 10
	Water permeability mod.Kf	mm/min	60-400
4	**pH value, salt content**		
	pH value (in $CaCl_2$)		6.0-8.5
	Intensive greening – salt content (water extract) [3]	g/l	$\leq$ 2.5
	Intensive greening – salt content (gypsum extract) [4]	g/l	$\leq$ 1.5
	Extensive greening – salt content (water extract) [3]	g/l	$\leq$ 3.5
	Extensive greening – salt content (gypsum extract) [4]	g/l	$\leq$ 2.5
5	**Organic matter**		
	Organic matter content	g/l	$\leq$ 40
6	**Nutrients** [5]		
	Plant available nutrients		
	- Nitrogen (N) (in $CaCl_2$)	mg/l	$\leq$ 80
	- Phosphorus (P_2O_5) (in CAL)	mg/l	$\leq$ 200
	- Potassium (K_2O) (in CAL)	mg/l	$\leq$ 700
	- Magnesium (Mg) (in $CaCl_2$)	mg/l	$\leq$ 200
	Plant available nutrients (in CAT)		
	- Nitrogen (N)	mg/l	$\leq$ 80
	- Phosphorus (P_2O_5)	mg/l	$\leq$ 50
	- Potassium (K_2O)	mg/l	$\leq$ 500
	- Magnesium (Mg)	mg/l	$\leq$ 200
7	**Foreign materials**		
	Diameter > 6 mm		

	- Tiles, glass, ceramic and similar	% mass	≤ 0.3
	- Metal, plastic	% mass	≤ 0.1
	Total area of plastic	cm²/l	≤ 10

Notes: [1)] The particle size diagram is to be entered into the specified particle size distribution range according to figure 4 (see section 12.2.2. of the FLL guidelines 2018); [2)] No requirements; [3)] The value should be as low as possible; [4)] To be proven if needed; [5)] Either in CAL / Calcium chloride or CAT

The tendency today is to use local soils and adapt them to the already researched and confirmed substrate characteristics for roof garden systems. Local soils have a better predisposition for the growth and development of autochthonous vegetation. (Dvorak, 2021) Along with a better ecological approach, the possible use of local soils also affects the financial aspect, reducing the price of the performance cost. Recent research shows that the minimum or recommended depth of a certain type of green roof can differ significantly at the regional and local level and significantly affect the success of vegetation development on the roof garden.

Roof garden system and fire protection

The fire resistance of roof gardens is an extremely important aspect, and it is a real and dangerous risk that is considered during the installation of a green roof itself. Minimizing the risk is possible by choosing the structure of the green roof and its components that help protect against fire damage, as well as planning access communications and zones that enable the approach of firefighters, the use of preventive systems and timely maintenance. Fire protection is legally defined nationally, and the FLL guidelines cannot be directly applied in Croatia. The elements that affect the penetration of heat to the structure of the building in the event of a fire are: different depths of the substrate, organic matter, height and density of plant material, amount of moisture, elements of the drainage accumulation layer, geotextiles, etc. The most vulnerable element to fires on the roof garden system is the most exposed, and that is the layer of plant material, that is, their poor maintenance and the amount of dry plant material on the green roof. Such materials are an easily flammable source of fire, since the amount of moisture in the dry material is significantly lower than the living parts of the plant. (Gerzhova et al., 2020) Minimizing the risk is possible by using types of fleshy leaves that contain a high proportion of water and taking into account the ratio of dry and fuel material between coniferous and deciduous vegetation. Deciduous vegetation sheds its leaves during the colder months and, unlike conifer needles, has no flammable resin. Therefore, when planning, it is necessary to consider the risks posed by coniferous species. (Jervis & Rein, 2015) The incorporation of paths or other design elements and the use of materials such as stone aggregate can prevent the spread of fire. Standards such as FLL include the installation of gravel barriers between the green roof surface and the roof elements. Such breaks act as a kind of fire barriers that break the continuity between combustible materials. (FLL, 2018) The factor preventing the spread of fire heat to the deeper layers of the system is the depth of the substrate itself. According to research in which the temperature on the surface of the bare substrate rose to 300°C, temperatures around 40°C were measured at the bottom of the green roof, which concludes that the substrate layer plays a key role in fire protection. Dry substrate is a very poor conductor of heat and does not direct it towards the deeper layers, which is most effectively achieved by substrates with a depth of ≥10 cm. Precisely because they are bad conductors of heat, it has a positive effect on their performance on green roofs because they provide insulation, and green roofs act as a barrier that reduces heat transfer. This slows down the transfer of heat, and can act on the spread and mitigation of fire intensity because it acts as a buffer zone between the roof structure and the building. (Gerzhova et al., 2019)

Principles of design and selection of plant species

We apply the principles of design that we use on overgrown terrain to roof garden systems as well, and include vegetation as an element with multiple purposes. The design principles we use on natural terrain we also apply to roof garden systems as well, and they include vegetation as an element with multiple purposes. The role of vegetation planning and design is particularly significant in the urban context and has an impact on the level of comfort, the experience of space, naturalness and aesthetics. This is precisely why the characteristics of plants such as size, grouping, form, transparency and position have the greatest influence on the design of the outdoor space. Vegetation plays a key role in defining the edges, the openness of the space, which is reflected in the shaping of volumes, surfaces and boundaries and providing spatial orientation, which shapes the experience of the city for the user, including recognition, safety and connection with the environment. (Pereković, 2016) The possible advantages of roof gardens are proportionally related to the choice of vegetation. The choice of plant species on green roofs is a process in which not only visual-aesthetic but also spatial-structural, functional and ecological-technical roles of vegetation are considered. The selection of plant species for a certain green roof depends primarily on the location, i.e. the climatic conditions, the intended system and purpose. In contrast to planting in a natural terrain, on roof gardens the priority is the functional rather than the visual aspect of the vegetation.

The reason for this is that in addition to the visual impression, green roof plant species must also have characteristics that will ensure their successful growth and development in locations where conditions are limited. Visually integrating roof garden surfaces on urban structures can help create softer lines and transitions between the urban, semi-natural and natural landscapes. In addition to habitat and the role of biodiversity, the color of used vegetation itself changes the perception of the urban environment. (Poorova et al.,2016) With color and texture variations, we introduce a visually diverse element into the urban environment, which can be attractive throughout several seasons. Visual design also includes combining different characteristics of plants such as height, size, texture and habit form. Manipulation of height with vegetation is a very effective method for layering the space, which creates additional dynamics in the space, and shares the function with the spatial-structural role of vegetation. Areas of green roofs, the design of which usually depends on the color and height of the vegetation, represent the first perceptual characteristics visible to residents from a greater distance. When compiling possible combinations of vegetation for green roofs, we recognize three basic categories; a) monocultures, b) simple plant mixtures or combinations, and c) complex plant communities. The above categories represent different approaches to the selection of vegetation, which differs in terms of maintenance requirements, biodiversity and function, therefore it is important to consider location conditions and user intentions. In order to enable the development of an ecologically and perceptually more complex sustainable roof garden, it is recommended to combine species from different categories, but taking into account biotic and abiotic needs. Designing roof gardens with monoculture visually provides a simple and uniform vegetation that is relatively easy to maintain given the same vegetation requirements. Its disadvantages can be susceptibility to pests and diseases that can quickly devastate them. (Shipton, 1977) Simple plant mixtures represent a combination of a certain number of plant species that are most often complemented by similar characteristics such as water requirements, substrate depth and the need for nutrients, and differ in the type of habitus (visual properties), the difference in vegetation cycles and durability (outside and in vegetation period). More complex mixtures with a larger number of species increase not only the visual perception but also the ecological habitat value. Complex plant communities mean the combination of several plant species that occur in autochthonous natural communities of an area. Often with a 'wild' appearance, such combinations try to imitate natural ecosystems and contribute to biodiversity in urban conditions. With a high degree of biodiversity, such plant communities are more resistant to pests and diseases than monocultures. They also compete as alternatives to extensive roofs with *Sedum sp.* species, due to the lower level of maintenance they require. The use of native species on green roofs is desirable, but roof garden systems are very challenging habitats for which in some cases there are no local plant communities that can survive in specific roof conditions especially climatic ones. Therefore, when choosing plant material, the knowledge of experts is necessary, since it is possible to establish substitute plant communities that will perform the function of the local flora just as well. (Dunnett & Kingsbury, 2004) It is often mentioned in the literature that moss is the most basic category of vegetation for green roofs, but as a rule, mosses are not implemented in a targeted manner, but appear on roofs through natural succession processes. As a pioneer species in the creation of habitats, by establishing a community, they can improve the chemical conditions of the soil, contributing to slower drying. Although they have significant properties in regulating storm water runoff and stabilizing temperature fluctuations, and their lack of true roots and drought tolerance make them ideal candidates for extensive green roofs, their quantitative role is small, especially compared to other types of vegetation. Mosses are non-vascular plants that have several desirable properties for use on green roofs, but with extremely specific conditions. Their ability to retain water, which can be up to 8-10 times greater than their weight, helps to slow down the runoff of precipitation from roofs, but quantitatively these amounts are still small. Also, mosses can help the nearby vascular plant species because their mass retains moisture in the substrate, which dries out more slowly. Maintaining moisture in the substrate also helps with significantly better cooling properties of buildings with green roofs and reduces sudden temperature fluctuations. (Andersonet al. 2010) The geophyte category includes perennial species of bulbs, tubers and rhizomes that allow them to survive extreme conditions through dormant periods. Geophytes usually bloom at the beginning of the growing season, and are often combined with other types of vegetation precisely to increase the visual, structural and ecological value of the roof garden. The use of annual plants in the form of meadow species is recommended due to their adaptability to extreme conditions and the ability to hibernate in the substrate in the form of seeds after the growing season. The advantage of annual meadows is the possibility of establishing a sustainable vegetation system. (Nagase & Dunnett, 2013) Planting with established plant species can be financially demanding, so sowing annual species in combination with *Sedum sp.* cuttings are a good alternative. Sowing annual meadows also contributes to sustainability, and is especially beneficial to various insects, especially pollinators. (Kircher, 2004) Increasing substrate depth increases the number of potential species, including grasses, perennials and woody species. Perennials are a category of plants that thrive at depths greater than 10 cm, ideally around 20 cm, due to the increased humidity of the substrate. (Dunnett & Nolan, 2004) Growth from the same root system every year, and the above-ground part of the plant, which can be herbaceous or woody, and which enables a significantly greater visual, structural and ecological value. Perennials increase resistance during the vegetation cycle and help to stabilize the substrate and prevent erosion, and after the establishment of plantations, lower maintenance requirements are ensured. Grasses are a category of plants that can be used as a supplement in combinations with other green roof plant species to increase complexity. The exception is the setting up of lawns on green roofs, by sowing or planting, which can be very demanding to maintain. On the other hand, due to their specific growth in turf form and a dense and intertwined root system, they have proven to be the type of vegetation that retains water the best and slows the runoff of rainwater on extensive types of green roofs the most. (Nagase & Dunnett, 2012) The selection of the type of grass depends on the climatic conditions of the region and the requirements foruse, but the number of species makes them available for use. Deciduous woody species such as smaller shrubs or ground covers can be used on substrates 15-25 cm deep. (Dunnet & Kingsbury, 2004) They represent species adapted to dry conditions characterized by a smaller, harder and waxy leaf or one covered with hairs. The coniferuos type of vegetation ensures the presence of green elements throughout the year, which contributes to the visual quality of green roofs, but compared to deciduous species, they contribute less to mitigating urban heat island effect, they are more sensitive to dry periods, and are a poor choice in terms of fire protection. (Dunnet & Kingsbury, 2004) Svakako je značajno razumjeti odnos supstrata, tipa vegetacije u kontekstu održivosti I bioraznolikosti I mogućnost zadržavanja oborina (Table 2).

Table 2. Average annual values of precipitation retention, i.e. coefficients of water permeability in relation to the thickness of the substrate and vegetation, according to FLL (FLL, 2018)

type of green roof	substrate thickness in cm	type of vegetation	average annual rainfall retention capacity in %	rainfall permeability coefficient Ψ_a
Extensive green roof	> 4-6	Sedum species	45	0,55
	> 6-10	Sedum species and meadow plants	50	0,50
	> 10-15	Sedum species, grasses, meadow plants, perennials	55	0,45
	> 15-20	Grasses, meadow plants, perennials	60	0,40
Intensive green roof	15-25	Lawn, smaller groundcovering shrubs, flowering shrubs, perenials	60	0,40
	25-50	Lawn, groundcovering shrubs, flowering shrubs, perenials, climbing plants	70	0,30
	> 50	Lawn, shrubs, trees, perenials, climbing plants	> 90	0,10

Plant types for extensive green roofs

The vegetation of green roofs should have several important characteristics: a) the ability to quickly establish a dense and stable cover under extreme conditions, b) the establishment of substrate coverage with vegetation in order to reduce wind erosion and substrate heating, c) a good ability to regenerate, so that plant areas are damaged under extreme conditions could recover independently without the need for replanting and d) the ability to effectively manage water, absorb and release it. Due to pronounced negative factors such as drought, low temperature and poor soil conditions, it is necessary to take into account scenarios of extreme conditions and the location of the habitat in the process of planning, design and selection of vegetation. An overview of the most common types for extensive green roofs can be found in Table 3. (Dunnet & Kingsbury, 2004, Getter & Rowe, 2006)

Table 3. The most common species used for planting on extensive and/or semi-intensive green roofs (Dunnet & Kingsbury, 2004; FLL, 2018)

Category	**Plant species**
Mosses and ferns	*Bryum argenteum, Tortella nitida, Trichostomum crispulum, Tortula muralis, Didymodon fallax, Grimmia lisae, Syntrichia laevipilla, Ceratodon purpureus, Tortula inermis, Cheilanthes sp., Davallia sp.*
Geophytes (bulbs, tubers, rhizomes)	*Tulipa tarda, Allium pulchellum, Allium schoenoprasum, Allium flavum, Allium atropurpureum, Allium caeruleum, Allium carinatum, Allium cernuum, Allium moly, Allium strictum, Allium vineale, Iris germanica, Iris variegata, Iris flavescens, Iris pumila, Iris tectorum, Crocus tommasinianus, Muscari neglectum, Muscari armeniacum, Muscari botryoides, Anthericum liliago, Anthericum ramosum, Babiana sp., Corydalis cheilanthifolia, Corydalis lutea, Oxalis acetosella, Vancouveria hexandra,*
Annual plants	*Gypsophila muralis, Linaria maroccana, Linum grandiflorum 'Rubrum', Eschscholzia californica, Tripleurospermum maritimum, Camassia quamash, Silene armeria, Lobularia maritima, Nemophila menziesii*
Perenials	*Sedum acre, Sedum album, Sedum floriferum, Sedum hispanicum, Sedum kamtschaticum, Sedum rupestre, Sedum sexangulare, Sedum spurium, Sempervivum tectorum, Sempervivum montanum, Sempervivum arachnoideum, Delosperma cooperi, Petrorhagia saxifraga, Saxifraga crustata, Saxifraga tridactylites, Lewisia cotyledon, Dianthus sp., Thymus sp., Campanula sp., Potentilla sp., Achillea sp., Anthemis sp., Aster sp., Solidago sp., Centaurea sp., Gypsophila repens, Verbascum phoeniceum, Verbascum chaixii, Pulsatilla vulgaris, Nepeta x faassenii, Calamintha nepeta, Acaena microphylla, Acaena buchananii, Acaena caesiiglauca, Acaena inermis, Acinos alpinus, Chiastophyllum oppositifolium, Euphorbia capitulata, Euphorbia cyparissias, Herniaria alpina, Jovibarba sobolifera, Sagina subulata, Alyssum argenteum, Alyssum montanum, Antennaria dioica, Armeria maritima, Armeria juniperifolia*
Grasses	*Festuca glauca, Festuca ovina, Festuca cinerea, Festuca punctoria, Festuca vivipara, Festuca rupicaprina, Festuca rupicola, Festuca valesiaca, Koeleria macrantha, Melica ciliata, Briza media, Briza minor, Stipa capillata, Stipa pennata, Festuca amethystina, Agrostis capillaris, Cynosurus cristatus, Festuca rubra, Poa pratensis, Trisetum flavescens,*

	Carex caryophyllea, Carex firma, Carex montana, Carex umbrosa, Helictotrichon sempervirens, Corynephorus canescens, Bouteloua curtipendula, Bouteloua gracilis
Deciduous woody plants	*Cytisus sp., Genista sp., Caragana sp., Ononis sp., Prunus tenella, Rosa pimpinellifolia, Rosa gallica, Salix lanata, Salix repens, Salix retusa*
Conferous	*Juniperus communis ssp. nana, Juniperus horizontalis, Juniperus procumbens, Pinus aristata, Pinus mugo*

Sedum sp. species are the most common choice because they form a very low and compact growth, which contributes to a dense and uniform texture. In addition to succulents, annual and perennial herbaceous plants, and also grasses, are common on extensive green roofs. Succulents on extensive systems have the advantage of low water requirements, while grasses and herbaceous plants can easily become more of a fire risk. Most types of succulents like *Sedum sp.* and *Sempervivum sp.* they function on the CAM (Crassulacean Acid Metabolism) principle, which sets them apart from other types of vegetation for the reason that such metabolism allows them to close their pores during the day to minimize moisture loss during high temperatures. Such an adaptation is specific to species adapted to arid climates, allowing them to open their stomata and take in CO_2 during the night when temperatures are lower. (Herrera, 2008) The problem with vegetation that functions on the basis of CAM is that by closing the stomata during the day, they partially or completely limit the effect of evapotranspiration. Evapotranspiration is an important effect of vegetation in urban areas that suffer from the UHI effect, because vegetation cools the immediate environment and lowers the air temperature by evaporating water. Therefore, when choosing vegetation for a green roof, it is also important to consider vegetation whose physiological and morphological characteristics are more favorable for cooling the space by evapotranspiration instead of exclusively CAM vegetation where possible. (Monteiro et al., 2016, Solcerova et al., 2017) Species that are autochthonous to the region and are common in natural succession processes can come into contact with the substrate planned for the green roof through wind or animals and establish natural communities by themselves. This simple form of greening can be achieved by targeted substrate preparation with nutrients and seeds to facilitate the succession process. Mosses are often used in combination with *Sedum sp.* species due to similar characters of form and height and tolerance to extreme conditions. (Heim et al., 2014) Their ability to survive large fluctuation in humidity is a very useful adaptation that allows them to recover from drought by rehydrating even after they have completely dried out. Species such as *Tortula muralis* and *Bryum argenteum* are gaining popularity precisely because of their sustainable way of managing water, which is a particularly important characteristic for the Mediterranean climate. (Cruz et al., 2019) Geophytes can successfully survive on extensive green roofs, above 10 cm depth. The most common choice are *Tulipa sp.* and *Allium sp*. plants, which were observed as the most successful species in research at locations in Germany. *Tulipa sp.* species has proven to be a successful choice for shallow substrates with a depth of 5 cm, which makes them a good choice where only lightweight extensive green roof systems can be installed. (Nagase & Dunnett, 2013) Geophytes' tolerance to drought, together with the lower need for maintenance, resistance and aesthetic characteristics secured a place on the green roof. Most species of annual plants, perennials and grasses were taken from dry habitats. Shallow substrates (4-6 cm) can often accommodate very specialized types of perennials such as succulents. Planting succulents on a deeper substrate is possible, but they are subject to elimination from more dominant species that suit a deeper substrate. Most *Sedum sp.* and *Sempervivum sp.* species inhabit habitats with low rainfall, well-drained substrates and a limited number of other species. *Sedum acre* and *Sedum rupestre* are capable of independently inhabiting green roofs in areas of northern Europe. *Sedum sp.* species can create interesting mosaics whose aesthetic quality is recognizable all year round, especially in the flowering period between May and July. Species such as *Sedum album* and *Sedum floriferum* change their leaf color from green to red in the dry and winter periods. *Sempervivum sp.* species are slower growing, more sensitive to excess moisture and, due to the way they grow, are not suitable for larger areas as a monoculture on green roofs. The most commonly used species include *Sempervivum tectorum* and *Sempervivum arachnoideum*. *Delosperma sp.* it has excellent tolerance to the dry period, but due to its sensitivity to low temperatures, it is recommended for planting in the coastal area of Croatia. The depth of the substrate from 6 to 10 cm is mainly suitable for herbaceous species of low pillowy or creeping growth, and they form aesthetically and structurally interesting textures. *Dianthus, Alyssum, Potentilla, Campanula sp.* etc. species which create meadow-like habitats can contribute to biodiversity. As a vertical accent, species like *Iris sp.*, *Sisychrinium sp.* and *Verbascum sp.* are common. On substrates with a depth of 12 cm, it is possible to use species of dry meadows such as *Achillea sp., Anthemis sp., Centaurea sp.,* etc. In grass mixtures, we usually only encounter those species that are adapted to grow on deeper, extensive roofs, the depth of which ranges from 6 -10 cm. Mixtures of non-native grasses that are better adapted to the conditions are common, such as *Festuca sp., Poa sp.* and species like *Agrostis capillaris*.

Distribution and number of species on the extensive type of roof

There are four main methods of establishing vegetation on green roofs that are used today: a) direct application of seeds and cuttings, b) planting plants grown in containers or plugs, c) placing vegetation mats and d) spontaneous colonization. The methods of establishing vegetation on extensive roofs differ depending on the purpose, climate conditions and type of vegetation, as well as temperature, humidity and quality of the substrate.

Green roofs are designed to support specific species selected by humans, but also to minimize the growth and development of invasive species. Understanding the biotic and abiotic factors that affect plant populations and their dynamics is the solution to creating successful habitats on green roof surfaces. The limitation is unavailable data at the regional and local level and insufficiently researched species in extreme conditions, and a neglected component of the dynamics of plant populations. Interactions change seasonally, and most often involve competition for nutrients and more favorable conditions. It is also necessary to consider ecological needs in order to avoid the establishment of communities where the most common interaction between plants is competition for nutrients. Otherwise, the dominance of allochthonous monocultures is possible. The distribution of species also depends on the height of the vegetation. Certain species can cover less dominant ones and prevent reproduction for further spread on the roof surface, and over the years cause a decrease in the number of established species and or plant communities. At the same time, these same species can provide shade for lower species during extreme conditions if they are compatible in exploiting limited resources, the need for the amount of light and the competition of the root system. Sustainable systems are able to support high biodiversity communities and enable successional processes. When it comes to the distribution of species on green roofs, it should be emphasized that populations move differently in natural habitats and conditions compared to green roofs, and are not affected by the same factors. Fragmentation of the habitat at such heights (although we also have green roof systems at the same level as the natural terrain) also affects the distribution and number of species present due to the distance from other green roofs and classic green areas. It is necessary to consider ways of mitigating these distances in order to improve the biodiversity of the habitat. The use of drought tolerant species such as *Sedum sp.* is a practice that has dominated for a long time when planting on green roofs of shallow substrates. However, despite the success of such species, experts increasingly turn to the selection of species of natural communities of dry habitats whose substrate depth ranges from 6 to 15 cm, and with this, the depth of the substrate on roofs increases. In northern and central Europe, the most diverse species come from shallow alkaline soils. Their characteristics are low growth, visual diversity and tolerance to drought, which in combination with indigenous local communities of dry habitats make excellent mixtures of high biodiversity, adaptable to the habitat and durable. The establishment of such plant communities forms a healthy extensive roof system, ecologically resistant and sustainable.

