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The 70th Anniversary  
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Forest Research  
Institute  
(1945-2015)

250 Years  
of Organised  
Forestry in  
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# The 70th Anniversary of the Croatian Forest Research Institute (1945-2015)

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The Croatian Forest Research Institute, one of the founders and publishers of the South-east European forestry (SEEFOR) journal, this year celebrates its 70<sup>th</sup> anniversary. The celebration of this anniversary in fact marks the beginning of the development of the institutions of scientific research in forestry in the Republic of Croatia (1945-2015), and it follows the 250<sup>th</sup> anniversary of the establishment of first forestry offices in Croatia. Although the first scientific research in the Croatian forestry was undertaken as far as in 1860, with the establishment of the Higher Forest and Management School in Križevci, only after the end of the Second World War (1945) did the Ministry of Agriculture and Forestry in Croatia start with the organization of the forestry service and research. The earliest document that shows the existence of a research institution is the Decree of the Ministry of Agriculture and Forestry, in which Zlatko Vajda, PhD was appointed an officer of 5<sup>th</sup> order in the Department of Practical Forest Research. In 1950, the main administration of forestry in Peoples Republic of Croatia became

the new founder and it changed the name into the Institute for Forestry and Game Research. In 1962 the University of Zagreb decided to include the Institute as a part of the Faculty of Forestry in Zagreb, and changed its name into the Institute of Forestry Research. It was active under this name until 1968, when the name was changed into the Institute for Forestry Research in the Socialist Republic of Croatia. The agriculture and forestry chamber of the PR of Croatia on 28<sup>th</sup> March 1959 in Rijeka founded the Forest Seeds Control Department, and on 11<sup>th</sup> June 1960 in Jastrebarsko founded the Institute for Conifers, whose name was in 1964 changed into the Yugoslav Institute for Conifers. All three of the established scientific institutes- the Institute for Forestry Research in Zagreb, the Forest Seeds Control Department in Rijeka and the Yugoslav Institute for Conifers in Jastrebarsko -were in 1974 united into the Forest Research Institute, Jastrebarsko.

The Forest Research Institute in Jastrebarsko as a scientific-research organization, with the

introduction of the Public Institutions Act (NN 76/92) became a public institution for which the republic of Croatia acquired the founding rights, and with the Scientific Research Acts Law (NN 96/93) it continued with its work as a public scientific institute and a property of Croatia. With the further development including the establishment of the Research Centres in Vinkovci, Varaždin and Pazin, it changed its name into the Croatian Forest Research Institute (2009).

During the last 70 years, the scientific institutions in Croatian forestry changed their structures, names and headquarters, but their fundamental goals, their role and their importance in the forestry remained the same: the development of forest science and the intensification of scientific findings in forestry, also closely connected to the operational forestry in the implementation of the results of research, as well as the improvement of management, protection and regeneration of forests and the preservation of biological diversity in forests, the development and the specialization of young scientists and researchers, cooperation with Croatian and foreign institutions and organizations, the investment in buildings and the purchase of new scientific equipment, the advancement of publishing and the international visibility of scientific results, and the popularization of science and forestry in the society.

This whole period will only formally be divided into several sub periods starting directly after the end of the Second World War, when the first institutions were established. At that time, experienced experts soon to be retired were working at the Institute and operational forestry was undertaking great harvesting activities, because of the restoration and industrialization. The experts from the Institute solved the ongoing problems in the operational forestry and largely contributed to the development of post-war forestry. Even though the scientific plans were modest, 22 books were published in that period. What followed was the period from 1950 to 1960, characterized by new problems in the forest tending, the expansion of conifers'

area of distribution and the intensification of the production of planting material. The way of funding the Institute was improved, new young assistants were employed, and programmes for long-term research were made. The next period lasting until 1974 brought changes, including the direct connection to the operational forestry by directly funding scientific research. When making medium-term research plans, along with the Fund for Scientific Work, operational forestry also took part, through the Business Association and directly. The projects and tasks became more complex and research was conducted on: silvicultural properties of forest trees, forest protection, soil, vegetation, improvement of forest seed husbandry and nursery production, the zoning of seed areas, physiology and nutrition, genetics and the breeding of forest trees, the forest types, and the rationalization of work. Young staff were specialized in Croatia as well as abroad. The uniting of three institutes at the beginning of 1974 brought the stabilization of funding and of scientific topics, which were dealt with at the Institute with more detail. Applied and scientific projects were successfully contracted and the publication of the results increased. Until 1990, the Institute primarily worked on broadening of the distribution area, the improvement of the establishment of conifer forest cultures and plantations, and researching the natural regeneration of forests, along with the development of physiology and nutrition, forest tree breeding and undertaking typological research. The rapid development of research topics and the specialization of scientists was stopped on 10 February 1998, when Nikola Komlenović PhD, scientific advisor, Branimir Mayer PhD (scientific advisor), Petar Rastovski PhD (scientific advisor), Zlatko Perić MSc (scientific assistant), Goran Bušić MSc (scientific assistant), and prof. Ante Krstinić PhD, a professor at the Faculty of Forestry in Zagreb, were killed in a car accident. Great changes had to be made within the main researchers and it was almost impossible to fill the gap that was created. Only about ten years later with the improvement of young researchers who came to the Institute after the accident was it possible to

continue with all of the research and ensure the new progress. In all of the next competitions for projects by the Ministry of Education the Institute ensured a sufficient number of projects. Equally successful were the research programmes and numerous projects as a part of the Programme of Scientific and Research Work in Croatian Forests funded by the General Forests' Benefits Fund (OKFŠ).

In the next period, the system of funding the projects by the Ministry of Education, Technology and IT was significantly improved, and until 1995 the Institute led 14 scientific projects. After that, in the period up until 2005, while continuing with the development of the methods of research, the new subjects were included, such as the research in protected areas (national and nature parks), and the improvement of biomass production in Croatian forests, but also the research of Mediterranean forests. Therefore the project "The Growth and Development of Special Purpose Forests" was one among the 7 projects funded by the Ministry of Science, Technology and IT. What followed was the initiative of regional development, together with the Ministry of Science, Education and Sports, Croatian Forests, local government, counties, towns and municipalities, established the Research Centre for Lowland Forests in Vinkovci, the Research Centre for Private and Urban Forests in Varaždin, and the "Josip Ressel" Research Centre for General Forests' Benefits in Pazin. This was the response to the request by operational forestry for connecting the research with its intensifying, but also to the attempts of the Ministry of Science, Education and Sports to enable the employment of young scientists outside of Zagreb.

The Croatian Forestry Institute is today the leading scientific research institution in the field of forestry in Croatia. It has developed a good and long-lasting collaboration with a number of prestigious institutes from several European countries (Austria, Germany, France, Spain, Denmark and Finland), and with countries from the region (Slovenia, Serbia, Slovakia, Bosnia and Herzegovina, Hungary, Macedonia, Montenegro and Albania). The Institute is a member of

IUFRO organization, as well as of the European Forestry Institute, and hosts its regional office for southeast Europe hosts as Department for International Scientific Cooperation EFISEE. It is also a member of ELI Institute for Ecology, and EVOLTREE network for the research of the adaptation of forest trees to climate changes.

The Institute is organized in six Research Divisions (Figure 1), three Regional Research Centres and two Departments (Common Affairs Services and Department for Nursery Production), five laboratories are organized in one inter-division accredited Department for Laboratory Research. The Institute has 88 employees, 22 of which are PhDs. In its nursery covering 22 ha and in the two greenhouses the Institute grows ornamental plants, and undertakes field research for the needs of scientific projects.

The Institute publishes two scientific journals: *Radovi* (<http://www.sumins.hr/radovi/>) and *South-east European forestry*, established by the international contract between eight faculties and institutes of forestry in the region ([www.seefor.eu](http://www.seefor.eu)).

For already five years the Institute has organized The Open Days, covering various different topics: Our Forests, Urban Forests, Forests and Water, Forests and Health and Forests and Fire. On that one day up to 800 children of different ages visit the Institute, where they are taught about science and forestry in a popular and entertaining way.

Based on the Development Strategy, the Croatian Forest Research Institute passed a subject-based evaluation for 2013-2020, as well as the process of reaccreditation, with excellent results, which confirmed it to be one of the four best scientific institutes in Croatia. The Institute has been carrying out four projects of Croatian Scientific Foundation (2014-2017): Estimating and Forecasting Forest Ecosystem Productivity by Integrating Field Measurements, Remote Sensing and Modelling (EFFEctivity), Advanced Forest Environmental Services Assessment (AFORENSA), Conservation of genetic resources of forest trees in light of climate changes (CONFORCLIM), and Defoliators as invasive forest pests in changing climate conditions (DIFPEST), because of which

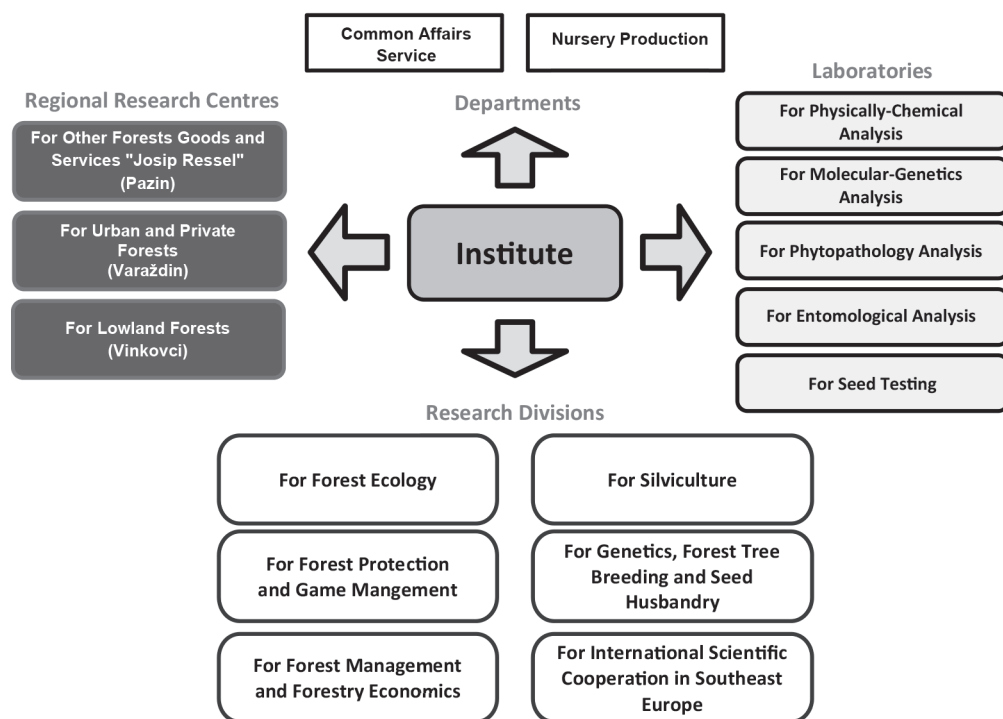


FIGURE 1. Organization of the Croatian Forest Research Institute

three new PhD candidates and one post-PhD candidate were employed.

An important feature of the Institute's role are the projects, which are continuously aimed for professional use, the Ministry of Agriculture, Administration for Forestry, hunting and wood industry, which in this period have acquired their legal foundation. Those are Diagnostic and prognostic service for forest pests (IPP), Monitoring of Damage to Forest Ecosystems (ICP), the Survey of Quarantine Pests, the Seed Bank (SJEMŠTED), and the Forest Trees' Genes Bank (GENBANK). Within these projects, the laboratories were renovated and equipped, and the building of the Seed Bank was built, in which the seeds of economically most valuable forest trees' species in Croatia are kept.

In this period the Institute also participated in several international projects preaccession funds INTERREG IIIB Cadeses CARBON-PRO

Carbon balance drafting and new resources management tools according to Kyoto Protocol (2006-2007), 7th Framework Programme FP7 Rok-For Sustainable Forest Management Providing Renewable Energy, Sustainable Construction and Bio-Based Products (2011-13), IPA AMF Adriatic Model Forest (2012-14), IPA HOLISTIC Adriatic Holistic Forest Fire Protection (2014-16) and two ERA-net projects FORESTERRA MedWildFireLab Global Change Impacts on Wildland Fire Behaviour and Uses in Mediterranean Forest Ecosystems (2014-16) and INFORMED Integrated research on Forest Resilience and Management in the Mediterranean (2015-17). Croatian Forest Research Institute is scientific and financial leader of COST action TN1401 CAPABAL Capacity Building in Forest Policy and Governance in Western Balkan Region with 16 EU countries participating in the Action.



# Regeneration of *Abies cephalonica* Loudon after a Large Fire in Central Greece

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## Abstract

**Background and Purpose:** *Abies cephalonica* Loudon (Greek fir) is a dominant tree species of the mountainous Greece that forms productive forests. Wildfires in fir forests were not considered a major threat but in recent decades, fir ecosystems in Greece have experienced large crown fires with subsequent ecological and economical losses.

**Materials and Methods:** This study was designed to aid our understanding of Greek fir recovery after fire. In Central Greece, 12 years after a large fire, fir regeneration and site factors were studied in 143 sampling plots located in 10 transects.

**Results:** Fir regeneration density decreased abruptly with distance from the remnant stands and followed the negative exponential curve with decreasing regeneration abundance from the border of the unburned zone. Abiotic factors such as elevation, aspect, slope steepness and ground cover type could not significantly explain any variability in fir regeneration density.

**Conclusions:** Our results showed that distance from a seed source was the most important variable in explaining fir regeneration. Moreover, the density of fir regeneration seemed adequate to provide stocking for a future fir forest.

**Keywords:** wildfire, seedling density, seed source, elevation, aspect, slope, groundcover

## INTRODUCTION

*Abies cephalonica* Loudon (Greek fir) is widely distributed in the mountains of central and southern Greece, at altitudes between 700 and 1900 m. Greek fir is a commercially

valuable timber and fuel wood species that is also grown for Christmas trees and planted as an ornamental.

During most of the 20<sup>th</sup> century, large fires in Greek fir forests were not a common observation. The complex topography and the

high spatial heterogeneity of mountainous Greece, probably, resulted in variable fuels and burning conditions that favored a non-uniform fire behavior. Nevertheless, after many years of fire exclusion and land abandonment, Greek fir dominated and created pure stands, increasing the likelihood of large fires. Consequently, in recent years, the mountain forest ecosystems dominated by Greek fir are affected by an increasing number of wildfires of unusual size and severity [1].

This change in Greek fir fire trends could have been related to summer drought episodes that have increased in the relatively more humid and colder regions of Greece [2]. In the mountainous Mediterranean forests, alterations in composition and flammability trends are foreseen due to changes in drought stress and fire frequency [3]. For many mountain forests, fire is likely to become almost as important for shaping the landscape as the direct effects of climate change [4]. Paleoecological data show that the thermophilous Mediterranean fir stands became extinct during the last 5000 years when anthropogenic fire and browsing disturbance increased [5]. In many occasions, the fire-intolerant fir forests were replaced by fire-tolerant forest species like oaks and the current distribution of *Abies* species in Europe has been reduced in areas less affected by fire [6].

The fire traits of Greek fir include a thin and flammable bark that provides little insulation for the cambium, shallow roots which are susceptible to soil heating, low crown base branches and flammable foliage [2]. Moreover, Greek fir has a tendency to grow in dense stands, many of which are of old age and increased mortality episodes have resulted in large amounts of dead fuels.

Greek fir is vulnerable to fire as it does not produce serotinous cones and does not maintain a seed bank when summer wildfires occur [7]. Greek fir reaches reproductive maturity at around 50 years and cone production peaks at the age of 100-140 years. Seed availability varies considerably between

years due to masting and seed dispersal usually begins in October. Since the window of opportunity for seedling establishment is short and germination rates are low, the first years after a fire are crucial for successful tree recruitment [8].

The response of fir ecosystems to fire may vary with fire frequency, size and severity, associated vegetation in the pre- and post-fire community, site conditions, distance to remnant stands, browsing and post-fire management. The rate of seedling establishment is quite variable and depends on the proximity of the seed source and seed production during the post-fire years [9]. Site conditions can be important during the early postfire years but tend to decrease with increasing time elapsed since fire, as canopy composition is getting more similar to pre-fire structure [9].

In many areas affected by stand-replacing fire, the natural return of fir forests is uncertain and opinions for postfire management differ. Arianoutsou *et al.* [10] concluded that the postfire recovery of Greek fir would be a slow process where unburned patches would play an important role. For the same site, Ganatsas *et al.* [11] suggested that without human intervention, shrubs and herbaceous species would dominate and planting could be the only solution for the reintroduction of Greek fir.

The capacity of Greek fir to regenerate after large stand-replacing fires is not well studied and this study was designed to aid our understanding of Greek fir recovery after fire with the aim to assist decision making in postfire management. The specific objectives were to evaluate the extent to which site factors affect fir regeneration after fire and identify the most important ones.

## MATERIALS AND METHODS

### Site Description

Our study area was located in the mountain fir forests of Central Greece (39°04'40"N,

21°57'08"E). The site has an oro-Mediterranean climate with cold and moist winters and relatively dry summers. The dry season lasts on average 2.5 months, from mid-June to the end of August. Meteorological data, for the last 18 years, from the nearby weather station of Fourná (39°03'32"N, 21°52'39"E) show that the mean annual temperature was 10.7°C and the mean annual rainfall was 1131 mm. The soils were mainly luvizols and acrisols developed on limestone and flysch.

Before fire, Greek fir was the dominant tree species while *Juniperus oxycedrus* L., *Quercus frainetto* Ten., *Quercus pubescens* Willd., *Ilex aquifolium* L. and *Crataegus monogyna* Jacq., were the main understorey woody species. The Greek fir tree (dbh > 10 cm) density of unburned stands was estimated from 13 randomly selected rectangular plots (40x10 m) and resulted in a mean density of 1125 mature fir trees·ha<sup>-1</sup> (range 720-1634 trees·ha<sup>-1</sup>).

On the 21<sup>th</sup> of September 2000, a wildfire started and burned 3100 ha of fir forest. Immediately after fire, the burned trees were logged and the site was left to regenerate naturally without any planting. In the burned area most understory woody species regenerated by resprouting, with the exception of juniper, but their density was very low. After fire, the main herbaceous plants found in the burned area were *Pteridium aquilinum* (L.) Kuhn, *Fragaria vesca* L., *Brachypodium sylvaticum* (Huds.) Beauv., *Digitalis ferruginea* L., *Hypericum perforatum* L., and various species of *Achillea*, *Aegilops*, *Agrostis*, *Bromus*, *Poa*, *Cirsium*, *Phlomis*, *Thymus*, *Trifolium* and *Vicia*.

### Sampling Design

In the summer of 2012, fir regeneration was recorded along 10 transects, following a systematic sampling where transects were separated by a distance of 100 m. Each transect started from an unburned forest edge and extended into the burn at a right angle until the distance from a point in the fire perimeter was equal to the distance from the starting point of transect (220-550 m). The center of the

initial sampling plots was located 10 m away from the stems of the first remnant fir trees, in order to exclude any crown effect. Along transects, circular sampling plots of 5 m radius (area = 78.5 m<sup>2</sup>) were located every 30 m, with a total of 143.

At each sampling plot, fir regeneration was assessed by counting the number of well-established individual plants (height > 20 cm). Furthermore, the dominant groundcover type was visually characterized at 10 points (every 1 m) along the main 10 m diameter of each sampling plot and the results were transformed to percentages [12]. The four groundcover types were: 1) bare mineral soil or stones, 2) dead plant biomass (litter or downed herbs and trees), 3) other competing vegetation (herbaceous or woody plants), 4) fir plants. Furthermore, in each sampling plot, we recorded the distance from a seed source (m), slope steepness (%), prevailing aspect (North, East, South, West) and elevation (meters above sea level) with the aid of a measure tape, GPS, clinometer, compass and a laser distance meter (LaserAce, MDL, UK).

### Data Analysis

The relationship between postfire fir recruitment and site factors (distance from seed source, altitude, slope steepness, aspect and groundcover) was investigated using linear regression analysis. Fir seedling density data were positively skewed and were log (x+1) transformed to meet the distributional assumptions. Model selection was performed using the Best Subsets tool of MINITAB 15 statistical software (Minitab Inc., State College, PA). Best Subsets compares all possible models using a specified set of predictors, and displays the best-fitting models that contain one predictor, two predictors, and so on. Analysis of variance followed by the Tukey's multiple comparison test were used to identify significant differences in seedling densities at various distances from the seed source. Analyses were conducted at a significance level  $p < 0.05$ .

**RESULTS**

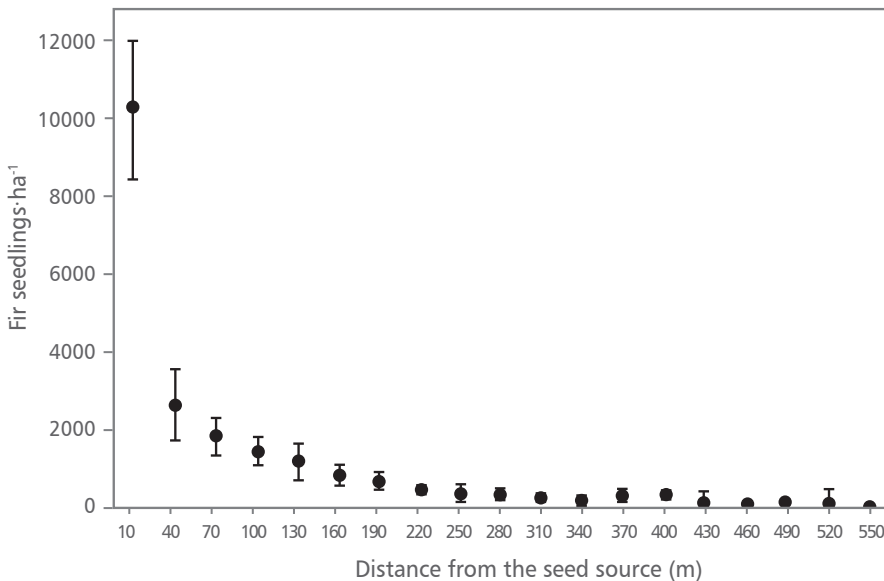
Regression analysis showed that only the distance to seed source was a significant and important predictor of fir regeneration density. This model explained 54% of the variation in the response variable. Adding more predictors to the model did not improve it. The model was:  $\log(\text{Seedling density} + 1) = 3.6719 - 0.004806 \text{ Distance (m) from seed source}$ .

Further regression analyses using the residuals of the main analysis and the other site factors as predictors resulted in models that explained very low percentages of the variation in seedling density and are not presented here.

Fir regeneration followed a negative exponential decline with distance from seed source (Figure 1). Overall, the mean density of fir was 1436 seedlings·ha<sup>-1</sup> (range 0-13843 seedlings·ha<sup>-1</sup>) and fir seedlings were found in 92% of the sampling plots. The analysis of variance revealed significant differences in seedling concentrations at various distances from the seed source. Ten meters away from the seed source, the mean density

of fir seedlings was 10084 seedlings·ha<sup>-1</sup> (range 5207-13843). At 40 m from the seed source, the mean number of fir seedling dropped sharply to 2591 seedlings·ha<sup>-1</sup>, then at 70 m to 1791 seedlings·ha<sup>-1</sup> and at 100 m to 1435 seedlings·ha<sup>-1</sup>. Beyond 100 m, the distance from seed source no longer resulted in significant differences in seedling density. Mean fir densities remained above 1000 seedlings·ha<sup>-1</sup> up to 130 m and dropped below 500 seedlings·ha<sup>-1</sup> beyond 220 m from seed source. Almost half of fir seedlings (49%) were found in the initial sampling plots at 10 m from the seed source while 70% of the total number of seedlings occurred within 70 m from the remnant stands.

The mean altitude of all sampling plots was 1305 m (range 1179-1399 m) while half of the sampling plots were located in a small altitudinal range of only 50 m (1300-1350 m) (Figure 2). High seedling densities were observed above 1330 m and low below 1250 m a.s.l.. Slope steepness varied from 0 to 47% (mean 24%) and no clear effect on fir regeneration density could be detected (Figure 3). Aspect had no

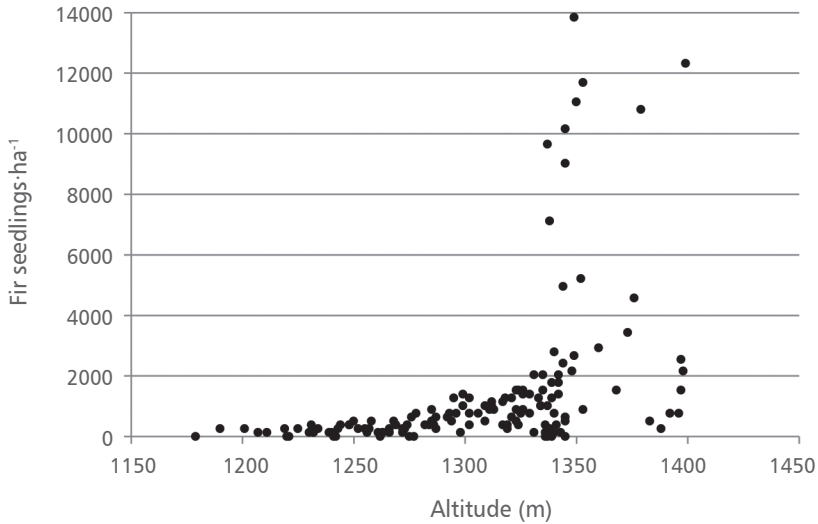


**FIGURE 1.** Fir seedling densities observed at various distances from the seed source. Dots represent the mean with 95% confidence intervals.

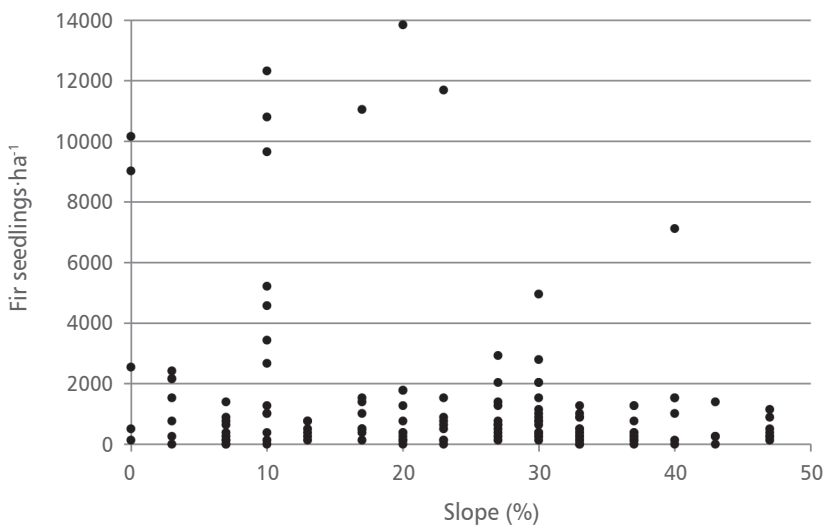
clear effect on fir regeneration density while most sampling plots were located on southern aspects and very few on western aspects (Figure 4).

Groundcover type was not an important predictor of fir regeneration. High fir regeneration densities were recorded mainly in the

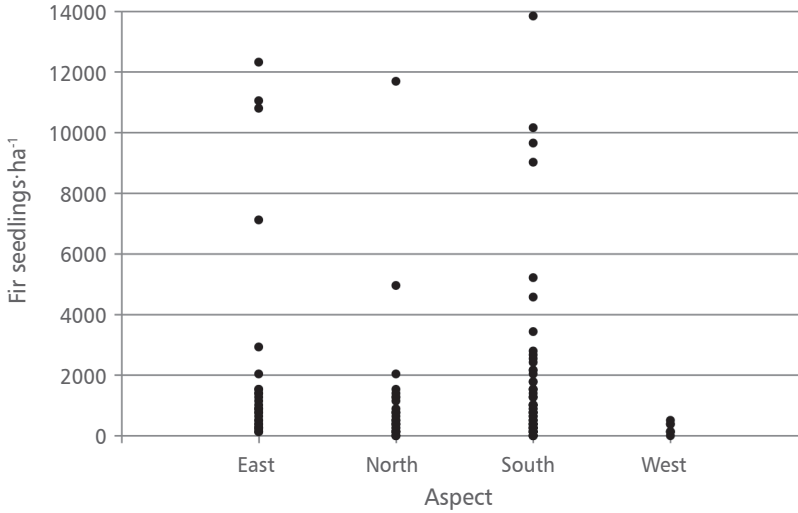
sampling plots with 10% of exposed soil or rocks (Figure 5). Most of the high seedling densities were observed in sampling plots that were covered by litter or woody debris at 20%. High fir seedling densities were observed in the few sampling plots with low competition from other vegetation.