Results and discussion

The technology and knowledge surrounding the implementation of green roofs is still in the early stages of knowledge despite centuries of presence of green roofs as elements of urban environments. However, in Germany and Europe, the wide application of extensive roofs during the last century contributed to a rich history of research. When considering previous research and guidelines for the implementation of green roofs, it is important to take into account the impact of the green roof system on the structure of the building itself; structural load, insulation, fire protection and protection of the building from possible negative impacts of the planned roof garden system. The selection of a suitable substrate and the vegetation itself are correlated, and if the goal is a sustainable solution, a general or schematic takeover of the roof garden system is not possible. National standards and regulations must be applied before selecting and implementing a green roof system. The component of the drainage-accumulation layers of the green roof and the possibility of water accumulation play a big role in the selection of vegetation and the depth of the substrate, and it is necessary to have a comprehensive solution proposal. Inadequate drainage can completely saturate the substrate and lead to rotting processes of the vegetation layer. Water regulation on the roof is directly related to: 1) particle size, 2) material compatibility, 3) environmental compatibility, 4) plant material compatibility, 5) fire protection characteristics, 6) stability and layer structure, 7) material settlement, 8) water permeability, 9) maximum capacity for water storage, 10) pH value, 11) salt content, and 12) resistance to weather conditions. For these reasons, it is recommended to select the material of the drainage layers based on knowledge of local weather conditions and the amount of annual precipitation, as well as the load-bearing capacity of the roof considering the different weights of the material. The selection of drainage layer material can also be influenced by the plant material, considering the different needs of plants for water and moisture. The depth and type of substrate should correspond to the specific requirements of the plants, and the chemical, physical and biological properties should be adapted accordingly. The substrate should be structurally stable, store and release water in accordance with the selected vegetation. Also, depending on the type of planned vegetation, it is necessary to pay attention to the following properties: 1) environmental compatibility, 2) compatibility of plant species, 3) arrangement of particles, 4) content of organic matter, 5) resistance to weather conditions, 6) water permeability, 7) maximum capacity for water storage, 8) air content, 9) pH value, 10) nutrient content, 11) adsorption capacity, 12) germination of seeds / regenerative parts of the plant / reproduction, 13) proportion of foreign substances and 14) subsidence of the substrate. When using organic matter in the substrate mixture for extensive green roofs, it is important to take into account the rate of decomposition, because it affects the structure of the substrate itself, and therefore it is used in very small amounts in the substrate of extensive roofs or it is completely replaced with mineral components. For this reason, commercial green roof substrates contain very little, if any, organic matter and are replaced by inorganic mineral components that perform the same function as a traditional substrate. Green roofs are sustainable green surfaces that can be implemented in such a way that they do not require additional irrigation. With various combinations of plant communities and substrate types, it is advisable to create green roof systems that use only precipitation and rainwater as a method of irrigation, but classic forms of irrigation are certainly possible on a green roof system. Their frequency is higher on newly implemented green roofs, due to the possibility of dry periods or intensive green roof systems. The method of planting plant material on the surface of the green roof must correspond to the biological characteristics of: individual plant species, different forms of vegetation and the general goal of greening. Dry and wet sowing options are possible, which can be combined with cuttings or the cuttings themselves can be used. It is also possible to lay pre-grown vegetation mats, plant individual plants or install grass mats. Maintenance is also necessary for extensive green roofs, but at a lower intensity compared to other types of green roofs. The maintenance process on extensive green roof systems may include the following; supply of nutrients, irrigation of steep roofs exposed to the sun (up to 40°), removal of vegetation, pruning and thinning, sowing in the case of thinned vegetation with seeds or cuttings of Sedum sp., transplanting to larger areas without sufficient cover, maintaining the edges of vegetation and non-vegetation elements, supplementing substrate in case of erosion, maintenance of drains and drainage corridors, protection of plants, maintenance of the irrigation system if it exists, checking the safety of elements of work at height.

Conclusion

Green roof systems have become a basic part of NBS initiatives, and thanks to the versatility and typology of roof gardens, and the number of possible types, they are becoming an increasingly available solution. The tendency is to increase the accumulation and self-sufficiency of green roofs, and the implementation of green and blue roofs within the same system has begun, that is, the accumulation and utilization of rainwater and/or gray water at the location itself. Many factors influence the success of the implementation of the green roof system, primarily the location, the choice of vegetation, the depth of the substrate and the purpose of the green roof. Functions of green roofs such as stormwater management, thermal insulation, energy efficiency and improvement of visual qualities are integrated into the process of planning, designing and implementing a green roof. Their role in modern urban environments is continuously researched and upgraded to test their effectiveness in solving urban problems such as space cooling and improving biodiversity. The effect that green roofs have on blue and green infrastructure can prove to be a very important green alternative in mitigating the effects of climate change faced by urban users. Understanding regional factors such as climate conditions, i.e. exposure to sun and wind, and oscillations between the highest and lowest annual temperatures and precipitation, is a key part of the process of selecting a suitable green roof system. The results of the analysis of microclimate conditions are necessary for the implementation of a sustainable system, the depth of the substrate and the type of vegetation of the green roof. A sustainability component can be integrated into the substrate layers of green roofs, primarily when using recycled or locally sourced materials. Adequate selection of autochthonous vegetation reduces the need for maintenance, irrigation and nutrients. Native vegetation also contributes to the realization of a higher level of biodiversity in the area and contributes to the ecological resistance of the green roof. When choosing species, it is necessary to consider the visual-aesthetic, spatial-structural, functional and ecological-technical roles of vegetation.

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THE “BIO-BAT” PROJECT: ASSESSING BAT DIVERSITY IN CENTRAL ITALY URBAN-RURAL INTERFACE

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Urban forests and green areas provide essential habitats to bats, offering roosting sites, foraging opportunities, and corridors for movement, contributing to their survival and population health. These green spaces play a crucial role in mitigating the negative impacts of urbanization on bat populations, such as habitat loss, fragmentation, and disturbance, promoting their long-term persistence in urban environments. Within the BIO-BAT Project, we investigated the bat diversity within the municipalities of Calenzano and Sesto Fiorentino, in the northern part of the metropolitan area of Firenze (Central Italy) between November 2023 and October 2024, in direct continuity with two protected areas (Monte Morello and Calvana Mountains). Using Audiomoth acoustic recorders, the research aimed at identifying bat species, assess their activity patterns, and evaluate their distribution across habitats, including forests, agricultural areas, urban zones, and industrial sites. A total of 596 recordings from 17 sites were analyzed, revealing the presence of 13 bat species, representing a significant portion of Italy's bat fauna. Notably, several species of conservation concern under the Annex II of the Habitats Directive, such as the Geoffrey’s bat (*Myotis emarginatus*) and the greater horseshoe bat (*Rhinolophus ferrumequinum*), were detected in the Calenzano municipality. The commonest species were the Savi's pipistrelle (*Hypsugo savii*) and the Kuhl's pipistrelle (*Pipistrellus kuhlii*), which are well-known urban exploiters. Additionally, species more typical of natural and forest habitats, such as the common noctule (*Nyctalus noctula*), Leisler's bat (*Nyctalus leisleri*), and Daubenton's bat (*Myotis daubentoni*), were recorded. A comparative analysis with the neighboring municipality of Sesto Fiorentino revealed a lower bat diversity, with a predominance of species adapted to human-modified environments. These findings highlight the importance of urban green spaces and forests as refuges for bats and emphasize the role of bats as valuable bioindicators of environmental health. The study underscores the need for continued bat monitoring and conservation efforts, particularly in light of the global decline of bat populations. By understanding the distribution and habitat requirements of bat species, we can develop effective greening strategies to protect these essential components of our ecosystems.

Keywords: Biodiversity conservation; Chiroptera; green areas; urban forests; urbanization.

Introduction

The current worldwide increasing urbanization significantly impacts biodiversity [1]. As cities expand, natural habitats are destroyed or fragmented, leading to habitat loss for many species, hindering their ability to adapt and thrive [2]. Pollution, noise, and light pollution from urban areas can further degrade biodiversity by stressing species and altering their behaviour [3]. Urbanization and human activities have also significantly impacted bat populations worldwide [4,5], besides few, well-adapted species [6]. As cities expand, bats lose their natural habitats, such as forests and caves, which provide essential shelter and food sources; in turn, habitat loss can lead to a decline in bat populations due to reduced breeding and foraging opportunities [5,6]. Additionally, the introduction of artificial light can disrupt bat natural navigation and hunting behaviors, leading to disorientation and increased mortality rates [7]. Human activities, such as deforestation and pesticide use, which are directly linked to urbanization processes, can also have detrimental effects on bats [8]. Deforestation and urban greening practices destroy bat habitats and reduces the availability of shelters and insects, which are the primary food source for many bat species, whereas pesticides and pollutants can contaminate food chains, poisoning both bats and their prey [5,9,10]. Furthermore, human disturbance, such as construction noise and human presence, can furtherly stress bats and interfere with their reproductive activities [11]. In response to these threats, many bat populations are facing decline and are at risk of extinction [5,9]. Conservation efforts are crucial to protect bats and their habitats, including habitat restoration, creating artificial roosting structures, and reducing the use of harmful pesticides, but also improving the effectiveness of ecological corridors [12,13].

Studying bat diversity in urban areas is crucial, as bats play a pivotal role in European ecosystems as insect predators. In particular, in urban environments, they can help control invertebrate pest populations, such as mosquitoes and moths, reducing the need for chemical pesticides, thus informing pest management strategies and promoting sustainable urban development [14]. Moreover, bats are sensitive indicators of environmental health, as their presence / absence may reveal information on habitat quality, air pollution, and the presence of contaminants [15]. By monitoring bat populations in urban areas, scientists can assess the overall health of these ecosystems and identify potential environmental threats [15]. To conclude, studying bat diversity can contribute to conservation efforts. By understanding factors influencing bat populations in cities, strategies can be developed to mitigate the negative impacts of urbanization and create bat-friendly urban environments, including artificial roosting structures, reducing light pollution, and promoting sustainable land use practices [16].

This study aimed to investigate the influence of urban development patterns on bat species richness in two contrasting urban areas situated on the northern outskirts of Florence, Italy. Specifically, we selected two urban areas characterized by a different urban development, one more interfaced with rural areas, the other more separated from the natural environment. Both areas are contiguous with two Natura2000 sites, both characterized by a high diversity of bat species. The aim of this study was to determine the species richness of bats in green areas (urban parks, roundabouts, tree-lined avenues, and olive groves) within the urban area, to determine their ecological function in maintaining bat diversity. We predicted that a more careful urban organization and good management of green areas are important contributors to maintaining a high level of bat species, including those less adapted to anthropized environments.

Materials and methods

We conducted our study in the northernmost part of the metropolitan city of Florence, encompassing the urban areas of two adjacent municipalities, Calenzano and Sesto Fiorentino.

Calenzano (43.852°N, 11.160°E; mean altitude, 69 m a.s.l; about 18.000 inhabitants in 2023) is situated within the Mugello Valley, a hilly region characterized by hilly landscapes and extensive greenery. This geographical location contributes to its significantly more natural environment compared to Sesto Fiorentino. The municipality boasts a substantial expanse of agricultural land, forests, and parks, fostering a predominantly rural character.

Sesto Fiorentino (43.831°N, 11.199°E; mean altitude, 54 m a.s.l; about 49.000 inhabitants in 2023), in contrast, is a more urbanized area due to its direct proximity to Florence, the regional capital, and its denser population. As a consequence, it exhibits a more developed infrastructure, including industrial zones and commercial centers. Although it incorporates green spaces, its overall character is predominantly urban and industrial.

Both municipalities border two Natura2000 sites, Monte Morello (Natura2000 code: IT5140008) and the Monti della Calvana (IT5150001), which are characterized by a rich diversity of flora and fauna [17,18,19].

Low-cost energy-efficient recorders (©AudioMoth) were placed in 14 green areas in Calenzano and 11 in Sesto Fiorentino (Figure 1). Acoustic data were first filtered in Kaleidoscope vers. 5.3.8 (©Wildlife Acoustics, Massachusetts, USA) in order to remove noise. Files were then assigned to species by using the "Tadarida" programming procedure, and manually confirmed by inspecting files with Audacity vers. 3.0.0 (©GNU General Public Licence, Boston, Massachusetts, USA). When species assignment was inconsistent, we classified files to genus (*Plecotus* spp., *Myotis* spp.) or phonic group (e.g., 'nyctaloid' comprising *Nyctalus* spp. and *Eptesicus* spp.).

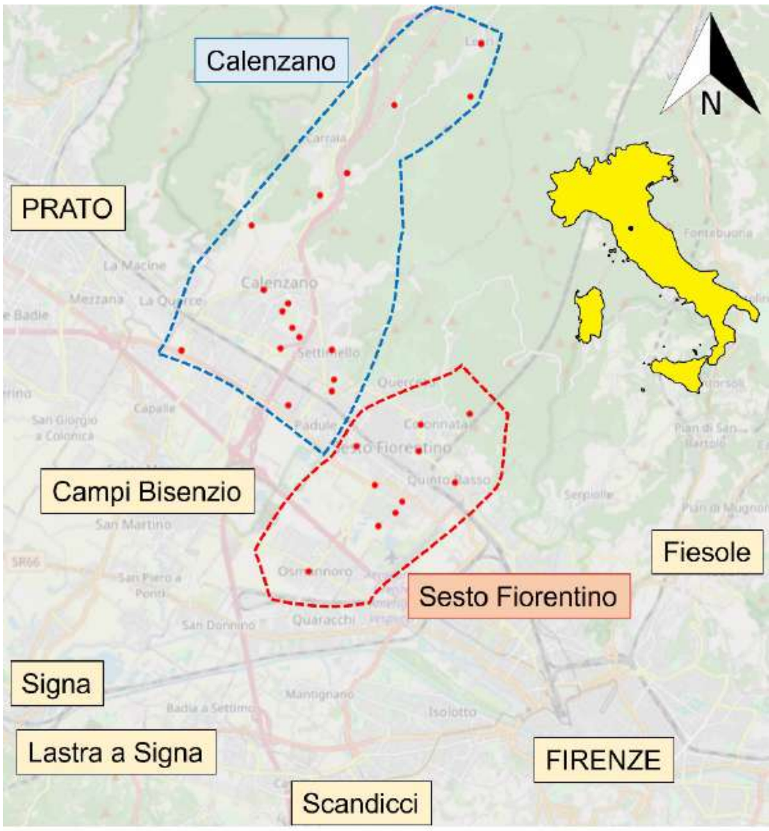

Figure 1. Urban survey sites (red dots) in the municipalities of Sesto Fiorentino (blue dotted line) and Calenzano (red dotted line).

Results and Discussion

From the analysis of the recordings (N = 596 in Calenzano, N = 519 in Sesto Fiorentino), 13 bat species were identified (out of the 34 officially present in Italy), some of which are included in Annex II of Directive 92/43/EEC, whose presence allows the designation of "Special Conservation Areas (SCAs)". All species were found in the urban area of Calenzano, whereas only 8 were detected also in Sesto Fiorentino. In particular, species listed in the Annex II of the Habitats Directive, i.e. the Geoffroy's bat *Myotis emarginatus* and the greater horseshoe bat *Rhinolophus ferrumequinum*, were found to be more widespread in Calenzano, and recorded in only one site in Sesto Fiorentino. The most common species are Savi's pipistrelle *Hypsugo savii* and Kuhl's pipistrelle *Pipistrellus kuhlii*, species particularly adapted to urban ecosystems. Species typical of natural environments and forests have also been recorded, mostly in Calenzano, such as the noctule *Nyctalus noctula*, the Leisler's bat *Nyctalus leisleri*, the Daubenton's bat *Myotis daubentoni*, the cryptic bat *Myotis crypticus*, the Nathusius' pipistrelle *Pipistrellus nathusii*, the common pipistrelle *Pipistrellus pipistrellus* and grey long-eared bat *Plecotus austriacus*.
The results of this study showed a significant difference in bat diversity between Calenzano and Sesto Fiorentino. The higher species richness and prevalence of protected species in Calenzano can be attributed to its more natural landscape, which provides a variety of habitats suitable for different bat species. The presence of bat species adapted to urban environments and also to forests and natural areas in both municipalities highlights the importance of urban green spaces for bat conservation. Therefore, our findings have important implications for urban planning and conservation efforts, as suggesting that the

preservation of natural habitats and the creation of high-quality green spaces within urban areas are crucial for maintaining bat diversity and supporting their ecological roles. Furthermore, the use of low-cost monitoring techniques like AudioMoth recorders can provide valuable data for bat conservation and management. Future research could explore the factors influencing bat distribution and abundance within urban environments, e.g. by investigating the impact of land use changes, habitat fragmentation, and human disturbance on bat populations. Additionally, studying the foraging behaviour and prey availability for bats in different urban habitats could provide further insights into their ecological requirements.

Conclusion

The present study showed that bat populations in the municipality of Calenzano exhibit a remarkable diversity, despite the global decline of bat species, with respect to the more urbanised municipality of Sesto Fiorentino. This finding underscores the importance of bat conservation and highlights their potential as effective bioindicators. Given the ease of bat monitoring and their sensitivity to environmental changes, they can provide valuable insights into habitat quality, biodiversity, and ecological processes, particularly in urban and industrial areas. As anthropogenic activities continue to degrade ecosystems, bats can serve as early warning signals of environmental stress, guiding targeted conservation efforts and sustainable development strategies.

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ESTABLISHMENT AND DEVELOPMENT OF LABORATORY FOR ADAPTED FOREST REPRODUCTIVE MATERIAL (LABADAPT)

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Introduction

Forest reproductive material (FRM) is crucial for all activities related to silviculture and is one of the backbones of forest ecosystem preservation. The most important activities include afforestation of available areas, restoration (e.g. burned areas, landfills, quarries, protective belts), prestoration, artificial forest regeneration (assistance to natural regeneration processes), and nature protection. Furthermore, FRM forms the basis of silvicultural activities and contributes to maintaining the forest's good condition and naturalness (Perić et al. 2013; Đodan et al. 2023). Increasing pressures on forests and their ecosystems create new challenges. This creates a gap between the current knowledge, awareness, readiness and resources and those desired and needed to tackle the new challenges posed by climate change. Future forest stands may survive and remain productive only if they have enough adaptive potential to cope with extreme events related to climate change (Kramer 2016). Recently, one of the crucial challenges has been the lack of FRM, especially high-quality planting material adapted to future threats. The adapted FRM includes seeds, seedlings, cuttings, and naturally regenerated plants better adapted to the forthcoming climate changes, abiotic and biotic threats, and socio-economic circumstances (Đodan and Perić 2022). Quality FRM is often a key factor for successful forest implementation and habitat restoration programs and for creating healthy, functional, sustainable, and resilient ecosystems (Đodan et al. 2023). It is also the backbone for implementing the newly adopted Law on nature restoration (Regulation EU 2024/1991). In the face of growing uncertainties, it is crucial to have high-quality FRM, especially high-quality seedlings, to preserve our naturally structured forest (Ministry of Agriculture 2016) and high biological diversity (EEA 2007). Seedling quality can be determined by various morphological and physiological properties (Davis and Pinto 2021). However, the most important ones are those that maximize their chances of survival and growth in their habitat conditions (Ritchie et al. 2010; De Souza et al. 2021). To determine the quality of the seedling as accurately as possible, it is necessary to integrate a combination of both morphological and physiological properties (Folk and Grossnickle 1997; Seletković et al. 2009). More than 30 methods are available in practical forestry for determining seedling quality (Mohammed 1997), however only a certain number of properties are determined in practical forestry (Haase and Davis 2017; Grossnickle and MacDonald 2018b). Therefore, a laboratory for adapted FRM at the Croatian Forestry Research Institute (CFRI) is initiated as a part of the project "Establishment and Development of LABoratory for ADAPTed Forest reproductive material (LABADAPT)" financed by the Ministry of Science and Education in the frame of NextGenerationEU Recovery and Resilience Facility – a financial instrument offering grants and loans to support reforms and investments in the EU Member States is. LABADAPT project aims to establish and develop infrastructure and protocols for testing adapted forest reproductive material. It will provide the infrastructure and supporting environment for testing various morphological and physiological seedling properties. The need for adapted FRM increases continuously, which is evident in practical forestry (Đodan et al. 2023). The need for more detailed planting material testing arose within the project Expert supervision of nursery production in Croatia (project details reviewed in Đodan i Perić 2022). Therefore, the establishment of the laboratory for adapted FRM in the LABADAPT project will serve as crucial support to the efforts of The Republic of Croatia to provide an adequate FRM and certification system. Project LABADAPT started in January 2024 (the project duration is 48 months, project leader: Dr. Martina Đodan, PhD). The project aims to I.) develop laboratory methods for testing forest planting material, II.) test the quality of forest planting material produced in the nurseries in the Republic of Croatia, III.) identify the needs and determine the guidelines for enhancement of the production and quality of forest planting material for the whole country, IV.) test new methods of production of adapted forest planting material, V.) digitalise activities in a seed bank, VI.) test new methods of FRM storage, VII.) test the quality of seed currently stored in seed bank. This paper will outline the importance of determining the morphological and physiological seedlings' properties for increasing their chances of survival and growth which will be carried out in the new laboratory for the adapted FRM.

A review of the qualitative seedlings' properties

Determination of morphological seedlings' properties

Apart from the seedlings' height and stem diameter, which are considered the most useful properties for determining the quality of seedlings because of a good correlation with survival and growth after out-planting (Dey and Parker 1997; Haase 2008), the laboratory will also make it possible to assess the quality of the root system. Several authors (Rose et al. 1997; Davis and Jacobs 2005; Tsakaldimi et al. 2005) have argued that root morphology may provide a more effective indicator of seedling quality than height and stem diameter. A High-quality root system is crucial for the survival and growth of seedlings in the field (Grossnickle and MacDonald 2018 a,b). A quality root system readily takes up water and nutrients and provides structural support to the seedling (Grossnickle and Ivetić 2022). The quality of roots and other parts of the seedling will be analysed using a scanner and specific software to examine the morphology, topology, structure, and color of the cleaned seedling surface. Additionally, a specific software will be used for precise positioning and analysis of the roots and other fragments of the seedling. This technology will also be used to determine the root volume, which is one of the key factors in avoiding stress in the early phase after transplanting seedlings to the field, especially during the summer dry period (Tsakaldimi et al. 2005).

Laboratory will be equipped with a dryer (max. temperature 300 °C) with a minimum of 100 litres capacity. The mass of dried seedlings or seedling fragments will be measured using high-precision scales. The mass determination is important both during seedling production and field planting (Grossnickle and Macdonald 2018b). Generally, the greater the mass of dry seedlings or seedling fragments, the greater the chances for the seedling's survival and growth in the field. Determination of the mass is carried out by using samples of the live seedlings or their parts immediately after collection, followed by measuring the mass after drying in the dryer for 48 hours (Drvodelić and Oršanić 2019).

Finally, laboratory experiments will provide insight into potential correlations and dependencies between the morphological and physiological properties of seedlings under various silvicultural regimes.

Determination of physiological properties of seedlings

The survival and growth of seedlings in the field depend largely on their ability to adapt to the habitat's conditions, i.e. physiological properties (Grossnickle 2012). As early as 1984, the importance of seedling physiology was recognized when Faulconer and Thompson indicated that seedling physiology is the main carrier of seedling quality. Therefore, physiological properties can be considered more reliable quality indicators than morphological ones (Seletković et al. 2011). Climate change causes shifts in the site's suitability for forest tree species (Dolos et al. 2015) but also increases the resistance of some individuals, provenances and forest tree species towards growing threats (Fady et al. 2016). Therefore, the adaptability of the physiological systems of different species and their provenances will be examined in the laboratory. This will bring us new conclusions about the species' suitability for the various site conditions (site-specific seedlings). The survival and growth of seedlings are mostly affected by their ability to cope with a water deficit and adapt to a wide range of air temperature changes (Grossnickle 2018). In the laboratory, the use of a 1200-litre capacity climate chamber will demonstrate the adaptability of the seedling's physiological system. The climate chamber will be able to demonstrate those and many more various conditions using the associated climate chamber software and licenses.

The use of a pressure bomb (pressure chamber, Scholander bomb) to measure leaves transpiration helps determine the rate of water loss from a plant. Moisture stress can be the most damaging off all the stresses (Drvodelić and Oršanić 2019) because it damages the photosynthetic system and causes disturbance in the physiological processes, affecting seedlings' growth and survival (Haase 2008). The effects of irrigation in nurseries have so far been mainly researched through measurement and determination of the morphological properties of seedling quality such as height and stem diameter (Drvodelić and Oršanić 2019), using a pressure bomb we can obtain more precise data on the water needs of the seedlings.

Photosynthetic processes in seedlings are highly sensitive to environmental stresses and reflect normal developmental and seasonal changes (Mohammed et al. 1995). Evaluating photosynthetic performance is an effective way to monitor how seedlings respond to various environmental factors, as photosynthesis is highly affected by changes in temperature, water availability, and light intensity (Perks et al. 2001). One of the determining factors of photosynthetic efficiency is the amount of available light (Jose et al. 2003). It is known that plants respond to ultraviolet (UV) radiation in their environment. Bright light, especially its integral UV part, can cause stress reactions in the seedling, triggering metabolic processes and metabolism changes (Müller-Xing et al. 2014; Trigona et al. 2023). In the laboratory, we will use a device to measure the amount of UV radiation and detect excessive light exposure in seedlings. With devices for measuring photosynthetic activity and the Leaf Area Index (LAI) it will be possible to get more precise data on photosynthetic processes in the seedlings (Zhu et al. 2013; Fang et al. 2019) important for seedlings' adaptation to the site.In addition to assessing the various properties of seedlings, we will also measure the presence of oxygen (O_2) in the soil using a device to gauge the quantity of O_2. This is important because O_2 and water are requiredfor the growth and development of roots (Drvodelić and Oršanić 2019). The law of the minimum (Justus Von Liebig 1873) states that the growth of a species is not determined by the total resources but is strongly determined by the resource that is needed and insufficient (minimally available) and then it becomes a limiting factor of growth. However, nursery production occurs under highly controlled conditions, making all resources highly dependent on silvicultural activities (Pinto and Davis 2021). Therefore, resources can be needed and insufficient, so while determining the quality of produced seedlings all resources must be considered.

All data collected for property determination will be automatically downloaded, stored, and used to create a metadatabase.

Conclusion

The European forests, forestry and forest sector, of which the Republic of Croatia is an important part, will face significant challenges: I.) more frequent extreme weather conditions for planted seedlings, II.) increasing demand for more areas to be planted, III.) limited seed availability that may hinder the seedling production of many forest tree species, and IV.) maladaptation of forest tree species and populations to novel conditions. Most European countries have recommendations or guidelines for selecting species and provenances that can be used in each site or zone. However, these recommendations are mostly based on present or past climatic conditions. To meet global objectives and enhance the forest, forestry, and related sectors in adapting to climate change, a high-quality FRM is essential. Unintentional use of inappropriate FRM eventually leads to forest degradation and loss of ecological, financial and social benefits. Therefore, integration and action of all stakeholders involved in silviculture is needed to tackle upcoming threats. Higher-quality seedlings do not guarantee survival and growth in the field, but they have a significantly greater chance of survival and growth. The establishment of LABADPT will advance the science of silviculture, nature protection and agroforestry, improve expert supervision, and ensure the production of high-quality seedlings to meet the needs of the entire Republic of Croatia.

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PRESTORATION OF POPLAR PLANTATIONS WITHIN CROSS-BORDER COOPERATION OF CROATIA AND SERBIA

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Introduction

Increased occurrence of forest damages poses a threat to forest productivity and consequently decreases biomass and wood products production as well as complex impacts on the market. Moreover, damages can decrease ecosystem services and forest biodiversity. Anthropogenic influence, depopulation of areas, and lack of silvicultural measures, resources and manpower are some of the examples which significantly add to the restoration complexity and lower management options. Despite the traditional good management of forest resources, which supports natural forest tree species composition, the current global changes force an increasingly active role of society in the forest restoration (Tolić 1995) and pint to the need of assisted species and provenance migration (Schüler and Chakraborty 2021).

Forest restoration is a long-term, expensive and often complex process. It is becoming increasingly challenging under today's altered site conditions (Jacobs et al. 2015), and it depends on nursery production and good silvicultural practices (Gladstone and Ledig 1990; Grossnickle 2000, Đodan et al. 2023). Moreover, new findings indicate a shift in site suitability for forest tree species in Europe (e.g. Dolos et al. 2015), but also the increased resistance of some individuals, provenances and forest tree species towards growing threats (Fady et al. 2016). For example, it is important to focus on species that are believed to be adapted to predicted climate extremes such as longer periods of drought (Schüler and Chakraborty 2021). This gives a more optimistic view since the use of adapted forest reproductive material (FRM) can enhance stand resilience (Đodan et al. 2023). Prestoration is a new term, which combines our efforts (activities) on forest restoration and adaptation. It includes climate projections and forthcoming threats to better counteract the predicted global changes. To preserve and increase forest biodiversity and ecosystems services, it is important to consider all forest restoration aspects, such as economic, social and ecological impacts. Additional human efforts are necessary, especially in prestoration activities, where the goal is finding new, innovative solutions for forest restoration to enhance forest resilience (Đodan and Perić 2022).

One of the largest projects addressing this issue is the H2020 SUPERB (Systemic Solutions for Upscaling of Urgent Ecosystem Restoration for Forest-Related Biodiversity and Ecosystem Services) funded by European commission under Green Deal call. Among its goals, the Green Deal SUPERB project tackles the influences of restoration and prestoration activities on forest biodiversity and ecosystem services. It also aims to restore forest landscapes across Europe and showcase different restoration activities throughout 12 demonstration areas. SUPERB involves a large network of scientists and stakeholders and through practical and scientific knowledge, seeks to develop strategies that will ensure the long-term resilience of forest ecosystems to climate change and other pressures. One of the twelve demonstration areas is Croatian and Serbian border river floodplain area split equally between Croatia and Serbia. Thus, broader demonstration area consists of Croatian and Serbian monitoring and restoration plots, while the paper will present only Croatian activities within SUPERB project. All activities are harmonised and complementary, especially methodological approaches, aiming to showcase the basic barriers, enablers and existing knowledge, as well as innovative approach to prestoration. Moreover, it aims to facilitate upscaling restoration activities on larger scales in the future. Paper presents basic goals, methodology, overview of established trial plots and scientific activities initiated in the Croatian part of the demonstration area.

Research area

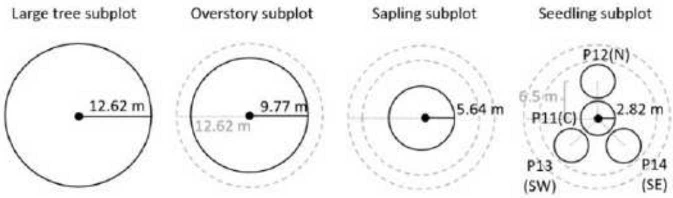
Within SUPERB project, cross-border demonstration area was selected in the vicinity of the City of Osijek. Historically, the area was partially cleared for agricultural and urban development purposes, followed by changes in water management due to dam construction and river regulation. Basic aim of demonstration trial plots is to enhance biodiversity, increase forest productivity and value of ecosystem services of the area. Thus, replacement of over matured non-native poplar plantations (*Populus x canadensis* Moench.), which were mainly used for intensive wood production and left unmanaged after the Civil war due to landmines were selected as one example of prestoration potential of the area. Now, the area is cleared of landmines, and management is possible again. The forests are owned mostly by state and managed by Croatian Forests Ltd, Zagreb. The resilience of these poplar plantations, which became popular starting in the 1920s either through afforestation of agricultural land or planting poplars after clear-cutting of natural mixed stands is very low. Climate changes and other threats negatively influenced poplar plantations and recently have only added to the management complexity. Namely, prolonged, more frequent and more intensified droughts, temperature increase, invasive plant species, pests and diseases, lack of forest reproductive material and manpower. In the long term, climate change may severely alter flooding regime in the sense of increase of winter and decrease of spring flooding owing to reduced snow melt. Invasive non-native tree (e.g. *Acer negundo* L., *Prunus serotina* Ehrh.) and plant species (e.g. *Echinocystis lobata* (Michx.) Torr. et Gray) already present a serious obstacle for natural regeneration and tree growth, also negatively impact tree species biodiversity of the area.

Research design

Current Croatian restoration area, monitored by three levels of trial plots, covers 270 ha. Monitoring includes monitoring of restoration activities conducted during the last three decades and initiation of prestoration activities on 53,5 ha. Research includes initial monitoring in the stands prior to prestoration, restoration stands where restoration activities are planned through planting activities and monitoring sites including cronosequence of restored stands in the area in the last four decades. A special methodology for project implementation and restoration success indicators was adopted.

Initial assessment

Before the start of any restoration activities on the plots, a detailed measurements of forest structure and composition together with measurement of biodiversity according to SUPERB project methodology was conducted. The purpose of the initial forest inventory is to describe the state of the forest trees (dead wood as well) and to be able to assess future effects (success and failures) of the measures on the ecosystem biodiversity and functions. Final aim is to identify the main indicators of restoration/prestoration success. The observations are conducted and recorded at plot and subplot scale, in a sample design laid out over the restored areas involving several aspects of the stand structure (Đodan et. Galić 2022) (adult trees, saplings and seedlings, Figure 1). Monitoring includes measurements of diameter at breast height, height of trees and tree-related microhabitats.



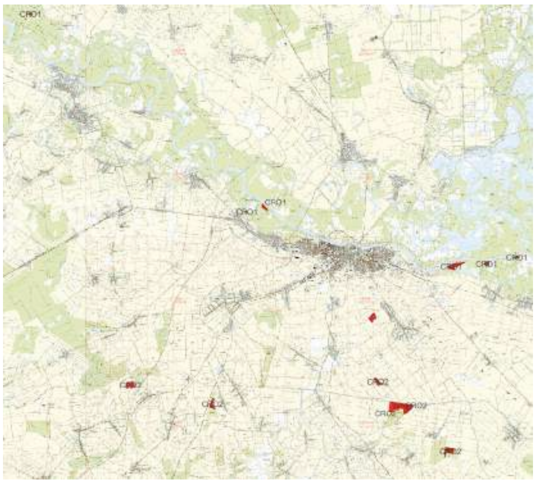
Field plot design for multilayered forest following a circular subplot design.

1. One large tree subplot (12.62 m radius) where only large adult trees (>27 cm dbh) will be measured;
2. One overstory subplot (9.77 m radius) where all adult trees (> 7cm dbh) will be measured;
3. One sapling subplot (5.64 m radius) where the smaller trees (saplings) will be measured and deadwood biomass will be assessed;
4. Four seedling subplots (2.82 m radius) where seedlings will be counted.
5. In addition, aground vegetation inventory of non-tree species will be done over 1-m quadrats within seedling subplots.

Figure 1. Experimental desing for cronosequence monitoring according to methodology adopted in the frame of SUPERB project (WP 6) (Đodan and Galić 2022)

Restoration trial plots

To successfully test forest restoration planting was carried out and the first results were recorded. Restoration plots cover two restoration sites located around the city of Osijek along the river Drava (departments “1a” and “95b”) with a total area of 53.5 ha (Figure 2). Croatian Forests Ltd. is acting as a linked third party in the project and conducts restoration on restoration plots, while Croatian Forest Research Institute (CFRI) conducts monitoring activities. In specific, the restoration requires removing all recently devitalised non-native poplar plantations and replacing with more resistant and economically interesting late successional mixed species stands. The poplar monocultures have been replaced with native species (*Quercus robur* L., 5,000 seedlings per ha, *Prunus avium* L., 100 per ha). Seedling type, age and provenance have been determined according to available seed resources and the availability in the nurseries (Croatian Forests Ltd.). This replacement of tree species composition is prescribed by forest management plans for selected demo area, while detailed description of restoration plan is publicly available (Đodan et. Galić 2022). Even though preferred silvicultural method is shelterwood in general, in the case of replacement of poplars removal of all trees is necessary. It prevents spreading of unwanted non-native poplar regeneration on the aimed replacement area, which can hinder growth and development of aimed natural tree species, thus decreasing the cost of clearing and tending measures. In addition, aimed mixed species stands will have natural species composition with intent to increase the tree species diversity. At the first site restoration was carried out during 2022/2023, while the second restoration site will be restored in autumn 2023/2024. Pretreatment consists of removal of overmatured poplar trees and shrubs (if needed), tree marking, initial tree measurements, dispatching of cut poplar trees with the use of mechanisation, seedling production (nurseries, Croatian Forests Ltd.), hand digging holes for seedlings and fencing. Seedlings after out planting have been measured in the April of 2024, immediately after planting activities took place. Natural regeneration of other tree species is expected and will be supported during the tending of young stands (e.g. *Ulmus* sp., *Tilia* sp.). Protection of seedlings has been done by fencing (protection against wildlife - grazing). Posttreatment will be conducted to secure survival, growth and development of young stands. It includes replacement of plants which died out, weeding, control of herbivores, monitoring of stand health and pests.



a) b)

Figure 2: a) Croatian trial plots are set in 15 forest stands (monitoring and restoration), b) restoration stand prior to initial assessment

Cronosequence plots

Monitoring plots (cronosequence plots) include 13 monitoring stands scattered around the city of Osijek alongside the river Drava (Figure 2). Monitoring stands are mainly referred as forest patches, which used to be part of a larger riparian ecosystem. Due to extensive land use, the damming and the regulation of rivers, climate changes, the vitality of trees has declined, and those plantations were already restored. Six out of the 13 monitoring stands are under the nature protection (Natura 2000, regional park, etc.). Monitoring methodology is similar to measurements in initial assessment plots, with the difference in basic plot desing. At each site, multiple stands represent the restoration chronosequence and within each stand up to 5 inventory plots were selected. The chronosequence consists of at least 6 stands within the same site (two sites according to soil type), covering a gradient from "damaged or degraded state/before restoration" to a "reference ecosystem" representing the target restoration state (Figure 3). It also includes different stages after restoration, covering more than 30 years gradient. To make sure that a gradient is chosen within a homogeneous site, we have checked soil type, slope, aspect and elevation on a topographic map and tried to select them rather close to each other. During the inventory, causes of tree mortality are determined, biodiversity is evaluated, and the herbaceous plant layers are monitored. Tree-related microhabitats as indicators for the potential presence of certain taxa of forest-dwelling species shall be surveyed following the catalogue developed by Kraus et al. (2016). Additionally, soil carbon and biological activity or soil diversification are assessed during the first two years of the project.

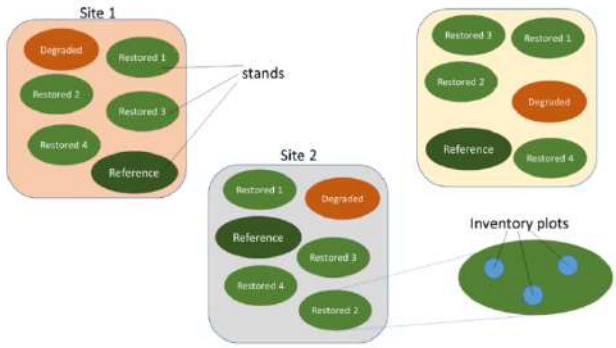


Figure 3 The chronosequence consists of 6 stands within the same site

Challenges in restoration

Monitoring of restoration on demonstration plots are much more than measuring tree growth and microhabitats. It is about recognizing basic obstacles and enabling factors, both related to current restoration plots and the areas in which restoration and prestoration will have significant role in the future. First results indicate increase of ecosystem services and biodiversity, as well as potential for increase of more valuable timber products. It should be considered that these are preliminary measurements and conclusions pointing to the need to finalise the project activities to be able to make conclusive statements. Nevertheless, some important findings of this complex study have already been revealed by the data, and those are the once on barriers to successful forest restoration/prestoration.

The first of the barriers are FRM issues, so often highlighted as crucial to majority of silvicultural interventions. From irregular or reduced seed production, lower seed quality, lack of planning of production cycle, type/age of seedlings as far as insufficient support to species and provenance assisted migration. When seed production is irregular or diminished, the speed and extent of forest restoration are limited and often cannot be planned but need to be adjusted according to the fluctuating seed yield. For instance, under normal conditions, the pedunculate oak (*Quercus robur* L.) mast years occur every 3 to 5 years. However, due to climate change and other threats, the balance within ecosystems has been disrupted, leading to a decline in the vitality of pedunculate oak stands. As a result, seed production has become increasingly irregular and weakened, negatively affecting natural regeneration. Factors contributing to reduced seed yield include late spring frosts, high humidity during pollination, summer drought, and harmful insect populations (Gradečki-Poštenjak et al. 2011.). The number of available tree species and the amounts of available FRM, especially adapted FRM, are quite limited, thus disabling increase of the number of tree species when doing active restoration. Moreover, if prestoration is regarded, then even greater barriers have been observed since prestoration is actively dependent upon assisted tree species and provenance migration. Even the possibilities of FRM quality are limited, since there is no infrastructure or protocols available in Croatia to conduct detailed physiological and morphological tests.

Secondly, legislation could be crucial barrier for restoration and mostly for prestoration which depends upon assisted migration. It can slow down or hinder the restoration process sometimes preventing the use of more adapted tree species and provenances. One of the barriers is also the overlapping nature or even gaps in regulations, sometimes confronting each other (e.g. forestry regulations and nature protection regulations). Additionally, there are limited capacities for monitoring and enforcement, which leads to delays or inadequate implementation of necessary measures, insufficient resources for restoration, and a lack of flexibility in legal regulations. One of the major results of the SUPERB demonstration area set in Croatia is the need to harmonise legislative efforts, both current and forthcoming (e.g. Law on nature restoration, Regulation on FRM). Given the constantly changing habitat conditions, a rapid and continuous adaptation of strategies is required to facilitate optimal forest restoration, as well as the preservation of biodiversity and native species in this dynamic environment.

Upscaling plans

SUPERB demonstration area covers much more than 50 hectares. Even though non-native poplar plantations in Forest district Osijek occupy 5,855.17 hectares upscaling potential reaches far beyond this amount. It includes all areas, stakeholders and time span, which are or will be in a need of restoration and prestoration by mixed species stands dominated by pedunculate oak at presence and in the forthcoming future. Recent storm event in July 2023 clearly demonstrated how quick and devastating disturbances can be in financial, social and ecological sense. Therefore, when all barriers and enablers to successful upscaling of restoration/prestoration activities will be identified, SUPERB will develop and disseminate plans and guidelines for successful restoration beyond the demonstration area and after the project’s completion. The application of the project outcomes is expected to cover a larger area, extend over longer timeframes, involve a greater number of stakeholders, and support new programs and markets. By restoring as much area as possible, we will significantly improve the health of our forests, enhance stand resilience to climatic conditions, increase the value of timber products, and increase the amount of carbon sequestered, thereby mitigating the negative impacts of climate change. The restoration will also lead to increased biodiversity and contribute to the protection and preservation of our natural forests. The outcome will include social, ecological and economic benefits, such as job creation and timber resources as a source of income. To achieve these goals, in addition to the restoration itself, regular monitoring of restored stands, thinning, transplanting, and other management activities will be necessary to ensure the sustainable development of forest stands.

Conclusions

The SUPERB project's adaptive restoration action in Croatia has revealed the complexity of restoration in a poor and depopulated area, which involves addressing stressors, necessary restoration measures, and legislation, all set against the backdrop of challenging socio-economic circumstances. The SUPERB project is one of the largest European initiatives focused on forest restoration. Moreover, the final demonstration area findings will have impact beyond the narrow restoration area (restoration plans are publicly available, while upscaling plan and upscaling roadmap are being prepared). Plans for upscaling forest restoration are crucial as they can help adapt to and mitigate climate change, help conserve biodiversity and improve ecosystems, while also providing economic benefits, improving human well-being and reducing poverty. By scaling up forest restoration efforts, we can help create a more sustainable future by sequestering more carbon dioxide, preventing forest loss and preserving natural habitats for countless species, ultimately contributing to global sustainability goals. SUPERB aims to provide tools and knowledge for joining adaptation and restoration under the new concept of prestoration. It also facilitates reaching full upscaling potential, which extends beyond time and spatial scales of the project. The goal is to preserve forests in the face of climate change, habitat degradation and other adverse impacts. Superb seeks to transfer knowledge, skills, and insights to a variety of stakeholders, demonstrating that successful restoration is achievable despite the various challenges that may arise. The key lies in learning, conducting research, and adapting good practices and methods based on the results, by doing so, these negative effects can also be mitigated. Furthermore, it is not sufficient to simply restore the forest; continuous monitoring, thinning, and other necessary management activities are essential to maintain sustainable forest stands.

Acknowledgments

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DYNAMICS OF ARTIFICIAL REGENERATION AND ESTABLISHMENT OF NEW FORESTS IN SERBIA FOR THE PERIOD 2017-2023

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Abstract: In the context of escalating impacts of climate change, improvements in forest conditions, increased carbon sequestration, and environmental improvements, increasing the forest area has become a critical task within forestry. This study aims to analyze the trends in afforestation over the past six years using statistical data.

Analysis of available data reveals significant disparities between afforestation efforts in state-owned versus privately owned forests. In state-owned forests, afforestation with hardwoods is higher by 52.4% compared to conifers, and the largest number of used seedlings are oaks (*Quercus* sp.), followed by poplars (*Populus* sp.), which are followed to a much smaller extent by black locust (*Robinia pseudoacacia* L.), other hardwood and softwood species, and finally beech (*Fagus sylvatica* L.). Among conifers, spruce (*Picea* sp.) and Austrian black pine (*Pinus nigra* J.F. Arnold) dominate, followed by Scots pine (*Pinus sylvestris* L.), fir (*Abies alba* Mill.), Weymouth pine (*Pinus strobus* L.) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) with a smaller number of seedlings. In private forests, conifers are afforested 15.6% more, spruce (*Picea* sp.) and Austrian pine (*Pinus nigra* L.) also dominate, while in hardwoods this role is taken over by poplar (*Populus* sp.) and black locust (*Robinia pseudoacacia* L.).

Year-over-year analysis demonstrates the varying extents and intensities of reforestation efforts, providing insights into the dynamic nature of these initiatives. These findings can inform more detailed analyses that support forest management plans, thereby contributing to enhanced afforestation strategies and subsequently expanding forested areas.

Keywords: afforestation, private forests, restoration, state forests

Introduction

Afforestation can transform vulnerable forests into diverse, productive, and climate-resilient forests (Bolte et al., 2009; Reyer et al., 2015). Increasing forest area through afforestation is not only desirable but also mandated by the Law on Forestry ("Official Gazette of the Republic of Serbia," Nos. 30/2010, 93/2012, 89/2015, and 95/2018). Article 80, paragraph 1, specifies that the budget of the Republic of Serbia, as well as the budget of autonomous provinces, must allocate funds for measures aimed at "increasing the degree of forest cover through afforestation." Also, meliorative measures, in addition to increasing the quality of forests, imply stopping degradation and reducing the surface of degraded lands, they should take into consideration the application of afforestation technologies, which guarantee satisfactory success while optimizing costs (Isajev *et al*., 2010).

In the context of adapting to and mitigating climate change, afforestation is a crucial tool. It is especially important in modern conditions, considering that it is a cost-effective and easily accessible option for mitigating the negative effects of climate change. In this context, the economic potential of afforestation as an adaptation strategy to climate change and its role in mitigating climate change was assessed before using dedicated forestry models and integrated assessment models (Calvin *et al*., 2014; Humpenöder *et al*., 2014; Doelman *et al*., 2020, etc.). It is of great importance that afforestation can be used as a tool that can be actively used to mitigate climate change through carbon sequestration.

At the beginning of the 19th century, forest coverage in Serbia was around 3,300,000 ha, and in 1912, the area of Serbia was 4,830,260 ha, which represents about 2/3 of the territory under forest (Vučićević, S., 2008). The attitude towards forests at the beginning of the 19th century led to Serbia having 1.5 million ha under forests at the beginning of the 20th century. In 100 years, about 1.8 million ha of forest area was removed. According to the data of the Ministry of Agriculture, Forestry and Water Management – Directorate of Forests of the Republic of Serbia, the trend of increasing areas under forest is going in a positive direction (https://upravazasume.gov.rs).

Previous authors who analyzed the trend of afforestation in the Republic of Serbia point to a pronounced negative trend of forested areas in the 21st century. In the period 2002-2021, the afforested area decreased by 5.39% on average per year, with 5.71% for broadleaves and 4.70% for conifers (Ćirković-Mitrović *et al*., 2022). This happened to the greatest extent as a consequence of reduced investments in forestry (Ivetić, 2015). Along with the negative trend of afforestation, a big problem is the success of the implementation of these measures, which has been significantly reduced due to inadequate afforestation technology and the implementation of silvicultural measures after the afforestation (Ivetić, 2015).