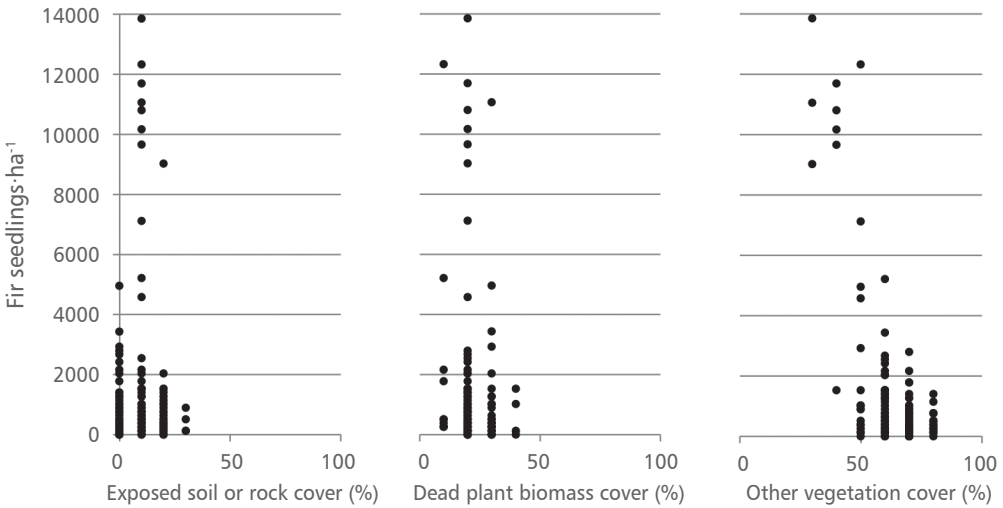
**FIGURE 2.** Scatterplot of fir seedling densities against altitude above sea level



**FIGURE 3.** Fir seedling densities observed at different slope gradients. Dots represent individual values.



**FIGURE 4.** Fir seedling densities observed at different aspects. Dots represent individual values.



**FIGURE 5.** Fir seedling densities observed at different levels of groundcover type (exposed soil or rock, dead plant biomass, other vegetation). Dots represent individual values.

**DISCUSSION**

In this study, the distance from a seed source was the most important variable in explaining fir regeneration when compared to other site variables, a common observation in similar

studies [13, 14]. Greek fir regeneration density decreased abruptly with distance from remnant stands and followed the negative exponential curve with decreasing regeneration abundance from the border of the unburned zone, a typical pattern of postfire fir regeneration

[12, 15]. Mature trees that survive fire and remnant stands are the only seed sources for colonization of burned sites [16] and the high concentration of seedlings near the remnant stands is a common observation for non-serotinous conifers [14].

Twelve years after fire, the mean density of fir seedlings was 1436 stems·ha<sup>-1</sup> which is close to and even higher than the fir density of the surrounding unburned stands (1125 stems·ha<sup>-1</sup>). Similar studies report a mean seedling density from 362 stems·ha<sup>-1</sup> [17] to 1603 stems·ha<sup>-1</sup> [18]. However, two studies on Greek fir regeneration reported that, 2 and 3 years after fire, no fir seedlings were observed although a considerable amount of healthy seeds was counted on the ground at distances up to 70 m from remnant trees [11, 16]. It seems that time since fire is positively correlated with seedling abundance for late successional conifer species such as firs that will dominate in the absence of fire [19]. Fir seedlings, usually, establish within 5 years of the fire [20] and while fir recruitment can be low during the first 50 years, it increases to higher levels thereafter by advancing waves of regeneration when the pioneering firs mature [21, 22].

Although seedling density is higher at forest edges, the natural fir regeneration can be abundant at distances greater than 200 m or 400 m from a seed source before declining farther away [18, 23, 24]. In our site, the mean fir densities remained above 500 seedlings·ha<sup>-1</sup> up to 220 m from the remnant stands and even at distances greater than 400 m, some fir seedlings were recorded. The observed seedling densities could lead to the creation of a mature fir forest within 50 years after fire that will cover most of the burned area. By the end of this century, the whole burned area is expected to be naturally reforested and occupied by a mature fir forest.

The relationship between fir regeneration and altitude was difficult to interpret, as many environmental variables covaried with altitude, the altitude range was small (almost 200 m) and the high seedling densities observed in the higher altitudes were mainly due to their

proximity to seed sources. Post-fire conifer regeneration usually increases across the elevation gradient, possibly due to increasing moisture availability as a result of an increase in precipitation and decrease in temperature at higher elevations [8, 17, 25]. Moreover, slope steepness was not considered an important abiotic factor of fir regeneration as has also been reported elsewhere [18, 19].

Aspect had no clear effect on fir regeneration density and it was not an important predictor as has also been reported by Donato *et al.* [18]. However, other studies show that a northern aspect favors fir regeneration [22, 26]. Moisture stress can be a critical factor limiting conifer regeneration in dry coniferous forests and is usually higher in sites of high solar radiation [14, 17, 21, 25]. Our observations can be partially explained due to the fact that Greek fir is considered one of the best drought-adapted European firs [27]. Moreover, our site was not a dry area but a relatively moist one with high rainfall and deep soils that can support good fir regeneration even in areas with high solar radiation.

Fir regeneration was high in locations with a low percentage of exposed soil or rocks. Seed germination and seedling survival after disturbance are generally favored by a thin organic layer and exposed mineral soil [28]. However, such seedbeds are usually found in the interior of a fire, away from the seed source which is the most important variable affecting fir regeneration [29]. As a result, tree recruitment can be even negatively related to the availability of exposed mineral soil in the first years after fire [25].

The effect of dead plant biomass on fir regeneration was not clear although most high seedling densities were observed in sampling plots covered with litter or woody debris at 20-30%. Following fire, fir establishment can be higher near woody debris than other microsite features [21]. The positive impact of coarse woody debris on plant regeneration can be attributed to its protective role from high temperatures and excessive water loss [14].

Greek fir establishment was low where

the herbaceous and woody plant cover was dense. The competing vegetation were mainly herbaceous plants and after fire the window of opportunity for conifer recruitment was probably very short as the rapid spread of herbaceous species increased competition and reduced the availability of favorable seedbeds [25].

## CONCLUSIONS

In conclusion, after a large stand-replacing fire, Greek fir regeneration depended mainly on the distance from seed source. Abiotic factors such as elevation, aspect, slope steepness and ground cover type could not significantly explain any variability in fir regeneration density.

Moreover, the density of fir regeneration seemed adequate to provide stocking for a future fir forest. However, our conclusions are valid only for our site, one of the best sites for fir growth in Greece. In less favorable areas, fir regeneration could be delayed or possibly replaced by shrubs or hardwoods.

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# The Effects of Exposure, Elevation and Tree Age on Seed Characteristics of *Fagus orientalis* Lipsky

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## Abstract

**Background and Purpose:** Natural or artificial regeneration, rehabilitation, and conversion from coppice to high forest are important practices in Oriental beech (*Fagus orientalis* Lipsky) forests in Turkey. Studies of the seeds of this species have increased in number because mast years are infrequent and seed germination is inhibited by dormancy. In this study we quantified the effects of tree age (40-59, 60-79 and 80-99 years), stand exposure (north, west, east and south) and elevation (600 and 800 m a.s.l.) on seed characteristics (germination, moisture content, and weight) of Oriental beech.

**Material and Methods:** The seeds used in this study were collected from natural beech forest at Kumluca, Bartın, in the western Black Sea region of Turkey. Experiments were carried out in the laboratory and the greenhouse of Bartın University.

**Results:** Seed germination and moisture content varied significantly by elevation, and seed germination was strongly influenced by elevation. Moisture content was 14% at 600 m and 16% at 800 m. The effects of elevation and tree age on 100 seed weights were not significant but exposure had a significant effect. The highest 100 seed weight was recorded for trees on southern exposures and the highest germination percentage of 82% was recorded for trees on northern exposures.

**Conclusions:** In conclusion, since oriental beech seedlings are produced by generative propagation method, seeds should be harvested in optimum distribution area of beech, from average ages and phenotypically plus tree.

**Keywords:** Oriental beech, natural forest, tree age, elevation, exposure, seed characteristics

## INTRODUCTION

One of the most abundant and economically important hardwood genera in northern hemisphere temperate forests is *Fagus* [1]. In Turkey, Oriental beech (*Fagus orientalis* Lipsky) is a common hardwood tree species that regenerates naturally in Turkish forests where species diversity is rich due to the variety of growing conditions. It is a shade-tolerant species, and optimum growth conditions for Oriental beech are on the north-facing slopes of the North Anatolian orogenic belt in the Black Sea Region and the Istranca Mountains in Thrace. Oriental beech forests in Turkey cover 1810079 ha, composing nearly 8.5% of the country's total forest area [2]. The elevation range of Oriental beech in the Balkans is 10-800 m above sea level (m a.s.l.). In Turkey, it grows at 1500-1700 m a.s.l. in Black Sea inner-valleys and up to 2000 m a.s.l. in the Aegean mountains [3-7]. Most Oriental beech forests are distributed in northern Turkey. However, relict Oriental beech forests are distributed in the Eastern Mediterranean region of Turkey, including the provinces of Adana, Osmaniye, Hatay, and Kahramanmaraş [8]. Oriental beech forests in Turkey usually have mixed structure with Nordmann fir (*Abies nordmanniana* (Steven) Spach, Uludag fir (*A. nordmanniana* (Stev.) Spach. subsp. *bornmuelleriana* (Mattf.) Coode&Cullen), Kazdagı fir (*A. equitrojani* Aschers et Sinten), Scotch pine (*Pinus sylvestris* L.), Anatolian black pine (*P. nigra* Arnold subsp. *pallasiana* (Lamb.) Holmboe) and Oriental spruce (*Picea orientalis* (L.) Link.) at higher elevations, but there are also pure Oriental beech stands that cover large areas and are connected by corridors [4-6, 9]. Oriental beech forests, however, have been degraded, especially near villages and transport corridors [10]. Plus trees are very difficult to find in these areas. These circumstances can negatively affect natural regeneration practices. In this species, seedling establishment and growth rate depend on the seed tree, habitat conditions, and especially on the light factor [11]. Many studies on beech regeneration have

been conducted in pure beech forests [12-14]. Studies on the regeneration of many plant species, especially beech, are usually correlated with light and weather conditions.

Ozel [14] stated that the group regeneration works in Bartın and Devrek in Turkey failed and that the areas that had lost the natural regeneration conditions needed regenerating by planting under the shelter of trees. Moreover, the seed is a very important material for outdoor plantation conditions, rehabilitation and conversion to high forest practices. Due to a lack of seed orchards for Oriental beech, the selected seed stands have been used in Turkey [15]. Generally, mast years occur every 3-5 years [12, 16], 5-6 years [4] for Oriental beech. However, Tosun [17] studied the seed yield of Oriental beech at Bolu (Turkey) and in the period of eleven years observed one mast year, two good years, three moderate years and five weak years. Suner [12] indicates that lower seed productivity exists in Oriental beech stands on calcareous bedrocks, whereas the seed year occurs more frequently and abundantly. Consequently, investigations on the seeds of this species have been on the increase because of rare mast years and germination problems caused by physiological dormancy [18, 19]. Moreover, Rezaei *et al.* [20] stated that oriental beechnuts are deeply dormant seeds that can germinate after 8-19 weeks of cold stratification. Besides, there were various studies on seed characteristics [21-25] and the storability of beechnuts [18, 26]. Thus, the aim of this study is to provide new information for seed technology of Oriental beech by examining the effect of tree age, stand exposures and elevations on seed characteristics.

## MATERIAL AND METHODS

### Material

Oriental beech belongs to the *Fagaceae* family. It is a shade-tolerant species [27]. Seedlings are tolerant to shade and grow under trees slowly, and therefore they exhibit growth with more speed in the juvenile age.

The seedlings of the *Fagus orientalis* L. can grow in gravel soil and gravel-silt and are able to expand their own roots. The optimum pH of this species is 5.5 in the periods, and beech seedling that expand its roots, may be spherical. The density of seedlings has a direct impact on the form of the seedlings [3, 4, 6, 9].

### Sampling Sites

The seeds of Oriental beech were collected in a natural forest in Kumluca - Bartın (41°30'5" - 41°20'27" N, 32°23'46" - 32°33'44" E) located in the northern part of Turkey (Figure 1). Oriental beech appears naturally from 400-1000 m a.s.l., especially in the Kumluca forest region [28]. Kumluca Oriental beech forests area was separated into two blocks on the basis of 600 m and 800 m elevations. In each block, 3 plots, in size of 100 m<sup>2</sup>, were taken according to age groups (40-59, 60-79 and 80-99 ages). In each plot, 3 trees with 150 m intervals were selected for having their seeds collected. The collected seeds were sampled, labelled and put in plastic bags. In addition, to determine the effect of the exposure factor, the seeds were obtained from 3 trees only from 60-79 ages group according to north, west, east and south exposures.

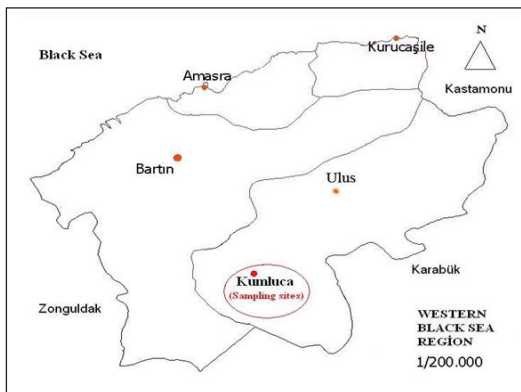


FIGURE 1. The locations of sampling sites at Kumluca-Bartın in Turkey

### Studies under the Laboratory Conditions

The seeds, collected from these sites, were stored frozen until laboratory studies were

initiated. In order to find out the amount of filled seeds, whole seeds were immersed in water and the ones floating on the surface of the water were separated. The filled seeds were chosen as 3×100 seed patterns for each sampled tree. In order to determine the weight of 100 seeds of Oriental beech, an average of three groups of 100 seeds was weighted. In the method for testing moisture content; three seeds group were separated and weighted for their wet and dry weights. The moisture contents ( $W_A$ ) were calculated [9] as follows:

$$W_A = (A-E) \cdot 100/A$$

where A is the wet weight and E is the dry weight.

The seeds were stratified in moisture-sand in a cool room at 4±0.5°C for 90 days [9, 27].

### Studies under the Greenhouse Conditions

The number of 3×100 seeds for each sampled tree sowed at ¼ sand+ ¼ clayey soil + ½ peat in the depth of 2-3 times of its size in the greenhouse in April. Following the sowing, the medium was thoroughly watered and kept damp all the time. Following the sowing date, the greenhouse temperature was kept at 25°C and 85% humidity. The germination period was completed in 45 days.

### Statistical Analysis

All experiments were based on completely random designs with three replicates, using 100 seeds in each replicate. The germination percentage was calculated as the proportion of the germinant of the total number of seeds. Arcsin ( $p$ )<sup>1/2</sup> transformed germination data were subjected to analysis of variance using computer software package SPSS 9.0 and Duncan's multiple range test was performed for significant effects.

## RESULTS AND DISCUSSION

### The Impact of Elevation

The results of ANOVA for the effect of elevation factor on seed germination and mois-

**TABLE 1.** Analyses of variance for elevation factor

Source of variation	D.F.	Characters		
		Germination percentage	100 seeds weight	Moisture content
Between groups	1	306.363	17.980	15.839
Within groups	16	32.941	7.510	0.997
F value		9.30***	2.39 <sup>NS</sup>	15.89***

D.F. - degrees of freedom

\*\*\* - correlation is significant at the 0.001 level; <sup>NS</sup> - non significant

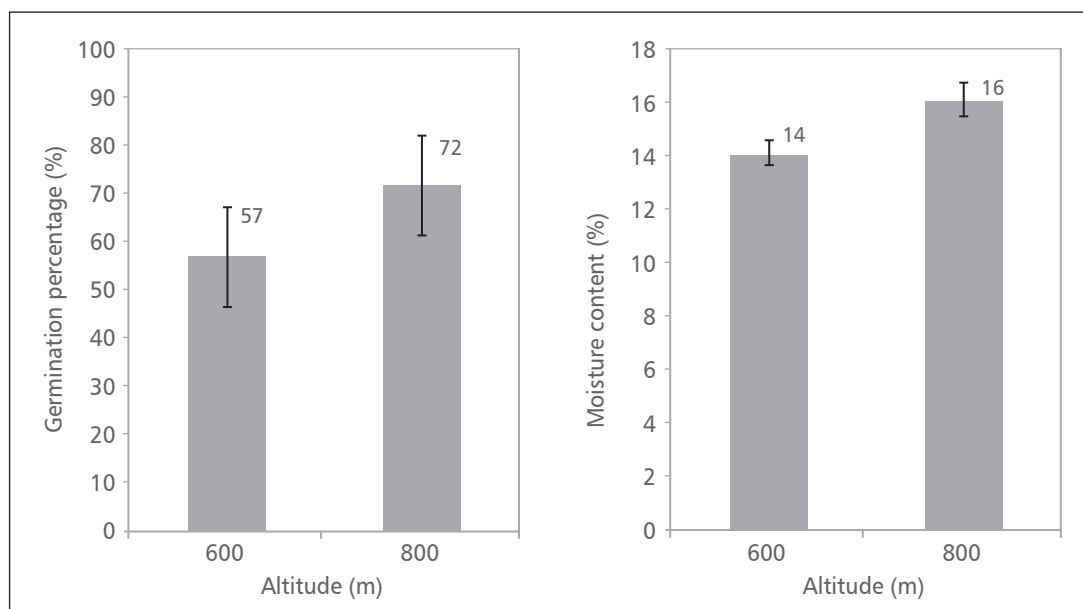
ture content showed significant differences at 0.001 confidence level (Table 1).

The elevation factor was especially very effective on the seed germination of Oriental beech. As a matter of fact, while the seeds collected from 800 m elevation showed 72% germination, other seeds, collected from 600 m elevation, presented 57% germination. Also, the moisture contents were found to be 14% for 600 m and 16% for 800 m elevation (Figure 2).

Saatcioglu [9] says that Oriental beech has fairly low moisture content (10.6-12.0%). In this study, the result obtained was very similar.

It is informed that high germination as 80% was obtained from low moisture content [9]. However, the high germination ratio in this study was obtained from high moisture content. The germination percentage of 800 m elevation was higher than of 600 m elevation. The 800 m elevation degree has nearly optimum distribution elevation for Oriental beech in this ecologic region. According to Vahid *et al.*, most of the seed production in Oriental beech takes place in 750 m above sea level [23].

In this study, 100 seed weight of 600 m and 800 m were 29.63 g and 27.63 g respectively. Oriental beech has heavy seeds and rarely has

**FIGURE 2.** The elevations factor on seed germination and moisture content

**TABLE 2.** Analysis of variance of tree ages factor

Source of variation	D.F.	Characters		
		Germination percentage	100 seeds weight	Moisture content
Between groups	2	260.013	9.384	1.285
Within groups	15	20.893	7.958	1.948
F value		12.445***	1.179 <sup>NS</sup>	0.660 <sup>NS</sup>

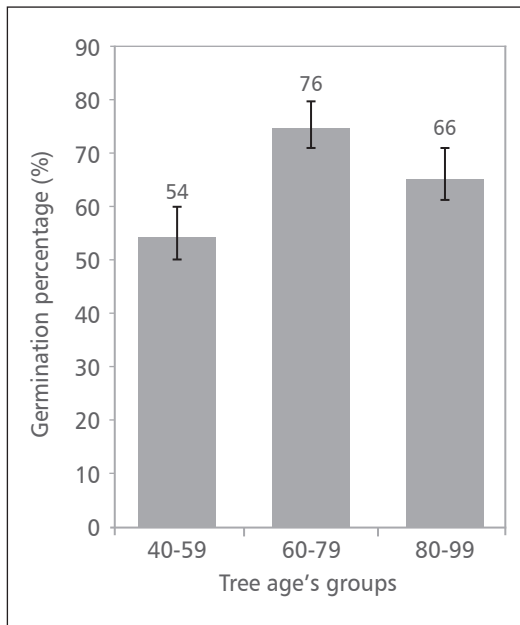
D.F. - degrees of freedom

\*\*\* - correlation is significant at the 0.001 level; <sup>NS</sup> - non significant

a mast year compared to some other forest trees. Saatcioglu and Urgenc [29] informed that the weight of 1000 seeds of Oriental beech was 273.1 g (215.3-316.7). These results are in accordance with the information by Saatcioglu and Urgenc [29], as well as by Gezer and Yucedag [19].

### The Impact of Tree Age

According to the results of ANOVA, tree age factor had a significant effect on seed germination at 0.01 confidence level (Table 2).

**FIGURE 3.** The effects of tree age's factor on germination percentage

The highest and lowest germination percentages were determined as 76% and 54% on trees aged 60-79 and 40-59 respectively (Figure 3).

Farmer [30] informed that *Fagaceae* languish 2 or 3 decades before flowering. Older trees also may not reproduce if they are in the understory of stand. Therefore, the amount of seeds is first of all a function of the number, the age, the size and the physiological condition of the trees in the stand. Espahbodi *et al.* [31] informed that more fertile seeds in terms of quality and quantity were obtained from middle aged trees. Although Fennessy [32] said that high quality fertile seeds increased from higher age to middle age, especially the beech and the oak tree, in this study, it was apparent that tree ages of beech merely affected the seed germination positively. On the other hand, Espahbodi *et al.* [31] confirmed that there was high germination on *Sorbus* seeds which were obtained from middle aged trees. Similar results to ours were also found on fir species by numerous researchers [33].

### The Impact of Exposure

According to the results of ANOVA, the exposure factor had a significant effect on seed germination at 0.001 confidence level, on moisture content at 0.01 confidence level and on 100 seed weight at 0.05 confidence level (Table 3).

The results of Duncan test - the variation coefficient, the average value, homogenous groups of the exposure factor on the germina-

**TABLE 3.** The result of ANOVA for exposure factor on seed of Oriental beech

Source of variation	D.F.	Characters		
		Germination percentage	100 seeds weight	Moisture content
Between groups	3	142.528	48.028	7.845
Within groups	20	0.268	13.055	1.131
F value		531.992***	3.679*	6.936**

D.F. - degrees of freedom

\*\*\* - correlation is significant at the 0.001 level;\*\* - correlation is significant at the 0.01

\* - correlation is significant at the 0.05 level

tion percentage, 100 seed weight and the moisture content are presented in Table 4.

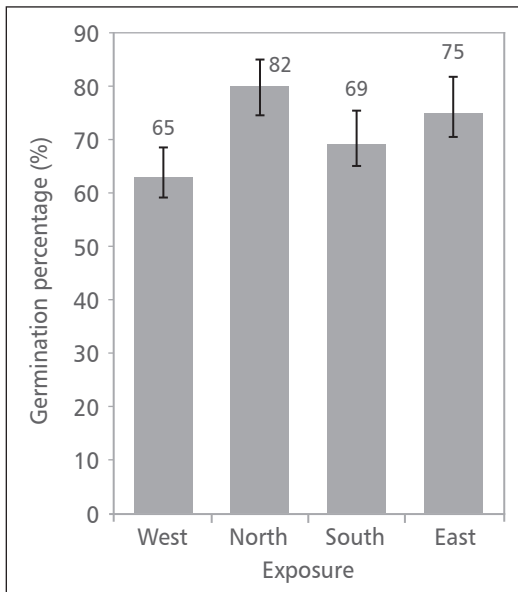
The highest germination percentage of 82% was obtained from the northern exposure, while the lowest germination was 65% from the western exposure (Figure 4).

This result is in accordance with the result of the research of the Oriental beech in Mazandaran Forests. It is concluded that the highest amount of collected seeds is related to the northern exposure of all studied trees [23].

**TABLE 4.** Average value, variation coefficient (CV) and homogeneity groups of exposure factor on seed characteristics of Oriental beech

Exposure	Variation coefficient (CV)	Averages value	Homogeneity groups
Germination percentage (%)			
North	1.03	82	a
East	0.69	75	b
South	0.99	69	c
West	0.70	65	d
100 seed weight (g)			
South	8.88	31.37	a
North	14.29	26.93	ab
West	5.05	26.92	ab
East	21.42	24.60	b
Moisture content (%)			
South	5.78	24.19	a
West	1.89	22.24	b
North	4.06	21.96	b
East	5.82	21.67	b





**FIGURE 4.** The effects of exposure factor on germination percentage

While the effects of elevation and the tree age factors on 100 seed weight had no significance, the effect of the exposure factor had showed significant noteworthy results. As a matter of fact, while the highest 100 seed weight was obtained from the southern exposure, the lowest 100 seed weight was

obtained from the eastern exposure. Soltani [34] informed that there were positive relations between the seed weight and the time of overcoming seed dormancy in beechnuts. This information was given by various researchers [35, 36]. However, this does not mean that the highest germination percentage cannot be obtained by earlier overcoming of seed germination. Furthermore, in this study, the highest germination percentage was supplied by the average seed weight and by northern exposure. From the observation of the samplings, it was determined that the beech trees in the northern exposure were healthier than others. In addition, technical staff of the general directorate of forestry said that the cavity of beech wood in the northern exposure was smaller than in other exposures.

## CONCLUSION

Since Oriental beech seedlings are produced by the generative propagation method, seeds should be harvested in the optimum distribution area of beech, which is 800 m a.s.l., from average ages (60-79 years old) and in the northern exposure for the highest germination percentage.

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# Growth-Climate Response of Young Turkey Oak (*Quercus cerris* L.) Coppice Forest Stands along Longitudinal Gradient in Albania

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## Abstract

**Background and Purpose:** Turkey oak (*Quercus cerris* L.) is the most widespread species in Albania and less investigated from dendroclimatological point of view. Previous studies have reported that *Q. cerris* is sensitive to the environment when growing at different latitudes and ecological conditions. Based on this fact we have explored the response of different *Q. cerris* populations located along the longitudinal gradient.

**Materials and Methods:** The stem discs were sampled from six sites (Kukes, Diber, Rreshen, Ulez, Elbasan, Belsh) along longitudinal gradient ranging from north-east to central Albania. All oak forests stands grow under the influence of specific local Mediterranean climate. Tree-ring widths were measured to the nearest 0.001 mm using a linear table, LINTAB and the TSAP-Win program. Following the standard dendrochronological procedures residual tree-ring width chronologies were built for each site. Statistical parameters commonly used in dendrochronology were calculated for each site chronology. Relations between the tree-ring chronologies were explored using Hierarchical Factor Classification (HFC) and Principal Component Analysis (PCA), while the radial growth-climate relationship was analyzed through correlation analysis using a 19-month window from April in the year prior to tree-ring formation (year  $t - 1$ ) until October in the year of growth (year  $t$ ).

**Results and Conclusions:** The length of the site chronologies ranged from 16 to 36 years, with the Elbasan site chronology being the longest and the Belsh site chronology the shortest one. Trees at lower elevation were younger than trees at higher elevation. Statistical parameters (mean sensitivity (MS) and auto correlation (AC)) of site chronologies were different among them and lower values of AC1 showed a weaker dependence of radial growth from climatic conditions of the previous growing year. Principal component analysis showed that Belsh, Rreshen and Elbasan site chronologies were clustered in the first principal component accounted 36.6% of the total variance. The HFC indicated grouping of the site chronologies in two clusters, where Belsh, Rreshen and Elbasan site chronologies were grouped in the first cluster while Kukes, Diber and Ulez in the second one, confirming the results previously provided from

PCA. The dendroclimatological analyses showed that the positive moisture balance in current April and previous September was the dominant climatic factor favoring the radial growth of *Q. cerris* in Albania.

**Keywords:** dendroclimatology, principal component analysis, hierarchical cluster analysis, correlation analysis

## INTRODUCTION

The genus *Quercus* is one of the most important clades of woody angiosperms in the Central and Western Europe, in terms of species diversity, ecological dominance, and economic value [1]. Turkey oak as one of the species of this genus is grown in England, France, Italy, Balkan Peninsula, as well as in Albania. The genus *Quercus* in Albania is mainly represented by Turkey oak (*Quercus cerris* L.), Italian oak (*Quercus frainetto* Ten.), Pedunculate oak (*Quercus robur* L.) and Sessile oak (*Quercus petraea* Liebl.). *Q. cerris* is the most widespread species in Albania, covering 132910 ha (30.8% of overall forest area) with a standing volume of 2.5 million m<sup>3</sup>, dominated by young forest stands ranging from the age of 10 to 20 years [2]. Oak forests in Albania are managed in two ways: coppice and high forests. However, there are some forests under mixed management. In general, about 74% of oak forests are coppice and 26% are high forests. Because they are managed as coppice, oak forests are often clear cut and managed with short rotation. This is the reason why they are so young.

Among the deciduous oaks, Turkey oak is less investigated from a dendrochronological point of view. In Albania, there are several studies related to this species focusing on silvicultural aspects [3], but there are no chronologies available, nor any study about radial growth and its response to climate.