Considering the previously mentioned observations, this paper aims to analyze the trends in afforestation across various periods and tree species.

Material and Methods

The afforested area data from the Republic Institute for Statistics of the Republic of Serbia were analyzed. Two distinct periods were examined, along with the entire 14-year span, which served as the basis for predictions for the period 2024-2028. The first period covers 2010 to 2016, and the second spans 2017 to 2023.

The analysis includes beech (*Fagus sylvatica* L.), oak (*Quercus* sp.), poplar (*Populus* sp.), black locust (*Robinia pseudoacacia* L.), as well as other broadleaves. In case of conifers the analysis was performed for spruce (*Picea* sp.), fir (*Abies alba* Mill.), Scots pine (*Pinus sylvestris* L.), Austrian pine (*Pinus nigra* L.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), larch (*Larix* Mill.), and other conifers.

The collection of data for the specified studied period of 14 years, was adequately sorted within the Microsoft Office software package.

Sorting was done into 4 regions: Belgrade, Vojvodina, Šumadija and Western Serbia, and Southern and Eastern Serbia region. The data was compiled based on the specified types of trees and a division was made based on ownership into the private and state sectors. The data sorted in this way were processed and the results were presented in the form of graphs with associated explanations.

Results and discussion

Forest resources represent the basis of our lives and the entire economic activity. Their depletion is a major challenge for the economic development of both developed and developing economies (Singh et al. 2024). Afforestation efforts on degraded lands positively affect various soil properties and improve general habitat conditions (Korkanç, 2014). It is also of great importance that afforestation (artificial regeneration and establishment of new forests) causes fundamental changes in the structure and functioning of ecosystems, including changes in available light, microclimate, production, nutrient cycling, and water balance, all of which can affect biodiversity (Elmarsdóttiret et al. 2008).

The volume of afforestation for the period 2010–2016

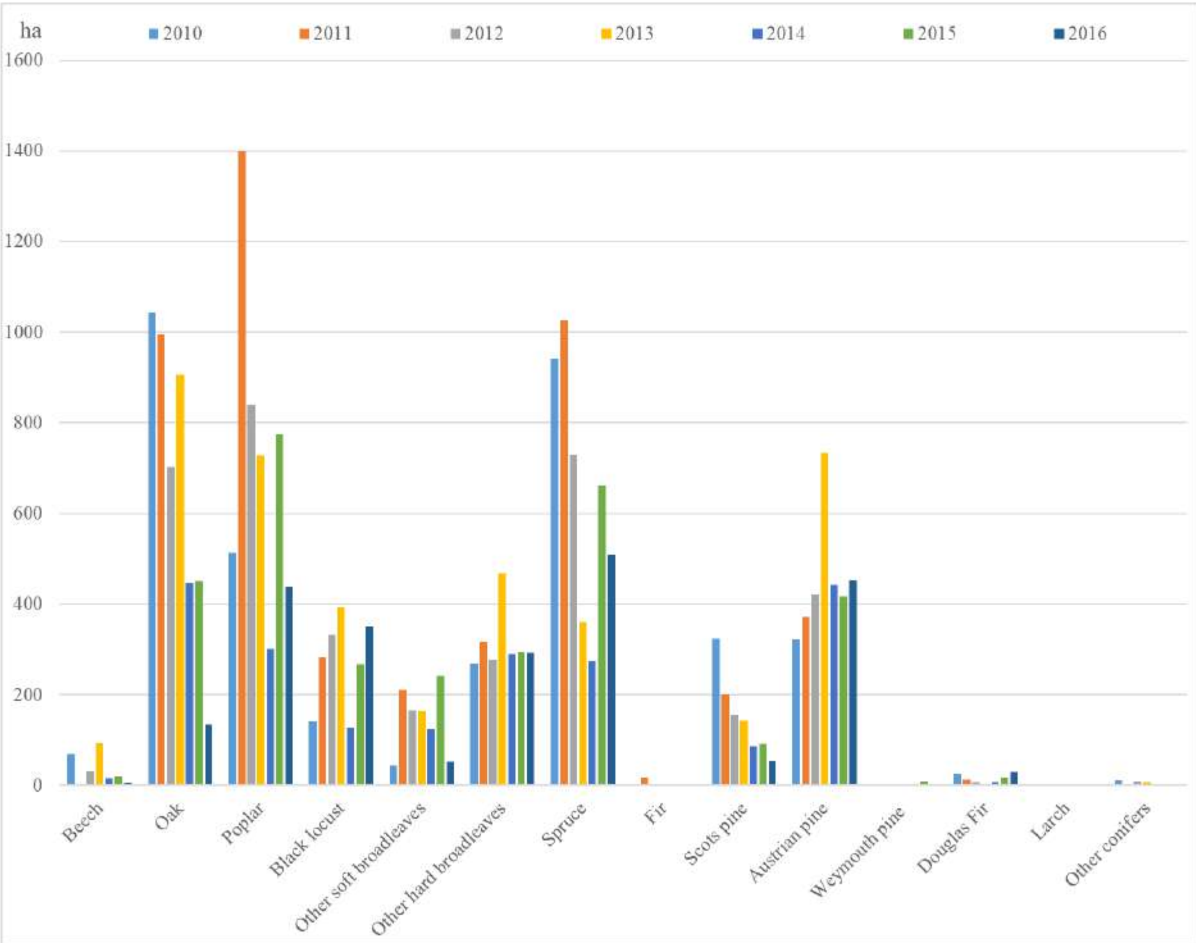

Figure 1: Presentation of the results of the volume of afforestation in hectares for the period 2010-2016

Figure 1 shows the dynamics of afforestation of different types of trees for the period 2010-2016. A total of 23920 ha was reforested, of which 20792 ha in the state sector and 3128 ha in the private sector. On average, 3417.15 ha of afforestation was realized annually. In all types of trees, except for Austrian pine, black locust, and Euroamerican poplars, a trend of decreasing the volume of afforestation is observed, which is in line with the general trend of decreasing the afforested area.

The most intensive afforestation with broadleaves species was carried out in the areas where afforestation was planned with oaks and poplars, while the least afforestation was carried out in places where beech and black locust were planned. In conifers, the largest area is forested with spruce and Austrian pine, and the smallest with larch and Weymouth pine.

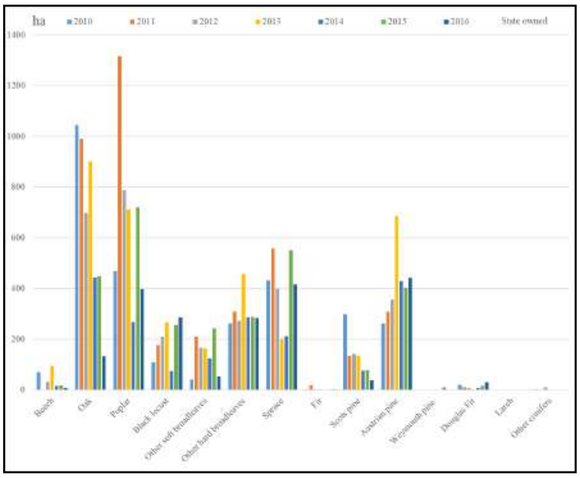

Figure 2: Presentation of the results of the volume of afforestation in hectares for the period 2010-2016 in state-owned sector

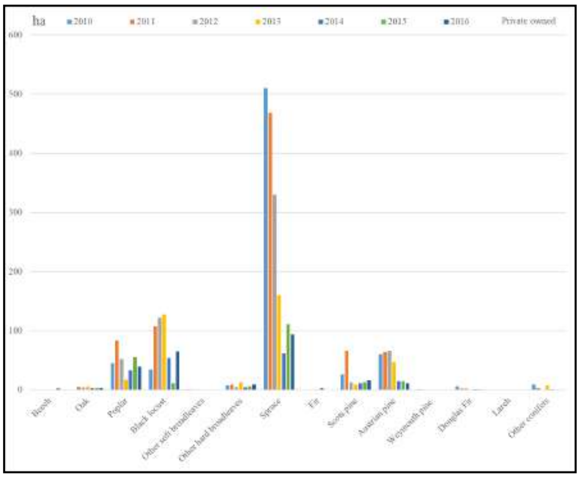

Figure 3: Presentation of the results of the volume of afforestation in hectares for the period 2010-2016 in private-owned sector

Figure 2 and 3 present differences in state-owned and private-owned sectors for period 2010-2016. There is significantly larger amount of afforested area in state-owned sector compared to the private sector. Afforestation efforts in the state-owned sector are more diverse and focused on long-term sustainable species, whereas the private sector primarily focuses on spruce and black locust, likely due to their resilience and suitability for specific terrains.

The volume of afforestation for the period 2017–2023

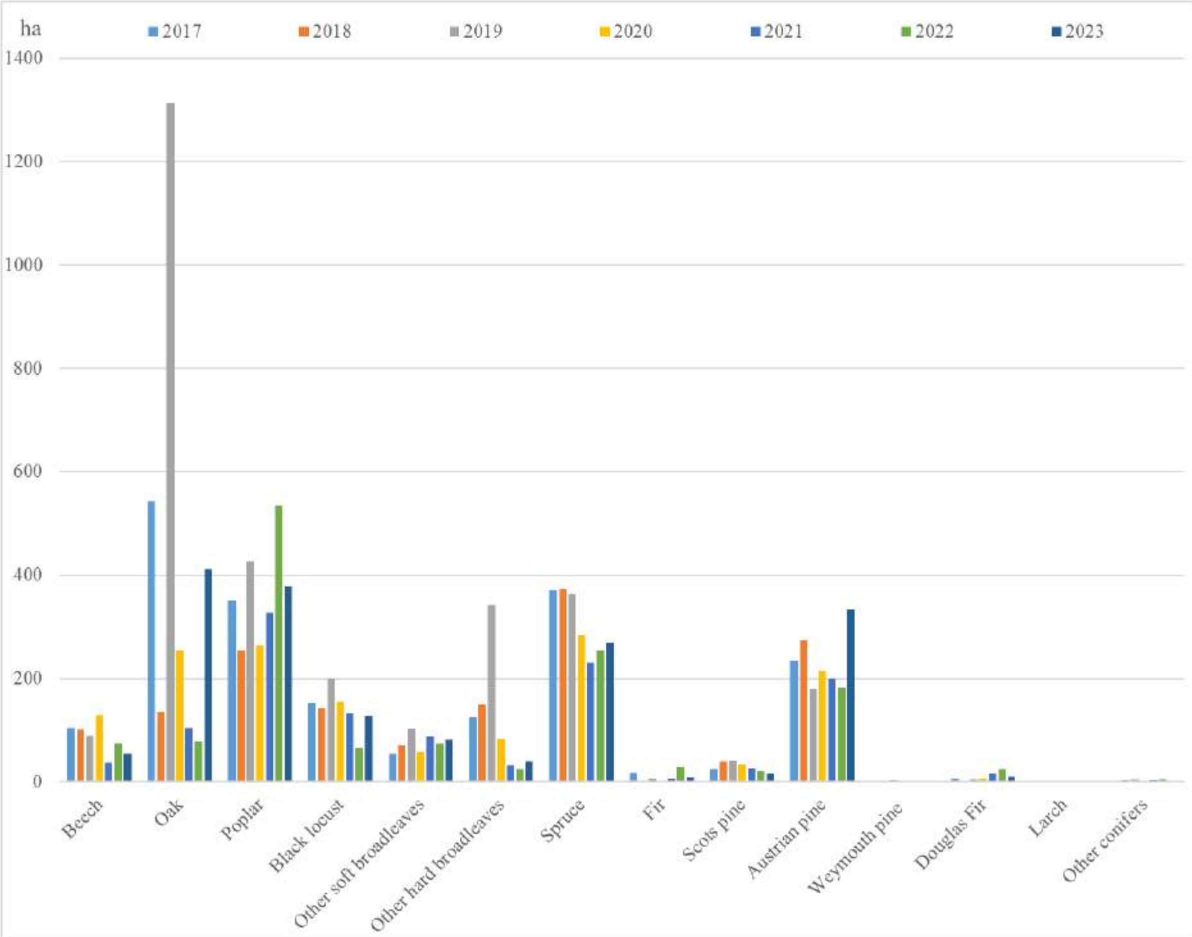

Figure 4: Presentation of the results of the volume of afforestation in hectares for the period 2017-2023

Figure 2 presents the dynamics of afforestation of different types of trees for the period 2017-2023. In that period, a total of 12,386 ha was reforested, of which 11,807 ha were in the state sector and 579 ha in the private sector. On average, 1769.42 ha of afforestation were realized annually. As in the case of the previous analyzed period, in all types of trees, except for Austrian pine, black locust, and Euroamerican poplars, there is a noticeable trend of decreasing the extent of afforestation. The intensity of afforestation in the areas is identical to the period 2010-2016. The largest percentage of afforestation areas belongs to oaks and poplars, the smallest to beech and black locust, among conifers the highest percentage also belongs to spruce and Austrian pine, while the smallest percentage belongs to larch and Weymouth pine.

This is in accordance with the general trend of afforestation in Serbia, that is, the use of Euroamerican poplars dominates to manage forests with a short rotation and pronounced economic benefits. In addition, black locust has proven to be a very practical species for afforestation of hilly areas, while Austrian pine is dominantly used on inaccessible and erosive terrains, and generally hilly and mountainous terrains.

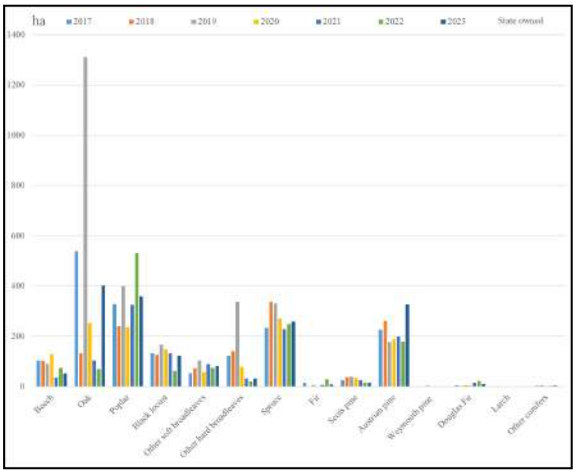

Figure 5: Presentation of the results of the volume of afforestation in hectares for the period 2017-2023 in state-owned sector

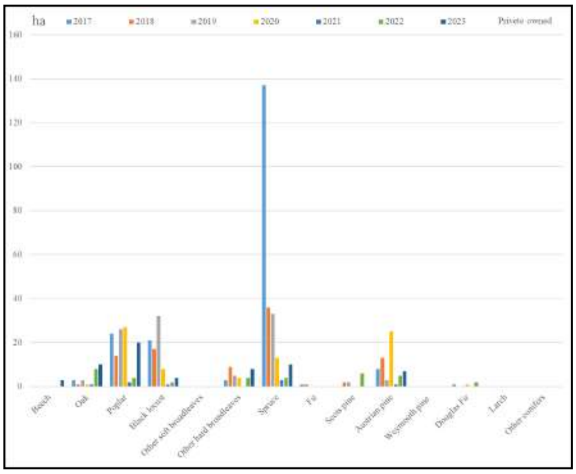

Figure 6: Presentation of the results of the volume of afforestation in hectares for the period 2017-2023 in private-owned sector

Figure 5 and 6 present differences in state-owned and private-owned sectors for period 2017-2023. Similar to the 2010–2016 period, there is a significantly larger afforested area in the state-owned sector compared to the private-owned sector. In the state-owned sector, the data indicate a slight change in species selection for afforestation purposes. Compared to the 2010–2016 period, the usage of poplar has decreased, whereas the usage of oak has increased. In the private-owned sector, there are no significant changes, with the usage of spruce still being dominant.

Conclusion

Within the period 2010-2016, a total of 23920 ha was reforested, of which 20792 ha in the state sector and 3128 ha in the private sector. On average, 3417.15 ha of afforestation was realized annually. In the period 2017-2023, a total of 12,386 ha was reforested, of which 11,807 ha were in the state sector and 579 ha were in the private sector. On average, 1769.42 ha of afforestation were realized annually.

Data from this paper can be summed up in few categories:

1. Dominance of certain tree species:
    - Broadleaf species like oaks and Euroamerican poplars dominate afforestation efforts, highlighting their economic importance and adaptability to short-rotation forestry practices.
    - Among conifers, spruce and Austrian pine are the most commonly used species, likely due to their resilience and suitability for specific terrains.
2. Sectoral distribution:
    - The majority of afforestation is within the state-owned sector, accounting for a significant portion of afforested areas in both periods. The private-owned sector's contribution remains relatively small but consistent.
3. Species-specific trends:
    - Species such as black locust, Austrian pine, and Euroamerican poplars showed an increase in afforestation, contrasting with the overall decline for other species.
    - Black locust is favored for its practicality in afforesting hilly regions, while Austrian pine is suited for mountainous and erosive terrains.

These trends emphasize the need for a balanced afforestation strategy that considers ecological sustainability, economic viability, and the challenges posed by decreasing total amount of afforested areas.

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FEMALE STUDENTS' PERSPECTIVES ON FORESTRY CAREERS IN SERBIA

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Introduction

Forestry plays a crucial role in the creation of green jobs and contributes significantly to the transition to a green economy by providing employment opportunities that benefit the environment and conserve natural resources (Olson, 2011). Besides providing green jobs, the forestry sector contributes to a low-carbon economy and promotes sustainability through the use of renewable resources and the creation of employment opportunities in rural areas (Kaur, Sharma, 2016). Sustainable forest management practices not only provide timber resources, but also contribute to biodiversity conservation, climate change mitigation and the creation of meaningful employment opportunities (Imbrenda et al., 2023).

However, challenges such as the low income in forestry compared to environmental protection tasks make it clear that targeted government support is needed to eliminate this inequality and strengthen the position of forestry employees in the labor market (Maitah et al., 2016). In recent years, over half a million people have been employed in the forestry sector in Europe, which supports various rural economic activities, with the largest workforces in Poland, Romania, Sweden and Germany. The number of jobs in forestry in the EU decreased slightly by 0.2% in the period 2008-2018. On the other hand, employment in Hungary and Poland increased significantly, while in Croatia it decreased by 55% (Imbrenda et al., 2023).

The Serbian forest sector (which consists of the subsectors: “forestry and logging”; “wood manufacture”; “paper manufacture”) has faced significant downsizing, primarily due to the contraction in the industry, although recent trends point to a recovery in employment. Between 1990 and 2015, the number of employees in the Serbian forest sector fell by 38%, with the forestry and logging sub-sector in particular recording a 25 % decline. Women's employment in the forest sector fell slightly from 28% to 27% between 1990 and 2010, but has recently risen again in absolute terms. However, the overall proportion of women in “forestry and logging” subsector has remained relatively stable, fluctuating between 14% and 16% in the period 1990-2015 (Fem4Forest, 2021).

A productive and dedicated workforce is essential for the success of forest-based organizations and the effective practice of forestry. These organizations rely on a well-educated, highly skilled and motivated workforce. Both industry employers and government agencies have high expectations of their new employees and expect university programs to adequately prepare future forestry professionals (Michael, Dasmohapatra, 2001).

However, higher education institutions in forestry are facing declining student enrollment in forestry and natural resource programs. This low student enrollment could result in fewer well-trained forest managers. Therefore, it is crucial to find ways to retain the students who choose to enroll in these academic programs so that a capable workforce is available in the future (Holmes, 2019). While the number of graduates in forestry technician training and forestry engineering bachelor's programs in Serbia fluctuated between 2010 and 2020, the proportion of women varied, but generally remained in the minority. It is noteworthy that female participation has reached some peaks, indicating a potential growth of gender diversity in this field (Fem4Forest, 2021). Studies in other countries in Europe showed similar trend as it was found that "*the share of female students vary from 15% in Norway to almost 50% in Iceland, with the other countries being placed in between*" (Sjølie, 2020).

Given the challenges of the sector, targeted efforts are essential to improve women's participation and ensure equal opportunities, thereby strengthening the forestry workforce and contributing to sustainable development. The Interreg project Fem2forests (https://interreg-danube.eu/projects/fem2forests), which began in January 2024, involves 15 partners from 9 countries. It focuses on making the forestry sector more attractive to girls and young women by raising public awareness, increasing promotion and providing more opportunities for practical learning and mentoring. This paper aims to explore the perspectives, motivations and interests of female students in Serbia, identify the key factors influencing their decision to pursue a career in forestry, and highlight the challenges and barriers they face.

Material and Methods

Data collection was conducted in April 2024 using an online survey via the Google Forms platform. This method enabled the efficient collection of responses from a large number of participants in a relatively short period.

Respondents were contacted with the assistance of:

- Teaching staff from the University of Belgrade-Faculty of Forestry and other faculties,
- Teaching staff from secondary schools,
- Educators in the Secondary Schools Student Dormitory "Jelica Milovanović".

This approach ensured access to various groups of participants, covering a broader population and allowing the collection of different data relevant to the research.

According to the joint project's methodology, the respondents were:

- Female students from secondary forestry schools (grades I-IV);
- Female undergraduate students at the Faculty of Forestry (years I-IV);
- Female students from other secondary schools (grades I-IV);
- Female undergraduate students at other faculties (years I-IV).

The study involved 105 participants, divided into two main groups:

1) 57 female forestry students (secondary schools and Faculty of Forestry);
2) 48 female students from other secondary schools and faculties.

Participation in the study was voluntarily and respondents were assured anonymity in line with good research practice. This ensured that the identity of the participants was protected, which fostered an environment of trust and encouraged honest and open responses.

In the data analysis phase, we used both Excel and SPSS (IBM SPSS Statistics ver. 19) to process and analyze the collected data. Descriptive statistics were applied to non-categorical variables. For categorical variables, frequency analysis was performed to examine the distribution and count of each category to gain insight into the prevalence and patterns within the data set.

Results and discussion

The majority of respondents (35%) attend a secondary school specializing in forestry. About 1/4 (22%) of respondents attend a secondary school that does not specialize in forestry. Almost 1/5 of the respondents (19%) have studied forestry at the faculty level. In addition, about 1/4 of the respondents (24%) have a background in non-forestry faculties. However, previous research found that exposure to forestry in high school was not a statistically significant factor in forestry university students' decisions to major in forestry or a related natural resource degree program (Bal et al., 2020).

The results show that personal interests and additional education and training opportunities are among the most important influencing factors for many respondents. In particular, the highest number of forestry students (42%) and the majority of other students (62%) cited personal interests as an “important” or “very important” influence on their career choice. Also, 32% of forestry students and 65% of other students indicated that additional education and training opportunities were “important” or “very important” factors. In contrast, the career choice of friends and family expectations generally had less influence. For almost 3/4 of both forestry students (74%) and other students (75%), the choice of friends had no influence. Similarly, 47% of forestry students and 33% of other students stated that family expectations had no influence. Furthermore, a previous study emphasizes that a connection to nature and concern for the environment significantly influence students' interest in pursuing studies in natural resources, including forestry. The study highlights the need to create a diverse and inclusive environment to attract students from different backgrounds (Moreno et al., 2020). The influence of family or friends overall was low in previous research, as well (Bal et al., 2020).

The decision to study forestry is influenced by a variety of factors ranging from family guidance and personal passion to academic attractiveness and social awareness. Around half of respondents (51%) cited love and passion for nature and the forest as their main motivation for pursuing forestry education. Previous research also shows that women are motivated to enter forestry because of their passion for the environment and their interest in working with nature (Larasatie et al., 2020). Almost 1/3 (32%) emphasized the importance of meaningful work and saw forestry as a way to have a positive impact on the environment and society. The results show that environmental protection (58%) and forest ecology (39%) are of most interest to students wishing to study forestry. However, a significant number of respondents are also driven by practical aspects such as forest management (21%) and timber harvesting (21%). According to previous research, a majority of students pursuing forestry degrees cited reasons such as a passion for the outdoors, hands-on learning, making a societal impact, and interest in forest management. Practical field skills were also a key motivator for many of the students surveyed (McGown, 2015).

The results show a diverse spectrum of career aspirations among forestry students. The majority (60%) expressed an interest in nature conservation, while other popular career fields include forest management (32%), research (26%), climate change protection and mitigation (25%), urban forestry (25%) and sustainable forestry (21%). Previous research has found that the growth and demand of the green economy have expanded job opportunities in the forestry sector. These opportunities now extend beyond traditional silviculture and forest management positions to include areas such as recreation, urban forestry, and fire management (Holmes, 2019). In terms of career paths, another study showed that students from 82 countries are most interested in working in forest-related nature and biodiversity conservation, while their interest was lowest in forest-related industry and non-forest-related fields (Owour et al., 2023).

The fact that 92% of non-forestry students had not initially considered forestry as a career option indicates a possible lack of awareness and exposure to the field. Only 8% of respondents had considered forestry as a career option before their current education. Those who have not considered forestry as a career often say: “I have other interests”, “I did not know what opportunities there were in this field”, “I did not know it existed”, and similar.

Similar findings emerge from previous research, where many participants reported that they did not initially choose forestry due to a lack of awareness about it as an academic program, since forestry-related courses were not offered in their high schools (Hubbard, 2014). Only 17% of non-forestry students stated that they were aware of career opportunities in the forestry sector. A remarkable 83% of respondents in this research admitted that they knew little about career opportunities in forestry despite the fact the job opportunities "*within forestry and natural resource management fields are numerous and much more varied than may initially be assumed*" (Grebner et al., 2022). In addition, most non-forestry students (73%) have no experience in forestry activities. However, 27% have participated in forestry activities (e.g. tree planting events, field trips to forests or nature reserves, forest clean-ups, art projects inspired by forests, etc.).

The survey examines what potential (non-forestry) students would like to have in order to consider a career in forestry (Table 1).

Table 1. Desired resources and knowledge when considering a forestry education

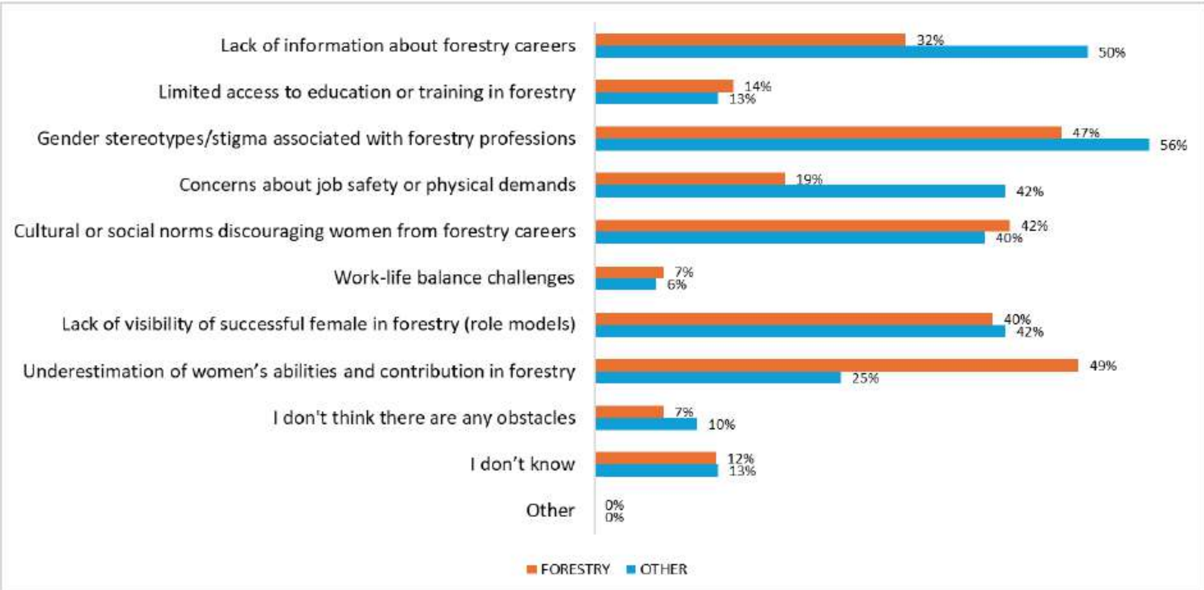
	Number	**Share**
Access to informational materials about forestry careers (i.e. what forestry jobs involve).	24	50%
Guidance from career counsellors familiar with forestry professions.	17	35%
Opportunities for job shadowing or internships in forestry-related fields.	20	42%
Networking events with professionals working in the forestry sector.	8	17%
Forestry-related workshop or field trip.	12	25%
Access to a mentor from the forestry sector.	10	21%
Seeing more role models (especially women) in forestry.	19	40%
Online platforms or databases showcasing forestry job opportunities and requirements.	20	42%
Virtual reality simulations allowing individuals to experience various forestry job roles firsthand.	8	17%
Gamified learning modules and challenges related to forestry careers.	5	10%
Information via social media.	19	40%
Other	3	6%
Number of respondents =n	48	

Half of the respondents expressed a desire for access to detailed information material on forestry careers. For 42% of respondents, opportunities for job shadowing or internships in forestry-related fields would be valuable. The same number of respondents would appreciate online platforms or databases presenting forestry job opportunities and requirements. The importance of seeing more role models, especially women, in forestry was emphasized by 40%. Around 1/3 (35%) of respondents would appreciate guidance from career counselors familiar with forestry careers. A quarter of respondents would like to take part in workshops or excursions on the subject of forestry. The survey results provide valuable insights into the factors that could increase the attractiveness of forestry as a career choice among non-forestry students: appropriate payment (67%), better image of foresters (42%), understanding, conserving and managing valuable natural resources (40%), etc. Previous research has shown that while forestry professionals recognize the benefits of sustainable forest management, incoming students and the general public often have a limited understanding of forestry. Many are misled by misrepresentations of forestry practices on social media, leading to confusion and misinformation (McGown, 2015).

The misconceptions/stereotypes about careers in forestry are numerous. The majority of forestry students (65%) and other students (54%) say that one of the biggest stereotypes among their peers is that forestry is not for women. This is in line with previous research findings that "*although most respondents agreed that there is better gender diversity in forestry higher education and government, the forest sector is still perceived to be a male dominated profession into which it is hard to attract women*" (Larasatie et al., 2020). Also, almost half of the respondents (49% of forestry and 48% of other students) believe that their peers think that opportunities in forestry are limited and mainly involve cutting down trees. This simplistic view ignores the multiple roles within forestry, such as conservation, ecosystem management and sustainable forestry practices. Another key misconception held by 44% forestry and 46% other students is that their peers believe a career in forestry does not require higher education. This is a critical misconception, as many positions in forestry require specialized knowledge and training, often obtained through higher education in forestry or environmental science. Half of forestry students (51%) and 38% of other students say that their peers think that all foresters are involved in manual logging activities ("all foresters are lumberjacks"). Similar misconceptions were identified in previous research, which found that "*the forest sector is usually associated with "people out in the woods and chopping down trees" or "lumberjack stereotype with big bushy beard", holding a "chainsaw", "or fighting fires or working for warehouse or cruising plots*" (Larasatie et al., 2020). The same study points out that women entering the forest sector are often expected to show toughness and to prove themselves all the time and adopt behaviors traditionally associated with men, including wearing certain clothing, which could be one of the reasons why women are not exactly pursuing a career in forestry (Hubbard, 2014; Larasatie et al., 2020).

The results of the survey show that several main barriers prevent girls from pursuing a degree and career in forestry (Graph 1).

Graph 1. The main barriers for girls to study in the forestry sector



A significant number of respondents (32% of forestry and 50% of other students) feel that there is a lack of information about forestry careers. This suggests that many girls are not aware of the wide range of opportunities in this field. As well, gender stereotypes and stigmatization are major obstacles, since 47% of respondents from the forestry group and 56% of other respondents mentioned this problem. Cultural and social norms that discourage women from pursuing forestry careers are cited by 42% of forestry respondents and 40% of others. The lack of visible female role models in forestry is seen as a barrier by 40% of forestry respondents and 42% of other respondents. A significant barrier cited by 49% of forestry respondents and 25% of others is the underestimation of the skills and contributions of women in forestry. Previous research points out that it is crucial to get more women into leadership positions in the forestry sector. Showcasing successful women can inspire others, and empowering women can attract more women to the forestry sector. However, these efforts are seen as a circular challenge: greater representation of women is necessary to attract more women to the field (Larasatie et al., 2020).

The survey shows clear differences in the perception of forestry professions between forestry and non-forestry students. While 40% of forestry students believe that forestry professions are perceived favorably, only 10% of other students do. Conversely, 12% of forestry students believe that forestry professions are perceived negatively, while 25% of other students share this view. Indifference is noted by 18% of forestry students compared to 38% of other students. Finally, 30% of forestry students and 27% of other students are uncertain about the perception of forestry professions in their communities. Previous research suggests that the forestry sector needs to improve its image and communication of its diverse positive aspects. The sector should promote itself beyond traditional perceptions and emphasize the human dimension, nature conservation and ecological contribution, rather than focusing solely on resource extraction and logging (Larasatie et al., 2020). Initiatives such as mentoring programs, networking events and showcasing successful women in forestry can help address these challenges (Wager, 2022, Larasatie et al., 2020).

Conclusions

Personal interests and additional educational opportunities are decisive factors that influence career choices in forestry. The career aspirations of forestry students are diverse: nature conservation is the most popular choice, followed by forest management, research and climate protection. This reflects a broad spectrum of interests within the forestry field.

There is a considerable lack of awareness about forestry careers among the respondents. Many non-forestry students were unaware of career opportunities in forestry prior to their current education. Also, girls and young women are not sufficiently aware of the diverse opportunities that forestry offers.

Misconceptions include beliefs that forestry is not suitable for women, that it primarily involves tree cutting, and that it does not require higher education. Forestry is still often perceived as a profession involving hard physical labor in the forest.

Barriers that prevent female students from pursuing forestry careers include lack of information, gender stereotypes and cultural norms. The lack of visible female role models and the perceived underestimation of women's abilities in forestry are important problems.

Thus, raising awareness of the different aspects of forestry, improving public perception and providing more opportunities for hands-on learning are crucial steps to attract more young women to forestry careers in Serbia. Furthermore, forestry needs to be better promoted as a sector that contributes to sustainable development and offers various opportunities. In this context, a systemic approach is needed: forestry, along with nature conservation, encompasses natural resource management and ecology and should be emphasized and promoted as a “green” job opportunity. Also, there is a need to promote successful women in forestry so that young girls can see realistic examples of career paths.

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LANDSCAPE ECOLOGY ANALYSIS OF THE KORENICA HUNTING GROUND

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1. Introduction

Processes that shape and maintain the landscape – geomorphology, natural disturbances, and human impacts – occur and repeat over time on landscape mosaics of various sizes, thereby creating distinct, recognizable visual and cultural units – the identity of the landscape (Ndubisi, 2002). Most of us have an intuitive sense of the concept of landscape, and in every case, this sense includes the variant elements that make up the landscape, its change over time, and the impact of ecological dynamics on the landscape (Farina, 1998). Landscape ecology is a discipline motivated by the need to understand the development and dynamics of patterns in ecological phenomena, the role of disturbances in ecosystems, and the characteristics of the spatial and temporal scale of ecological events (Urban et al., 1987). It offers new concepts, theories, and methods that reveal the importance of spatial patterns (both anthropogenic and natural) in the dynamics of ecosystem interactions. Ecosystems are connected by the flow of energy, species, and minerals throughout the landscape mosaic, and in the case of disturbances, anthropogenic or natural, changes occur in structure and function. From a landscape planning perspective, anthropogenic interventions in space cause disturbances within existing natural ecosystems, so it is important to understand the causes and consequences of actions in space, as well as how they alter and impact the flow of energy, species, and minerals in ecological structures for responsible planning.

Ecologists use hierarchical levels to structure ecological knowledge – from the smallest unit of the organism, then populations, communities, ecosystems, landscapes, biomes, biogeographical regions, and finally the largest, the biosphere (Ndubisi, 2002). Landscape ecology studies the horizontal spatial layer at the landscape level – its structure, function, and changes, where functions and changes are reflected through the structure. Forman and Godron propose the patch-corridor-matrix spatial framework to describe the functional components of any landscape – urban or rural, and in doing so, emphasize the homogeneity of the landscape (Ndubisi, 2002). Furthermore, they emphasize that this spatial framework is increasingly being used as a tool for describing landscapes and their structure in landscape ecological planning and design.

Forman and Godron (1986), who were particularly influential in connecting landscape ecology with landscape planning, state that landscape ecology has a unique role in our lives; the quality of the landscape we live in largely depends on its understanding. When we focus on landscape heterogeneity, it becomes possible to see how ecosystems are intertwined – an action "here and now" causes an effect "there and then." Since the system is interconnected, it is crucial to understand the spatial relationships between landscape elements; the flow of species, energy, and materials; and the ecological dynamics of the landscape mosaic. Thus, understanding landscape ecology results in practical tools for decision-making in landscape planning and design. People reflect their needs in the spatial structures of landscapes, and therefore, landscape ecology tools provide knowledge to optimize the natural-anthropogenic relationship, thereby contributing to sustainable development.

This work is based on a landscape ecological analysis of the Korenica hunting ground, analyzing individual structural elements of the landscape according to Forman and Godron's method. Geographically, the hunting ground is part of the central Dinaric mountain area of Croatia, and as a relatively small region, it differentiates as the Mountainous Croatia. It is located in the Lika region, which is characterized as a basin within the mountain boundaries of Velebit – Velika Kapela – Mala Kapela – Plješivica, with frequent occurrences of karst fields that, besides being important socio-economic centers, are also geological phenomena (Krbavsko, Gacko, Ličko, which is the largest in Croatia, etc.) (Magaš, 2013). Climatically, it belongs to the continental and mountainous climate region, which is moderately warm and humid with warm summers, and phytogeographically, it belongs to the Eurosiberian-North American region. Lika is home to the Plitvice Lakes National Park, whose boundary continues to the area of the hunting ground that is the subject of analysis. It is also the area of several significant landscapes – Gacko Polje, Dabarsko Polje, Risovac-Grabovača, and Bijeli Potoci-Kamensko.

Among the settlements, two towns stand out, both located in karst fields: the largest and capital of Lika, Gospić, in Ličko Polje, and Otočac in Gacko Polje. According to Magaš (2013), they represent demographic and economic centers (Figure 1). Villages are more numerous and are typically situated in rows along the edges of karst fields, while hamlets, which are the most numerous type of settlement, are scattered near villages and across karst fields.

The objective of this paper was to assess and explore the potential application of landscape ecology analysis and principles within landscape planning. Additionally, it aimed to establish the significance of comprehending the complex cause-and-effect relationships between ecosystem functioning and anthropogenic interventions by employing the landscape ecological analysis method developed by Forman and Godron, specifically applied to the Korenica hunting ground.

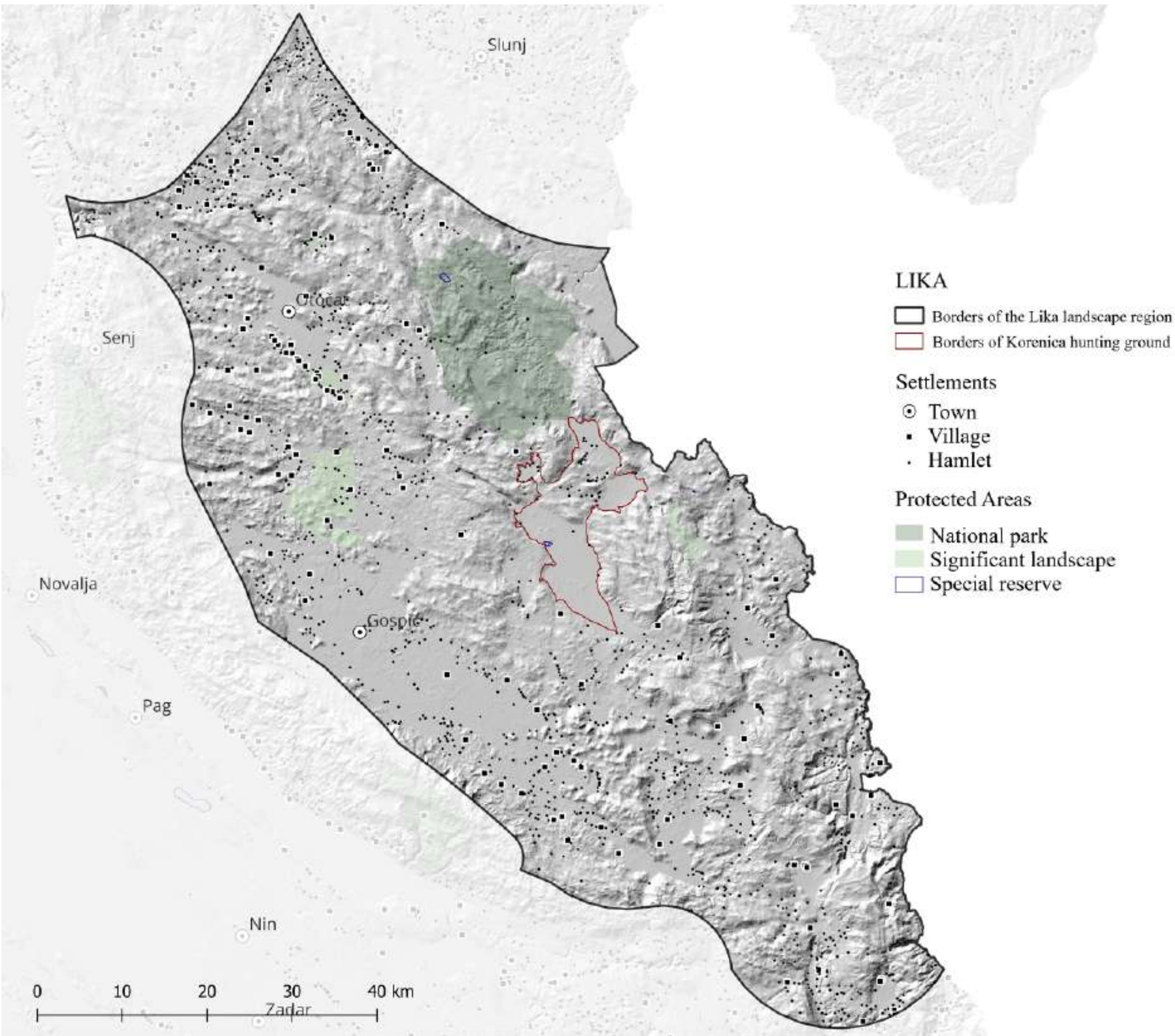

Figure 1. Lika landscape region.

2. Theoretical Framework

Landscape ecology is the youngest branch of ecology, which evolved in the years following World War II in Central and Eastern European countries (Schreiber, 1990). It has developed into a distinct, unique, dynamic, and integrated global science, with its roots deeply embedded in geography, geobotany, and land management (Farina, 1998). In the late 1930s, German biogeographer Carl Troll first coined the term landscape ecology while studying land-use and development issues in East Africa, inspired and motivated by spatial patterns observed in aerial photographs and their potential for landscape interpretation (Naveh and Lieberman, 1994). Essentially, landscape ecology has combined the spatial (horizontal) approach of geographers with the functional (vertical) approach of ecologists (Naveh and Lieberman, 1994; Forman and Godron, 1986; Farina, 1998). Troll (1971) defined landscape ecology as a field concerned with the complex causal relationship between communities and their environment in a given landscape area, with the task of providing a functional analysis of the landscape that will present the numerous multi-layered and reciprocal interdependencies of landscape elements (Troll, 1950/2007).

In the 1960s, landscape ecology was introduced as a scientific tool in landscape planning and management. Buchwald (1963, in Naveh and Liberman, 1994) argued that it plays an important role in overcoming the tensions between modern society and its landscapes arising from the increasing demands of industry and the natural potential of the land (Naveh and Lieberman, 1994). Today, global ecological issues are more prevalent, and their negative impacts are more strongly felt. This has led to the development of the concept of sustainable development (environmental, social, and economic protection), which can be achieved by applying landscape ecological methods and principles, as the landscape provides the context for such development (Žmire, 2016). The contemporary emergence of landscapes as suitable subjects for ecological studies stems from three main factors: (1) large-scale environmental issues and land management problems, (2) the development of new concepts related to scale in ecology, and (3) technological advancements, including the wide availability of spatial data, computers, and software for manipulating those data, as well as rapid and continuous growth in computing power (Turner et al., 2001). Consequently, modern landscape ecology methods are often used to explain complex phenomena, such as disease transmission, where it has been established that landscape configuration influences and is one of the relevant factors in the transmission of viruses within zoocenoses. It also determines the impact of biocenoses on the structure and spatial configuration of landscapes, as well as the influence of landscape structure on the phenological phases of phytocoenoses (Farina, 1998).

Landscape ecology is a spatially determined ecology that studies the structure and dynamics of spatial mosaics and their ecological causes and consequences, and it can be applied at any level of hierarchical organization or at various scales of resolution (Wiens, 2005). Its primary goal is to understand the patterns, mechanisms, and consequences of spatial heterogeneity, while its ultimate goal is to provide a scientific basis and practical guidelines for the development and maintenance of ecologically, economically, and socially sustainable landscapes (Wu, 2007). According to Forman and Godron (1986), the landscape is shaped under the influence of three mechanisms: specific geomorphological processes over a long period, colonization patterns of organisms, and local disturbances of individual ecosystems over a short period. Consequently, they determine that landscape ecology studies three characteristics of the landscape (1) Structure – the spatial relationship between spatial components, specifically: the distribution of energy, materials, and species with respect to size, shape, abundance, types, and configurations of ecosystems; (2) Function – the interaction between spatial components; i.e., the flow of energy, materials, and species among constituent ecosystems and (3) Landscape change – changes in the structure and function of spatial components of the ecological mosaic over time.

The landscape structure is a characteristic within which, once identified, it is possible to see existing and predict future functions and changes within the mosaic. The functions and changes of the landscape encompass the processes and roles that take place within the structure, with changes playing an essential role within the structure and function of the landscape. Changes refer to disturbances and fragmentations of the landscape, both natural and/or anthropogenic in origin. For example, fires caused by natural and/or anthropogenic activity are disturbances in the landscape that cause a series of consequential processes – the disappearance of undecomposed biomass, the influx of nutrients into the system, colonization by new pioneer species, and impact on the distribution of species. The construction of anthropogenic structures, such as roads, causes habitat fragmentation, i.e., the transformation of a continuous habitat into remnants or fragments that vary in number, shape, and size, resulting in habitat loss, reduced genetic pool, population number and range, and increased spread of invasive species and predation on allochthonous flora and fauna. Thus, studying landscapes through these three characteristics structures the approach to understanding the area and the process of interest, and for planning and design purposes, it provides a holistic understanding of the landscape of interest, enabling informed decision-making towards sustainable development.

Mediterranean, tropical, desert, and alpine landscapes are examples of distinctly different spatial situations. Despite their extreme differences, they all share a common fundamental structure. The structure of the landscape, or the spatial arrangement of landscape elements, can be classified into two layers: the vertical layer includes relief, soil, vegetation, animals, and human structures, while according to Forman and Godron (1981, 1986), the horizontal layer includes the spatial arrangement and relationships of landscape elements – patches and corridors within the matrix (Figure 2). Matrix represents the largest and most connected element of the landscape, playing the primary role in its functioning, although its extent may be irregular. Patches represent areas of irregular shape that differ in appearance from their surroundings by their shape, size, heterogeneity, and edge characteristics. Corridors are narrow linear strips of land that differ from their surroundings, with their main characteristics being width, connectivity, and quality.