The natural stands of this species in Albania grow under the Mediterranean climate conditions, characterized by dry and hot summers and wet and cold winters, both periods unfavorable for growth [4-6]. Oak forests in low altitude sites face a lack of moisture during summer and the water shortage during these months of the growing season reduces the radial growth.

Tree ring analysis is considered as a powerful tool for the identification of the most important relations between the tree radial growth and the climate [7-9]. In dendrochronological studies the inter-annual response of radial growth to local climate variations is evaluated through the study of the relation between the total tree-ring width and the climate data [10]. Tree-ring responses of Turkey oak to climate fluctuations have been widely studied in Italy [11, 12], Serbia [13], Slovakia [14], etc.

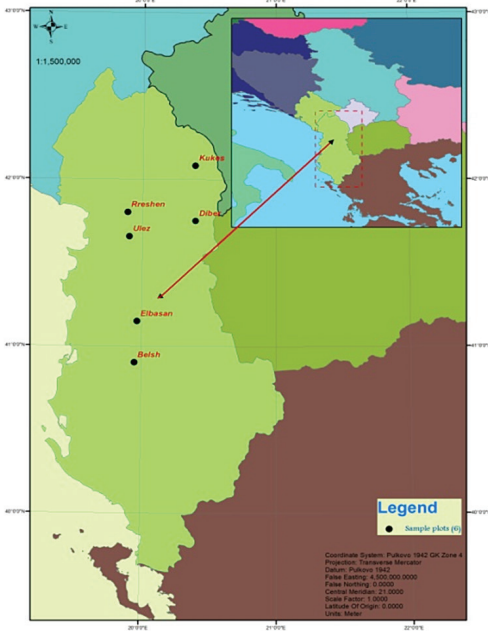
Previous studies have also reported that Turkey oak is sensitive to local climatic conditions and to those sites located at different altitudes [15]. This characteristic initiated the idea to investigate the response of the radial growth of young *Q. cerris* populations located along the longitudinal gradient to different local climate conditions. Because all Turkey oak forest stands are conventionally managed as coppice in the climate-growth analysis, we were constrained in selection of such young oak stands.

Therefore the objectives of this study were: i) to develop first *Q. cerris* chronologies for different sites, ii) to study the radial growth-climate relationship along the longitudinal gradient and iii) to study the response of the radial growth of *Q. cerris* to local and regional climate.

## MATERIALS AND METHODS

### Research Locations

The study was carried out in six sites along longitudinal gradient (Figure 1). In the north-east part our sampling sites were Kukes (KUK) and Diber (DIB), in the northern central part of Albania we chose Ulza (UL) and Rreshen (RESH), while from southern-central Albania we took samples from Graceni (ELB) and Belsh (BEL). All studied sites represent the natural



**FIGURE 1.** The location of sampling sites in the map

habitats of mixed forest stands of Turkish oak (*Q. cerris* L.) and Macedonian oak (*Q. frainetto* Ten.) managed intensively as coppice for a long time. The study sites are located in different exposition and in a broad altitudinal range from 240 m until 692 m a.s.l (Table 1). Natural understory vegetation consists of common hornbeam (*Carpinus betulus* L.), common juniper (*Juniperus communis* L.), common

hawthorn (*Crataegus monogyna* Jacq.) and herbaceous vegetation. These forest stands are grown on brown soils formed on limestone bedrock. Such soils have medium thickness with a sub-clay structure.

**Climate of the Studied Areas**

The KUK site is in a hilly area and it has a Mediterranean climate [16] with harsh winters and abundant rainfall. The mean annual temperature is 11.2°C, with long-term mean total annual rainfall reaching the value of 850 mm. The coldest month is January (-3°C), while the warmest month is July (21.9°C). Precipitation has a non-uniform distribution, with the highest amount of precipitation during winter (321.7 mm) and the lowest amount during summer (100 mm) (Figure 2).

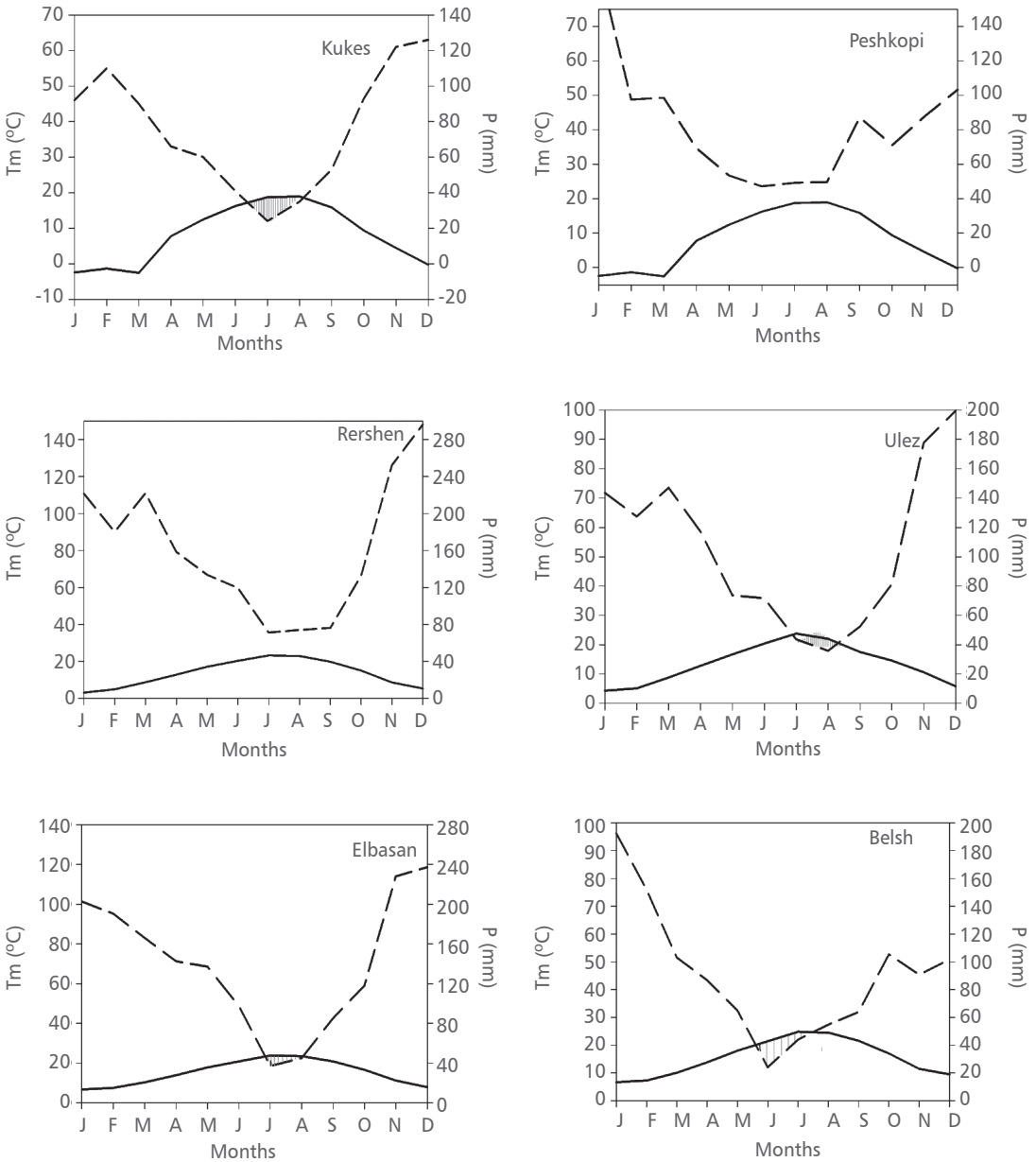
The DIB site is also in a hilly area and it has a Mediterranean climate [16] with a mean annual temperature of 8.2°C. The coldest month in this site is January (-2.4°C) and the warmest is August (19°C). The amount of precipitation is 990 mm per year on average. The majority of precipitation falls in winter (366.9 mm) where January is the wettest month (175.6 mm), whereas the driest month is July (49.2 mm). For both sites snow is a common phenomenon during the winter period.

The RRESH and UL Turkey oak natural stands are growing under the influence of the northern central hilly area with a Mediterranean climate. The long-term mean annual temperature in both sites is 13.5°C. The lowest recorded

**TABLE 1.** Characteristics (mean ± standard deviation) of the sampled trees and altitude of the studied sites

Site	Altitude (m a.s.l.)	DBH (cm)	H (m)	Age (years)
Kukës (KUK)	365	6.9 ± 4.5	6.0 ± 2.2	28.0 ± 7.0
Diber (DIB)	616	10.7 ± 8.2	7.0 ± 2.5	22.0 ± 5.0
Rreshen (RRESH)	240	7.0 ± 4.1	4.5 ± 1.7	18.0 ± 6.0
Ulez (UL)	241	6.9 ± 2.9	7.0 ± 2.5	21.0 ± 6.0
Elbasan (ELB)	692	20.7 ± 12.9	15.5 ± 3.1	36.0 ± 7.0
Belsh (BEL)	136	9.4 ± 5.9	7.4 ± 3.5	16.0 ± 1.0

DBH - diameter at breast height; H - tree height



**FIGURE 2.** Climate diagrams of the studied sites along longitudinal gradient. Dashed lines indicate monthly total precipitation and solid lines mean monthly temperatures.

temperature for RRESH and UL sites is 3.1°C and 4.3°C in January respectively, while the warmest month is July (23.2°C and 23.8°C respectively). The long-term mean annual sum of precipitation is 1937 mm for RRESH and 1271 mm for UL site.

The wettest month for both sites is December (296.2 mm (RRESH) and 199.7 mm (UL)), while July (71.1 mm) and August (35.8 mm) are the driest months for RRESH and UL. The snow is a temporal phenomenon in both sites during

winter, affecting the moisture soil balance, especially in the start of the cambial activity of Turkey oak species.

The ELB and BEL sites have a southern central Mediterranean climate. The mean annual temperature is 12.2°C and 15.5°C for ELB and BEL respectively. The minimum temperature for ELB is 5.2°C (January) and the maximum 21.7°C (August), while for BEL the lowest temperatures is 6.7°C (January) and the highest (24.8°C) was recorded in July. The total amount of precipitation is 1271.2 mm (ELB) and 1745 mm (BEL) per year. The highest amount of precipitation in both sites falls during winter and spring, with November, December, January and February being the wettest months and summer months being the driest. Summer months are drier in BEL site where the long-term monthly sum precipitation recorded was 122.6 mm, whereas in ELB site the amount of summer precipitation was 216.7 mm on average. The driest months are July and August for ELB and June and July for BEL site. Snow is common for ELB site, lasting on average 17 days and usually falling in January or February, but is not a common phenomenon for BEL site.

### Tree Sampling

Dominant healthy trees with no damage were selected for sampling at all study sites. The core sampling at first five sites was carried out during the period from October to November 2012, while the samples from Belshi area were taken in February 2014. From 8 to 18 sample trees were cut at each site. Three stem discs from the bole, middle and top of the stem were taken from each tree. The stem discs were air dried and sanded until the tree-ring patterns were perfectly visible. Tree-ring width (TRW) was measured to the nearest 0.001 mm using a linear table, LINTAB (Frank Rinn S.A, Heidelberg, Germany) and the TSAP-Win program. For each sampled tree, height (H) was measured with Vertex, while diameter at breast height (DBH) with caliper.

TRW series were cross-dated and checked visually and statistically using PAST- 4™ software ([www.sciem.com](http://www.sciem.com)). Firstly, visual on-screen

comparison was done after TRW measuring and then statistical cross-dating was conducted by calculating statistical parameters, such as the t-value according to Baillie and Pilcher ( $t_{BP}$ ) [17] and Gleichlaufigkeit coefficient (GLK%) [18]. Additional quality control was applied using the COFECHA program [19].

The cross-dated ring width series were detrended and standardized to remove age-related growth trends [7, 20]. The standardisation was performed with the ARSTAN software [21]. A negative exponential function was fitted to the raw data and then the original values were subtracted from the fitted ones. The residual series were averaged using a bi-weight robust mean function to construct residual chronologies of TRW for each site.

Statistical parameters commonly used in dendrochronology were calculated: 1) mean sensitivity (MS), which is a measure of the relative change in ring widths between successive years, 2) the first order autocorrelation (AC1), which reflects the influence of the previous year's growth on current growth with a time lag of one year [7] and 3) expressed population signal (EPS), which quantifies how well a chronology based on a finite number of trees represents the hypothetical perfect or true chronology. The first two parameters indicate the sensitivity of the species growth to environmental factors, while the EPS was used to evaluate the climatic signal of each chronology [22].

Considering the relatively young age of the trees, we used the residual chronology (RES), since the RES contains more high-frequency signals and is better in examining the growth-climate relationship in the study area. In the following analyses the period 1998-2012 which is common to all chronologies has been taken into account. In this common period all studied chronologies reached an EPS higher than the theoretical threshold of 0.85.

### Multidimensional Analysis

The relations between the tree-ring chronologies were explored using hierarchical factor classification and principal component analysis. The first analysis made a classification of the



series based on their similarity, while the last one made an ordination of the RW series based on their variance. The Principal Component Analysis (PCA) was run for the ordination [23, 24]. We applied the Varimax with Kaiser Normalization rotation method and, according to Kaiser-Guttman's rule [25], we decided to take into account only the principal components with eigenvalues larger than 1.

Moreover, for series classification we applied the Hierarchical Factor Classification (HFC) [26]. We performed HFC to classify Turkey oak site chronologies into meaningful groups and subgroups. The HFC clustered the oak radial growth chronologies based on their similarity and variance homogeneity. Thus, one cluster or node is established by merging two or more oak chronologies. The HFC can simultaneously be both a classification and a factor analysis tool and therefore different clusters of grouped chronologies can be distinguished [27, 28]. At each cluster node, the first component represents what the merging groups of chronologies have in common, whereas the second one represents the main differences between the clusters [27]. In the HFC analysis, the fusion level is a measure of the diversity of the groups obtained by cutting the dendrogram at each level. The PCA and HFC analyses were performed using SPSS 17 software (SPSS inc).

## Growth – Climate Relationship

The radial growth-climate relationship was analyzed through correlation analysis. We had access over two sources of climate data – local meteorological information from meteorological stations of Kukesi, Peshkopi, Burreli, Elbasani, Belshi and Shkodra which were used to produce climatic diagrams of the study sites (Figure 2) and a gridded CRU TS 3.1 temperature and precipitation dataset for the 1901-2009 period with a grid resolution of  $0.5 \times 0.5^\circ$  [29]. The CRU dataset used in this study was extracted from the database using the KNMI Climate Explorer web page (<http://climexp.knmi.nl/>). We extracted climate dataset for the region encompassed by the coordinates  $40^\circ 25' - 42^\circ 25' N$  and  $19^\circ 25' - 20^\circ 25' E$ .

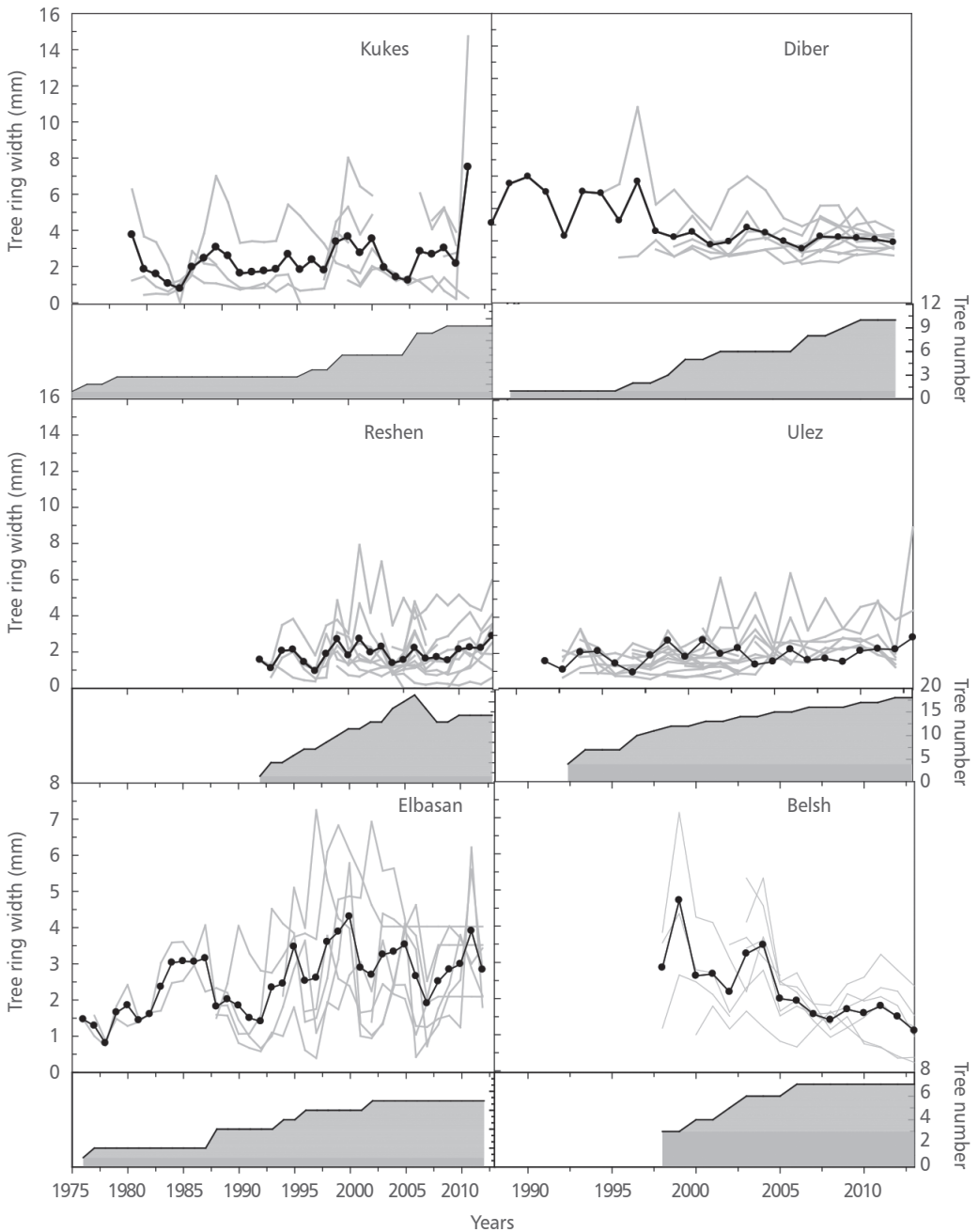
When we compared local climate datasets with the gridded CRU TS 3.1 dataset, it was ascertained that these climate data were not sufficiently reliable, mainly due to the lack of homogenization, missing values and brevity. Therefore, we took these datasets only as a reference, to which the CRU TS 3.1 dataset was compared and checked for anomalies. To describe the climatic signal of Turkey oak populations along the longitudinal gradient, Pearson's correlations were computed between the RW standard chronologies for each sampled site and between monthly total precipitation (P) and mean monthly temperature (T) for the common period 1998 - 2012, using a 19-month window from April in the year prior to tree-ring formation (year  $t - 1$ ) until October in the year of growth (year  $t$ ). In addition, correlation functions were calculated between the three chronologies represented by the first principal components (PC1) and regional climate data. For this analysis we built a regional climatic dataset by averaging the values of climatic variables for the region encompassed by the coordinates  $40^\circ 25' - 42^\circ 25' N$  and  $19^\circ 25' - 20^\circ 25' E$ . The regional climatic dataset was then used to study the radial growth climate relationship at the regional scale [30]. Significance and confidence intervals of Pearson's correlation coefficients were calculated using SPSS 17 software.

## RESULTS

### Characteristics of the Site Chronologies

The length of the chronologies ranged from 16 to 36 years, with the ELB site chronology being the longest and the BEL chronology the shortest one (Figure 3). Trees at lower elevations were younger than trees at higher elevations. The height and diameter at breast height varied between sites reaching the highest value in ELB site and the lowest in RESH site (Table 1).

The mean correlation among trees (MC) which ranks from 0.16 (DIB, 22 years) to 0.47 (KUK, 28 years) was considered significant at the  $p < 0.05$ . Statistical parameters (MS



**FIGURE 3.** Raw tree-ring width chronologies of Turkey oak (*Quercus cerris* L.) (grey lines) and the arithmetic mean (black circle solid lines) of studied sites. Upper part of the figure is the raw tree-ring width chronology of the sampled site; lower part shows sample replication.

and AC1, Table 2) indicate heterogeneous values within the six chronologies, with UL site chronology displaying the lowest mean sensitivity and DIB site chronology showing the lowest first-order autocorrelation. All chronologies showed a lower value of AC1 indicating a weaker dependence of current oak radial growth than of the growth of the previous year. The EPS for all site chronologies was higher than the critical value of 0.85 on the common period 1998-2012 (Table 2).

### Multidimensional Analysis

The PCA was run for each site chronology (Figure 4a). It is well known that the most relevant numerical result of PCA is the percentage inertia as a measure of the importance of a component. In the RW analysis, the first principal component explains 36.64% of the total inertia and the second component contributes up to 58.8%. The principal component analysis showed that BE, RRE and ELB site chronologies clustered in the first principal component accounted 36.6% of the whole variance, while UL site chronology contributes in the second principal components with 22.2%.

The RW chronologies dendrogram obtained from the HFC analysis (Figure 4b) has grouped the existing site chronologies in two clusters.

RRESH, BEL and ELB site chronologies have been grouped in the first cluster while KUK, DIB and UL have been grouped in the second one.

### Growth-Climate Relationship

Correlation analysis showed that the most distant sites showed a higher sensitivity and a reverse response against temperature (Figure 5). Most of the sites showed significant negative correlation with current June temperatures as well as with current September and October precipitation. Ring width chronologies from RRESH and UL sites showed significant and positive relationship with current July temperature. Considering the temperature impact on tree radial growth, especially during summer months, the tree-ring width of sampled trees at UL and BEL sites was negatively affected by high June temperatures. Moreover, previous July and August temperatures showed a significant positive correlation with RW growth of trees in these sites.

The correlation analysis pointed out a significant positive correlation of radial growth with April precipitation, highlighting the importance of water in early wood growth. The correlation function performed for the first component (PC1) of RW confirmed the positive influence of current June and previous

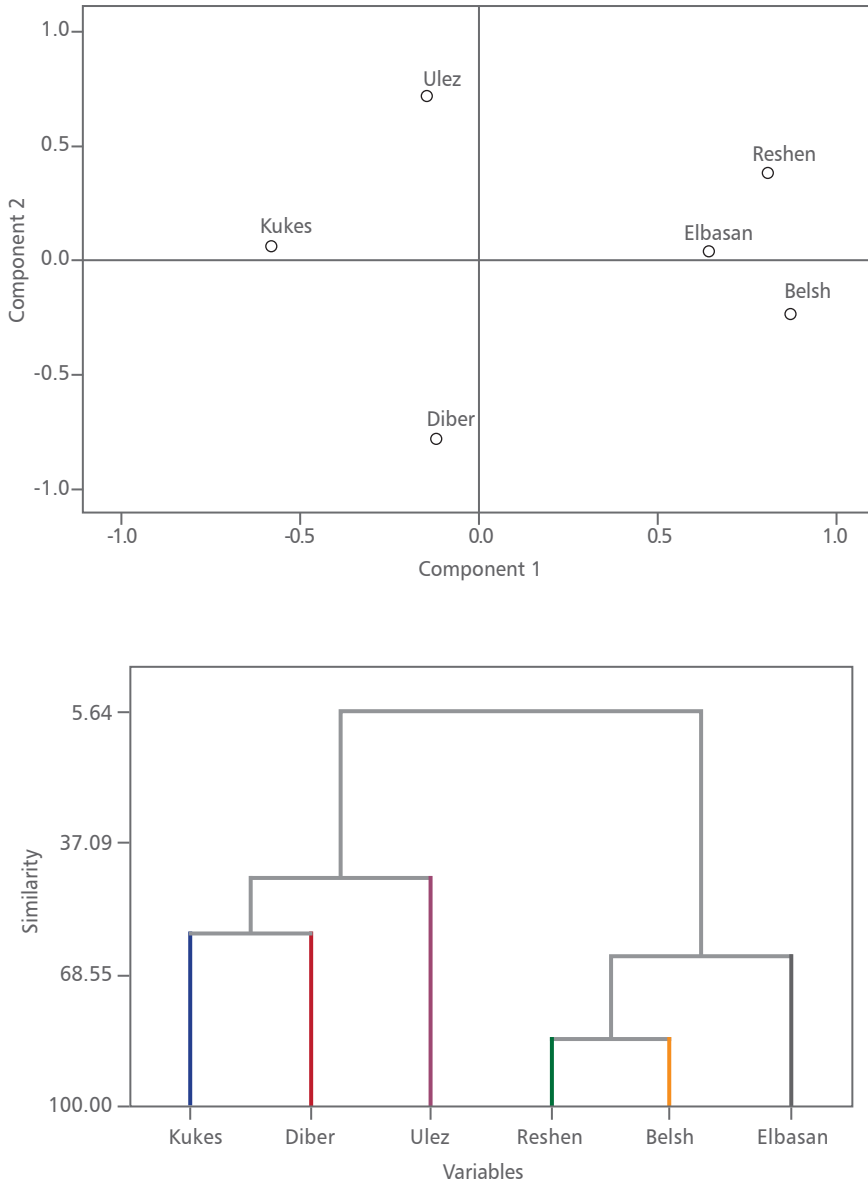
**TABLE 2.** Statistical parameters of site chronologies for each sampled site

Site	Total tree-ring width				
	MC	MS	AC1	Corr	EPS > 0.85
Kukes (KUK)	0.47	0.25	0.54	-0.58	1990-2012
Diber (DIB)	0.16	0.30	0.27	-0.12	1995-2012
Rreshen (RRESH)	0.35	0.29	0.48	0.81	1998-2012
Ulez (UL)	0.17	0.19	0.51	-0.15	1997-2012
Elbasan (ELB)	0.21	0.35	0.44	0.64	1980-2012
Belsh (BEL)	0.46	0.25	0.56	0.87	1998-2013

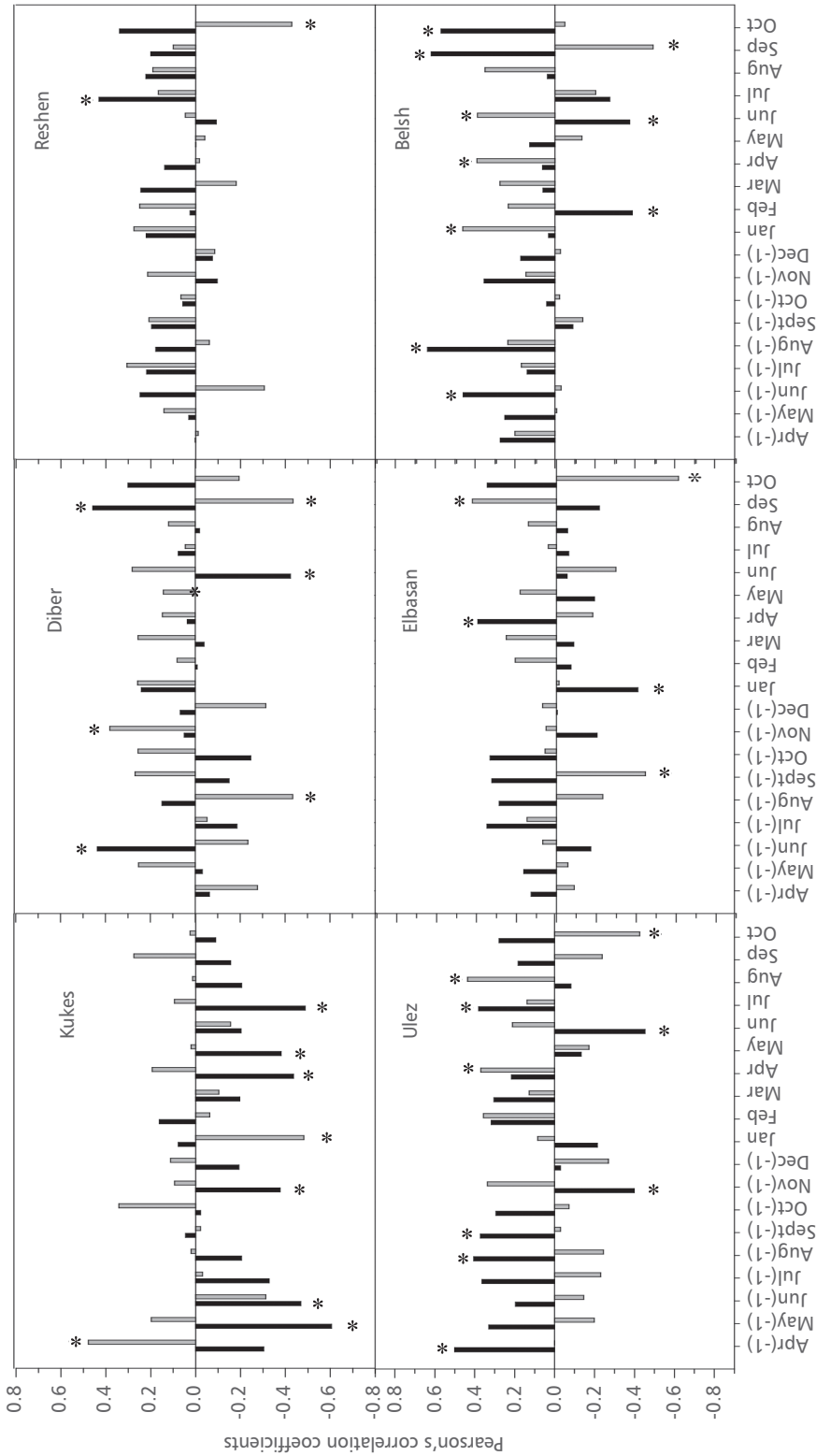
MC - mean correlation between trees; MS - mean sensitivity; AC1 - First order Autocorrelation; Corr - Correlation between site chronology and first principal component; EPS - Expressed Population Signal

July and September precipitation over radial growth at a regional scale. Negative correlation found between PC1 and July temperature of

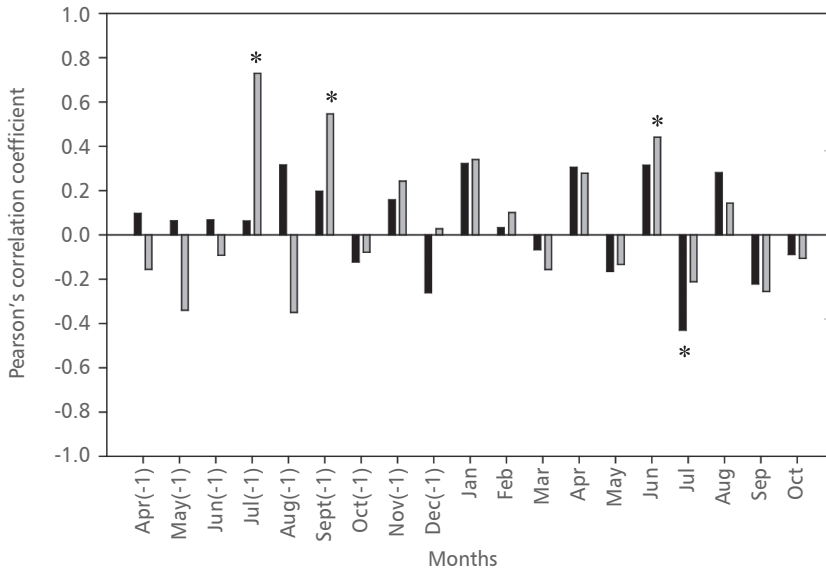
the current growing year was consistent with the same relationship found for KUK site (Figure 6).