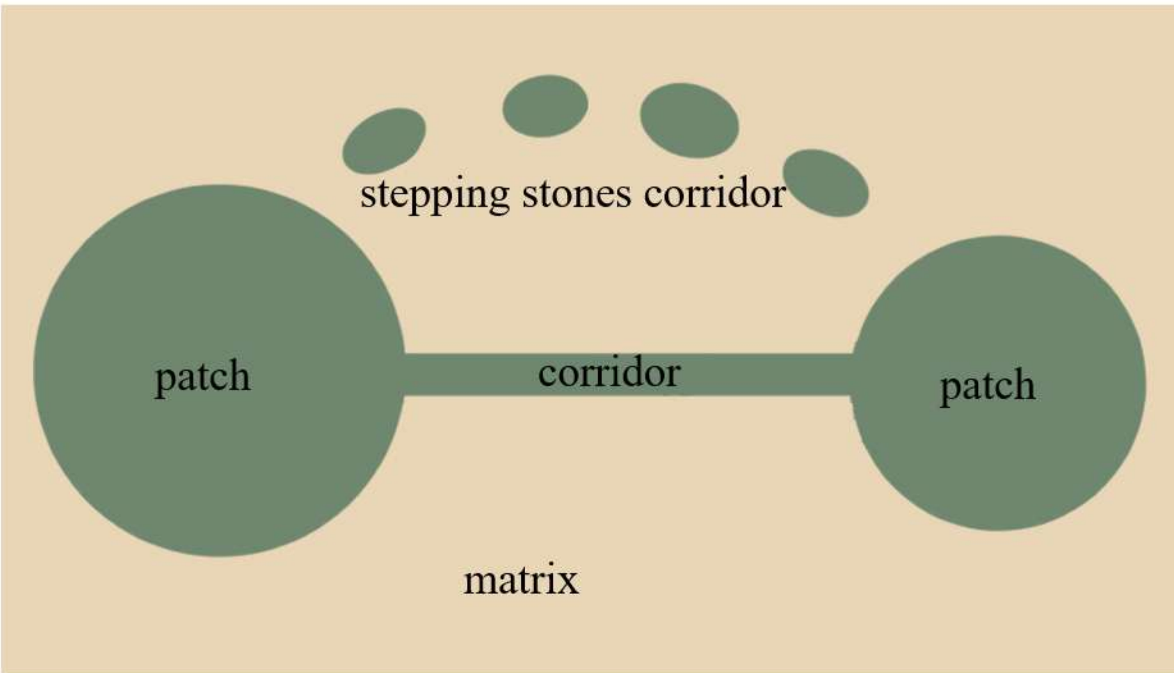

Figure 2. Schematic representation of landscape elements

By mapping and analyzing the structural landscape elements through landscape ecological analysis according to Forman and Godron, it is possible to gain insight into the functions and changes within the landscape, as well as the mutual influence of natural and/or anthropogenic activities on the surrounding mosaic.

Ndubisi (2002) states that, given how landscapes are modified by natural and human disturbances, the structural framework of patches-corridors-matrix theoretically allows for speculation about the impacts on landscape structure and vice versa, and suggests that this is a promising way to understand spatial relationships within the landscape. Forman (1995) studies the processes of landscape change that lead to different spatial arrangements of patches, corridors, and the matrix, as well as their ecological consequences, to create sustainable landscapes. Therefore, the broader the ecological knowledge applied in analyzing landscape structure, the broader the meaning of the analysis and understanding of space will be in terms of understanding the dynamics of energy, species, and material flow. On the other hand, applying basic ecological knowledge gives the analysis a narrower connotation in understanding the relationships of spatial structures. Forman and Godron have been particularly influential in linking landscape ecology with landscape design and planning, and their structural framework of patches-corridors-matrix has been frequently used to describe landscapes and their structure within landscape planning and design projects.

3. Methodology

3.1. Application of Landscape Ecological Analysis by Forman and Godron to the Korenica Hunting Ground

In this paper, landscape ecological analysis was conducted using Forman and Godron's method, which is based on mapping and describing the structural components of the landscape (matrix, patches, corridors). The analysis process involved the following steps: (1) Collecting spatial data characteristic for determining the structural elements of the landscape for the area of the Korenica hunting ground; (2) Input and analysis of data, and determination and classification of structural landscape components according to the National Habitat Classification (NHC) in the QGIS software, (3) Creation of maps of the matrix, natural and anthropogenic patches, corridors, and a composite representation at a scale of 1:100,000.

Description of the Korenica hunting ground area through individual structural components considering the landscape ecological context (Figure 3). Data used to define landscape structures are spatial data on habitats according to the NHC, obtained from Bioportal. CORINE land cover and use data were used exclusively for data comparison, and additional data on areas of succession and forest classification were used for a more detailed characterization of natural patches. For further comparison of the obtained spatial data with the current situation and better interpretation of the results, a digital orthophoto from 2019 and 2020 was used, as well as KML formats of spatial data for input and verification on Google Earth Pro satellite images from 2023.

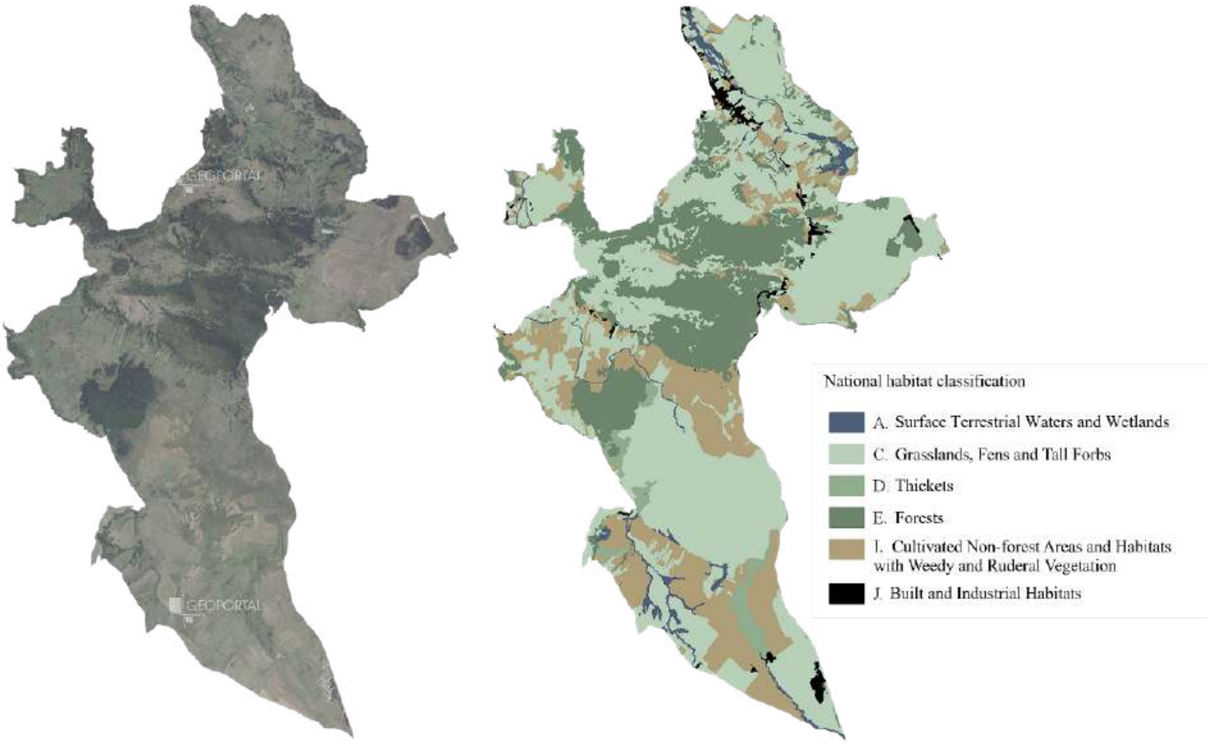

Figure 3. Korenica hunting ground (left) and a representation of the covered habitats according to the National habitat classification (NHC) (right).

3.2. Basic Characteristics of the Korenica Hunting Ground

A landscape ecological analysis was applied to the Korenica hunting area, covering a total of 16,318 hectares, located in the Lika-Senj County of the Republic of Croatia (Figure 4). More precisely, it belongs to the Krbava region of the Lika area (Magaš, 2013).

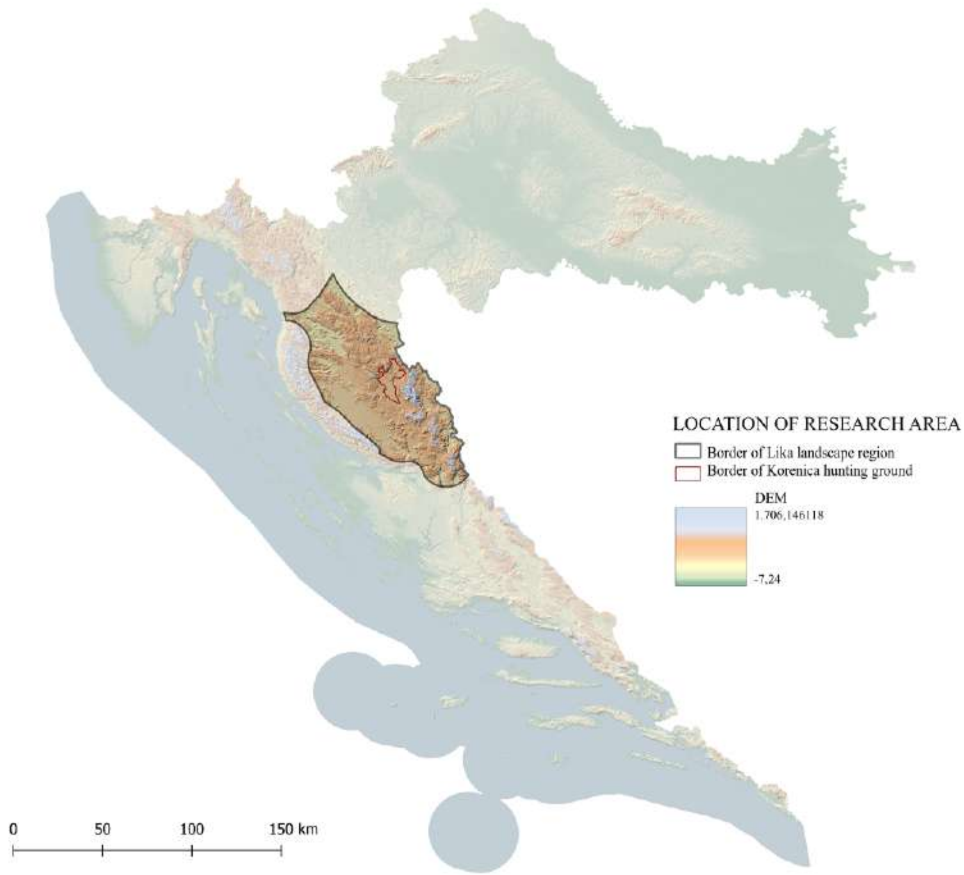

Figure 4. Geographical location of Lika landscape region and Korenica hunting ground in the Republic of Croatia.

The hunting area is a karst region with a geological-lithological substrate composed of brown siliceous sands that lie directly on a karstified limestone base (Hunting Management Plan, Vodolšak, 2016). Due to the permeability of the sedimentary rocks of the lithological substrate, underground water networks are present, while the surface rivers Krbava and Krbavica are sinking rivers with a constant surface flow. Additionally, seasonal watercourses that dry up periodically are common. There are also closed karst fields, geological phenomena, which are at the highest elevations in Croatia – above 500 m: Krbavsko Polje and Bjelopolje. Krbavsko Polje is the most prominent geomorphological flattened zone of the Krbava-Lika area and the hunting area itself. It is an extremely karstic field with shallow soils on a gravel substrate and frequent flooding, making it only partially suitable for arable farming, thus influencing the occurrence of spatial patterns and the development and presence of hygrophilous phytocoenoses (Magaš, 2013). The relief is defined by dominant plateaus covered with meadows, grasslands, pastures, as well as cultivated and abandoned agricultural areas, while the central part is hilly and covered with forest vegetation.

A special reserve called 'Laudonov Gaj' is present – a protected area of forest vegetation consisting of oak trees older than 270 years, which is a valuable, rare, and significant remnant of oak forests (Figure 5). According to Dasović (2007), Laudonov Gaj is a natural forest patch of anthropogenic origin, created in the mid-18th century through a reforestation project aimed at stabilizing the shifting sands of Krbavsko Polje. Pedunculate oak (*Quercus robur* L.) saplings were chosen for the project, as this species is well-suited to floodplain habitats with high groundwater levels. In the mid-19th century, conifers were planted on the peripheral parts of the special reserve with the same purpose of stabilizing the shifting sands, and they are notable as the only group of conifers within the hunting area.

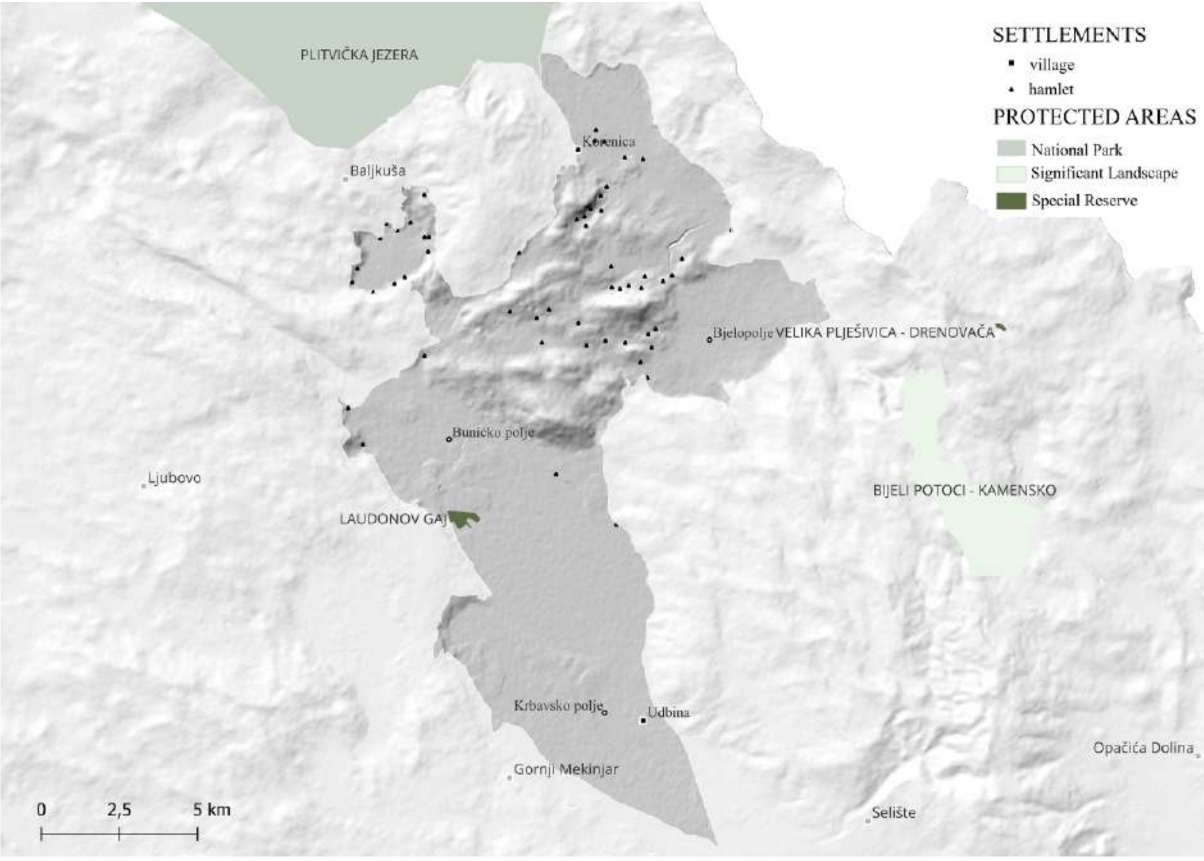

Figure 5. Digital Elevation Model (DEM) with settlements and protected areas within the research area

Anthropogenic impact is relatively minor compared to the existing natural features, and there are no significant infrastructural elements. The most prevalent anthropogenic factor is agricultural land, and according to the Hunting Management Plan (Vodolšak, 2016), the area is characterized by significant road accessibility, particularly the state road D1, which passes through the hunting area and is especially busy during the warmer part of the year (from May to October). Settlements are not predominant, with hamlets being the most common, evenly scattered and concentrated at the foothills of the hilly part of the northern half of the area. The most significant settlements are the larger villages of Korenica at the northern edge of the area and Udbina at the southern edge, both located along the D1 road.

The hunting area is confirmed or potential habitat for endangered and strictly protected animal species such as the wolf, lynx, brown bear, wildcat, short-toed eagle, peregrine falcon, honey buzzard, western capercaillie, and corncrake (Vodolšak, 2016). Therefore, it has an ecological function for the protection and preservation of the biological and ecological balance of natural wildlife habitats and the maintenance of the genetic diversity of native species.

4. Results

4.1. Matrix

The matrix (Figure 6) is defined by habitat class C according to the National habitat classification – Grasslands, fens and tall herb vegetation; a group of habitats whose plant component is primarily composed of perennial herbaceous plants, among which semi-shrubs are also often found.

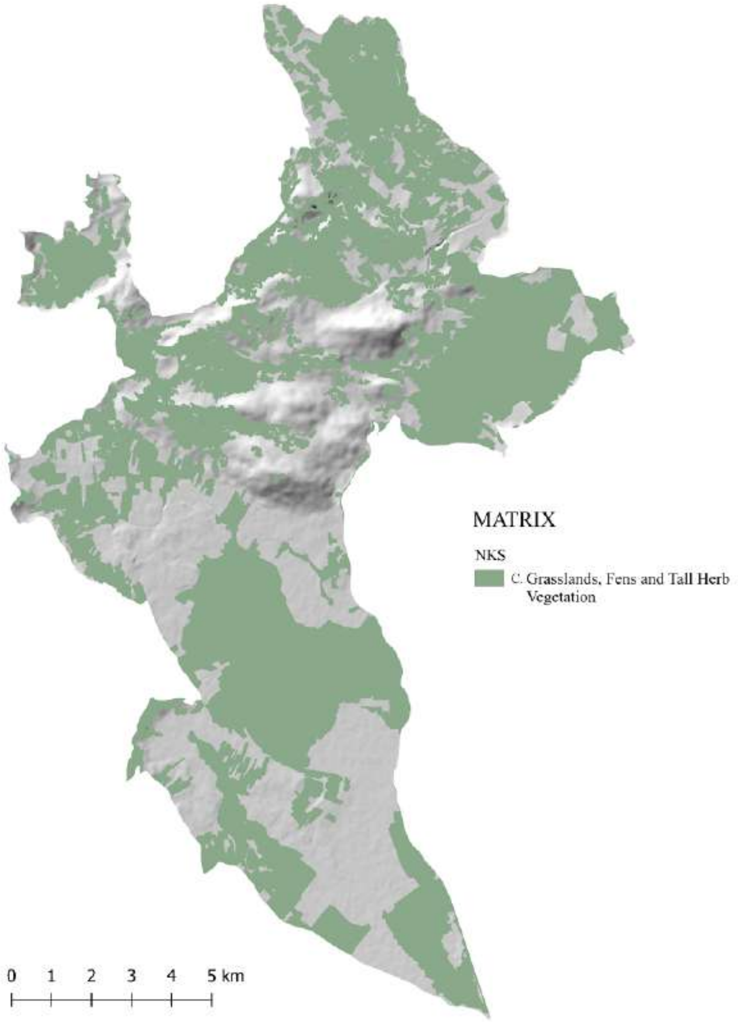

Figure 6: Matrix of the research area obtained from Landscape Ecology Analysis

The matrix consists of a mosaic of grasslands, meadows, and pastures, which occupy mostly flat areas of the hunting area. The stands of the matrix are predominantly grasslands of various associations and alliances, with the largest area covered by Scorzonera grasslands, alliance *Scorzonerion villosae*, Horvatić 1949. Grasslands, pastures, and hay meadows indicate the presence of livestock farming activities, and according to data from the Game Management Plan of the Korenica hunting area (2016), mowing is done twice a year, while grazing lasts from late autumn until snow cover. It can be concluded that the rate of livestock farming is low, with no intensive grazing or soil exploitation, and as a result, there is no occurrence of nitrophilous plants – the soil has stable and unchanged edaphic factors, and consequently, there is no change in the cover. Meadows are often the result of abandoned agricultural farms and pastures, and if this trend continues in the future, succession processes and then forest encroachment are possible. Mesophilic hay meadows (Alliance *Arrhenatherion elatioris* Br.-Bl. 1926), hygrophilic permanently wet meadows of Central Europe (Alliance *Molinion caeruleae* Koch 1926), and hay meadows of the common reed and Panonian vetch (As. *Molinio-Lathyretum pannonici* Horvatić 1963) occur exclusively near aquatic habitats of rivers, streams, and seasonally flooded areas.

4.2. Patches

Natural patches are predominantly composed of forests, primarily deciduous, which cover the hilly terrain of the hunting area (Figure 7). From a phytobiological standpoint (Bertović, 1975), the hunting area is located in a zone characterized by the climazonal community of sessile oak and common hornbeam (Querco-Carpinetum Croaticum Ht.) of the southern geographical variant. In the tree layer, fruit trees are present, as well as several shrub species such as *Ligustrum vulgare* L., *Corylus avellana* L., *Prunus spinose* L., *Lonicera carpifolium* L., and others.

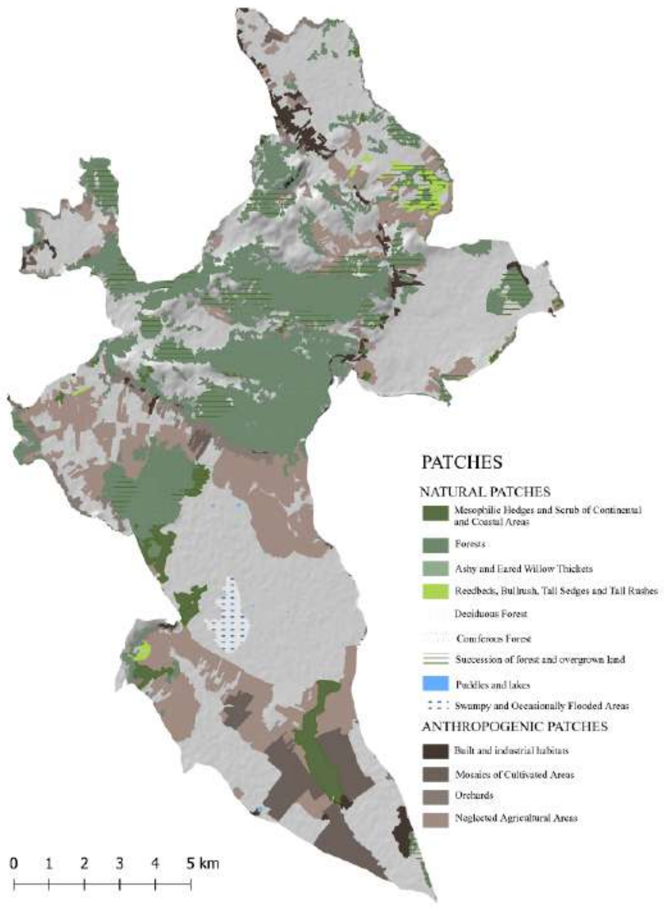

Figure 7. Patches of the research area obtained from Landscape Ecology Analysis

In the central, southwestern part of the hunting area, within Krbavsko Polje, there is a natural patch of anthropogenic origin – the special reserve "Laudonov Gaj" – a pedunculate oak forest (*Quercus robur* L.) adapted to the floodplain habitat of Krbavsko Polje. On the peripheral parts and clearings of the special reserve, the pioneer conifer species *Pinus sylvestris* and *Pinus nigra* L. were introduced, and today they represent the only conifer patch within the hunting area, contrasting with Laudonov Gaj, with which it shares a boundary, resulting in a clearly recognizable edge. Furthermore, the shrubby species *Sarothamnus scoparius* L. was also introduced along the conifer edge, and it has persisted to this day, naturally regenerating. The natural patches of anthropogenic origin – the pedunculate oak and conifer forests – are significant in that they were planted in the area to stabilize the shifting sands of Krbavsko Polje, and thus, in modern times and the future, prevent their spread.