**FIGURE 4.** The multidimensional analysis between site chronologies in the sampled sites (a - the pattern of the chronologies on the planes spanned by the first two principal components of PCA-s; b - the dendrogram created by HFC of the site chronologies of the tree-ring width).



**FIGURE 5.** The correlation function between residual site chronologies and monthly sum of precipitation (grey bars) and mean monthly temperatures (black bars). The black asterisks indicate significant correlation at  $p < 0.05$ .



**FIGURE 6.** Correlation functions between PC1 of the first PCA on sites chronologies for total tree ring width and monthly sum of precipitation (grey bars) and mean monthly temperatures (black bars). The black asterisks indicate significant correlation at  $p < 0.05$ .

## DISCUSSION

Our study provides evidence about the growth of young *Q. cerris* coppice forest stands along longitudinal gradient in Albania. These forest stands were from 16 to 36 years old, which caused a limitation in the climate-growth analysis because of the short time span. Statistical parameters (MS and AC1) corresponding to six different chronologies showed heterogeneous values according to the longitudinal spatial distribution. Therefore, we can infer that entire young Turkey oak populations have a non uniform sensitivity to local environmental conditions. The low values of first-order autocorrelation indicated a weak persistent lag effect in the growth of young oaks from previous year's radial growth.

The multidimensional analysis showed that ring width chronologies of BEL and RESH sites contributed more to the first axis than ELB chronology. It suggests the existence of a common climate signal in all chronologies

mentioned above. On the other hand, the UL chronology had a higher contribution to the second axis compared to DIB and KUK chronologies. The most distant location of DIB and KUK chronologies regarding to both axes indicate the lack of common signals as found in other sites.

The correlation analysis showed that radial growth of Turkey oak at distant sites was more sensitive against temperature. Our analysis showed a contrasting relationship between KUK, DIB and BEL chronologies with previous June temperatures, but DIB and BEL chronologies showed the same negative relationship with current June temperatures. It implies that high temperatures in the early summer may induce increased water stress and a subsequent decrease in radial growth due to elevated water loss associated with evapotranspiration and soil water evaporation. The negative effect of June temperatures on young oak growth has been previously observed in several localities in Continental Europe [31, 32], in northern

Spain [33,34] and also in Mediterranean region [35,36]. The positive relation found with April precipitation indicates that the abundant precipitation during this month favors the development of the ring at the beginning of the growing season.

In addition, the positive relation of PC1 with June precipitation shows that water balance in this month is critical for phenology and auxology of deciduous oaks because of the internal flow requirements for ring-porous wood [30, 37]. At the regional scale, the positive correlation with previous September, shows that precipitation during this month is very important for soil water recharge affecting directly the Turkey oak latewood formation and indirectly the early wood formation in the successive growing year. A previous autumn signal has been identified in other dendroclimatic studies of deciduous oaks, especially with regard to the earlywood formation [30]. This positive link has been previously observed in other studies [35, 38, 39] and could be ecophysiologicaly meaningful. Autumn moisture can have also a direct positive effect on root growth [40], so when the new growth cycle begins, in the following year the tree will have a much larger root structure and will therefore be in a position to grow more [41].

## CONCLUSION

The approach used in this study highlighted the short-term climatic responses of young *Q. cerris* forest stands along longitudinal gradient. Young growth oaks showed a climatic response that was typical for this species not only to Albania but also in some Mediterranean countries and Continental Western Europe, with a negative relationship to current June temperatures. This study also showed that positive moisture balance in current April and previous September has favored the radial growth of this species in Albania.

This study is an important first step in understanding and anticipating the responses of young growth coppice stands to climate and a good basis to explore the age effect on climate - growth relationships which could also reflect physiological changes related to ageing.

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# Leaf Morphology Variation of *Populus nigra* L. in Natural Populations along the Rivers in Croatia and Bosnia and Herzegovina

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## Abstract

**Background and Purpose:** The aim was to determine the morphological differences between the hairy type of European black poplar (*Populus nigra* subsp. *caudina*) and the typical type from the riparian forests populations as well as between the river systems. Hairy black poplar spreads in a mosaic pattern across the Submediterranean climatic type along the River Neretva and the typical European black poplar is growing on alluvial soils along large rivers in the territory of Croatia and Bosnia and Herzegovina.

**Material and Methods:** Samples for leaf morphometric analysis were collected in 17 natural populations of European black poplar along six rivers in Croatia and Bosnia and Herzegovina.

**Results:** Discriminant analyses have determined that in the differentiation of population groups largely contribute some characters such as the distance between the leaf widest part and the leaf base (DBW) and the petiole length (PL). The differences between populations and analysed groups, as well as the differences between populations belonging to a particular river system, were confirmed for all studied characteristics.

**Conclusions:** Significant differences have been determined between the typical and the hairy type of European black poplar in the studied morphological traits and these dissimilarities are in accordance with the climatic differences in respective habitats of continental riparian forests and the Submediterranean type of climate. Populations sampled in the lower course of the River Neretva, which correspond to the hairy type of the European black poplar, have smaller leaves and a greater angle between the first lower lateral vein and the midrib.

**Keywords:** leaf morphological traits, European black poplar, *Populus nigra* subsp. *caudina*, genetic variability

## INTRODUCTION

The conservation of genetic resources of some forest tree species, particularly the endangered ones, should have high priority in any large-scale programme of forest ecosystem management. The European black poplar (*Populus nigra* L.) is a species growing on alluvial soils along large rivers in the temperate zones in Europe, Asia and Northern Africa [1]. In the territory of the Republic of Croatia, the European black poplar grows in riparian floodplain forests along rivers Mura, Sava, Drava and the Danube. Its presence in the total forest area of Croatia is small, yet their universal role is significant. Because of continuous human activities in the European black poplar's natural habitats, its range has been reduced to individual, partly separated areas only, although for the European conditions the habitats are very well preserved riparian forests [2]. The overall length of rivers and creeks in Bosnia and Herzegovina is around 11000 km, but the habitats of European black poplar are limited only to the valleys of bigger rivers, such as Drina, Bosna, Una, Sana and Neretva with the total length of 1200 km [3]. In Herzegovina, along the River Neretva, a special subspecies of black poplar can be found, known as the hairy poplar (*P. nigra* subsp. *caudina*), which is characterised by distinctly hairy shoots and leaves [4]. Hairiness retains on one-year and two-year-old shoots and partially on older shoots as well. Apart from the leaves and shoots, the petioles of inflorescence and fruits are also permanently hairy. So far, these poplars have been recorded in two more southern Balkan areas, in southern Macedonia and in Thessaly in central Greece, while some populations from Albania and Turkey have also attributed to this taxon [5-7]. The studied population of the hairy type of black poplar is found in the Submediterranean type of climate. This climate is characterised by mild winters and dry summers. The value of gene conservation in these populations may be crucial for the whole species with regard to the trends of climate changes [8-11]. The survival of the European black poplar, as an autochthonous species, has been endangered also by the cultivation

of superior hybrids of Euroamerican poplars (*P. × canadensis*) and Eastern Cottonwood (*P. deltoides*) clones within the area of its natural range. Some of these imported clones were female and their flowering was synchronized with the European black poplar. Crossing between these various poplar species is possible and the question is whether in the European black poplar juvenile populations there is the introgression of the Eastern Cottonwood genes or the Euroamerican hybrid genes [12-14]. The genetic resources of the European black poplar are also threaten by a non-planned land use along the rivers, the regulation of river flows, gravel pits, dumps, and the lack of legislative regulations.

In this study the intention is, apart from hairiness, to find out which dissimilarities can be found in other morphological traits of the leaves of the typical and hairy black poplar, as well as between the populations and the rivers' systems. It also examines the importance of the hairy type of the European black poplar concerning conservation and the breeding of poplars with regard to the increasing effect of climate changes [15].

## MATERIALS AND METHODS

The samples for leaf morphometric analysis were collected in 17 natural populations of the European black poplar in the area of Croatia and Bosnia and Herzegovina (Figure 1). In Croatia, the investigations were conducted among five populations along the basin of the River Drava (Žirovnjak, Trščana, Bobrovac, Šučurica and Topolje) and three populations along the River Sava (Jarun, Zaprešić 1, Zaprešić 2) from 5 to 15 years old. In Bosnia and Herzegovina the samples were collected in nine populations from four rivers: one population was along the River Drina (Tegare), two populations were collected along the River Vrbas and the River Bosna (Zlavast, Banja Luka, Bilješevo, Dobojo) and four populations were sampled along the River Neretva (Ovčari, Počitelj, Čapljinina, Metković), all being at the age up to mature trees (30 years).



FIGURE 1. The location of the analyzed *Populus nigra* populations (after Vanden Broeck 2003)

To simplify the processing of data, the studied populations were divided into four separate groups according to the ecogeographical zones. Groups CRO-01 and CRO-02 belong to Croatian rivers (Drava and Sava). Group B&H-01 is com-

posed of six populations from the inner part of Bosnia and Herzegovina and group B&H-02 consists of three populations that were sampled in the lower course of the River Neretva in the Submediterranean region. Leaf samples were collected from short fertile shoots and in total the analysis included 514 trees and 6513 leaves, from which 32565 data have been measured and statistically processed (Table 1).

The fully developed, undamaged European black poplar leaves have been investigated [16]. Within one tree the leaf dimorphism and the seasonal heterophylly in poplars was also found [17, 18]. The leaves were taken only from short shoots only in the central part of tree crown, which proved to give a more reliable description of the studied morphological properties than the leaves from the long shoots [19-21].

The measurements of the following morphological parameters have been taken: leaf blade length (LBL), leaf blade width (LBW), petiole length (PL), the angle between the first lower lateral vein and the midrib ( $\alpha$ ) and the distance

TABLE 1. Sampled populations

Group	Population	Locality	N	n	Latitude	Longitude	Altitude (m)
CRO-01	Drava 1	Žirovnjak	60	300	46°19' N	16°43' E	139
	Drava 2	Trščana	60	300	46°15' N	16°44' E	149
	Drava 3	Bobrovac	60	300	45°48' N	17°47' E	102
	Drava 4	Šučurica	60	300	45°46' N	18°11' E	88
	Drava 5	Topolje	60	300	45°40' N	18°30' E	99
CRO-02	Sava 1	Jarun	52	260	45°46' N	15°55' E	117
	Sava 2	Zaprešić 1	33	165	45°50' N	15°46' E	131
	Sava 3	Zaprešić 2	40	200	45°49' N	15°48' E	125
B&H-01	Bosna 1	Bilješevo	10	500	44°07' N	18°00' E	357
	Bosna 2	Doboj	10	500	44°45' N	18°06' E	141
	Vrbas 1	Zlavast	10	500	44°01' N	17°29' E	580
	Vrbas 2	Banja Luka	10	500	44°46' N	17°13' E	154
	Drina 1	Tegare	11	550	44°08' N	19°26' E	180
	Neretva 1	Ovčari	10	488	43°41' N	17°59' E	330
B&H-02	Neretva 2	Počitelj	7	300	43°08' N	17°43' E	15
	Neretva 3	Čapljina	13	650	43°07' N	17°42' E	10
	Neretva 4	Metković	8	400	43°03' N	17°41' E	3

N - number of sampled trees; n - number of sampled leaves

between the leaf widest part and the leaf base (DBW), as shown in Figure 2.

Measured morphological traits were summarized using descriptive statistical parameters, with standard algorithms using descriptive statistical analysis [22]. Data are presented following univariate statistical parameters: arithmetical mean (M), standard deviation (SD) and the coefficient of variation (CV). Statistically significant differences between the investigated objects were determined using univariate nonparametric Kruskal-Wallis analysis of variance tests (Kruskal-Wallis ANOVA).

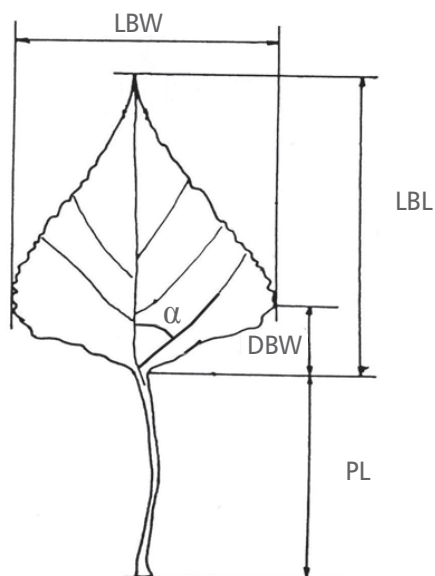
The analysis was carried out on the entire sample in a manner that examined differences between all populations, among the groups CRO-01, CRO-02, B&H-01 and B&H-02, between the groups CRO-01 and CRO-02, groups B&H-01 and B&H-02 and between the populations on the basis of belonging to a particular river system.

According to McGarigal *et al.* [23], multivariate statistical methods were used (the

principal components analysis and the discriminant analysis) to show the similarities or the differences between the studied populations and groups on the basis of the measured morphological characteristics of leaves. In both analyses the arithmetic means of each population for each parameter were used. Five principal components in total were derived by principal component analysis. The first two of them were selected by Kaiser criterion (eigenvalue greater than 1) for further analysis of relations between populations [24]. When it comes to the interpretation of the results, the first two principal components are shown in the graph to determine the trend of the relationship between the populations. To determine the features that best distinguish predefined groups, a discriminant analysis was carried out. These statistical analyses were performed using the statistical program STATISTICA 8.0 [25].

## RESULTS

The results of the descriptive statistical analysis in Table 2 are shown according to the populations, and in Table 3 are listed according to the investigated groups. The results show that the Croatian population from the group CRO-01 has the highest average values for the traits such as leaf blade length (LBL), leaf blade width (LBW), the distance between the leaf widest part and the leaf base (DBW) and the petiole length (PL). On the contrary, the highest average values for the trait angle between the first lower lateral vein and the midrib ( $\alpha$ ) were peculiar to populations from the group CRO-2. The two groups of studied populations from Bosnia and Herzegovina had larger and wider leaf blades (LBL, LBW) and longer petioles (PL) that characterized populations from the inner part of Bosnia and Herzegovina (group B&H-01). Populations which were sampled in the lower course of the River Neretva (group of B&H-02), except that they had smaller leaves, also had the higher values for the angle between the first lower lateral vein and the midrib ( $\alpha$ ). Also it can be seen that the highest degree of



**FIGURE 2.** The investigated leaf morphological traits (LBL - leaf blade length, LBW - leaf blade width, DBW - distance between the leaf widest part and the leaf base, PL - petiole length,  $\alpha$  - angle between the first lower lateral vein and the midrib).

**TABLE 2.** Descriptive statistics of the measured morphological traits at the population level

Population	LBL (cm)		LBW (cm)		DBW (cm)		PL (cm)		$\alpha$ (°)	
	M $\pm$ SD	CV	M $\pm$ SD	CV	M $\pm$ SD	CV	M $\pm$ SD	CV	M $\pm$ SD	CV
Drava 1	5.5 $\pm$ 1.18	21.5	4.5 $\pm$ 0.97	21.3	2.0 $\pm$ 0.43	21.5	3.2 $\pm$ 0.99	30.9	38.9 $\pm$ 5.56	14.3
Drava 2	5.5 $\pm$ 1.06	19.4	4.2 $\pm$ 0.87	20.8	2.0 $\pm$ 0.43	21.2	3.0 $\pm$ 0.86	29.1	36.9 $\pm$ 5.21	14.1
Drava 3	4.5 $\pm$ 1.14	25.4	3.9 $\pm$ 0.91	23.7	1.7 $\pm$ 0.42	24.9	2.9 $\pm$ 0.94	33.1	39.7 $\pm$ 6.37	16.0
Drava 4	5.1 $\pm$ 1.06	20.6	4.4 $\pm$ 0.95	21.7	1.9 $\pm$ 0.45	23.2	3.0 $\pm$ 0.93	31.2	39.8 $\pm$ 6.56	16.5
Drava 5	4.6 $\pm$ 0.99	21.5	4.0 $\pm$ 0.83	21.0	1.8 $\pm$ 0.39	22.0	2.9 $\pm$ 0.95	32.8	40.0 $\pm$ 6.06	15.1
Sava 1	4.4 $\pm$ 1.09	24.8	3.3 $\pm$ 0.94	28.1	1.7 $\pm$ 0.46	27.2	2.2 $\pm$ 0.81	37.3	34.4 $\pm$ 7.77	22.6
Sava 2	4.9 $\pm$ 1.10	22.2	3.8 $\pm$ 0.95	25.1	1.9 $\pm$ 0.53	27.2	2.4 $\pm$ 0.79	33.0	33.2 $\pm$ 8.25	24.8
Sava 3	4.9 $\pm$ 1.11	22.7	3.6 $\pm$ 0.85	23.3	1.8 $\pm$ 0.52	28.3	2.5 $\pm$ 0.85	33.8	32.8 $\pm$ 8.36	25.5
Bosna 1	6.3 $\pm$ 0.86	13.6	4.8 $\pm$ 0.57	12.0	1.7 $\pm$ 0.28	16.0	3.8 $\pm$ 0.74	19.4	42.7 $\pm$ 7.72	18.1
Bosna 2	6.4 $\pm$ 1.15	17.9	4.9 $\pm$ 0.81	16.3	1.8 $\pm$ 0.39	21.2	3.7 $\pm$ 0.86	22.9	42.3 $\pm$ 6.44	15.2
Vrbaš 1	6.1 $\pm$ 0.70	11.4	4.8 $\pm$ 0.50	10.4	1.9 $\pm$ 0.30	15.7	3.9 $\pm$ 0.62	16.0	39.7 $\pm$ 5.44	13.7
Vrbaš 2	6.3 $\pm$ 0.77	12.2	4.7 $\pm$ 0.57	12.1	1.7 $\pm$ 0.27	15.8	3.7 $\pm$ 0.77	20.8	42.0 $\pm$ 8.15	19.4
Drina	6.7 $\pm$ 0.92	13.6	4.8 $\pm$ 0.66	13.7	1.9 $\pm$ 0.35	18.8	3.8 $\pm$ 0.88	23.3	41.3 $\pm$ 6.14	14.9
Neretva 1	6.0 $\pm$ 0.72	12.0	4.6 $\pm$ 0.53	11.6	1.7 $\pm$ 0.25	15.1	3.3 $\pm$ 0.65	19.5	41.0 $\pm$ 8.43	20.6
Neretva 2	5.1 $\pm$ 0.71	13.9	3.7 $\pm$ 0.54	14.7	1.7 $\pm$ 0.41	24.3	2.3 $\pm$ 0.69	30.1	45.7 $\pm$ 6.75	14.8
Neretva 3	5.0 $\pm$ 0.67	13.5	3.6 $\pm$ 0.48	13.5	1.6 $\pm$ 0.25	16.1	2.0 $\pm$ 0.46	23.0	43.3 $\pm$ 6.43	14.8
Neretva 4	5.1 $\pm$ 0.94	18.5	3.8 $\pm$ 0.64	16.8	1.7 $\pm$ 0.38	22.4	2.3 $\pm$ 0.54	23.2	45.7 $\pm$ 6.26	13.7

LBL - leaf blade length; LBW - leaf blade width; DBW - distance between the leaf widest part and the leaf base; PL - petiole length;  $\alpha$  - angle between the first lower lateral vein and the midrib; M - average value; SD - standard deviation; CV - coefficient of variation (%)

variability shows the trait of petiole length (PL) as it is shown in Tables 2 and 3. Higher variability for all measured characters for Croatian river populations was determined for the group CRO-02 (the River Sava) and in Bosnia and Herzegovina for the group B&H-02 (lower part of the River

Neretva), except for the trait angle between the first lower lateral vein and the midrib ( $\alpha$ ), which shows a slightly greater degree of variability.

The differences between populations and analysed groups, as well as the differences between the populations belonging to a particular

**TABLE 3.** Descriptive statistics of the measured morphological traits at the group level

Population	LBL (cm)		LBW (cm)		DBW (cm)		PL (cm)		$\alpha$ (°)	
	M $\pm$ SD	CV	M $\pm$ SD	CV	M $\pm$ SD	CV	M $\pm$ SD	CV	M $\pm$ SD	CV
CRO-01	5.0 $\pm$ 1.16	23.1	4.2 $\pm$ 0.94	22.5	1.9 $\pm$ 0.44	23.4	3.0 $\pm$ 0.94	31.6	39.1 $\pm$ 6.07	15.6
CRO-02	4.7 $\pm$ 1.13	24.1	3.5 $\pm$ 0.93	26.2	1.8 $\pm$ 0.51	28.1	2.3 $\pm$ 0.83	35.5	33.6 $\pm$ 8.11	24.2
B&H-01	6.3 $\pm$ 0.90	14.2	4.8 $\pm$ 0.62	13.1	1.8 $\pm$ 0.32	18.0	3.7 $\pm$ 0.78	21.0	41.5 $\pm$ 7.19	17.3
B&H-02	5.0 $\pm$ 0.77	15.3	3.7 $\pm$ 0.55	15.1	1.6 $\pm$ 0.34	20.8	2.2 $\pm$ 0.57	26.4	44.6 $\pm$ 6.56	14.7
B&H-02	5.0 $\pm$ 0.77	15.3	3.7 $\pm$ 0.55	15.1	1.6 $\pm$ 0.34	20.8	2.2 $\pm$ 0.57	26.4	44.6 $\pm$ 6.56	14.7

LBL - leaf blade length; LBW - leaf blade width; DBW - distance between the leaf widest part and the leaf base; PL - petiole length;  $\alpha$  - angle between the first lower lateral vein and the midrib; M - average value; SD - standard deviation; CV - coefficient of variation (%)

river system, were confirmed for all studied characteristics. Table 4 shows the results of non-parametric univariate analysis of variance (Kruskal-Wallis ANOVA). Analysed populations and groups, as well as the populations belonging to a particular river system, differ from each other with statistical significance level of 0.0001 for all the measured characters of leaves.

The results of the principal component analysis show that the cumulative variability captured for the first two PC-axes is approximately 90%, with the largest share of the first PC-axis of approximately 60% (Table 5). The remaining three axes, whose eigenvalue is less than 1 in

total variability, contribute to only about 10% and can be ignored. The contribution of each of the measured morphological characters to the each of the calculated PC-axis is shown in Table 6. Figure 3 shows the relation between studied populations based on the first and second principal components. From the Table 6 we can assume that all of the original variables are positively correlated with the first principal component, regarding that from the right side of the diagram populations with larger leaves are grouped and on the left side populations with smaller leaves. The distribution of the studied populations along the second PC-axis largely

**TABLE 4.** Kruskal-Wallis ANOVA for the measured morphological traits

Analyse 1 – population: H (16, N = 6513);  $p = 0,0001$ ;  $\chi^2 = \text{Chi-Square test (df=16)}$ .

Analyse 2 – river: H (5, N= 6513);  $p = 0,0001$ ;  $\chi^2 = \text{Chi-Square test (df = 5)}$ .

Analyse 3 – group: N = 6513);  $p = 0,0001$ ;  $\chi^2 = \text{Chi-Square test (df=3)}$ .

Analyse 4 – CRO-01/CRO-02: H (1, N = 2125);  $p = 0,0001$ ;  $\chi^2 = \text{Chi-Square test (df=1)}$ .

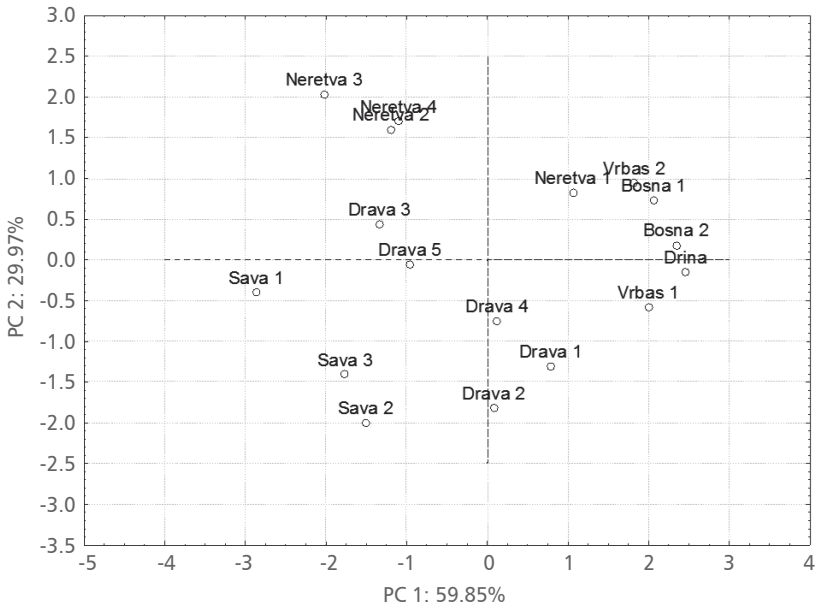
Analyse 5 – B&H-01/ B&H-02: H (1, N = 4388);  $p = 0,0001$ ;  $\chi^2 = \text{Chi-Square test (df=1)}$ .

Analyse	Kruskal-Wallis test	Morphological trait				
		LBL	LBW	DBW	PL	$\alpha$ (°)
Population	H	2592.36	2433.03	691.07	2992.43	1114.49
	$\chi^2$	2027.61	2131.20	504.51	2451.45	713.73
River	H	2052.17	1815.07	384.41	2346.392	932.15
	$\chi^2$	1555.63	1549.97	284.91	1910.51	575.99
Group	H	2304.53	2263.87	328.65	2835.83	977.52
	$\chi^2$	1871.01	2044.90	233.19	2323.13	584.83
CRO-01/	H	41.68	197.18	17.73	204.18	293.32
CRO-02	$\chi^2$	36.62	133.44	6.97	149.31	273.09
B&H-01/	H	1518.47	1899.93	223.40	2275.50	148.79
B&H-02	$\chi^2$	1044.38	1418.36	158.40	1589.24	88.97

LBL - leaf blade length; LBW - leaf blade width; DBW - distance between the leaf widest part and the leaf base; PL - petiole length;  $\alpha$  - angle between the first lower lateral vein and the midrib

**TABLE 5.** Eigenvalues, the percent of variance and cumulative variance

Main components	Eigenvalue	% Total Variance	Cumulative Eigenvalue	Cumulative %
1	2.992682	59.85364	2.992682	59.8536
2	1.498491	29.96981	4.491173	89.8235
3	0.352645	7.05290	4.843818	96.8764
4	0.136580	2.73159	4.980397	99.6079
5	0.019603	0.39205	5.000000	100.0000



**FIGURE 3.** Scatterplot of the PC analysis for 17 studied populations of *Populus nigra*. Particular values for the first PC are on the x axis, and for the second PC on the y axis.

contributes to a positive correlation for the trait angle between the first lower lateral vein and the midrib ( $\alpha$ ), and a negative correlation for the character distance between the leaf widest part and the leaf base (DBW).

The discriminant analysis was used to determine which leaf morphological characters best separate the studied groups. The results of the discriminant analysis suggest that the variability between the experimental groups is significant (Wilk's  $\lambda = 0.00032$ ;  $F(15, 25) = 29.336$ ;  $p < 0.00001$ ). For five varia-

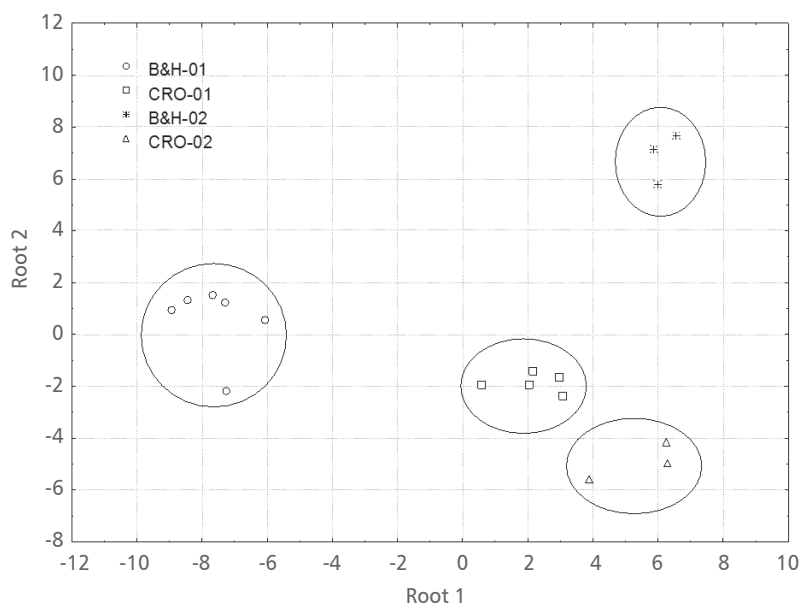
bles and four groups of canonical analysis, three discriminant functions were obtained. From the means of the canonical variables it is evident that the discriminant function 1 in the fullest extent particularly distinguishes group B&H-01 from other studied groups (Figure 4, Table 7). In the Table 8 it is evident that in this differentiation largely contribute characters such as the distance between the leaf widest part and the leaf base (DBW) and petiole length (PL). Since this table contains the eigenvalue and the cumulative proportion of the explained

**TABLE 6.** Factor-variable correlations (factor loadings)

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
LBL	0.546315	0.080723	-0.151271	0.804953	0.155539
LBW	0.571610	-0.040310	-0.016851	-0.235424	-0.784812
PL	0.554369	-0.074487	-0.276459	-0.531439	0.572949
DBW	0.165978	-0.711838	0.668192	0.060142	0.125061
$\alpha$ (°)	0.199824	0.692529	0.673739	-0.102872	0.126362

LBL - leaf blade length; LBW - leaf blade width; DBW - distance between the leaf widest part and the leaf base; PL - petiole length;  $\alpha$  - angle between the first lower lateral vein and the midrib





**FIGURE 4.** Scatterplot of the canonical scores for four studied groups of *Populus nigra*. Particular values for the first discriminant function are on the x axis, and for the second function on the y axis

variation for each discriminant function, it can be determined that the discriminant function 1 contains 68.2% of the explained variability. The discriminant function 2, which together with discriminant function 1 explains 95.9% of the variability, clearly distinguished group of B&H-02 from the other three studied populations which were sampled on the River Sava and which belong to the group CRO-02 (Figure 4, Table 7). Variables  $\alpha$  (the angle between the first lower lateral vein and the midrib) and LBL (leaf blade length) largely contribute in the differentiation between these two groups, as it is shown in Table 8. The third discriminant function, which explains only 4.1% of the total variability, best

distinguished the two groups of Croatian population belonging to the basin of the River Drava and the River Sava (Table 7).

## DISCUSSION

Interpopulation variability and the variation between the river systems were statistically significant for all the investigated morphological traits that correspond to the previous research in the juvenile populations of the European black poplar along the River Drava and the River Sava [19, 20, 26]. Some of the morphological parameters, such as petiole length (PL), show a statistically important difference between the investigated populations, and have also been determined in previous investigations [26]. Interpopulation variability in the Danube basin was in most of morphological characters between 10 and 20% [27]. The highest contribution of variation between populations had a number of veins on the left and the right blade side. The highest contribution of variation within the populations

**TABLE 7.** Means of canonical variables

Group	Root 1	Root 2	Root 3
B&H-01	-7.60668	0.57274	-0.43766
CRO-01	2.15751	-1.86648	2.01742
B&H-02	6.14944	6.86594	-0.45985
CRO-02	5.46808	-4.90061	-2.02719

**TABLE 8.** Standardised coefficients for canonical variables

Variable	Root 1	Root 2	Root 3
LBL	-0.67926	1.00828	-1.42333
LBW	-0.55689	-0.59956	0.81317
PL	-1.05147	-0.43264	0.13713
DBW	1.82785	0.19787	0.82135
$\alpha$ (°)	0.36774	1.13227	0.40122
Eigenvalue	44.12233	17.91218	2.65094
Cumulative proportion of explained variation	0.68211	0.95902	1.00000

LBL - leaf blade length; LBW - leaf blade width; DBW - distance between the leaf widest part and the leaf base; PL - petiole length;  $\alpha$  - angle between the first lower lateral vein and the midrib

(more than 50%) had a distance between the base of the leaf blade and the widest part [27]. This character corresponds to the results of the interpopulation differentiation in our study. The mentioned characters is under a genetic control and is less influenced by the environmental changes, so it could serve to estimate the intrapopulation and interpopulation variability, as well as to show the introgression of the genes of other poplar species in local population of the European black poplar. The morphological variation of seven leaf characters from 84 trees of eastern cottonwood (*Populus deltoides* Bartr. ex. Marsh. var. *deltoides*) from nine populations in Ontario was also studied [28]. Leaf characters showed significant variation among populations ( $P \leq 0.003$ ) and the number of veins showed a significant correlation with the longitude ( $r=0.81$ ,  $P < 0.009$ ) and the latitude ( $r=-0.69$ ,  $P < 0.05$ ). None of the other leaf characters were related to latitude and longitude.

The genetic diversity of ten black poplar populations along the main river systems across Slovenia and Croatia (rivers Soča, Sava, Drava and Mura) was revealed by using six microsatellite loci [29]. The results of this study indicate that the gene pool of the remaining *Populus nigra* populations in this territory is still well preserved. In natural populations, the introgression of the genes of *Populus deltoides* was very low. One of the populations along the River Drava

(Suhopolje, near locality Šučurica, population Drava 4 in Table 1 and 2) was distinct from the others and clustered alone as a separate group.

The assessment of the population genetic structure of the *Populus nigra* L. along the rivers revealed a significant spatial genetic structure which was probably caused by a limited gene flow. This helps to explain the reduced diversity in the juveniles [30-32].

The results of the research of the European black poplar leaf morphological variability in the natural stands along the Sava and Drava show that in the studied progeny the phenotypes resemble each other and mostly prevail among the European black poplar [19, 20, 29] with no introgression with *P. deltoides* or poplar hybrids. These results confirm the researches of Van den Broeck *et al.* [1, 34] carried out in Belgium. By studying the poplar flowering phenology in clonal archives they noted that the male poplar clone (*P. nigra* var. *italica*) and 22 clones of *P. × canadensis* flower earlier than the studied clones of local European black poplar (*P. nigra*). Based on this finding, they concluded that there is no great danger of the introgression of the American black poplar genes into the European black poplar genom, but also that the introgression of genes should not be excluded completely, having in mind that considerable variations in the poplar flowering phenology have been noticed.

Leaf morphometric characters have significantly contributed in the intra- and interpopulation variability of *Populus nigra* populations in the basin of the River Danube [27]. The discriminative model with all characters achieved less than 60.38% of correct allocation due to a high variability of examined characters within populations. The results of morphological analysis do not entirely support the results of the molecular analysis, but they generally support the similarity among neighbouring populations and, according to this, the results provide a proper selection and the use of forest reproductive material.

Hairy subspecies of European black poplar (*Populus nigra* subsp. *caudina*) spreads in a mosaic pattern across a warm and dry area of Herzegovina along the River Neretva, while along the rivers in the region of central and mountainous Bosnian belt only individual trees are mixed with the typical black poplar. It can be assumed that the area of its current distribution range is what has been left of its former and probably much larger population [6]. The habitat of the hairy European black poplar in Herzegovina is the consequence of ecogeographical conditions and of rather historical factors and their effect on the development of flora in that area. This belief leads to the idea that the current distribution of the hairy black poplar is a result of a recent spread of the black poplar from the North (from the River Drava and the River Sava, Groups CRO-01 and CRO-02) toward the South. Individual trees of the hairy black poplar in northern and central Bosnia grow at higher altitudes, in the colder and more humid mountainous region [4]. The isolated Submediterranean populations (Group B&H-02), growing along the River Neretva, can be found at 15 m a.s.l. The average temperature during vegetation period ranges between 15.9°C and 16.9°C for populations from inner Bosnia and Herzegovina (Group B&H-01) and is 20.7°C for populations from the lower course of the River Neretva (Group B&H-02). Ecological and climate conditions along the River Neretva are completely different with characteristically

long, warm and dry summers (with the average precipitation of 365 mm in vegetation period). Moreover, these conditions are characterised by a distinctive fluctuation in the water level, as well as the saturation of the pedological layer with lime-stones. We can assume that the hairy black poplar found along the River Neretva is a remnant of an earlier continuous land area, spreading from Greece to Herzegovina via Macedonia and Albania. The hairy type of black poplar (*P. nigra* subsp. *caudina*) growing along the River Neretva differs considerably from the black poplar in the riparian populations along the European rivers. This rare hairy type of *P. nigra* is considered to be a xeromorphic form in the Submediterranean region [15].

In a preliminary attempt to assess the taxonomic status of *P. nigra* subsp. *caudina* ten trees from each of seven populations were studied for nuclear and chloroplast markers that have shown differences between species, subspecies, and single clones in *Populus* [15, 35-37]. One of the populations was a pure hairy type, three were hybrids and the others were typical spp. *nigra*. The results show no indication that the hairy type is a separate taxonomic unit because all the polymorphisms are the same as the ones of the European black poplars from Bosnia and Herzegovina, whether they are hairy or not. These results do not support the hypothesis of a possible tertiary relict [6] but rather they support the classification of the hairy type as a xeromorphic ecotype of the European black poplar. For the characterised strong trichome development on petioles of *P. nigra* subsp. *caudina*, candidate genes were analysed in order to understand the basis of its distinctness as well as the genetic variation [38, 39].

There are significant differences between the typical and hairy types of European black poplar in the studied morphological traits and these differences are in accordance with the climate differences in respective habitats of continental riparian forests and Submediterranean type of climate.

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# Mycorrhizal Fungal Community of Poplars Growing on Pyrite Tailings Contaminated Site near the River Timok

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## Abstract

**Background and Purpose:** Mycorrhizal fungi are of high importance for functioning of forest ecosystems and they could be used as indicators of environmental stress. The aim of this research was to analyze ectomycorrhizal community structure and to determine root colonization rate with ectomycorrhizal, arbuscular mycorrhizal and endophytic fungi of poplars growing on pyrite tailings contaminated site near the river Timok (Eastern Serbia).

**Materials and Methods:** Identification of ectomycorrhizal types was performed by combining morphological and anatomical characterization of ectomycorrhizae with molecular identification approach, based on sequencing of the nuclear ITS rRNA region. Also, colonization of poplar roots with ectomycorrhizal, arbuscular mycorrhizal and dark septated endophytic fungi were analysed with intersection method.

**Results and Conclusions:** Physico-chemical analyses of soil from studied site showed unfavourable water properties of soil, relatively low pH and high content of heavy metals (copper and zinc). In investigated samples only four different ectomycorrhizal fungi were found. To the species level were identified *Thelephora terrestris* and *Tomentella ellisi*, while two types remained unidentified. Type *Thelephora terrestris* made up 89% of all ectomycorrhizal roots on studied site. Consequently total values of Species richness index and Shannon-Weaver diversity index were 0.80 and 0.43, respectively. No structures of arbuscular mycorrhizal fungi were recorded. Unfavourable environmental conditions prevailing on investigated site caused decrease of ectomycorrhizal types diversity. Our findings point out that mycorrhizal fungal community could be used as an appropriate indicator of environmental changes.

**Keywords:** ectomycorrhiza, molecular identification, poplars, Timok, pyrite tailing

## INTRODUCTION

Mining complex in the vicinity of Bor (Eastern Serbia) represents a considerable source of environmental pollution. Soil from a large area near the river Timok was contaminated by flotation tailing composed mainly of pyrite ( $\text{FeS}_2$ ). Consequently vegetation in this area suffered abiotic stress induced by a low pH, high content of copper and lead, deficiency of soil organic matter and severe deficiency of the available mineral nutrients [1].

Poplars are woody species suitable for phytoremediation purposes, because they can extract or incorporate into their aboveground tissues or stabilize in their root systems numerous contaminants from soil [2, 3]. They are also well adapted to a broad range of climatic conditions and soils, have deep root systems, cycle large amounts of water and grow rapidly producing large amount of biomass [2-4]. Soil microorganisms such as mycorrhizal fungi could have important role in phytoremediation because they can modify bioavailability of heavy metals and/or increase plant growth [5]. Poplars can make functional associations with both ectomycorrhizal (ECM) and arbuscular mycorrhizal (AM) fungi [6]. Since these two mycorrhizal forms are known to prefer different climate and soil conditions such as nutrient content, pH and C/N ratio [7], dual colonization enables poplars to have broader ecological valence.

Mycorrhizal fungi facilitate the establishment and survival of vegetation under stress condition providing nutrients and water otherwise not accessible for plants [8]. With a net of their hyphae they can stabilize the tailing material and improve soil structure while with compounds produced by the extraradical mycelium can accumulate or chelate heavy metals [9]. In order to be efficient for use in phytoremediation techniques, mycorrhizal fungus has to satisfy two important conditions: tolerance to high concentration of heavy metals in soil and good functional compatibility with a plant used in phytoremediation [10].

Functional compatibility and stress tolerance

in mycorrhiza are species specific and depend on both partner [11] therefore the information on the ECM community structure can provide valuable information about physiology of forest trees and functioning of forest ecosystems [12].

The aim of this research was to analyze ectomycorrhizal community structure and to determine root colonization rate with ectomycorrhizal, arbuscular mycorrhizal and endophytic fungi of poplars growing on pyrite tailings contaminated site near the river Timok. This information could be helpful in further research on creating inoculum for afforestation of sites contaminated with pyrite tailings.

## MATERIAL AND METHODS

### Physico-Chemical Properties of Soil from the Pyrite Tailings Contaminated Site Timok

Physical and chemical properties were determined in the surface layer of the soil (up to 30 cm). The following soil characteristics were analyzed: particle size distribution (%) by the international B-pipette method with the preparation in sodium pyrophosphate [13], determination of soil textural classes based on particle size distribution by using Atteberg classification,  $\text{CaCO}_3$  percentage (%) was measured volumetrically by using Scheibler's calcimeter [14] and pH in  $\text{H}_2\text{O}$  and KCl were determined by electrometric method with combined electrode on Radiometer pH meter. Concentrations of heavy metals in soil were determined with Atomic Absorption Spectrophotometer (VARIAN AAS 240 FS). All analysis were performed in the laboratory of Soil Science in the Institute of Lowland Forestry and Environment in Novi Sad.

### Site

Mycorrhizal fine roots were isolated from soil samples collected in the river land of the river Timok (N 44°00'29.96'', E 22°21'54.48'', 228 m a.s.l.) located about 20 km from Zaječar town, in Eastern Serbia. Site was covered with naturally grown cca 40 years old poplar trees



(*Populus alba* L., *P. nigra* L., *P. tremula* L. and their hybrids) mixed with *Amorpha fruticosa* L., *Betula pendula* Roth and *Alnus glutinosa* L. Climate is temperate continental with the average annual precipitation in the area of 581.4 mm. Average temperature of the air in January is  $-0.2^{\circ}\text{C}$ , in July  $22.4^{\circ}\text{C}$ , while the average yearly temperature is  $11^{\circ}\text{C}$  (The Republic Hydro-meteorological Service of Serbia <http://www.hidmet.gov.rs/>).

### Sampling

Five mature white poplar trees were randomly selected for sampling. For ECM community analysis two soil samples per tree were taken in July 2010, at a distance of about 1m from the tree trunk. A soil core of 274 ml volume and 18 cm deep was used for taking standardized samples [15]. In total, ten soil core samples were collected and kept stored at  $4^{\circ}\text{C}$  for up to three months. Prior to mycorrhizal analysis each soil core was submerged in cold tap water to loosen the soil structure. Roots were carefully washed from soil and vital ECM root tips were separated from old, nonturgid and nonmycorrhizal (ONN) root tips in water under a dissecting microscope. For the determination of root length colonization with ECM, AM and endophytic (END) fungi five additional soil samples per plant (in total 25) were taken in August 2011 using the same sampling approach.

### Identification of Ectomycorrhizae

ECM types were identified by combining morphological and anatomical approach with molecular methods performed at the laboratory of the Department of Forest Physiology and Genetics in Slovenian Forestry Institute in Ljubljana, Slovenia.

Morphological and anatomical characteristics of each ECM type were assessed by a binocular Olympus SZX 12 (light source Olympus Highlight 3100, daylight filter) and microscope Olympus BX 51 (magnification 100-2000 x) following methodology proposed by Agerer [16] and Kraigher [17], and ECM descriptions published in Agerer [18], Agerer *et al.* [19], and Agerer and Rambold [20]. All fine root tips

were manually counted under the stereomicroscope. Based on the presence and abundance of emanating elements, ECM types were also classified into the exploration types proposed by Agerer [21].

Molecular identification was based on nucleotide sequencing of ITS (Internal Transcribed Spacer) regions in nuclear ribosomal DNA. This molecular marker is considered as the best for fungi identification [22]. After DNA extraction from 5-20 root tips with a PlantDNAeasy Mini Kit (Qiagen, Hilden, Germany) from each ECM type ITS region was amplified with ITS 1f and ITS 4 primer pair [23]. DNA fragments were separated in and excised from agarose gel and purified with Wizard<sup>®</sup> SV Gel and PCR Clean-up System (Promega Corporation, Madison, WI, USA). Sequencing was performed commercially at Macrogen Inc. (Seoul, Rep. of Korea). Species, genus or family of ectomycorrhizal fungi were determined by comparing sequences to the ones deposited in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/index.html>) and Unite databases [24].

### Root Colonization

Before evaluation, poplar's fine roots from 5 soil samples were isolated from soil, separated from the roots of herbaceous species by means of visual inspection and jointed together in one sample. Extracted roots were gently washed and cleared in 10% potassium hydroxide and stained with Trypan blue in lactoglycerol according to Kormanik and McGraw [25] and Karliński *et al.* [26]. Colonization of poplar roots by ECM, AM and END fungi was evaluated using the intersection method by McGonigle *et al.* [27] modified by Karliński *et al.* [26] at 200 $\times$  magnification. A minimum of 200 line intersections per subsample (microscopic slide) were scored for the presence of AM structures (hyphae, vesicles, arbuscules, and coils), ECM or END fungi. Cross section without fungal structures was counted as "empty root". The results are presented as a percentage of root length colonized i.e. partition of number of particular fungal structures in total number of cross sections.

## Data Analysis

Diversity indices (Shannon-Weaver index, Species richness index, Evenness, Equitability and Berger-Parker index) were calculated per sample and per site in the way that ECM community data were pooled after formulas given by Atlas and Bartha [28]:

- Species richness (d):  $d = (S-1) / \log(10)N$ , where S - number of ECM types, N - number of all mycorrhizal tips;

- Shannon Weaver diversity index (H):

$$H = C/N (N \cdot \log N - \sum n_i \cdot \log n_i),$$

where C - 2,3, N - number of all mycorrhizal tips,  $n_i$  - number of mycorrhizal tips of individual ECM type;

- Evenness (e);  $e = H / \log S$ ,

where H - Shannon Weaver diversity index, S - number of ECM types;

- Equitability (J):  $J = H / H_{\max}$ , where H - Shannon Weaver diversity index,  $H_{\max}$  - theoretical maximal H assuming each ECM type was represented with one mycorrhizal tip;

- Berger-Parker evenness index (BP):

$$BP = 1 - (N_{\max} / N),$$

where  $N_{\max}$  = number of mycorrhizal tips of the most frequent ECM type, N=number of all mycorrhizal tips.

Relative abundance of ECM types was calculated as a ratio between the tips number of individual ECM type and total number of ECM tips.

## RESULTS

Physical and chemical analysis of examined soil samples from pyrite tailings contaminated site showed high content of total sand (91.4%), moderately acid pH (4.91) (according to Dugalić and Galić [29]) and very low concentration of nitrogen (0.06%) (Table 1). According to its granulometric content soil can be classified in the sand texture class. Also, it belongs to the group of technogenous soils, type deposol [30]. Origin of this soil type is related to the undeveloped

**TABLE 1.** Granulometric composition and some chemical properties of soil from Timok site

Coarse sand (%)	Fine sand (%)	Dust (%)	Clay (%)	Texture class	pH in H <sub>2</sub> O	pH in KCl	CaCO <sub>3</sub>	Carbon	Nitrogen	C/N
8.2	83.2	3.0	5.6	Sand	4.91	4.74	1.67	4.71	0.06	78.34

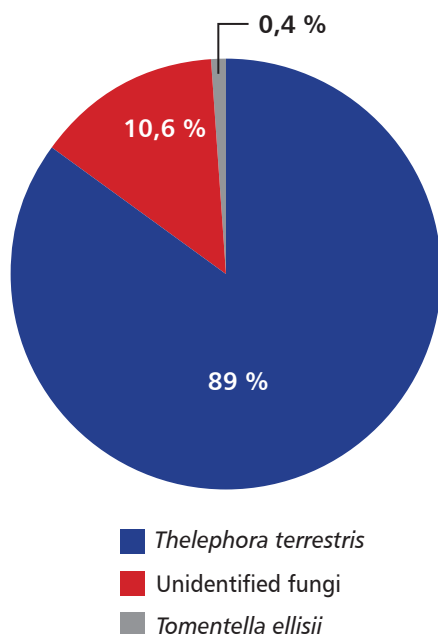
**TABLE 2.** Concentrations of heavy metals in soil from Timok site (with their maximum amounts allowed in the soil according to the National legislation)

Heavy metal	Concentration (ppm)	Maximum amount allowed in the soil (mg·kg <sup>-1</sup> )
Cr	28.3	100
Ni	14.4	50
Cd	3.3*	3.0
Pb	83.4	100
Cu	896.9*	100
Mn	147.5	/
Fe	58141.21	/
Zn	413.9*	300

\* Values higher than maximum allowed by National legislation

alluvial soil (fluvisol) but subsequently influenced by the spilling of pyrite tailings and  $\text{Fe}_2\text{S}$  deposition causing lower pH and contamination with heavy metals. Comparison of heavy metals content in soil from studied site with the National legislation limits [31] has shown that concentration of copper was almost 9x higher than its maximum allowed amount, while zinc was increased and cadmium was slightly above the allowed amount (Table 2).

Analyzing 34042 fine roots on Timok site four ECM types were recorded, while values of Species richness index and Shannon-Weaver index were 0.80 and 0.43, respectively. In average 1.4 ECM types, 550.6 vital mycorrhizal root tips and 3404 all fine roots were recorded per soil sample. Consequently, average values of diversity indices were extremely low (Table 3). In total, at site Timok four ECM types were recorded. Two of them were identified to the species level: *Thelephora terrestris* and *Tomentella ellisii*, while two ECM types remained unidentified (Table 4). *Thelephora terrestris* made up 89% of all ECM roots (Figure 1) and consequently medium distance exploration type made up almost 90% of all ECM roots on this site (data were not shown). Although both fungal groups, Ascomycota and Basidiomycota had two members, Basidiomycota was much more



**FIGURE 1.** Ectomycorrhizal fungi community structure of poplars from Timok site

abundant and made up 89.4% of all ECM roots (data not shown). In examined poplar roots no AM fungal structures were observed, while ECM and END fungi colonized 18.19% and 2.23% of root length, respectively (Table 5).

**TABLE 3.** Total values and average values per sample ( $\pm$  standard error) for number of ectomycorrhizal (ECM) types, number of vital ECM roots, old, nonturgescient and nonmycorrhizal roots, number of all roots, percentage of vital mycorrhizal roots and diversity indices on investigated site Timok (based on 10 samples)

Examined parameters	Total value for site	Average value per sample
Number of ECM types	4	1.3 $\pm$ 0.16
Number of vital mycorrhizal roots		550.6 $\pm$ 184.6
Old nonturgescient and nonmycorrhizal roots		2853.6 $\pm$ 892.2
Number of all roots		3404.2 $\pm$ 946.4
Percentage of vital mycorrhizal roots		19.7 $\pm$ 5.0
Species richness index (d)	0.80	0.144 $\pm$ 0.06
Shannon-Weaver index	0.43	0.138 $\pm$ 0.07
Equitability (J)	0.31	0.199 $\pm$ 0.10
Eveness (e)	0.71	0.461 $\pm$ 0.23
Berger-Parker index	0.11	0.045 $\pm$ 0.03

**TABLE 4.** Identified ectomycorrhizal fungi on the basis on the similarities with sequences given in the internet basis GenBank and UNITE and phylogenetic analyses

ECM type	Accession numbers of three best shot in GenBank and % of match and % of identity	Accession numbers of three best shot in UNITE and % of match and % of identity	Morphological-anatomical identification	Phylogenetical analysis
Unidentified type 1	<i>Meliniomyces</i> sp. <a href="#">KC007335.1</a> 99% 99%; Uncultured <i>Hebeloma</i> <a href="#">JQ724056.1</a> 99% 99%; Uncultured Helotiales <a href="#">DQ273322.1</a> 99-98%	<i>Mollisia benesuada</i>   Estonia <a href="#">UDB003038</a> ; <i>Crocicreas furvum</i>   Lithuania <a href="#">UDB003037</a> ; <i>Niptera dilutella</i>   Estonia <a href="#">UDB003005</a>	/	/
<i>Thelephora terrestris</i>	<i>Thelephora terrestris</i> <a href="#">JQ711980.1</a> 100-100%; <a href="#">HM189965.1</a> <i>Thelephora terrestris</i> 100-100%; <i>Thelephora terrestris</i> <a href="#">HQ406822.1</a> 100-100%	Thelephoraceae   Slovenia <a href="#">UDB008264</a> 100%; <i>Thelephora terrestris</i>   Estonia <a href="#">UDB003348</a> LOCKED by Urmas Kóljalg; <i>Thelephora terrestris</i>   Estonia <a href="#">UDB003346</a> 99%	<i>Thelephora terrestris</i>	<i>Thelephora terrestris</i>
<i>Tomentella ellisii</i>	<i>Tomentella ellisii</i> <a href="#">DQ068971.1</a> 100% 99%; Uncultured Thelephoraceae <a href="#">JN704829.1</a> 100% 99%; Uncultured ectomycorrhiza ( <i>Tomentella</i> ) clone <a href="#">EU700261.1</a> 97% 99%	<i>Tomentella ellisii</i>   Italy <a href="#">UDB016490</a> 95%; <i>Tomentella ellisii</i>   Estonia <a href="#">UDB000219</a> 96%; <i>Tomentella ellisii</i>   Finland <a href="#">UDB011603</a> LOCKED by Irja Saar	/	<i>Tomentella ellisii</i>
Unidentified type 2	Uncultured Pezizales clone P1_Contig_0290 <a href="#">JN704819.1</a> 100% 99%; Uncultured ectomycorrhizal fungus clone Riv-5 <a href="#">EF484935.1</a> 100% 99%; Uncultured ectomycorrhizal fungus clone unk1350 <a href="#">GU553372.1</a> 100% 99%	<i>Sphaerosporella brunnea</i>   Finland <a href="#">UDB000994</a> 94%; <i>Otidea alutacea</i>   Estonia <a href="#">UDB011428</a> 98%; <i>Rhizina undulata</i>   Finland <a href="#">UDB016153</a> 96%	/	/

**TABLE 5.** Average values ( $\pm$ standard error) of poplar roots colonization with ectomycorrhizal, arbuscular mycorrhizal and dark septated endophytic fungi at Timok site

Examined parameter	Average value
% Root length colonization with arbuscular mycorrhizal fungi	0
% Root length colonization with ectomycorrhizal fungi	18.19 $\pm$ 3.47
% Root length colonization with endophytic fungi	2.23 $\pm$ 0.50
% Root length colonization with other hyphae	1.80 $\pm$ 565
Arbuscular mycorrhizal fungi/Ectomycorrhizal fungi	0

## DISCUSSION

The main causes of extreme conditions at the pyrite tailings contaminated site near the river Timok were unfavourable water-air properties of analyzed soil, low pH and contamination with heavy metals copper and zinc. In such soils with high proportion of total sand, content of water available to the plants is low. Also, moderately acidic pH of the soil could not be favourable for while poplars that are known to prefer fluvisol soil type with slightly or moderately alkaline pH [32].