Forest edges are curved, predominantly irregular, and successional (soft), forming a permanent ecotone between the interior of forest patches and anthropogenic patches of cultivated and abandoned agricultural areas. Therefore, the forest edge is partially anthropogenic, meaning it has been influenced by agricultural activities, while successional processes are gradually taking over abandoned anthropogenic areas.

Water body patches are mostly small lakes, with the largest water patch being the seasonally flooded area of Krbavsko Polje, which is the habitat of hygrophilous hay meadows of common reed and Panonian vetch (As. Molinio-Lathyretum pannonici Horvatić 1963). It is important to note that spatial data show a very small patch of the flooded area of Krbavsko Polje, while in reality, seasonal flooding covers at least half (potentially more) of the total area of the field. Additionally, the area of Bjelopolje, a karst field in the upper eastern part of the hunting area, is also a floodplain and should be considered as such, even though it is not represented in the spatial data.

Patches of aquatic plant communities – reed beds, bulrushes, tall sedges, and rushes of the class Phragmito-Magnocaricetea Klika in Klika et Novák 1941 – are edge communities of lakes, rivers, streams, eutrophic ponds, and marshes, as well as shallow floodplains or areas with a high groundwater level, dominated by marsh species, mainly helophytes. Within the hunting area, they form a buffer zone around water bodies and are found in open areas due to the high groundwater level of the region.

The largest proportion of anthropogenic patches consists of abandoned agricultural areas overgrown with shrubby and/or herbaceous vegetation, followed by a mosaic of cultivated areas. Built and industrial habitats occupy small areas along roads (anthropogenic corridors) and can be considered to play a role in habitat fragmentation. Built-up and cultivated areas can also be observed near forest edges, which, in such situations, are anthropogenic, sharp, and straight.

4.3. Corridors

Corridors in this case (Figure 8) exemplify the importance of scale in landscape structure analysis – their understanding and interpretation depend on the scale, i.e., the extent of the observed area and the level of detail in representation. Corridors, observed at the given scale of 1:100,000, are predominantly composed of surface flows of rivers and streams, with their ecological roles primarily limited to the flow of energy, materials, and species of aquatic habitats. There is the presence of riparian zones of river (stream) corridors – a band of vegetation along streams or rivers that differs in appearance from the surrounding matrix (Forman, Godron 1986). The riparian zone of the area consists of hygrophilous vegetation along the river – the habitat of reed beds, bulrushes, tall sedges, and rushes of the class Phragmito-Magnocaricetea Klika in Klika et Novák 1941. In addition to ecologically significant riparian zones, there are also forest corridors and corridors of mesophilic hedgerows and thickets of continental, and exceptionally coastal, regions of the order Prunetalia spinosae Tx 1952, which develop as edge protective strips along roads and as hedgerows between agricultural areas. Seasonal watercourses can be considered permanent corridors as they develop specific vegetation/habitats distinct from the surrounding matrix.

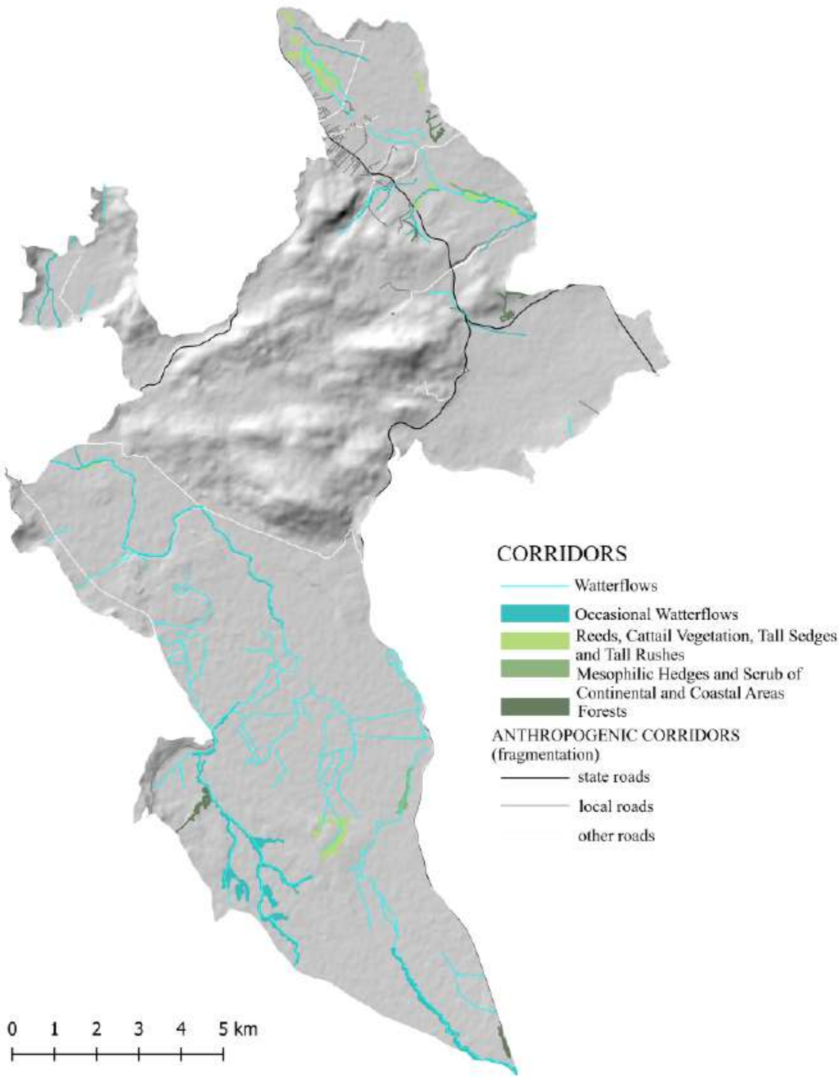

Figure 8. Corridors of the research area obtained from Landscape Ecology Analysis

Anthropogenic corridors are exclusively for human use, but they pose barriers and dangers to habitats (plant and animal life), causing disturbances from an ecological perspective. They play a role in habitat fragmentation, with the state road D1 having the greatest impact – it has the highest traffic frequency and divides the area/habitat into two units. However, if we examine the landscape structure at a larger scale – in this case, at a scale of 1:7,000 on a patch map – additional corridors within the forest edge become visible (Figure 9).

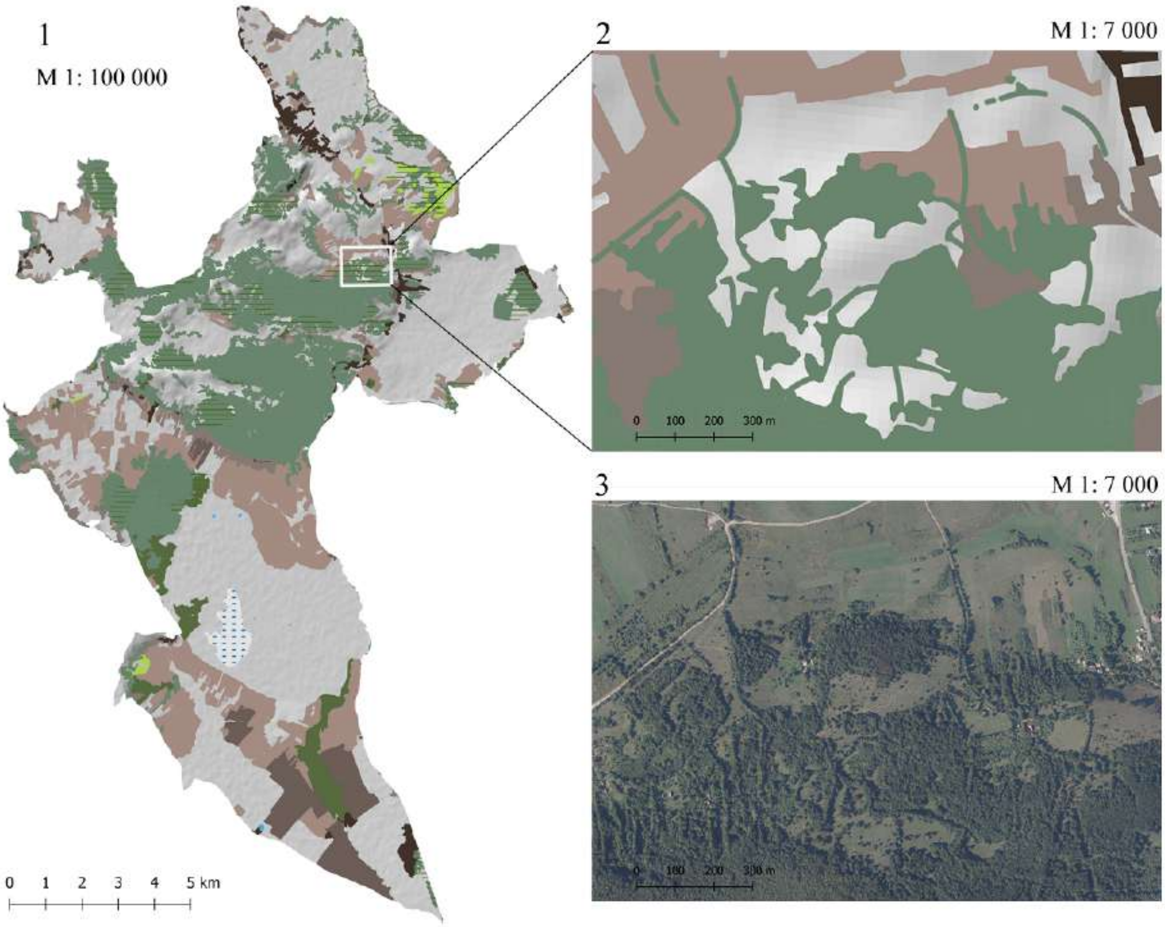

Figure 9. Structure of the landscape depending on the scale: 1. Patch map of the research area obtained from Landscape Ecology Analysis, 2. Enlarged view of the patch in M 1:7 000, 3. DOF view of the enlarged segment

Natural forest patches are surrounded by anthropogenic patches of cultivated and abandoned agricultural areas, as well as grasslands, meadows, and pastures of the matrix, which dictate their edge and edge effects (Figure 10). Analysis of Digital Orthophoto Maps and spatial data determined that forest edges are expanding successively as a result of the abandonment of agricultural activities or the reduction of arable land, creating new habitats that provide for new species while simultaneously creating a protective buffer for the forest – shielding the interior of the forest patch from local disturbances, such as windthrow.

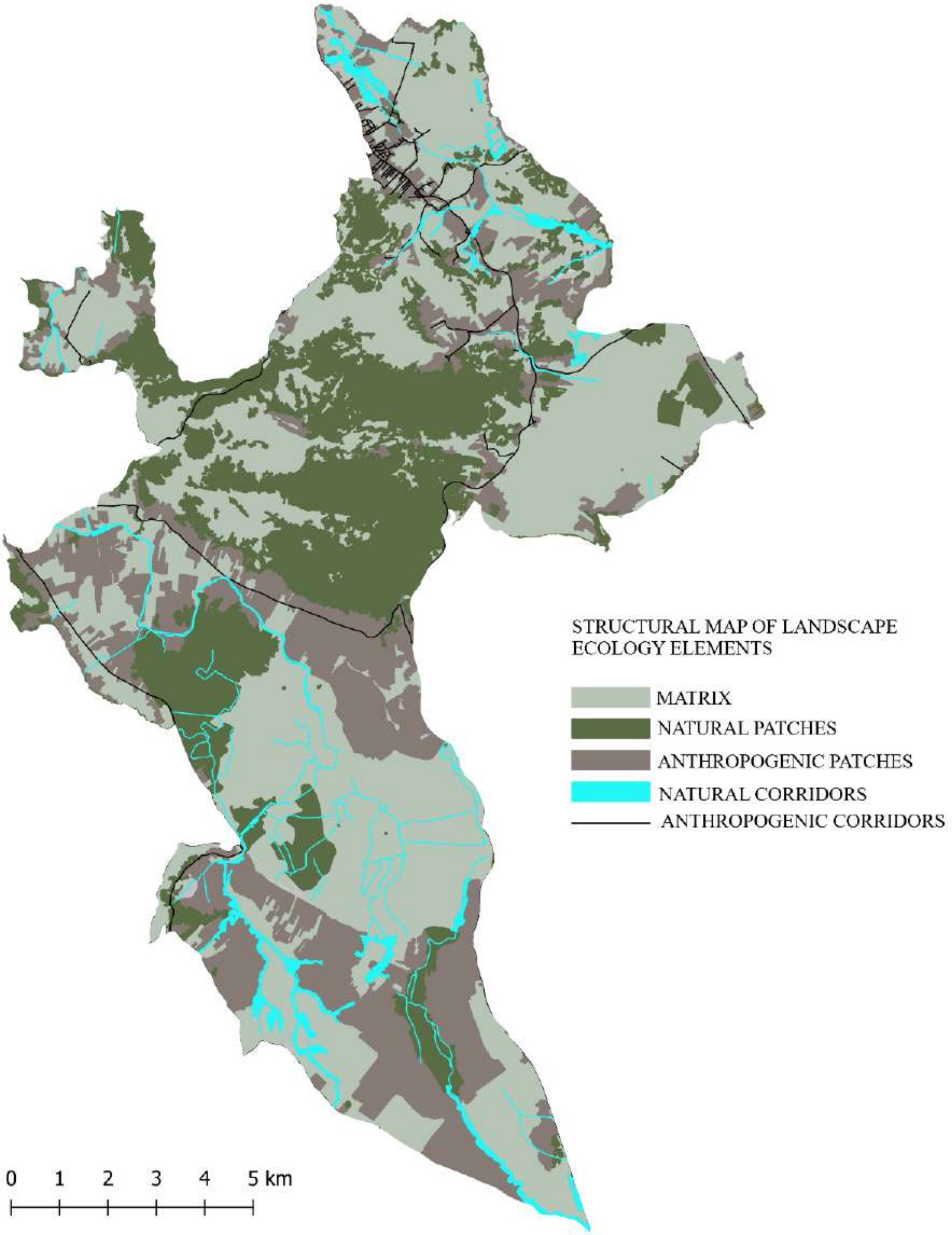

Figure 10. Structural map of the research area obtained from Landscape Ecology Analysis

5. Discussion

The reduction of local disturbances reduces the likelihood of forest clearings, which would consequently result in increased biodiversity within the patch. Grasslands, pastures, and hay meadows are largely the result of clearing the sessile oak and common hornbeam forests during the settlement of the area (Vodolšak, 2016), and given the current trend of progressive succession, there is potential for the forest to reclaim these areas. According to the Hunting Management Plan (Vodolšak, 2016), certain stands of the matrix are subject to extensive grazing, which maintains the natural state of the grasslands, as it resembles natural grazing that supports native phytocoenoses. Due to the aging and migration of the population, farms are being abandoned, creating the potential for pastures to be left untended. Consequently, in grasslands without grazing, a few species of tall grasses would dominate, displacing the existing flora and fauna and reducing plant species abundance. Since the hunting area serves as a habitat for wildlife – deer, roe deer, wild boar, hare, and brown bear – they play a role in maintaining the native habitats of the matrix through natural grazing, thereby reducing the potential spread of invasive grass species in the future.

Laudonov Gaj (pedunculate oak forest) and coniferous forests, as natural patches of anthropogenic origin, stabilized the shifting sands of Krbavsko Polje and simultaneously successfully prevented the subsequent expected process of desertification in the future. The reforestation action can be seen as a historical instance of landscape planning, but it raises the question of preserving shifting sands as a rare phenomenon in Croatia and a growing problem in certain desert regions of the world (desertification). It also brings into question whether it is possible to optimize the control of such areas in a way that prevents their spread but does not eliminate them entirely.

The Krbavsko Polje area only partially allows for arable farming activities due to the seasonal flooding caused by high groundwater levels, and thus the habitats of the flooded areas have been preserved in their natural state. Aquatic habitats – lakes, rivers, and seasonal streams – are sources of biodiversity that have not been disrupted by anthropogenic structures or activities, and they remain preserved and self-sustaining. Riparian zones (corridors) of rivers and lakes are particularly valuable due to their role in creating a protective buffer for water bodies, helping prevent erosion of the banks, and serving as rich habitats and corridors for various species. Within the matrix, at a larger scale of 1:7,000, stepping stone corridors can be observed, which greatly facilitate the movement/migration of animals from one patch to another across larger, unprotected areas (Figure 11).

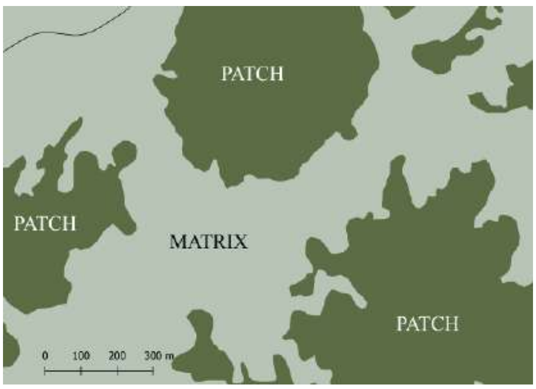

Figure 11. Spatial data (left) and DOF of the stepping stone corridor within the matrix (right).

Anthropogenic corridors, which are elements of fragmentation, consist of roads. The state road D1 passes through the area, dividing it into two sections, thereby affecting animal migrations. As a side effect along the roads, ruderal weed vegetation is expected, which takes over the habitat of existing native phytocoenoses. Considering the tertiary road that passes through the central part of the area, it can be said that the area is fragmented into three sections. It does not have a significant impact on animal migration, as traffic is slow and minimal, and the natural plant communities are not threatened by it because the road passes through already cultivated and abandoned agricultural areas.

6. Conclusion

The principles and methods of landscape ecology are crucial in sustainable planning and development, and understanding the structure, function, and causes, processes, and consequences of disturbances (both natural and anthropogenic) significantly contributes to professional and high-quality strategies and plans for implementing interventions or undertaking restorative measures for existing conditions.

In landscape planning, analysis based on determining landscape structure provides an understanding of the area/landscape as a functional unit and offers information on the mutual influences of existing landscape structures and/or anthropogenic interventions. Furthermore, with sufficient ecological knowledge, it opens up possibilities for landscape ecological projections of future interactions between existing landscape structures and implemented and/or planned anthropogenic interventions, although it is not possible to precisely predict natural disturbance regimes such as fires, floods, etc. It contributes to understanding the heterogeneity of the landscape, which results from interactions between human activities and natural processes, and its application in landscape planning processes offers the possibility of achieving balanced functions between the structural units of patch mosaics.

Therefore, structural ecological analysis in landscape planning is a useful foundation for preparing environmental impact assessments and strategic environmental impact assessments. Applying landscape metrics to the structural elements of the landscape and evaluating them serves as a tool in optimization processes using multi-criteria vulnerability analyses. It is also very important as a tool in studies, strategies, plans, programs, and projects of green infrastructure in urban areas or rural development – urban environments are continually expanding, and it is necessary to ensure connections between suburban, 'rural' natural patches or protected areas with preserved urban natural patches within the urban matrix. Planning stepping stone corridors within the urban matrix is extremely important as it ensures sustainable green infrastructure, linking 'green' urban elements with valuable elements often located outside the city. This contributes to the health and recreation of residents, biodiversity, ecological functions, naturalization of excessively built environments, and mitigation of the urban heat island effect in 'concrete canyons.' It is also possible to apply it to planning green bridge corridors or stepping stone corridors in areas where road infrastructure is planned, regardless of the density of construction or traffic frequency, or in restorative measures where road infrastructure already exists and causes habitat fragmentation/disturbance.

The definition and analysis of structural elements is also a useful foundation and method for creating descriptive analyses of a specific area. It can play a role in creating typological registers, catalogs, inventories, atlases, and landscape frameworks.

Knowledge of landscape ecology methods and principles has an important guiding role in landscape architecture – in planning and design processes and procedures, as they define a new and/or existing landscape that either is or will become a reflection of social consciousness and will dictate the environment, health, and future of all populations that inhabit it.

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IDENTIFICATION OF LANDSCAPE VALUES OF THE MUNICIPALITY KAPTOL

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Introduction

The municipality of Kaptol is a geographically and historically significant area in the north-east of Požega-Slavonia County with an area of 85.49 square kilometres. It consists of ten settlements: Alilovci, Bešinci, Češljakovci, Doljanovci, Golo Brdo, Kaptol, Komarovci, Novi Bešinci, Podgorje and Ramanovci. With a population of 3,472 inhabitants (as of 2011), the region is known for its rich cultural and natural heritage (Općina Kaptol, 2009.). Archaeological finds point to ahat Kaptol has been inhabited since the late Bronze Age. Traces of the Hallstatt culture have been found in the area, making it an important site for understanding early European civilizations (Potrebica, 2013.). In addition to its historical significance, Kaptol is part of the Papuk Nature Park, which is a UNESCO Global Geopark, adding further natural value to the area. Characterised by rolling hills, fertile valleys and dense forests, the landscape requires careful management to preserve its unique ecological, historical and cultural features.

Conservation and sustainable management of landscapes have become key elements of both urban and rural planning in the 21st century (Cerovac, 1994), especially in areas with significant natural and cultural values such as Kaptol. The main objective of this research is to identify the key landscape values of the municipality of Kaptol. This includes understanding the natural, ecological, aesthetic, cultural and historical elements that contribute to the region's landscape. By recognizing these values, this study aims to provide a basis for future conservation efforts and promote sustainable development that balances growth with the protection of ecological and cultural heritage (van Rogen, Zlatić, 2014). Identifying these landscape features is the first step in developing more effective management and planning strategies for the area. Planning that considers landscape qualities focuses on integrating aesthetic, social, historical, cultural, biological, environmental, and economic aspects (Gobster and Xiang, 2012; Sarlöv Herlin, p. 400). These elements must be identified, safeguarded, or even enhanced in future planning efforts (Stephenson, 2010). Consequently, it is crucial for spatial plans to include measures for landscape protection (Tomić Reljić et al., 2023). So, this study contributes to the broader discourse on landscape conservation by providing insights into the specific challenges and opportunities in Kaptol.

Figure 1. The sites of the Kaptol municipality, Author of the photographs: Marta Ljevar, 2023.

Material and Methods

The preparation of this paper began with a desk research of literature and cartographic representations of the selected area in order to deepen the concept of spatial planning, rural and sustainable development, the identification of elements, but also the identification of the character of the space through examples of similar areas. By studying maps of the municipality and reading historical records, the historical, natural and cultural heritage of the selected area have been researched. Once the desk research a field trip followed to get a deeper and more realistic insight into the terrain landscape characteristics. The cabinet's research and field trip were followed by processing of existing data and the creation of new data in the QuantumGis 3.30 program (hereafter: QGis) to create a database, which was later used to identify the landscape features of the municipality of Kaptol. The database was created from various sources that already contained existing georeferenced data (Tomić Reljić, Koščak Miočić-Stošić, Butula, Andlar, 2017)., but which in several cases had to be supplemented because they had not been created completely or accurately enough. Supplementing the database was based on notes from the field and using sources: digital orthophoto from 2014-2016, topographic map 1:25000 and OpenStreetMap. It was also a base for relief and visibility analysis. Then, all elements important for the identification of landscape values were mapped using the created database and different tools of the QGIS program. It is important to note that this paper does not deal with the landscape evaluation, but only aims to indicate them within the research area. However, it can serve as a basis for further analysis of the area and thus the evaluation of the landscape qualities.

Results and discussion

The relief analysis of the municipality of Kaptol began with a detailed hypsometric map, which shows that the altitude of the area is between 80 and 964 meters above sea level (Figure 2). Approximately half of the region is located in the lowland belt at an altitude of less than 300 meters. The northern part of the municipality, which rises towards the Papuk mountain, lies at an altitude of between 300 and 964 meters. This area is characterized by rolling hills covered with vineyards and, at higher altitudes, dense forests. The hypsometric map not only illustrates the differences in altitude within the region, but also shows how these physical differences affect land use and vegetation distribution. The lower, flatter areas are mainly used for agriculture, while the higher, steeper regions are covered with vineyards and forests.