Analysis of fine roots' number, values of diversity indices and relative abundances of fungi that form ECM association with poplars from site Timok enabled comparison of ECM fungal community from studied site with ones from other similar sites.

On site Timok average number of fine roots per 1 dm<sup>3</sup> of soil was 12425.3/dm<sup>3</sup>, while number of vital ECM roots was 2009.7/dm<sup>3</sup>. Recorded values were much lower in comparison with results of Krpata *et al.* [33] who counted 1735-4263 mycorrhizal root tips in 100 ml of soil in aspen stand from site contaminated with heavy metals. On the other hand, in the experimental field with increased ozone concentration in the air, average value of fine poplar roots was 2599.1/dm<sup>3</sup> in control treated with water and 4573.5/dm<sup>3</sup> in the antiozonant protected plants [34].

Comparison of Shannon-Weaver diversity index recorded on site Timok (0.43) with its common values, which are in the range 1.5-3.5 according to Urbančič and Kutnar [35], showed that diversity on studied site was decreased. Low diversity of ECM types supports the observation that pollution could cause disappearance of sensitive ECM fungi and increase abundance of tolerant ones, decreasing in that way its diversity [36]. High proportion of sand in the soil and low content of water available to the plants (data not shown) suggested unfavourable conditions for development of mycorrhizal community. In addition, adverse influence of drought conditions on mycorrhizal fungi was proven by Lodge [37].

Under extreme abiotic conditions on site Timok only four ECM types were recorded. Similar results were obtained in studies of ECM communities on the sites under the influence of stress factors. On the site contaminated with heavy metals, Regvar *et al.* [38] recorded 7 ECM types on birch. On the clone *Populus nigra* × *maximowiczii* cv. Max grown as short rotation crop, Hryniewicz *et al.* [39] found 5 ECM types. On the other hand, Krpata *et al.* [33] recorded 54 ECM types on aspen from the site contaminated with heavy metals. On two sites polluted with zinc, Mlecško [40] recorded 23 ECM types, and the same number of ECM types was observed on uranium polluted site [42].

In our work data were collected in summer 2010 and 4 ECM types (*Thelephora terrestris*, *Tomentella ellisii* and two unidentified types) were recorded. On the same site in winter 2009, Katanić *et al.* [41] recorded 6 ECM types: *Tricholoma sculpturatum* (Fr.) Quel., *Tuber puberulum* Berk. & Br., *Thelephora* sp., Helotiales sp., Sebaciniales sp. and Sordariomycetidae sp. Seasonal change in ECM community supports hypothesis of Koide *et al.* [43] that temporal distribution within ECM fungal community reduces competition among species.

Tomenteloid fungi belong to the most frequent and most abundant ECM partners of coniferous and broadleaved trees in woods of Europe and North America [44]. Kraigher and Al Sayegh Petkovšek [45] noted that, beside *Cenococcum geophilum*, fungus *Thelephora terrestris* is one of the rare fungi which form developed ECM in drought conditions. In addition, teleforoid fungi could play crucial role in forest ecosystems under the influence of stress [45]. Results from Timok are in accordance with their studies. Dominance of medium distance exploration type revealed in our study is concordant with results of Rudawska *et al.* [46] who recorded high proportion of this exploration type on the locality contaminated with heavy metals.

High intraspecies variability could be found in ECM fungi according to Cairney [47], while Johnson *et al.* [48] consider that groups of genotypes can affect ecosystem processes in the same way as species do. Within species,

individuals differ a great deal in important reproductive and functional characters. Since, ECM fungus *Thelephora terrestris* is adapted to extreme conditions on the site Timok it could be assumed that strain from Timok developed some physiological adaptations to the extreme environmental factors.

In preparation of mycorrhizal inoculum for afforestation of damaged or contaminated sites, autochthonous strains of fungi, well adapted to such environmental conditions, should be chosen [17, 49]. Strain of ECM fungus *Thelephora terrestris* from Timok could be proposed as a basis for creating inoculum for afforestation of this one or similar localities with extreme conditions.

## CONCLUSIONS

According to the presented results it could be concluded that physico-chemical soil properties of studied site were unfavourable considering poor water properties, relatively low pH and high content of heavy metals (copper and

zinc). Only four different ectomycorrhizal fungi were found from which fungus *Thelephora terrestris* made up 89% of all ectomycorrhizal roots. Total values of Species richness index and Shannon-Weaver diversity index were 0.80 and 0.43, respectively. No structures of arbuscular mycorrhizal fungi were recorded. The presented results suggest that described environmental conditions on investigated site caused decrease of ectomycorrhizal types diversity. Our findings point out that mycorrhizal fungal community could be used as an appropriate indicator of environmental changes.

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# The Influence of Snow Cover Changes on Red Deer (*Cervus elaphus* L.) Migrations in the Western Part of Gorski Kotar Region in Croatia

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## Abstract

**Background and Purpose:** Animal migrations are a direct result of reproduction, behaviour characteristics, predators, population density, disturbance, loss of habitat, climatic, vegetational and nutritional factors. The availability and accessibility of natural food in winter months is dependent on snow cover. The main objective of this study was to determine the migrational activities of red deer and to examine the dependency between migrations and climatic factors.

**Materials and Methods:** The study was carried out in the northwest Dinarid mountains, i.e. in the western part of Gorski Kotar region, which represents a large integral forest complex, distinctive due to its significant vertical drops, diverse relief characteristics and habitat conditions. Data on red deer migrations was collected over a 12 year period from hunting records, gamekeeper logs and records from game counting and monitoring.

**Results and Conclusions:** The results of the macroclimatic analysis show a statistically significant difference ( $p < 0.05$ ) between the monitored weather stations in the study area. The Klana site stood out as the most appropriate red deer winter habitat, based on climatic conditions. Climatic conditions play a key role in seasonal red deer migrations or non-migrations. The Crni Lug site was assessed to be the least favourable due to macroclimatic conditions (snow depth).

**Keywords:** snow cover, red deer (*Cervus elaphus* L.), migration, Gorski Kotar

## INTRODUCTION

Red deer (*Cervus elaphus* L.) is a common game animal in both the coastal and mountainous parts of the northwestern Dinarid mountain range (Gorski Kotar region) of Croatia, including areas near the Slovenian border. In the latter half of the 19<sup>th</sup> century, red deer almost completely vanished from this area, but was quickly reintroduced in Slovenia [1]. From here, it soon migrated back into the Gorski Kotar region. Therefore, it can be considered to be an indigenous species. The number of individuals and spatial distribution following reintroduction started to grow, slowly at first and much more rapidly in the second half of the 20<sup>th</sup> century, due to multiple factors: regulation of hunting legislation that protected large herbivore ungulates, systematic destruction of large predators, overgrown abandoned agricultural areas, intensive forest exploitation and intensive nutrition with game feeders [1].

As a result, deer inhabits large expanses of the forested area in the Gorski Kotar region in Croatia. Hunting management programmes have estimated the total population at 970 individuals in the western part of Gorski Kotar region [1].

In order to consider deer game management within modern ecological conditions, it is essential to stress that changes in the ecology affect game behaviour, as animals changing their rhythm and behavioural patterns accordingly. Knowing how these changes impact animal populations, it is possible to take adequate breeding measures on hunting grounds, remove negative consequences and drawbacks observed to cause disturbances in herds, and determine the tolerance limits between game animals and habitat.

It is important to know that deer favour certain pastures, that they enjoy a variety of herbal food, including fruits and seeds, and occupy different sites for feeding, rest and sleeping. They seek out specific shelters from predators, and open spaces for playing and mating. Access to water is particularly

important, especially in areas where does raises their young. It is a well known fact that deer often visit feeders during the winter months, and thus an appropriate spatial distribution of such structures is essential for their survival.

In order to meet these needs, deer have seasonal migrations, i.e. they have both winter and summer habitats and separate habitats for the mating season. It is also very important to know the spatial distribution of herds. It is well known that during their lifetime, deer inhabit a much smaller range than other migrating animals. Migration is movement from one area into another, though it is also important to note that it represents a periodical movement that includes returning to the starting area [2]. Partial migrations are those in which only a portion of the population migrates, while the remainder remains in the same period throughout the year. This is a typical occurrence in environments with high seasonal variation. This occurrence has been recorded for all indigenous deer species in Scandinavia, and also for small ruminants, such as roe deer *Capreolus capreolus* [3], large ruminants such as moose *Alces alces* [4], and also for red deer [5]. Seasonal migrations are a well-known occurrence among large herbivores, which is caused by the availability of food, and risks from predators [6]. Red deer in the temporal belt regularly migrate from higher elevations in summer to lower elevations in winter [3, 5, 7] in order to reach areas with better snow conditions [8].

The depth of the snow cover at high elevations spurs animals to migrate to lower elevations [3, 7, 9]. According to Mysterud *et al.* [10], the share of migrating deer in the total population is from 38 to 100%, and migrations are typical in areas with diverse relief and in high elevation areas.

Migrations in the Gorski Kotar region are the result of reproduction, behaviour characteristics, predators, population density, disturbance, loss of habitat, climatic, vegetational, and nutritional factors. Distances covered during migration may be shorter or longer, depending on the severity of climatic

conditions and food availability. Such seasonal migrations are related to climatic conditions, resulting in a range of habitats used by animals depending on the season.

The spatial behaviour of mammals is influenced by several factors: metabolic needs, body weight, nutritional habits and the mating season [11-13]. Habitat size is also related to a combination of other factors, such as age [14, 15], population density [16], predators and human interference [17]. The winter habitat is typically a more concentrated area in which animals are forced to move due to a lack of food [18].

Due to weather conditions, the environment can change through natural variations, either due to the availability of food due the depth of the snow cover, or due to the intensity of grazing and the season [19]. In dealing with the environment, deer can migrate towards summer or winter areas, and can cover great distances in doing so [20-22]. Vertical migrations from higher to lower elevations are a typical model of migration as a strategy in trying to meet energy needs [23]. Such migrations are caused by the depth of snow cover at high altitudes during winter and ultimately due to the change in seasons and the varying quality and quantity of available food for animals [24].

When the Bavarian Alps region becomes accessible following the snow melt, deer are known to temporarily and briefly migrate outside their usual small wintering areas. In the Alps, winter and rutting ranges are relatively small, averaging 113 ha and 134 ha, respectively, whereas the mean size of home ranges used from spring to autumn amounts is 386 ha [25].

Habitat structure and food quality and distribution have the greatest influence on habitat size. In this context, the metabolic ratio and food consumption are reduced in ruminants in moderate climates of the northern hemisphere during the winter period, as a period traditionally associated with weight loss [26]. In these areas, ruminants preserve energy during winter by reducing activities and movement within the limited habitat [25, 27-29].

When determining food availability, snow cover is a relevant factor that significantly reduces food availability and accessibility during the winter period. Therefore, during winter, snow cover and temperature are considered to be extremely important factors for determining spatial behaviour of many mountain ruminant species.

The strategy for compensating for difficult winter conditions could include migrations to lower valleys with less snow during winter and, most importantly, to the rich pastures at higher elevations during summer. Migration can have a strong influence on the spatial behaviour of each individual adopting this strategy: many populations of large ruminants migrate seasonally between separate life areas [30]. Alternative strategies such as migrations or non-migrations can occur within the same population due to environmental fluctuations and migration cost differences for specific individuals [4]. Usually, migrating ruminants in moderate climates, such as red deer, choose higher summer habitats and lower winter habitats [5].

It is commonly agreed that migration towards lower areas in the fall and early winter represents a strategy that involves finding winter habitats with smaller snow depths [8], due to the fact that deeper snow cover reduces food accessibility, increases energy consumption due to movement [31] and makes deer more vulnerable to predators [32].

In the Alps, during harsh winter conditions with deep snow cover, red deer select areas of alpine pastures under the tree line, where the snow is less deep, the insolation is greater and feed availability is good due to the fragmented distribution of the snow. Energy expenditures for thermoregulation are minimised by selecting areas with a southern or southwestern exposition, protected from strong winds. In years with less snow, red deer that are not fed prefer the security of forest habitats at lower elevations, and graze on the valley meadows.

Red deer receiving supplementary feeding have more constant ranges, and save energy

by combining the alpine pastures with feeding areas [33].

Fast spring migration in Norway is significantly different from other reports of slow deer spring migrations due to the gradual greenification on the elevation gradient [5, 18].

In the Republic of Croatia, ecological research on deer game populations has been conducted in the Podunavlje area [34]. Part of the study was focused on deer migrations and spatial separation of the sexes, except in the mating season when male and female individuals were together. Outside the mating season, herds of female units with calves occupy one part of the hunting ground, while males occupy another.

The exact snow cover depths at which animals will alter their life habits, i.e. migrate into more acceptable climate conditions regardless of nutrition quality, is known for all wild ungulate species. The critical snow cover depth that causes migration among red deer is from 50-60 cm [35].

The main objectives of this study were to determine the changes in snow cover depths that are significant for red deer migrations and to examine dependencies between migrations

and climatic factors (number of days with snow depths  $\geq 50$  cm and maximum daily snow depths) in the context of their changes in the western part of Gorski Kotar region.

## MATERIALS AND METHODS

### Study Area

The study was conducted in the western part of Gorski Kotar region. According to the Köppen classification, the study area is included in the Cfsbx climate type. This is a moderately warm rain climate, without dry periods. Based on the data from the Parg weather station, the average annual air temperature for the study area is 7°C, and the average precipitation is approximately 2000 mm [36]. Relief of the study area is extremely irregular and meuble.

Gorski Kotar region in Croatia represents a large integral forest complex, distinctive for its significant vertical drops, and diversity of relief characteristics and habitat conditions (Figure 1).

The study area includes a smaller section of the coastal region (Klana, Grobnik) which

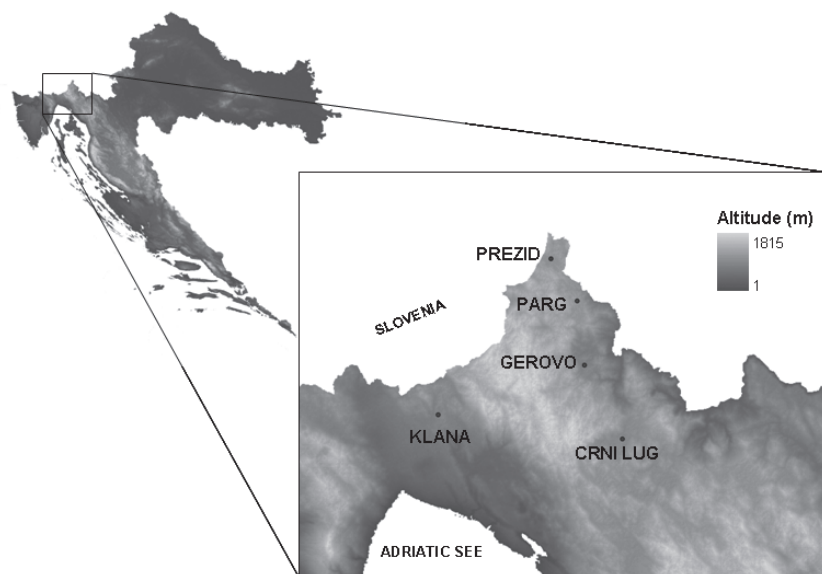


FIGURE 1. Study area

represents the winter habitat, and an inland region (Parg, Gerovo, Prezid, Crni Lug) from which the deer migrate.

### Snow Analysis

In the study area, data on the number of days with snow depths  $\geq 50$  cm and maximum daily snow depths ( $\geq 50$  cm) were analysed for the winter period from 1950 to 2011 from the weather stations Parg, Gerovo, Prezid, Crni Lug and Klana. A reduction or increase of climate elements was analysed using a linear trend, and the significance of the linear trend was tested with the Student's t-test. All climate data was processed using the KlimaSoft 2.0 software (Monachus informatika, Croatia). Statistical data analysis (regression analysis, Student's t-test) was conducted using Statistica 7.1 software [37] with a 5% significance level considered statistically significant.

### Game Observations

Hunting authorities are obliged by law to perform regular counts of all game species that permanently or temporarily inhabit hunting grounds [38, 39]. There are numerous known and acknowledged methods for determining the number of individuals. The most commonly used method for counting big game is observation and monitoring throughout the year, at several known locations. Data were collected on red deer migrations from the hunting records, gamekeeper logs and records from game counts and monitoring activities in the study area. Counts and monitoring the red deer population was carried out in an area of 75206 ha in the winter period, i.e. from October to December, in the years 2000 to 2011. Outgoing migrations were analysed to determine whether they were caused by snow cover, regardless of the number of migratory individuals. In total, 132 count records, hunting records and gamekeeper logs were analysed. The red deer population in the study area was assessed at 970 individuals [1].

Data from weather stations was analysed to determine the existence or non-existence

of a critical snow depth ( $\geq 50$  cm) for this study area during the winter months. Based on game counts, red deer monitoring data from gamekeeper logs and hunting records were analysed. If migration individuals were observed and the snow depth was  $> 50$  cm, this was assigned a score of 1, while if there was no migration and the snow depth was  $< 50$  cm, this was assigned a score of 0. Regression analysis was used to determine whether there was a correlation between red deer migrations and snow cover depth.

## RESULTS AND DISCUSSION

Based on many years of experience in monitoring red deer activities in the study area, particularly activities related to the start of the seasonal migration, it can be assumed that climate conditions, such as snow cover depth and duration, represent the main factor influencing migrations. Therefore, the focus of this study was directed at these climate conditions.

When considering the number of days with snow depth  $\geq 50$  cm, there was a significant difference between the Klana site, which represents the red deer winter habitat, and the inland sections of the western part of Gorski Kotar region (sites Gerovo, Crni Lug, Prezid, Parg) from where red deer game usually migrates.

The average number of days with snow depth  $\geq 50$  cm at the Klana site was 0.33 days, while at Prezid, Parg, Gerovo and Crni Lug area, this number varied from 10.18 to 17.25 days (Table 1). The Klana site had the fewest number of days with snow cover, which explains deer migrations from the inner areas of the western part of Gorski Kotar region, i.e. from the sites Gerovo, Crni Lug, Prezid and Parg.

The results in Table 2 clearly indicate that the area near the Klana weather station is the winter habitat with the smallest maximum daily snow depths. Maximum daily snow depths at the Klana area was 18.93 cm, as opposed to the

**TABLE 1.** Comparison of the mean number of days per winter season with snow cover  $\geq 50$  cm (period from 1950 to 2011)

Weather station	Mean $\pm$ Std. Dev.
Klana	0.33 $\pm$ 1.11 <sup>a</sup>
Gerovo	12.80 $\pm$ 17.91 <sup>bc</sup>
Crni Lug	17.25 $\pm$ 18.18 <sup>b</sup>
Prezid	10.18 $\pm$ 14.29 <sup>c</sup>
Parg	10.76 $\pm$ 13.61 <sup>c</sup>

Note: Values within a column marked with a different letter differ significantly ( $p < 0.05$ ); Std. Dev. - standard deviation

**TABLE 2.** Comparison of mean values of the maximum daily amount of snow cover (cm) for the period 1950 to 2011

Weather station	Mean $\pm$ Std. Dev.
Klana	18.93 $\pm$ 18.03 <sup>a</sup>
Gerovo	71.08 $\pm$ 40.85 <sup>b</sup>
Crni Lug	79.76 $\pm$ 37.91 <sup>b</sup>
Prezid	70.38 $\pm$ 46.75 <sup>b</sup>
Parg	38.47 $\pm$ 13.09 <sup>c</sup>

Note: Values within a column marked with a different letter differ significantly ( $p < 0.05$ ); Std. Dev. - standard deviation

maximum daily snow depths in the areas from which the red deer migrated, which ranged from 38.47 cm to 79.76 cm. It can be assumed that this was the reason for red deer migrations from the Prezid, Parg, Gerovo and Crni Lug areas into the winter habitats at Klana.

Based on the results of the study and analysis of macroclimate conditions (number of days with snow depths  $\geq 50$  cm and maximum daily snow depths), it is clear that there is a certain regularity, seen in the statistically significant difference between observed weather stations in the study area. The Klana site stands out from all other weather stations concerning the variables examined. As Table 2 shows, more favourable macroclimate conditions play the

main role in seasonal game migrations, in this case red deer migrations. Of all the weather stations, Crni Lug was shown to have the least favourable macroclimate conditions (snow cover depths).

Trends related to the number of days with snow and maximum daily snow depths in the northwest Dinarid region are negative. A significant reduction in the number of days with snow  $\geq 50$  cm was seen at the Crni Lug and Prezid sites, which are areas from which red deer usually migrate during winter months. A significant reduction in maximum daily snow depths was present at the sites Gerovo, Crni Lug and Prezid, which are also areas from which red deer usually migrate (Table 3).

**TABLE 3.** Linear trends number of days per winter season with snow  $\geq 50$  cm and maximum daily snow depth (cm) for the period 1950 to 2011

Weather station	Linear trend	Beta	B	t	p-level
Number of days with snow $\geq 50$ cm					
Klana	$Y = -0.0028x + 0.4181$	-0.0435	-0.0028	-0.3317	0.7412
Gerovo	$Y = -0.1026x + 15.931$	-0.1000	-0.1026	-0.7657	0.4469
Crni Lug	$Y = -0.2725x + 25.61$	-0.2616	-0.2725	-2.0643	0.0434*
Prezid	$Y = -0.2256x + 17.077$	-0.2757	-0.2256	-2.1844	0.0329*
Parg	$Y = 0.0087x + 10.502$	0.0111	0.0087	0.0847	0.9327
Maximum daily snow depth (cm)					
Klana	$Y = -0.0792x + 21.349$	-0.0767	-0.0792	-0.5858	0.5602
Gerovo	$Y = -0.6156x + 89.86$	-0.2632	-0.616	-2.0777	0.0421*
Crni Lug	$Y = -0.7452x + 102.49$	-0.3433	-0.745	-2.7837	0.0072*
Prezid	$Y = -0.8916x + 97.576$	-0.3330	-0.892	-2.6899	0.0093*
Parg	$Y = -0.087x + 41.08$	-0.1142	-0.0857	-0.8761	0.3845

\* significant at  $p < 0.05$

In recent years, the number of days with snow and the maximum daily snow depth values have declined, which will certainly reflect on the intensity of red deer migrations in the northwest Dinarid region. According to Malnar [1], in the period 2005 to 2009 at the sites Parg, Prezid, Crni Lug and Gerovo, the number of days with snow  $\geq 10$  cm was reduced by 20%, number of days with snow  $\geq 30$  cm was reduced by 38% and number of days with snow  $\geq 50$  cm was reduced by as much as 54% in comparison to reference period from 1961 to 1990.

Red deer migrations in the northwest Dinarid region have been much less frequent in recent years. The significant climate changes that have appeared, or will appear in the future, will have serious biological and ecological consequences on forest ecosystems and wildlife. Air temperature is a decisive factor for climate character. According to Ugarković and Tikvić [40], average annual air temperatures in the Gorski Kotar region had shown a statistically significant increase of 0.5°C to 1.0°C, for the period 1991 to 2007, in comparison to the reference period (1961-1990). In line with the increased average annual air temperatures and significantly positive trend in absolute maximum air temperatures, the number of warm days ( $\geq 25^\circ\text{C}$ ) per winter season has increased by 10-14 days and the number of hot days ( $\geq 30^\circ\text{C}$ ) by 1-3 days during the years 1991 to 2007, while snow precipitation has been declining.

Due to the increase in air temperature, snow cover depths in the northern hemisphere have been reduced by 10% in the last 20 years [41]. The average number of days per winter season with critical snow depths ( $\geq 50$  cm) was only 2 days for the Gerovo site, and not a single day for the Klana site (Table 4) for the period from 2000 to 2011. The inland sections of the Gorski Kotar region had shown only 5 years with critical snow depths ( $\geq 50$  cm). These data corroborate the reductions in red deer migration in the Gorski Kotar region obtained by observing and telemetric monitoring.

According to Hafner [35], the critical snow depths that caused migrational activities of

**TABLE 4.** Number of days per winter season with snow  $\geq 50$  cm at the weather stations Gerovo and Klana

Years	Weather stations	
	Gerovo	Klana
2000	0	0
2001	1	0
2002	0	0
2003	7	0
2004	22	0
2005	11	0
2006	0	0
2007	0	0
2008	0	0
2009	0	0
2010	47	0
2011	0	0
Average	7.3	0

the red deer were 50-60 cm. In the observed migration period, such critical depths were recorded in 5 years at the Gerovo site. The average maximum daily snow depths were 50 cm at the Gerovo site and 12.7 cm at the Klana site. Maximum daily snow depths in the northwest Dinarid region were the cause of lesser migrational activities of deer game (Table 5).

**TABLE 5.** Maximum daily snow depth (cm)

Years	Weather stations	
	Gerovo	Klana
2000	17	1
2001	56	28
2002	36	0
2003	57	18
2004	100	11
2005	61	34
2006	45	8
2007	37	3
2008	34	6
2009	41	8
2010	93	31
2011	23	4
Average	50	12.7



Reduced food availability and snow cover can reduce winter mobility in red deer [25, 33] and roe deer (*Capreolus capreolus* L.) [42, 43].

Multiple regression analysis showed that the number of snow days and the maximum daily snow depths ( $\geq 50$  cm) have a significant influence on red deer migrations. Snow as the climate factor explained 29.28% of red deer migrations in the study area (Table 6).

In the Italian Alps region, the time of migratory deer migrations is connected to snow cover, since this region is under the inverse influence (although weak) of snow precipitation intensity, and is more prominently correlated with winter snow precipitation, where only 2 cm of snow was enough to trigger the migration of migratory deer from their summer habitats into their winter habitats. On the other hand, stationary red deer are under a very mild influence of snow conditions, likely due to the lower elevation of their habitats as compared to migratory deer, which significantly reduces the snow influence [44].

Regional differences in topography, habitat accessibility or climate not only influence the share of migratory individuals and specific details related to the seasonal movement of deer game; they are likely the actual cause for the migrations. Such spatial behaviour confirms that red deer avoid snow, as estimated in other studies in mountainous environments [25, 33, 45], and that the presence of snow cover is the main factor in determining the fall migration of this species.

According to some researchers, seasonal migrants such as red deer have medium sized habitats [5]. Unlike red deer, roe deer is a stationary animal with a small habitat and few

migratory individuals [46]. Luccarini *et al.* [44] determined two types of behaviour of the red deer in Italian Alps - migratory and stationary. In that study, migratory and stationary individuals were equally distributed between sexes.

Stationary deer use areas of comparable size during spring, summer and fall, while their winter areas were considerably smaller. Migratory deer used large sized areas in the fall and the spring, considerably larger than their summer habitats, and substantially larger than those used in winter. Therefore, regardless of the selected strategy, winter habitats were significantly smaller.

These results support other findings of a strong influence of climate conditions on the spatial behaviour of ruminants, particularly of snow cover as the decisive factor in the winter period. Furthermore, the size of the winter habitat is dependent on the severity of winter: the deeper the snow, the smaller the habitat animals use [25].

According to Luccarini *et al.* [44], migratory and stationary animals in the Alps region show periodic vertical movements throughout the year, reaching the highest altitudes during summer and lowest during winter months. This aspect is emphasised in the case of migratory deer, which reached distant summer habitats, located at the highest altitudes, every year, while stationary deer simply increased their use of higher areas, but did not reach those altitudes used by migratory deer.

The deer monitored in the northwest Dinarid region showed little migratory activity due to warm winters, i.e. low number of days with critical snow depths (Table 4) and small maximum daily snow depths (Table 5).

**TABLE 6.** Multiple regression analysis of the dependencies between the number of snow days and maximum daily snow depths ( $\geq 50$  cm) and red deer migrations

	Beta	Std. Err. of Beta	B	Std. Err. of B	t (10)	p-level	Adjusted R <sup>2</sup>
Intercept			0.3750	0.1530	2.4494	0.0342	0.2928
Climatic factor (snow)	0.5976	0.2535	0.6250	0.2651	2.3570	0.0401	

## CONCLUSIONS

The macroclimatic conditions (number of days with snow depth  $\geq 50$  cm and maximum daily snow depths) showed a certain regularity, i.e. a statistically significant difference between the observed weather stations in the study area. The Klana weather station was shown to be the most favourable deer game winter habitat based on all the study variables. As for the other weather stations, Crni Lug was found to be the habitat with the least favourable macroclimatic conditions (snow depth).

Due to growing changes in climate and climatic conditions in the northwest Dinarid region, a new trend has emerged, with a decline in the number of snow days and maximum daily snow depths  $\geq 50$  cm. For

that reason, years without snow precipitation or without critical snow depths that would otherwise influence migrations, are becoming more frequent. Therefore, red deer have reduced the intensity of their migrations in the study area. However, in years with critical snow depths ( $\geq 50$  cm), migrations are still present. According to the results (Table 6), the monitored climatic factors (number of snow days and maximum daily snow depths  $\geq 50$  cm) have a significant influence on red deer migrations. These two factors alone explain 29% of red deer migrations in the study area. Naturally, migrations are also influenced by other factors such as food availability, which in turn is related to the amounts of snow, winter feeding, human activities and game disturbances, presence of predators, etc.

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# Experts' Perceptions of the Effects of Forest Biomass Harvesting on Sustainability in the Alpine Region

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## Abstract

**Background and Purpose:** In the EU political agenda, the use of forest biomass for energy has grown rapidly and significantly, in order to mitigate carbon dioxide emissions and reduce the energy dependence on fossil fuels of European member countries. The target of the EU climate and energy package is to raise the share of renewable energy consumption produced from renewable resources to 20% in 2020 (Directive 2009/28/EC). With regards to biomass energy, the supply of forest wood biomass is expected to rise by 45% (reference period: 2006-2020), in response to increasing demand for renewable sources. The increase of forest biomass supply could have both positive and negative effects on several forest ecosystem services (ESs) and local development. These effects should be assessed in a proper manner and taken into account when formulating management strategies. The aim of the paper is to assess the environmental, economic and social sustainability of forest biomass harvesting for energy, using the Figure of Merit (FoM) approach. **Materials and Methods:** Sustainability was assessed through a set of four indicators: two focused on experts' opinions regarding the effects of forest biomass harvesting and the other two focused on the cost-benefit analysis (potential energy obtained and costs for wood chips). The research was developed through four case studies located in the Alpine Region. A semi-structured questionnaire was administered

face-to-face to 32 selected experts. The perceived effects of forest biomass harvesting for energy on ESs and local development were evaluated by experts using a 5-point Likert scale (from “quite negative effect” to “quite positive effect”).

**Results:** All experts agree that forest biomass harvesting has a positive effect on forest products provision and local economic development (employment of local workforce, local entrepreneurship and market diversification), while the effects on other ESs are controversial (e.g. carbon sequestration, habitat quality, natural hazards protection and recreational values).

**Conclusions:** Therefore, it can be asserted that the effects of forest biomass harvesting on sustainability vary according to the local context. The results of FoM can support decision makers in order to analyze the environmental, social and economic sustainability of forest biomass harvesting for energy.

**Keywords:** ecosystem services, ecological effects, socio-economic effects, local development, Multi-Criteria Analysis, Alpine Space

## INTRODUCTION

At global level, renewable energies cover more than 13% of the world's energy demand [1] and they could meet more than 50% of the total energy demand by the middle of 21<sup>st</sup> century, if supported by appropriate energy policies and innovative renewable energy technologies development [2]. In the pool of renewable energies, wood and wood wastes cover about 47% of the gross consumption of all renewable energy in the European Union in 2011 and 67% of bioenergy use [3].

According to the European Union (EU) Forest Action Plan of the period 2007-2011 [4], the 27 EU member countries have a high energy potential from forests, despite the relevant constraints to wood mobilization [5]. EU Forest Action Plan considers the promotion of use of forest biomass for energy generation one of the 18 key actions to improve the competitiveness of the European forest sector [6]. Subsequently, the new EU Forest Strategy (2013) encourages to use forest resources in a manner that minimizes the negative impact on the environment and prioritizes the outputs that have higher added-value and are able to create job opportunities.

In 2005 around 98 million m<sup>3</sup> of wood overbark was removed from EU forests and used as fuelwood [7]. This wood quantity for energy purpose is foreseen to increase in future decades, because the targets of the EU climate and energy package are to raise the share of

renewable energy consumption produced from renewable resources to 20% in 2020 and to reduce the greenhouse gas (GHG) emissions by 20% compared to 1990 levels (Renewable Energy Directive 2009/28/EC). EUwood estimates that the EU's forest biomass supply would increase by 11% from 2010 to 2030, while the demand for forest biomass would rise by 73% [8]. Other studies evaluate an increase in the use of renewable biomass by 45% by volume between 2006 and 2020, this value representing 8% of expected total increase in renewable energy use in EU member countries [9, 10]. Instead, Nabuurs *et al.* [11] for the whole Europe estimate that forests could supply 729 million m<sup>3</sup> of wood overbark for energy use by 2060.

According to the UNCED's report “Our common future” (1987) - better known as Brundtland report [12] - the sustainable development can be defined as the kind of development that meets the needs of the present without compromising the ability of future generations to meet their own needs. However, this definition is more focused on the economic aspects of the sustainability concept, considering only marginally environmental and social aspects. Currently, the need to consider simultaneously these three perspectives is widely recognized; in this direction in recent decades two models have been developed, with the aim to analyze the interrelationships between the environmental, social and economic aspects

of sustainability [13]. The first model considers the three aspects as concentric ("concentric model"), while in the second model the three aspects are represented equally ("overlapping circles model"). According to the "concentric model" of sustainability, the economy sphere is a subset of the society sphere, and the latter is a subset of the environmental sphere. In the "overlapping circles model" of sustainability the three spheres are not concentric and the model considers in more detail the nature of each sphere and the reciprocal interactions. In the bioenergy sector the "overlapping circles model" is the most suitable model in order to assess the effects of biomass harvesting from forests considering simultaneously environmental, economic and social sustainability [14].

Environmental sustainability can be defined as "a condition of balance, resilience, and interconnectedness that allows human society to satisfy its needs while neither exceeding the capacity of its supporting ecosystems to continue to regenerate the services necessary to meet those needs nor by our actions diminishing biological diversity" [15]. Environmental sustainability refers to the concept of ecosystem services (ESs) first introduced in the early 80's by Ehrlich and Ehrlich [16]. In the 90's several authors analyzed this concept through different perspectives [17, 18]. Currently, ESs can be defined as the benefits obtained from nature that satisfy human needs [19, 20] such as provisioning services (e.g. food, fodder and timber), regulating services (e.g. air and water purification), cultural services (e.g., recreational opportunities), and supporting services (e.g. nutrient cycling). Many attempts have been made both to systematically categorize and to comprehensively list ESs [20-22].

Sustainability of economy - in the strict sense - is considered as the ability to maintain productivity [23]. In a broader sense, sustainable growth is the creation of a social and economic system which provides support to increase the real income, to improve the level of education, and to improve the quality of life [24, 25].

While, social sustainability is defined as a life-enhancing condition within communities and a process within communities that can achieve that condition [26]. The key aspects to consider in social sustainability are [27]: equity of the current generation access to fundamental services (e.g. health, education) and equity between generations, widespread people's participation to the decisions, a sense of community responsibility, respect for property rights. Social sustainability can be implemented incorporating perceptions, preferences and opinions of local community, stakeholders or public in general in the decision making process, following the basic principles of participatory approach [28].

Wood biomass from forests (e.g. harvesting residues as branches and tops, stumps and coarse roots, dead trees, etc.) could satisfy an interesting percentage of the energy demand through improving the efficiency of harvesting and mobilization technologies, increasing the use of biomass in the high forested areas and in the regions with a high rate of natural regeneration (i.e. mountain and marginal areas). In this potential future scenario an important aspect to take into account is the analysis of the sustainability of forest biomass harvesting for energy use, considering the effects on the environment and society. Starting from these considerations, the aim of the paper is to analyze the sustainability of forest biomass harvesting for energy purpose considering the environmental, economic and social sustainability according to the "overlapping circles model". The sustainability of forest biomass harvesting was analyzed through the use of the Figure of Merit (FoM), taking into consideration simultaneously the environmental, social and economic aspects of sustainability as well as expected from the "overlapping circles model". The research was developed in four case studies located in Alpine Region and involved in the Recharge.green project [29]. The case studies considered - Triglav National Park in Slovenia, Gesso-Vermenagna, Mis and Maè valleys in Italy - were chosen taking into account different environmental and socio-economic contexts, in order to test the method in various situations and to compare the results.

## MATERIALS AND METHODS

### Study Areas

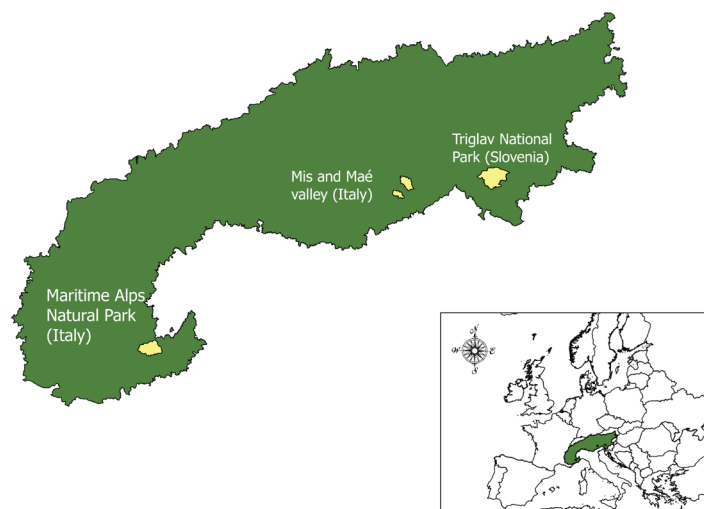
The sustainability of forest biomass harvesting was analyzed in four case studies, located in different parts of the Alps (Figure 1): Triglav National Park (Slovenia), Mis valley and Maè valley (Belluno Province, Italy) and valley (Cuneo Province, Italy). These four case studies were chosen in order to represent various Alpine conditions. The key variables used to select the study areas were: (1) forest cover, (2) percentage of land under protected-area status (from 100% of Triglav National Park to 45% of Maè valley); (3) tourist importance in terms of average annual number of visitors; (4) importance of primary sector for local economy (e.g. timber and hay production). Among the available data across the four study areas, these variables were chosen as proxies for many ecosystem services (in particular for provisioning, cultural and regulating services) [30].

The first study area is the Triglav National Park (TNP), located in the north-east part of Slovenia. TNP is the only national park in Slovenia and the current boundaries are established by a National Law of the 2010. TNP covers an area of almost 840 km<sup>2</sup> which is nearly four per cent of the Slovenian surface. The main land uses are:

forests (62%) and managed grasslands (10%). The typical forest types in the park are: European beech forests (27981 ha), dwarf mountain pine forests (11350 ha), Silver fir-European beech forests (4925 ha), and Silver fir-Norway spruce forests (4191 ha). The park provides a variety of ESs. On the one hand nature conservation, environment and cultural heritage protection as well as recreation and tourism (about 580000 tourists per year) are the most important ESs in TNP; on the other hand agriculture and forestry are important for the people living in the park.

Mis Valley covers an area of 11800 ha and it is crossed by Mis Stream (22 km long). It includes two municipalities and large part of the area is covered by the Dolomiti National Park (71% of Mis valley). Forest area covers about 8347 ha and the main forest categories are hornbeam and manna ash forests (2420 ha), European beech forests (2133 ha), dwarf mountain pine forests (1442 ha) and Norway spruce forests (533 ha). Considering the area covered by forest management unit plans, the mean growing stock is nearly 214 m<sup>3</sup>·ha<sup>-1</sup> with a current annual increment of 3.78 m<sup>3</sup>·ha<sup>-1</sup>·year<sup>-1</sup>. The tourism sector is less developed and the visitors are mainly concentrated in the area of the Dolomiti National Park.

Maè Valley covers an area of 23300 ha around



**FIGURE 1.** Geographical location of the study areas in the Alpine Region (source: Alpine Convention, SOIA database for alpine perimeter, EUROGEOGRAPHICS for administrative boundaries).



the Maè Stream (33 km long), and it includes four municipalities. The area is part of Natura2000 Network, Dolomiti UNESCO site and Dolomiti National Park (45% of total land area). In the past Maè valley was characterized by traditional use of wood for rural building structures, now strongly declined. Nowadays the use of wood for heating remains high for households' traditional activity. Regarding the forest area (18928 ha), the main forest categories are European beech forests (3963 ha), dwarf mountain pine forests (2532 ha) and mixed forests of Norway spruce and European beech (2167 ha). Considering the area under forest management unit plans, the mean growing stock is nearly  $208 \text{ m}^3 \cdot \text{ha}^{-1}$  with a average annual increment of  $3.50 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ . In this area the tourism sector is less developed with an average number of visitors per year equal to 40 000.

Gesso-Vermenagna valley is located in the north-western part of Italy (Piedmont Region), close to the French border. The study area includes seven municipalities. The land area is approximately 51500 ha of which about 32000 ha are located in protected areas (Maritime Alps Natural Park or Nature2000 sites). The main land uses are forests (42%) and pastures (33%). The main forest types are European beech forests with 11500 ha, chestnut forests with 2700 ha, and mixed forests (maple, linden and ash) with 1850 ha. The average standing stock is  $183 \text{ m}^3 \cdot \text{ha}^{-1}$ , with an average annual increment of  $7.73 \text{ m}^3 \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ . Gesso-Vermenagna valley is a mountainous area mainly based on the primary sector (about 22% of total firms), while the secondary sector (industry) is poorly developed.

The services sector is based on tourism with an average of 121000 visitors per year.

### Research Framework

The sustainability of forest biomass harvesting for energy production was assessed through a set of four indicators: (1) perceived effects of forest biomass harvesting on ESs; (2) perceived effects of forest biomass harvesting on local development; (3) potential energy obtained from forest biomass; (4) costs for energy production. The first two indicators focused on the local experts' opinions concerning the effects of forest biomass harvesting on ESs and local development, while the other two indicators focused on the cost-benefit analysis (potential energy obtained and costs for wood chips). The data of the first two indicators were collected through a semi-structured questionnaire, while the other two indicators were quantified using data from *ad hoc* survey and forest management plans.

The experts were identified by the researchers of the Recharge.green project and the local partners in a brainstorming session. The major selection criterion was their professional experience in one of the following sectors: forest management and planning, environment conservation, rural development and renewable energy development. Besides, the experts were chosen on the basis of their expertise and knowledge of the local context. The experts identified in each case study were mainly policy makers and technicians. At the end of this preliminary stage, a total of 32 experts - distributed as illustrated in Table 1 - were identified and directly contacted.

**TABLE 1.** Number of experts interviewed subdivided per study area

Country	Study area	N° experts
Italy	Mis valley (Veneto Region)	5
	Maè valley (Veneto Region)	6
	Gesso-Vermenagna valley (Piedmont Region)	8
Slovenia	Triglav National Park (Gonška Region)	13
<b>Total</b>		<b>32</b>

The semi-structured questionnaire administered to the experts was subdivided in 6 thematic sections and composed by 20 questions (19 close-end questions and one open-end question). In the present paper the data of the two thematic sections concerning forest biomass harvesting (effects on ESs and on local development) were used to elaborate the indicators of sustainability.

The semi-structured questionnaire was administered through face-to-face interviews to the local experts identified in the period September 2013 – July 2014. For each question, besides ticking the given answer, there was discussion, in order to collect comments and explanations for responses and to discuss the various related aspects with the experts. The discussion was assumed to help to interpret the quantitative results and, furthermore, it was helpful in case experts had difficulty in understanding the meaning of some words or concepts. In the present paper qualitative information collected during the interviews were used in the discussion of the results in order to explain and understand the differences between case studies.

### Figure of Merit and Set of Indicators

A useful and practical tool for a multi-criteria analysis of the sustainability of biomass harvesting for energy is represented by the figure of merit or FoM [31]. FoM tool allows the integration and the simultaneous comparison of several indicators of environmental, economic and social sustainability of bioenergy development. FoM tool is a simple and straightforward method that through a set of indicators allows to compare the performance of a device, system or method, relative to its alternatives. In literature, FoM is applied to assess the performance of different renewable energies (e.g. solar, wind, hydropower), on the basis of a set of performance indicators [32]. In this paper FoM was modified and adapted, in order to assess the performance of forest biomass harvesting for energy in different case studies considering the local characteristics. In other words, FoM tool was used in order to compare the performance of the same activity in different geographical and socio-economic contexts.

The first two indicators (perceived effects of forest biomass harvesting on ESs and on local development) were measured using data from questionnaires responses rated on a 5-point-Likert scale ranging from -2 to +2. The value of the other two indicators (potential energy obtained from forest biomass; costs for energy production) can range from 0 to an undefined upper limit. In these cases the range was calculated from the interval of variation given by the difference between the best and the worst performance of each case study.

For each indicator, the range of scores was divided into 10 equal classes. In this way, for the indicators 1 and 2 each class has a width equal to 0.4. For the indicators 3 and 4, the width of the classes was calculated dividing in 10 equal parts the range obtained from minimum to maximum value of the indicator. Finally, each indicator obtains a class-relative rank according to its performance from 1 (the best score) to 10 (the worst score) [1]. In the present study the same level of importance to each indicator has been given, without attributing different weights. The formula used to calculate the FoM was:

$$\text{FoM} = \text{Relative rank } I_1 * \text{Relative rank } I_2 * \text{Relative rank } I_3 * \text{Relative rank } I_4$$

where:

$I_1$  = perceived effects of forest biomass harvesting on ESs (range from -2 to 2);

$I_2$  = perceived effects of forest biomass harvesting on local development (range from -2 to 2);

$I_3$  = potential energy obtained from forest biomass considering tree composition of forest types ( $\text{MJ}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ )

$I_4$  = unit cost for energy production ( $\text{€}\cdot\text{t}^{-1}$  fresh chips).

The first indicator ( $I_1$ ) is associated to the perceived effects of forest biomass harvesting for energy on ESs. In this study six ESs provided by Alpine forests were considered and assessed, assuming the commensurability of these ESs (Table 2). This set of ESs reflects both the particular mountain ecosystems characteristics

and the decision making context (expanding renewable energies in general and bioenergy in particular for the local development) [33].

For each case study the experts evaluated the positive and negative effects of forest biomass harvesting using a 5-point Likert scale (-2 = quite negative effect, -1 = negative effect, 0 = no effect, 1 = positive effect, 2 = quite positive effect). The experts assessed the effects with special regards to the environmental characteristics of the local context. This indicator is considered as a benefit in case of positive effects and as an environmental cost in case of negative effects.

The second indicator ( $I_2$ ) used in the FoM has the purpose to evaluate - through the experts' opinions - the positive and negative perceived

effects of forest biomass harvesting on local development, considering the socio-economic characteristics of the study area. The effects were assessed using three dimensions related to different features of local development: economic, social and cultural. For each effect dimension were selected appropriate sub-indicators. The experts assessed the effects on each sub-indicator taking into account all aspects described in Table 3 in an aggregate way.

Economic indicators track the costs and business aspects of a process. When considering sectors such as renewable energies production, these indicators must go beyond conventional financial reporting to describe the creation of wealth and its distribution and reinvestment for future growth [34]. The hypothesis is that

**TABLE 2.** Alpine ecosystem goods and services considered in this paper (source: [20-22])

Ecosystem good and service	Definition adopted
<b>Provisioning services</b>	
Provision of forest and agricultural production	Products obtained directly from ecosystems such as agricultural products, forest products and aquaculture products. If relevant, could also include extractable products (e.g. mushrooms, natural medicines, peat, ...)
Provision of fresh or potable water	Provision of fresh or potable water including water filter function of soils
<b>Regulating and maintenance services</b>	
Protection against natural hazards	Mediation/Buffering of flows (mass, liquid, gaseous) for avoiding extreme events (such as floods, soil erosions, landslides, avalanches, storms, rock falls, ...)
Carbon sequestration in vegetation and soil	Amount of carbon sequestered by the ecosystem for regulating the global atmospheric composition
Ecological habitat quality	The overall habitat quality for wild plant and animal species, necessary for the function of ecosystem services mentioned above. Habitat quality is (mutually) dependent on nutrient cycling, seed dispersal and pollination. Also, the long term ecosystem stability (=resilience) and resistance against pests affecting human health and forest- or agricultural production are an expression of high ecological habitat quality
<b>Cultural services</b>	
Recreational values	Value for recreation (such as walking, hiking, skiing, climbing, boating, leisure fishing and leisure hunting), possibility for relaxation and silence in general

**TABLE 3.** Socio-economic effects on local development considered in this paper (source: [37-47])

Indicator	Ambit	Description and related issues
<b>Economic indicators</b>		
Local market diversification	Local economy	Allocation of resources over a large number of markets in an attempt to reduce risks of concentrating resources and to exploit the economies of flexibility [44]. Willingness to invest in renewable energies to diversify the market. System flexibility to react to market changes and to renewable energies price fluctuations.
Local entrepreneurship	Local economy	Propensity of the local population to initiate business enterprises'. Effects on business opportunities and productive diversification of the area.
Resource efficiency	Local economy	Use of natural resources, with the main purpose of minimising their input when producing a product or delivering a service. Amount of energy production with a less amount of non-renewable resource input.
<b>Social indicators</b>		
Employment of local workforce	Quality of life	Improving the economic development of local community. The installation, operation and maintenance of renewable energy technologies are generally of modest scales, so they create more employment, for the local workforce. Building the technical capacity of the local workforce.
Increasing income per capita	Quality of life	Income per capita is a positive variable of social welfare, and is often an effect of technical progress [41]. Payments to local farmers for hiring their land and "compensations" to the local community made by the owner of the renewable energy plant.
Tourism	Quality of life	Renewable energies development creates changes in the area and effects on tourism development. Attractiveness of the area for visitors is an indicator of social development.
Social and community aggregation	Social stability, involvement and legitimacy	Effects on the capacity to improve local people participation (i.e social and political empowerment, participative decision-making, participatory integrated assessment) Effect on social capital and on community capacity-building
Political stability	Social stability, involvement and legitimacy	Citizens' acceptance of the system or, in other words, the potential of conflicts induced by energy systems, and the citizens participation in the decision making process.
Human health	Health and safety	Health hazards for the local population linked to the renewable energies production (potential health impact due to severe accidents; health consequences of normal operations).
<b>Cultural indicators</b>		
Property rights and rights of use	Local traditions and values	Land and resource tenure, dependencies on foreign sources (e.g. financial investments, knowledge), customary rights

encouraging the use of forest biomass for energy purposes has positive economic effects at local level. In particular, the positive effects concern the market diversification (e.g. wood chips as efficient alternative to traditional fuelwood), the creation of new job opportunities in the forest-wood-energy chain, and the impetus for innovation and local entrepreneurship.

Social indicators are employed to assess both the technological impacts, and the effects of political strategies, interventions or plans. There are various models for the measurement of social impact and the discipline of social indicator research provides a vast list of works on which to base the choice and selection of appropriate indicators [35]. In the present work, social sub-indicators were used in order to take into account three main ambits: quality of life of people, socio-political stability and health and safety. The hypothesis is that the use of forest biomass for energy can potentially have positive effects on the quality of life of local communities (increase employment opportunities and income per capita), but - on the other hand - it can also have potentially negative effects on the health and safety of forest workers.

The term cultural indicator is a term developed by Gerbner [36] and refers to the elements that reflect our culture. The local culture can influence the rational choices of the people (i.e. political decision makers, managers, members of community) but, conversely, in a long term period the economic investments and the land use changes can influence the local culture. Consequently, the cultural indicators have the purpose to quantify the potential impacts of an investment on cultural aspects in a specific territory. Considering the roots of cultural aspects in the context, it is assumed that the use of forest biomass for energy purpose has no effect in the short and medium term on cultural indicator. The potential positive effects may be found only in the long term.

For the purposes of the present study, the authors selected 10 sub-indicators (3 economic indicators, 6 social indicators and 1 cultural indicator) in order to evaluate the effects of

forest biomass utilization for energy production on local development in selected study areas.

The 10 sub-indicators are described in Table 3, evidencing their economic, social and cultural impact dimension, the specific ambit of the impact, and the indicator features. The ambit of impact of forest biomass harvesting concerns: i) the impact on the local economy, ii) the impact on the quality of life, iii) the impact on the social stability, involvement and legitimacy, iv) the impact on health and safety and v) the impact on local traditions and values. The description of each indicator moves from the general definition to the specific issues related to forest biomass for energy purpose.

For each case study the local experts evaluated the effects of forest biomass harvesting on local development using a 5-point Likert scale (-2 = quite negative effect, -1 = negative effect, 0 = no effect, 1 = positive effect, 2 = quite positive effect").

The third indicator ( $I_3$ ) considers the annual potential primary energy that can be obtained from forest biomass in each case study considering as key variables the annual increment ( $m^3 \cdot ha^{-1} \cdot year^{-1}$ ) and the tree composition per forest types. The fuel has an amount of energy - called primary energy - that is converted through combustion in final energy to be used for heating or hot water for sanitary purposes [48]. For this analysis, the calorific values of the main Alpine tree species with a moisture content of 15% (dry wood) were considered (Table 4). Calorific value indicates the amount of heat that develops from the mass (weight) in its complete combustion with oxygen in a calorimeter standardize. In the last step, the calorific value of each tree species ( $kcal \cdot kg^{-1}$ ) was transformed in energy content ( $MJ \cdot kg^{-1}$ ) using the specific wood density. The energy content allowed to calculate the potential energy that can be obtained in a hectare of forest in each case study. Energy potential is considered as a benefit which can be transformed in monetary terms using the local market price.

The last indicator ( $I_4$ ) considers the costs per ton of wood chips, taking into account the

**TABLE 4.** Energy content of main tree species present in the case studies

Tree species	Wood density (kg·m <sup>-3</sup> )	Calorific value (kcal·kg <sup>-1</sup> )	Energy content (MJ·kg <sup>-1</sup> )
Norway spruce ( <i>Picea abies</i> )	450	4857	20.33
Silver fir ( <i>Abies alba</i> )	440	4650	19.47
Dwarf mountain pine ( <i>Pinus mugo</i> )	500	4130	17.29
Chestnut ( <i>Castanea sativa</i> )	580	4599	19.25
European beech ( <i>Fagus sylvatica</i> )	750	4617	19.33
Ash ( <i>Fraxinus excelsa</i> )	720	5350	22.40
Maple ( <i>Acer</i> spp.)	740	4607	19.29
Hop-hornbeam ( <i>Ostrya carpinifolia</i> )	820	4640	19.42
Alder ( <i>Alnus</i> spp.)	540	4440	18.59

<sup>1</sup> 4.186 coefficient used to obtain KJ from kcal. Source: modified by Ilarioni [49].

local productivity and costs. Four type of costs are considered in this indicator: harvesting costs, extraction costs using tractor with winch, chipping costs and transport costs (average distance around 25 km). Labour costs are included in each indicator. The main factor that influences machine cost estimation is the annual utilization rate of machinery; this factor depends mainly on technical reliability of machines, roads conditions, logistics [50]. The labour cost estimation is influenced by the local costs of life and the specific conditions of local workforce in forest sector.

## RESULTS

The sustainability of forest biomass harvesting for energy was assessed through a set of four indicators and in a synthetic way, using the figure of merit (FoM). Firstly, we show the results of each indicator and subsequently we evidence the results of FoM.

$I_1$  was calculated as the mean value of the experts' perceived effects of forest biomass harvesting on ESs (Table 5). The effects can be both negative and positive and are strongly influenced by the local context (e.g.

**TABLE 5.** Mean value of perceived effects of forest biomass harvesting on ESs ( $I_1$ ) by case study

ESs/Case study	Triglav National Park (n <sup>1</sup> =13)	Mis valley (n=5)	Maè valley (n=6)	Gesso-Vermenagna valley (n=8)
Forest products provision	1.31	1.20	1.50	0.29
Water provision	0.00	0.00	0.17	0.00
Natural Hazards Protection	-0.23	0.50	1.17	0.14
Carbon sequestration	-0.15	-0.20	0.50	0.00
Habitat quality	-0.62	1.00	1.33	-0.14
Recreational value	0.08	0.80	1.67	-0.29
<b>Indicator 1 (mean)</b>	<b>0.07</b>	<b>0.55</b>	<b>1.06</b>	<b>0.00</b>

<sup>1</sup> n=number of experts in the study area

presence of protected area, geomorphological and orographic conditions) and the forest management strategies adopted (e.g. silvicultural treatments). Results in Table 5 show that all experts considered positive the effect of forest biomass harvesting on forest products provision (e.g. timber and bioenergy production), with average values ranging from 0.29 in Gesso-Vermenagna valley and 1.50 in Maè valley. Most experts agreed that the removal of woody biomass has negligible effect on the provision of fresh or potable water (average values ranging from 0 to 0.17).