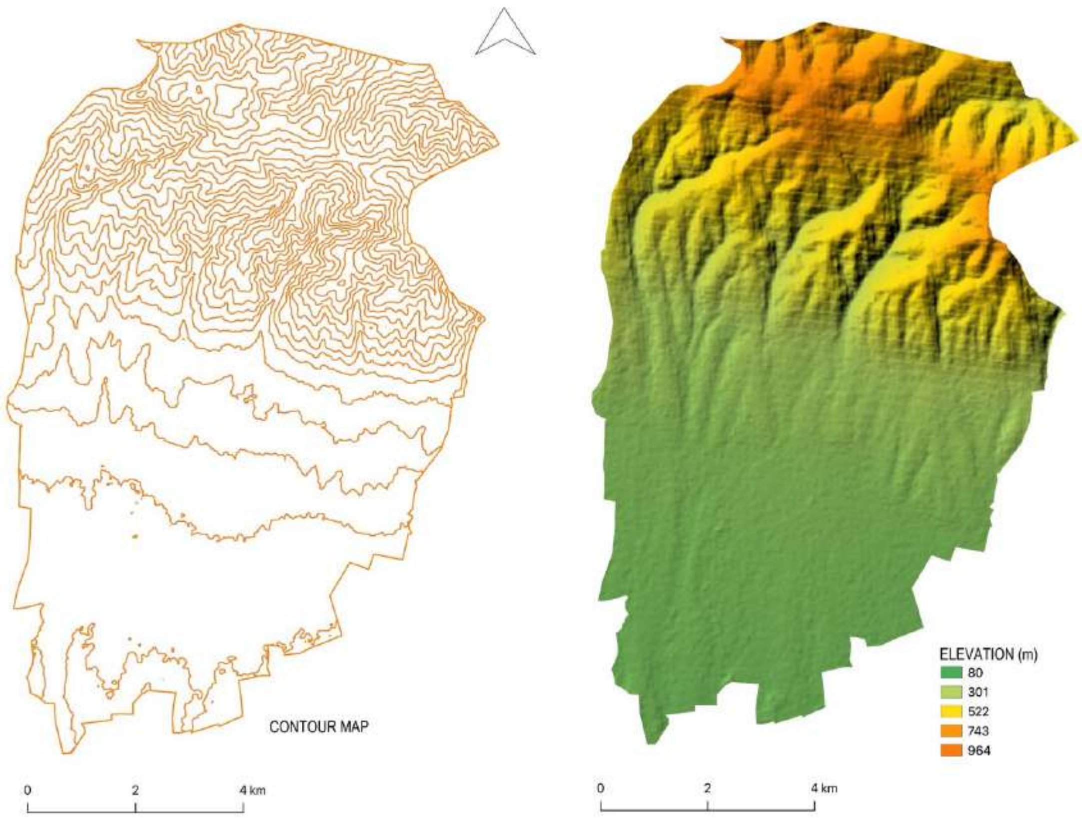

Figure 2. Cartographic representation of the hypsometry of Kaptol Municipality

The slope map further divides the landscape into zones based on gradient (Figure 3-left). It shows that the northern parts of the municipality, closer to Papuk, are steeper, exceeding gradients of 80-90% in some areas, while the southern regions are predominantly flat or gently sloping. These steep slopes, particularly in the northern areas, are subject to significant erosion, with landslides and surface runoff occurring during periods of heavy rainfall. The analysis of the slopes (Figure 3) provides important insights for land use planning and suggests that agricultural activities in these steep areas should be carefully managed to prevent soil erosion and degradation.

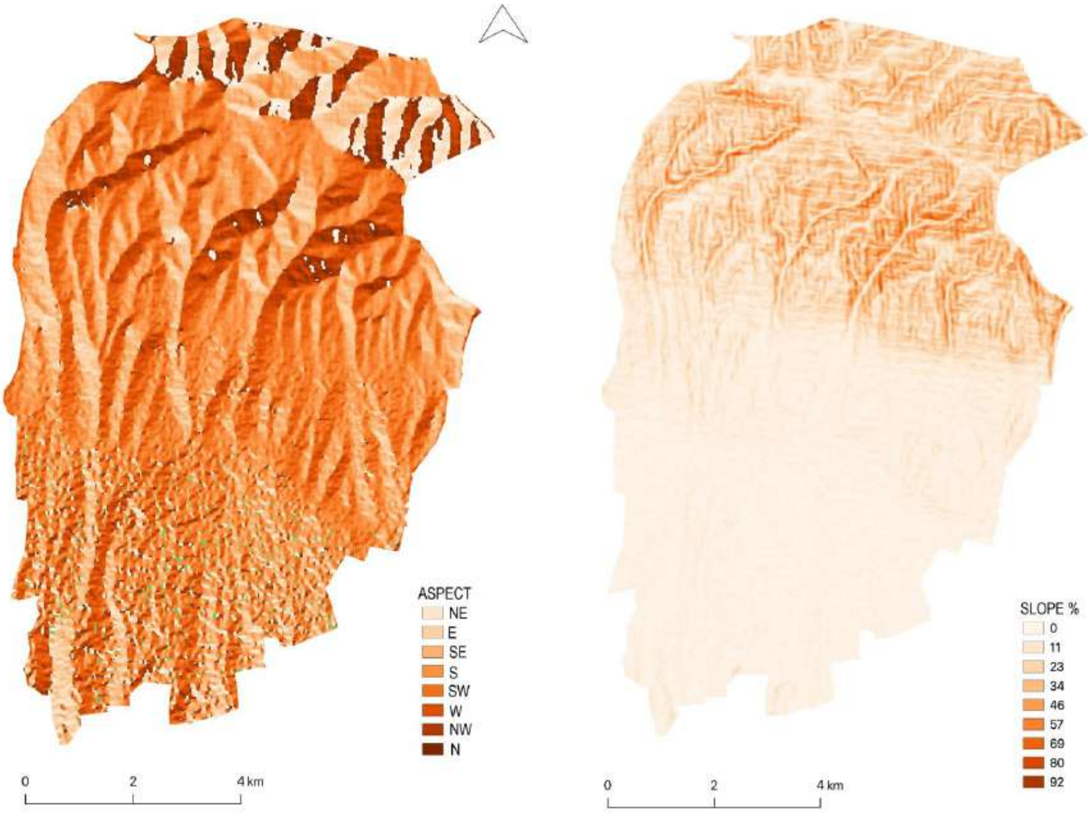

Figure 3: Cartographic presentation of the Kaptol Municipality slope (right) and aspect (left)

Analysis of the exposure revealed that most of the slopes of Kaptol face south and southwest, with some areas in the upper parts of the municipality facing north (Figure 3). The southern and southwestern exposures tend to be warmer and drier, which is particularly favourable for viticulture and fruit production, as the increased sunlight accelerates sugar production in these crops. The south-facing slopes of the Papuk Mountain are known for their excellent vineyards, which are among the most productive in the region. In contrast, the north-facing slopes are cooler and wetter, supporting the growth of dense forests, which are crucial for maintaining biodiversity.

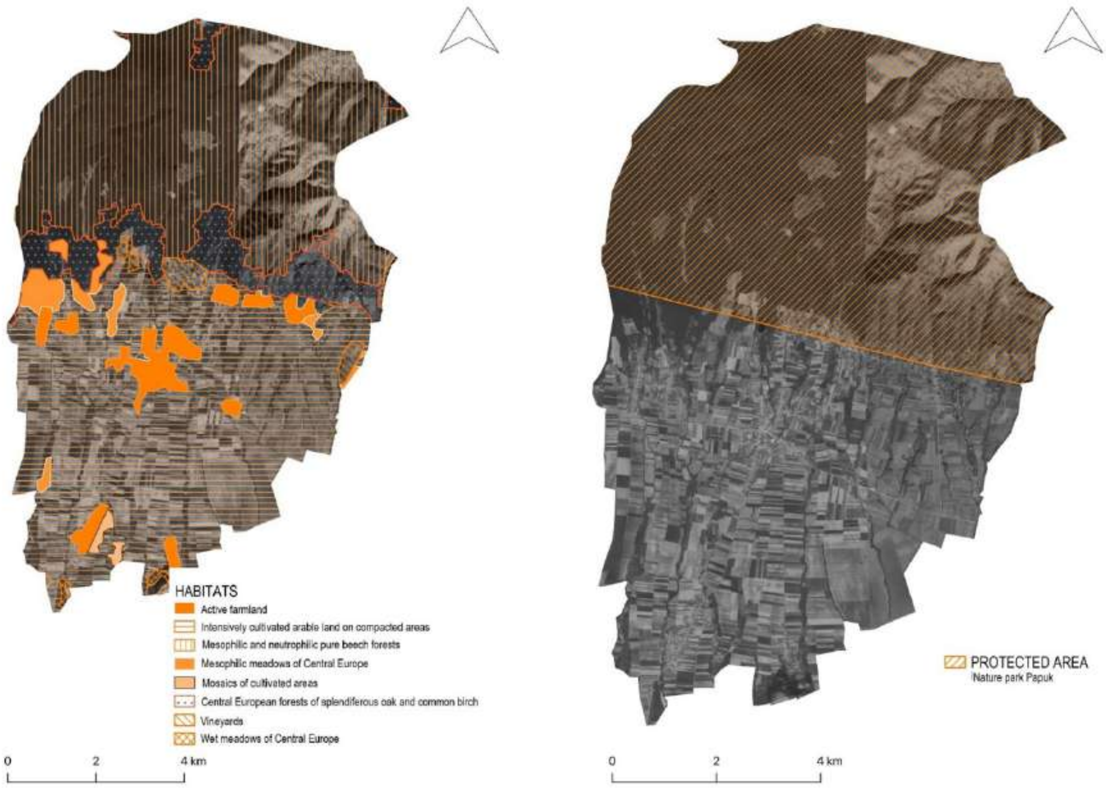

Figure 4: Cartographic representation of nature park Papuk (right) and terrestrial habitats of the municipality of Kaptol (left)

The natural values of Kaptol were analysed using various maps showing the forests, grasslands and water systems (Figure 4). The municipality's proximity to the Papuk Nature Park enriches it with natural beauty and biodiversity (Figure 4). The park itself is a protected area known for its unique geological formations and rich plant and animal life. Land cover analysis in Kaptol revealed a diverse range of ecosystems, with mixed deciduous forests dominating the northern highlands, while the southern lowlands are covered by agricultural fields and grasslands (Figure 4). This diversity of land cover not only supports a variety of habitats, but also contributes to the aesthetic value of the landscape, with contrasting textures and colours visible from different viewpoints.

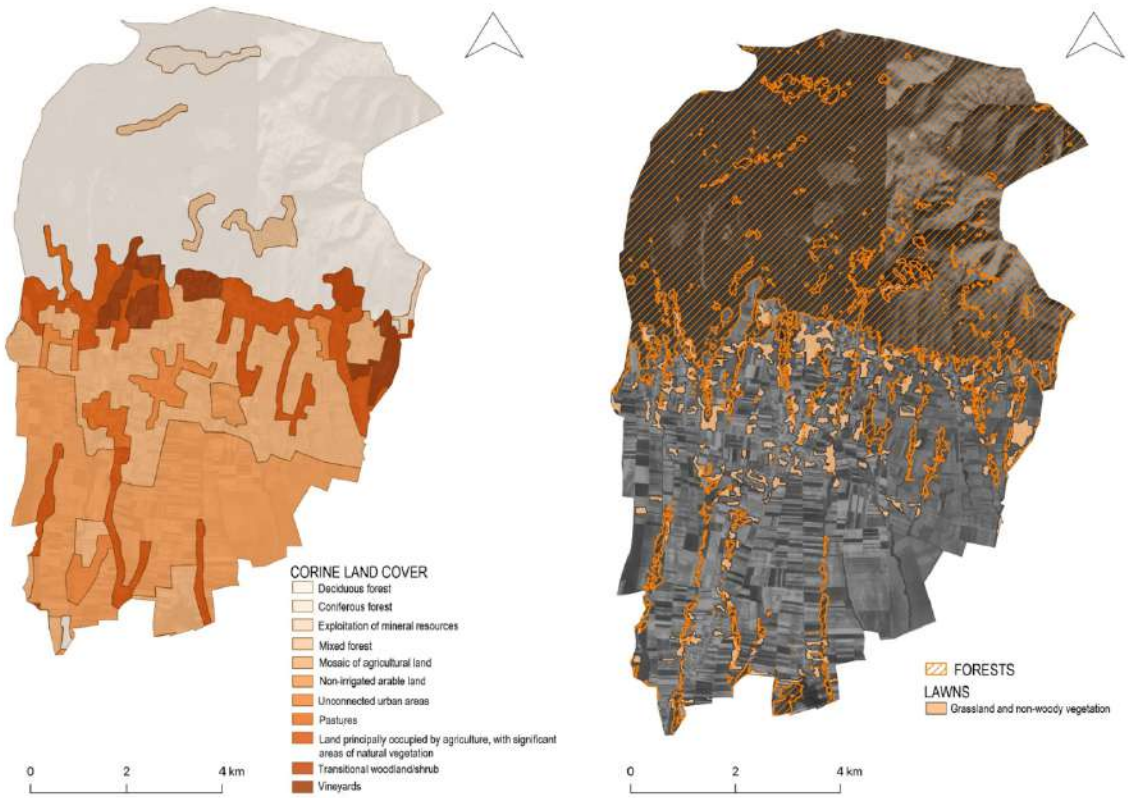

Figure 5: Cartographic representation of the Corine land cover and forests and grasslands of Kaptol municipality

One of the most important natural features identified was the presence of wetlands and watercourses (Figure 6). Kaptol is home to the artificial lake Bistra, which was created by damming the Kaptolčanka stream. This lake has become a popular recreational area for residents and visitors providing opportunities for fishing, hiking and nature observation. The water systems in Kaptol, including the streams and seasonal watercourses that originate in the Papuk mountains, play a crucial role in biodiversity and agriculture. However, these water systems also present a challenge in terms of managing erosion and maintaining water quality, especially in areas where agricultural runoff may contribute to pollution.

In the Municipality of Kaptol, the degradation points are mainly associated with illegal waste dumping and erosion in steep areas. The illegal dumpsites were identified in several locations (Figure 6), especially in rural areas where waste management systems are less effective. These landfills pose a significant threat to both the natural environment and the visual quality of the landscape.

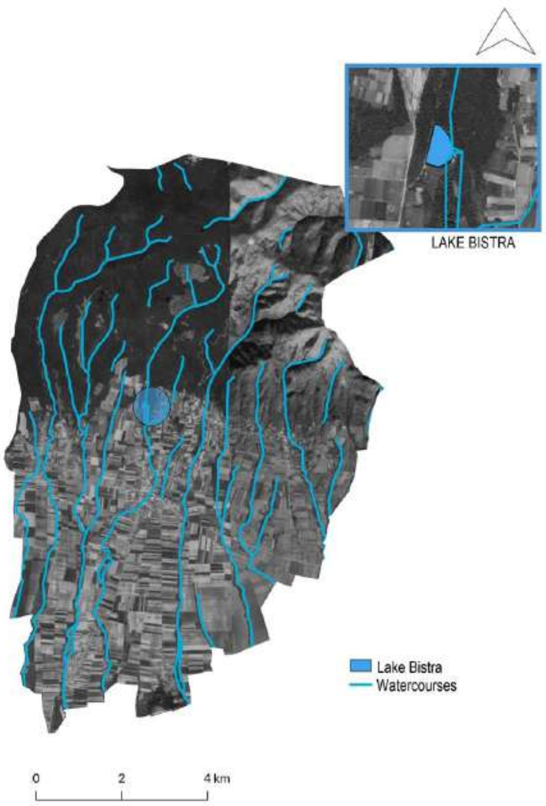

Figure 6: Cartographic representation of lakes and watercourses (left) wild waste disposal sites in Kaptol municipality (right)

The paper also examined the historical and cultural values of Kaptol, identifying several key archaeological sites and architectural landmarks (Figure 7). The most significant archaeological site is the Hallstatt culture necropoli**s** in Gradci, which dates back to the early Iron Age. This site includes numerous burial mounds and artifacts that provide insights into the lives and customs of the early inhabitants of the region. These archaeological sites, along with others such as the Čemernice tumuli, highlight the long history of human settlement in the region and underscore the importance of preserving these cultural landmarks (Potrebica, 2013.) In addition to its archaeological heritage, Kaptol is home to several architectural landmarks, including the Church of St. Peter and Paul, a baroque building from the 18th century, and the Old Town of Kaptol (Figure 8), a medieval fortress with a distinctive hexagonal layout. These structures not only contribute to the cultural identity of the region but also offer opportunities for tourism and education.

Figure 7: Cartographic presentation of archaeological sites, zones and cultural assets of Kaptol municipality

Figure 8: Old Town of Kaptol

The cultural heritage of Kaptol is intertwined with its natural landscape, with many of these historical sites located in areas of high scenic value, further enhancing their significance.

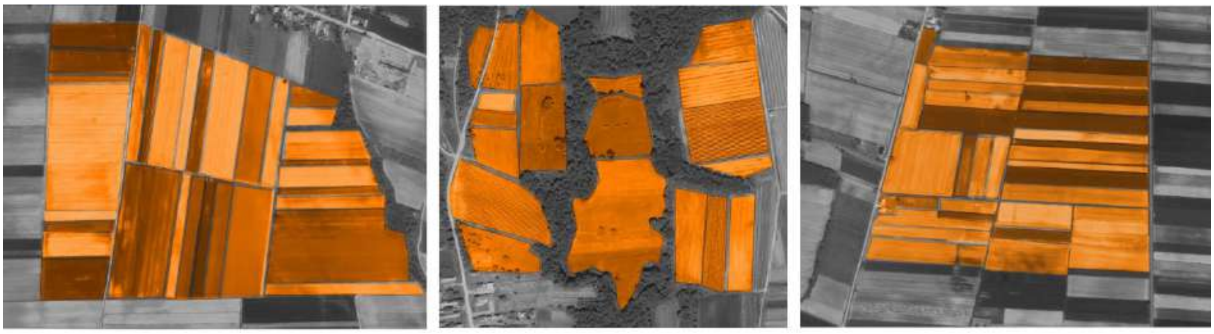

Figure 9: Landscape patterns of agricultural land

The visual-experiential featuress of Kaptol were analyzed using a combination of satellite imagery and GIS-based visibility assessments. The landscape is characterized by a mosaic of agricultural fields, forests, and water bodies, which together create a visually diverse and aesthetically pleasing environment (Figure 9). The patterns of the agricultural fields in Kaptol are particularly distinctive, with three main types identified: strict parallel patterns, natural organic forms, and geometrically combined patterns. These patterns, which are shaped by both the natural terrain and human activity, contribute to the overall visual character of the landscape. The strict parallel patterns are typically found in the flat lowlands, while the organic patterns follow the contours of the hills and forests.

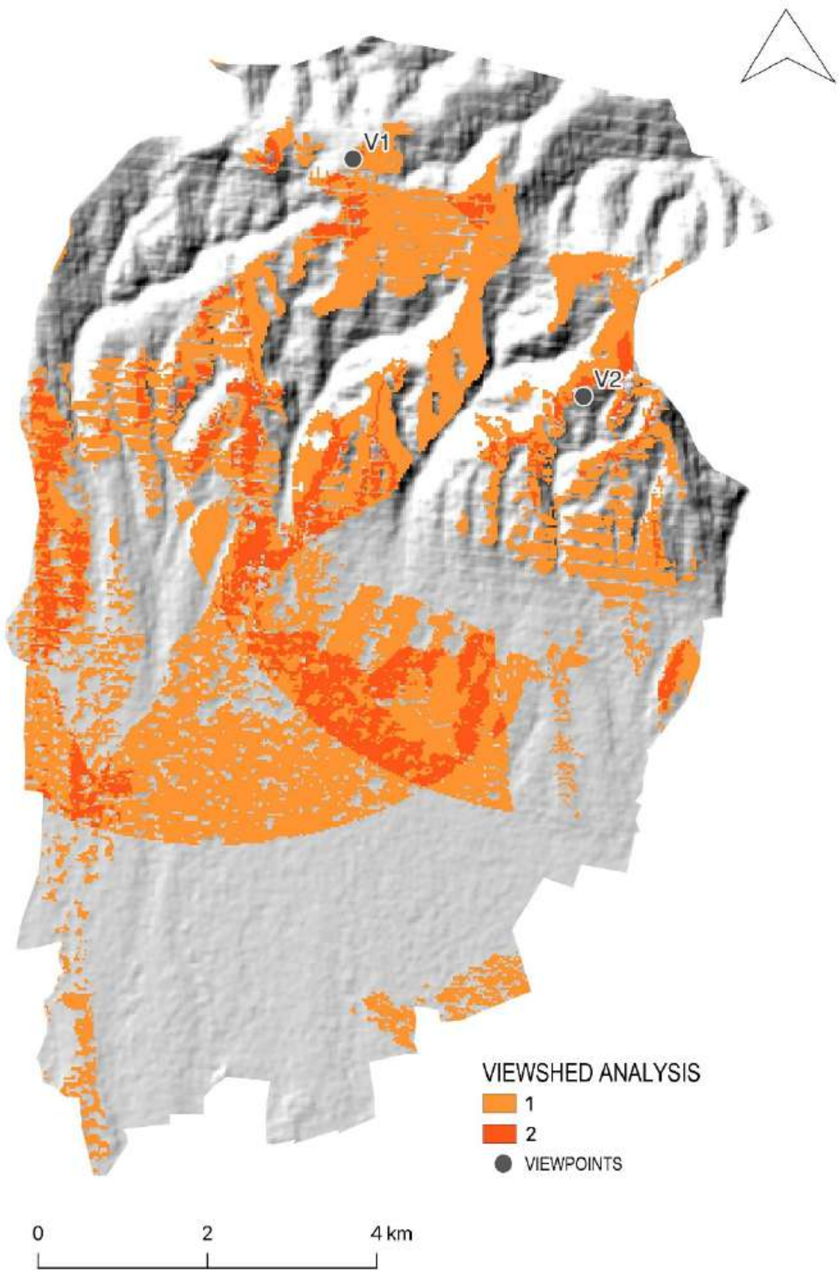

Figure 10: Cartographic representation of Viewshed analysis of Kaptol municipality

A viewshed analysis was conducted using QGIS to identify areas of high visibility and scenic frequency within the municipality (Figure 10). The analysis focused on two key viewpoints: a high elevation point within the Papuk mountains (V1) and the Lovačka baraka (V2), a popular destination for visitors. The results of the viewshed analysis revealed that a large part of the southern municipality is visible from these points, offering a wide panoramic view of the agricultural lowlands and the forested hills of Papuk. These views are important not only for their aesthetic value but also for their potential (Keleş, Atik, Bayrak, 2018). to promote ecotourism and recreational activities.

Conclusion

The municipality of Kaptol is a local self-government unit that is at the beginning of its development and is raising awareness of its values. The results of this paper reveal a region rich in natural, cultural, and visual resources, each of which contributes to the overall value of the landscape. The inclusion of part of Kaptol within the Papuk Nature Park enhances its ecological significance, while the archaeological sites provide a unique cultural depth that is rare in other parts of the country. It is also important to note that Kaptol is a municipality that is relying more and more on tourism and wine production over time, thus making it an ideal candidate for creating a landscape evaluation model for the purpose of examining these activities in order to contribute to a faster and more efficient development of the area. However, the study also highlights several challenges that need to be addressed to ensure sustainable management of the Kaptol's landscape. These include improved waste management strategies to combat illegal dumping, stricter regulations to prevent the degradation of natural habitats. Addressing these issues is critical for maintaining the integrity of the landscape and ensuring that future generations can continue to benefit from its ecological and cultural resources (von Haaren, 2014). In addition, the study provides a valuable framework for future research, particularly in the areas of landscape evaluation and conservation planning. By building on the identification of key landscape features, further studies can focus on the development of management plans that balance development and conservation and ensure that the landscape of Kaptol remains a place of both natural beauty and cultural significance.

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