The effects of biomass harvesting on the protection against natural hazards were considered as positive by the experts of the three case studies located in Italy, while negative (-0.23) by the experts of Triglav National Park. According to their answers during the interviews, the Slovenian experts took in consideration the fact that harvesting logging residues increases soil compaction and erosion in fine textured and moist soils, and this aspect is particularly relevant in protected areas (e.g. biotopes).

Concerning the effects on carbon sequestration in vegetation and soil, the experts of

Triglav National Park and Mis valley considered as negative the overall effect (-0.15, -0.20), while the experts of the other two case studies perceived a neutral or moderately positive effect.

The effects of forest biomass harvesting on habitat quality were perceived as positive by Mis valley (1.00) and Maè valley (1.33) experts, while by experts of the Triglav National Park and Gesso-Vermenagna valley were perceived as negative (-0.62 and -0.14).

Also the experts' opinions regarding the effects on recreational value show divergences among case studies. In this case, effects of biomass harvesting were judged negatively in Gesso-Vermenagna valley (-0.29), while were considered positive in Triglav National Park, Mis and Maè valleys (0.08, 0.80, 1.67).

The results of the second indicator ( $I_2$ ), concerning the perceived effects of biomass harvesting on local development, are shown in Table 6. Results evidence that the effects of forest biomass harvesting on local development were considered as positive in all case studies for almost all indicators. Four sub-indicators obtained scores over 1 in all case studies, which are the following: local market diversification,

**TABLE 6.** Mean value of perceived effects on local development ( $I_2$ ) by case study

	Triglav National Park (n=13)	Mis valley (n=5)	Maè valley (n=6)	Gesso- Vermenagna valley (n=8)
Local market diversification	1.31	1.40	1.33	1.14
Local entrepreneurship	1.46	1.60	1.50	1.29
Resource efficiency	1.15	1.40	1.50	1.00
Employment of local workforce	1.69	1.60	1.67	1.25
Increasing income per capita	1.31	1.20	1.00	1.14
Tourism	0.23	1.40	1.67	-0.29
Social and community aggregation	0.85	1.00	1.33	0.71
Political stability	0.23	1.00	1.17	0.43
Human health	0.00	1.00	1.00	-0.29
Property rights and rights of use	0.85	0.60	1.33	0.50
<b>Indicator 2 (mean)</b>	<b>0.91</b>	<b>1.22</b>	<b>1.35</b>	<b>0.69</b>

local entrepreneurship, resource efficiency, employment of local workforce, and increasing income per capita. These results evidence that all the experts considered the use of forest biomass for energy as an important resource for local economic development, while the social and cultural effects are more controversial. Concerning social indicators, two indicators of quality of life obtained a score over 1, while the effects of biomass harvesting on the third one (tourism) were considered negative by the experts of Gesso-Vermenagna valley (-0.29).

The indicators of social stability (social and community aggregation, political stability) obtained positive values in all case studies, while the experts of the Gesso-Vermenagna valley considered the activity of biomass harvesting as potentially negative on human health (-0.29). The effect of biomass harvesting on the property rights and rights of use was considered positive in all case studies.

The results of the third indicator ( $I_3$ ) show the potential energy obtained from forest biomass and are presented in Table 7. The differences in the potential energy from case studies are due to the annual increment and to the characteristics of tree species (wood density and energy content). Gesso-Vermenagna valley present the highest values of annual increment and many species with a high calorific value and wood density (e.g. European beech) form a significant part of the growing stock. On the other side, the low values of annual potential energy in Maè valley are mainly due to the high percentage of softwood species (Norway spruce and dwarf mountain pine).

Finally, the results of the fourth indicator ( $I_4$ ) are reported in Figure 2. The costs collected in the case studies are higher than the data from literature: according to Asikainen *et al.* [51] in Eastern Europe the harvesting, extraction,

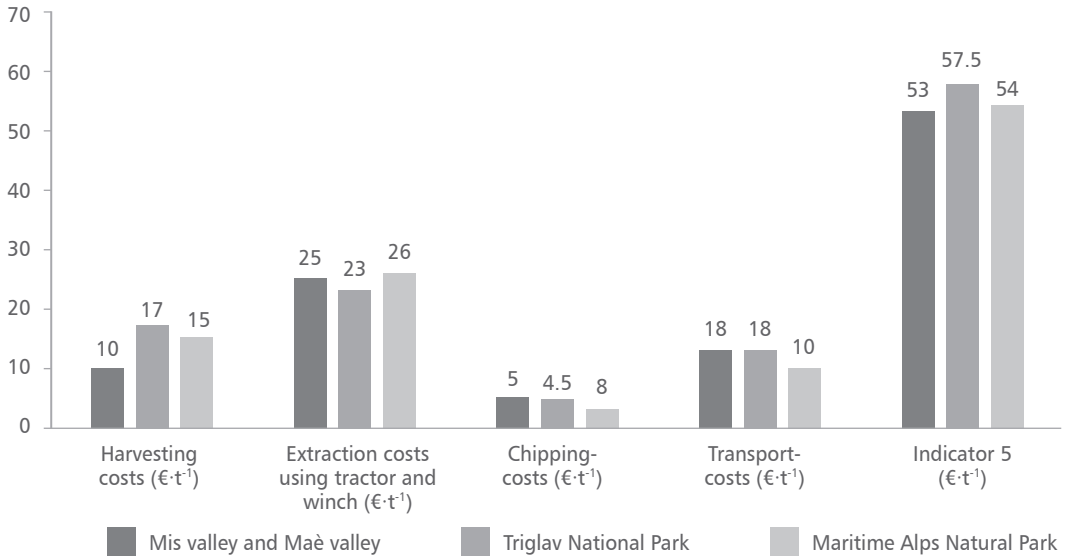
chipping and transport costs of logging residues vary between 20-25 €·m<sup>-3</sup>, while in Western Europe these costs vary between 30-35 €·m<sup>-3</sup>. These differences are due to the higher labour and fuel costs in Western European countries. In Veneto region (Mis and Maè valleys) the total costs (harvesting, extraction, chipping and transport costs) has been estimated at around 53 €·t<sup>-1</sup> fresh wood chips and this value is comparable with the value reported in local literature 65 €·t<sup>-1</sup> fresh wood chips [52]. The Triglav National Park case study shows costs slightly higher for harvesting (17 €·t<sup>-1</sup> fresh chips), while the Gesso-Vermenagna valley is in agreement with Mis and Mae Valley data (for harvesting 15 €·t<sup>-1</sup> fresh chips and for extraction 26 €·t<sup>-1</sup> fresh chips in Gesso-Vermenagna valley).

Finally, FoM was implemented with the support of the four above mentioned indicators (Table 8). From the theoretical point of view, in the present study FoM can vary from a minimum of 1 to a maximum of 10000. The results show that the lowest FoM is found for the case studies of Maè valley (FoM = 60) and Gesso-Vermenagna valley (FoM = 60), while the highest FoM is found for Triglav National Park (FoM = 1200). The greater sustainability of case studies located in Veneto Region is mainly influenced by results of the questionnaire, in particular by the fact that experts considered the forest biomass as an important resource for local development with few negative effects due to harvesting. In Triglav National Park the conservation objectives prevail. In the managed forests (2<sup>nd</sup> and 3<sup>rd</sup> protection zone) the forest management is more oriented to timber production than to wood for energy production. Forest biomass for energy production is a local product mainly used for home heating and deriving from private owned forests which prevail in the park.

**TABLE 7.** Annual potential energy ( $I_3$ ) by case study

	Triglav National Park	Mis valley	Maè valley	Gesso-Vermenagna valley
Potential energy (MJ·ha <sup>-1</sup> ·year <sup>-1</sup> )	55090	56700	39440	98680





**FIGURE 2.** Average costs for wood chips production included the labour costs by case study (source: [52-55])

**TABLE 8.** Results of figure of merit (FoM) for forest biomass harvesting by case study

	$I_1$	Relative Rank $I_1$	$I_2$	Relative Rank $I_2$	$I_3$	Relative Rank $I_3$	$I_4$	Relative Rank $I_4$	FoM
Triglav National Park (n=13)	0.07	5	0.91	3	55090	8	57.5	10	1200
Mis valley (n=5)	0.55	4	1.22	2	56700	8	53	1	64
Maè valley (n=6)	1.06	3	1.35	2	39440	10	53	1	60
Gesso-Vermenagna valley (n=8)	0.00	5	0.69	4	98680	1	54	3	60

## DISCUSSION

The results of the present research show that there are divergences between experts' opinions in the various study areas. These divergences are due to the different local economic, social and cultural contexts: perceptions and opinions regarding sustainability of forest biomass harvesting are deeply related to local conditions. In particular, concerning the perceived effects of biomass harvesting on forest products provision, probably the experts' evaluations take into consideration only the short-term effects of forest biomass harvesting. Conversely, the international

literature shows that the long-term effects of forest biomass harvesting are influenced by complex relationships between harvesting and site nutrients which include the magnitude of nutrient removal and the rate of nutrient cycling [56]. In particular, removal of site organic matter and its associated nutrients may also reduce the concentrations of base cations in soils and foliage and adversely affect future productivity, if site nutrient status is not managed [57].

Concerning the effects on the habitat quality, the differences among case studies seem to be due to the perspective of analysis assumed by single experts. Most of the Italian experts

declared that in their opinion removing the wood residues from forests decreases the risks of insect pollution, pests and fires, and increases the presence of different habitats. On the other side, the experts of Triglav National Park highlighted the negative effects of this activity on saproxylic insects and other deadwood-dependent organisms. Negative opinions of Triglav National Park experts may also be related to the fact that conservation objectives prevail within the park area; outside the Triglav National Park boundaries the opinions of experts could be different. In fact, Nijnik *et al.* [58] assert that the removal of residues and deadwood changes pest population and composition and affect their predator, while other authors evidence that the normal utilization of wood residues after forest operations has a negative impact on wildlife and biodiversity [14, 59]. This negative effect is due to the importance of deadwood components (standing snags, stumps, fallen logs, broken off tops and limbs, twigs) in order to supply food and cover resources for many wildlife species [60, 61]. In addition, Nijnik *et al.* [58] identified three types of negative effects of residue and deadwood extraction (logging residues attract species laying eggs in the piles, soil disturbance affects mosses and species reproducing in the vegetation, and deadwood extraction leads to habitat fragmentation for dependent species), and only one positive impact (removing stumps leads to an increase of sapling of deciduous species).

In addition, most of experts of Veneto Region (Mis and Maè valleys) affirmed that the long practice of close-to-nature forest management can always guarantee the preservation of forest habitat and - at the same time - the wood cutting can help the establishment of the right balance between open areas and the forest itself. This is important not only for recreational values but also for the preservation of habitat mosaic and for the major ecological variability. In general, we cannot always affirm that the harvesting operations, both of standing trees and other biomass, tend to reduce the structural complexity of a forest stand, but surely influence various physical features of the forest landscape and

consequently the recreational value. Moreover, forest biomass harvesting can have positive effects on biodiversity, but harvesting effects and deadwood removal can also produce negative effects on habitat. Management strategies and policy measures must take into consideration experts' opinions and evaluations, weighting and combining them in efficient ways.

Regarding the local development, the results highlight that economic, social and environmental dimensions derived from experts' evaluations must be combined from separate aspects to a unified and multifaceted issue. For example, on one side forest biomass harvesting is considered as a source for local development, and a crucial matter for local community aggregation; on the other side, experts focused their attention also on the possible impacts of biomass power plants, especially for what concerns atmospheric emissions.

In the case studies of Veneto Region, the high scores that experts assigned to the effects of biomass harvesting on employment, local entrepreneurship and community aggregation are related to the past tradition of wood cutting and working. In fact most of the experts perceived biomass harvesting as a practice that could implement again forest related activities, and this role is central as a source of job opportunities to develop the area and maintain people in their territory. Only two sub-indicators show divergences between case studies: human health and tourism. Most of Triglav National Park's experts during the interviews declared that in their opinion bioenergy development is not a tool to increase people participation in decision making process and tourism flows. Besides, around half of the Slovenian experts considered as neutral the effects on human health, while the Italian experts emphasized the positive effects of wood residues removal regards to the insects dangerous for human safety (e.g. *Thaumetopoea pityocampa* L. and *Thaumetopoea processionea* L.).

The two indicators related to the annual potential energy and costs are deeply related to local species characteristics ( $I_3$ ) and to the specific conditions of work in the forest sector ( $I_4$ ). These

indicators can be useful for decision makers in order to carry out a cost-benefit analysis of forest biomass for energy use. We can assert that the most important indicators of FoM are the effects of forest biomass harvesting on ESs and local development ( $I_1$  and  $I_2$ ) because they include all the most important environmental, economic and social aspects. In order to take in consideration the different relevance of indicators, in future applications of FoM to assess the sustainability of bioenergy the four indicators could be weighed differently giving more weight to the effects on ESs and local development.

## CONCLUSIONS

In conclusion, the results of our research show that, according to experts' opinions, forest biomass harvesting for energy has positive effects on most of the ESs. In the specific, environmental sustainability is important in order to maintain a certain standing stock in forest, a long term site productivity and a good level of biodiversity [62]. Economic sustainability is the engine for new business investments, the innovative technologies development and the local economic growth. In particular, the results of this study confirm that the forest biomass use for energy purpose could have positive effects on the creation of new job opportunities and on the diffusion of innovation. Social sustainability is fundamental for ensuring successful formulation and implementation of energy policy, in order to reduce conflicts and improving cooperation among the different groups of interest [63]. Besides, social sustainability is crucial in order to reduce the divergences between local community and decision makers, to increase the inclusiveness of participatory decision making process, and to decrease the conflicts between land users [64]. The results confirm that the forest biomass harvesting for energy could potentially have positive effects on the quality of life of local communities and social cohesion.

The proposed method based on FoM allows comparing and evaluating synthetically the

single case studies in order to highlight any critical points or inefficiencies. The advantages of the method are the simplicity of implementation and the possibility of taking into account simultaneously qualitative variables (e.g. experts' perceptions) and quantitative variables (e.g. costs). The combined analysis of social, economic and environmental sustainability can give clear guidance to decision makers to improve the efficiency of the use of forest biomass for energy, reducing the effects on the environment in general and the ESs in particular.

The main limit of the method is the inability to consider all relevant aspects from the political and technical point of view.

The future steps of the analysis will focus on an integration of the indicators to be used in the FoM in order to consider all aspects of forest biomass harvesting for energy. Besides, the method will be extended to a comparison of the different renewable energies in the case studies considered in the Recharge.green project.

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# Challenges and Opportunities in the Use of Marketing Tools and the Promotion of Non Wood Forest Products-Based Small and Medium Enterprises in the South East Europe

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## Abstract

**Background and Purpose:** The South-East Europe (SEE) region is facing a new market economy era where establishing new businesses is more than needed in all sectors. The forest sector, faced with new market emerging opportunities, is affected by the constant increase of a number of forest related enterprises. This paper describes the challenges and opportunities related to the use of marketing tools for improving business of the small and medium enterprises (SMEs) dealing with non-wood forest products (NWFPs) in SEE region. The research on this subject in the SEE region is scarce despite the rich biodiversity as a solid base for establishing eco-businesses.

**Materials and Methods:** The method used in this paper is quantitative, based on survey data collected from enterprises in the NWFPs sector followed by statistical data analyses using Statistical Package for Social Sciences (SPSS). The survey was conducted during 2011 and 2012 in four SEE countries: Croatia, Bosnia and Herzegovina, Serbia and Macedonia.

**Results:** The results showed that developed channels of distribution, branding and advertising of NWFPs are recognized in all countries as important and very important, but these marketing tools are used only few times per year. The majority of respondents pointed out advertising as the most frequently used and



as the most successful tool. Interviewees' future investments are to be focused on improving equipment for drying, packing, refrigerating and transport. Several entrepreneurs from Macedonia pointed out that they would invest in increasing the number of buying points and herb plantations while almost all Croatian entrepreneurs will invest in advertising, branding and promotion. Majority of respondents from Serbia are also of the opinion that financial resources should be invested in new equipment. The reasons for these investments can be summarized in reducing costs and increasing profit or in faster turnover.

**Conclusions:** The use of marketing tools is important because they have positive influence on sale, improve communication and cooperation between enterprises and with consumers, build reliable buyers and increase the number of consumers, which leads to higher profit and creates new opportunities for NWFPs based enterprises. Intensive use of promotion tools should be seen as an opportunity for these enterprises in the SEE region to overcome current challenges and improve their business. The main reason for this situation, as it was stated in the interviews, is the fact that most of the enterprises do not have a final product. Another reason is lack of funds, because of which the question: "If you have financial sources where you should invest?" was answered with promotion, branding and other marketing tools.

**Keywords:** non-wood forest products, small and medium enterprises, promotion, SEE region

## INTRODUCTION

Small and medium enterprises (SMEs) play a key role in the national, regional and global economy, and provide outsourcing products and services [1]. Products such as mushrooms, herbs, wild berries and other forest fruits (commonly known as non-wood forest products-NWFPs<sup>1</sup>) are an important aspect in the sustainable management of forests. Thus, in order to increase the contribution of this sector to the economic and social development, it is necessary to support the establishment and development of SMEs that base their business not only on wood, but also on NWFPs [2].

There are several factors that can influence the competitive advantage of SMEs. The literature emphasises "the importance of marketing, strategic positioning, and entrepreneurship as key factors in business survival and growth. The ability to identify and operate in a particular market niche enables the firm to exploit a range of specializations and offers protection from larger competitors" [4]. However, marketing in SMEs differs from these activities in large enterprises. Marketing in SMEs is characterised as "haphazard, informal, loose,

unstructured, spontaneous, reactive, built on as well as conforming to industry norms" [5].

This means that significant attention should be given to activities such as production, marketing and promotion. Promotion, as a business activity, is a significant tool for future development of every business, closely connected to increasing employment, poverty elevation, and income of the company [6-8]. Promotion is of great importance and can be a major advantage, especially if SMEs have limited financial resources, but good competences in promotion techniques. Powerful tools to reduce the problems of marketing access for SMEs are, in some cases, e-business and e-marketing.

Promotion is a part of marketing referring to the advertising and selling. It is a mechanism of communication: exchange of information between sellers and consumers [9]. The purpose of promotion is to get people to understand what one product is, what they can use it for, and what is the desire for it. A product or service means nothing unless the benefit of such a service can be communicated clearly to the target market [10]. To be effective, promotional efforts should contain a clear message targeted at a specific audience reached via an appropriate

<sup>1</sup> NWFPs are "goods of biological origin other than wood, derived from forests, other wooded land and trees outside forests" [3]. In this paper, as NWFPs are considered: mushrooms, medicinal and aromatic plants, wild berries and other forest fruits.

channel. Promotion may involve advertising, public relations, personal selling, and sales promotions [8-10].

Marketing definitions, such as marketing as "a social and managerial process by which individuals and groups obtain what they need through creating and exchanging products and values with others" [10] may not necessarily apply to the small firm context due to its unique characteristics. Existing literature has emphasized the need to develop and refine existing marketing models, which can be used to profile the marketing practices in small firms [11].

SMEs are known to be the heart of strong economies, as well as being the backbone of development in rural areas and small towns [12]. Small business marketing consists of business activities relating to: identifying a target market, analysing its potential, and delivering what it takes to satisfy the market [13]. Small firms typically have different requirements with respect to marketing, with their inherent characteristics impacting upon the willingness/ability of the owner/manager to use conventional and/or contemporary marketing tools [14].

Marketing activities such as advertising, public relationships (PR) or branding are not considered to be relevant for successful working of the SMEs, although personal word of mouth communication [15], the characteristics of owners/managers such as skills, abilities, resources [16], and on-going dialog with existing and new customers [15] seem to be a more acceptable practice. Other marketing characteristics of the SMEs are the limited specialist expertise (owners/managers tend to be generalists rather than specialists) and limited impact in the marketplace [17]. Building personal relationships is vital to the company's success and considerable time and effort are invested in maintaining good relations with regular clients [17].

The research on this subject, and especially comparisons between different countries in the SEE region are scarce. In order to ensure the economic benefits of these products, and their

sustainable use, special attention should be paid to marketing of NWFPs [18, 19]. Thus, the main goal of this research is to determine the challenges and opportunities of existing SMEs in the SEE region dealing with NWFPs related to promotion activities in order to improve their current business. The main research question is: "What are the main promotion activities of NWFPs based small and medium enterprises in the SEE region?".

## CONCEPTUAL FRAMEWORK

Businesses face the challenge with macro trends such as globalization, hyper-competition and the Internet [20] in finding ways to maintain prices and profitability [20]. For example the concept of total product [21] offer three components that can help small and medium sized firms to differentiate their product from those of competitors: quality, reputation and service [21].

Developing detailed framework for the marketing process, including an entire set of specific activities to be performed to meet management's strategic goals and objectives [22], is a part of facing marketing challenges for the SMEs. The main answers seem to be better segmentation, stronger branding, and superior customer relationship management [20].

The unique characteristics of SMEs do not perfectly fit into the traditional marketing theory [15, 17] because the SME owners/managers behave and think differently from conventional marketing decision-making practices in large companies [17].

Most SMEs will have a product or a service, which they will offer at a price and which they will promote through some kind of medium that reaches their market place [10] (Figure 1). It can be easily determined that SMEs marketing can be described under the frameworks of the 'four Ps'- '4Ps' [23].

For an SME practitioner to accept a concept such as the '4P's' it must have relevance, therefore, if a simple '4Ps' description is not relevant to an entrepreneur it will not be used [23].

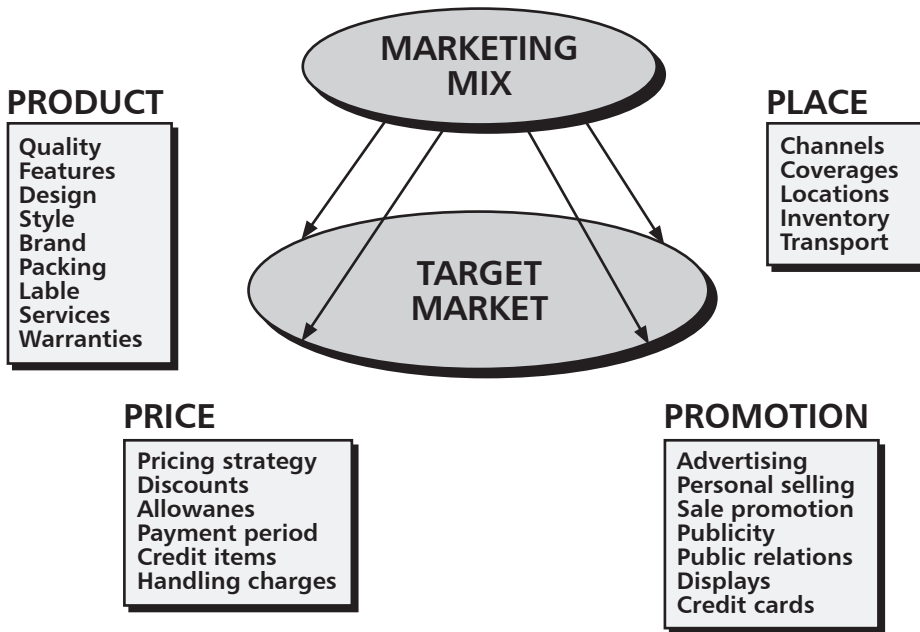


FIGURE 1. 4Ps in marketing (adapted from Kotler *et al.* [10])

However, marketing activities in SMEs will always be pragmatic, practical and relevant to the individual SME [22]. Resnick and Cheng [15] in their 4Ps model for SMEs marketing conclude that small businesses have personal relations with their customers, that they execute business in hand instead of planning, that services and goods are provided according to customers' demands and that they build long-time customer relationships.

## MATERIALS AND METHODS

The research in this paper is descriptive, because it describes and explains the state of the art of marketing tools usage in SMEs business dealing with NWFPs in the SEE region. Four countries, Bosnia and Herzegovina (B&H), Croatia, Macedonia and Serbia, participated in this research. These countries were selected because of their potential for NWFPs resources and SME sector based on these products [24].

Using door-to-door survey technique, primary data were collected from 30 companies from

B&H, 27 from Croatia, 36 from Macedonia and 91 from Serbia. The survey was part of the "Forest Policy and Economics Education and Research" (FOPER II) project and Collaborative Regional Research Teams (CRRTs) topic "Entrepreneurship, markets and marketing of non-timber forest products in SEE region". The necessary data were collected starting from August 2011 until March 2012. Due to the lack of repository system with a list of companies in the NWFPs based sector in the researched countries, the sample size was determined according to the list of registered companies gathered from governmental institutions and from companies willing to participate in this research. Therefore the sample size was not the same for each participating country.

The survey questionnaire consisted of 51 questions, grouped into six topics: 1) socio-demographic characteristics of respondents, 2) basic information about the enterprise, 3) purchasing of NWFPs, 4) processing of NWFPs, 5) selling and trade in NWFPs, 6) business analysis. For this paper seven questions related to promotion activities of small and medium

enterprises were further analysed and presented more comprehensively. The questionnaire included closed and open-ended questions.

The collected data were quantitatively analysed with Statistical Package for Social Sciences (SPSS) version 18, summarized by frequency distribution, selected measures of location and dispersion (mean and standard deviation) and presented in this paper.

## RESULTS

More than 70% of the respondents in all four countries find the use of marketing tools such as developed channels of distribution, familiar product (brand), advertising etc., in SME development as an important and very important segment (Figure 2).

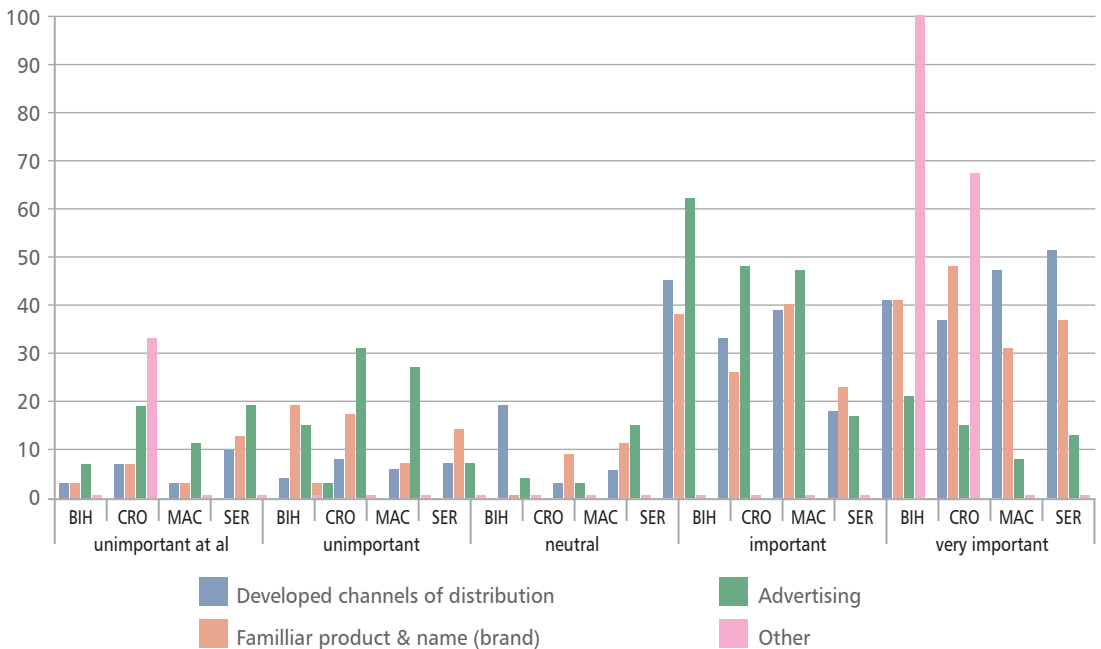
According to 86% of the respondents in B&H, marketing activities of developed channels of distribution are important and very important in this sector, followed by advertising with 83 % and familiar product/name (brand)

with 79% of respondents. They also stated that visiting and participating in fairs is very important.

On the contrary, in Croatia most of the respondents (74%) are of opinion that having familiar product/name (brand) is an important and very important marketing practise in NWFPs sector, followed by developing channels of distribution (70%) and advertising (63%).

Results related to the importance of marketing tools in Macedonia and Serbia are similar. The highest percentage of respondents from both countries (86% in Macedonia and 69% in Serbia) believe that the developed channels of distribution are important and very important as a marketing activity in this sector, followed by familiar product/name (brand) (71% in Macedonia and 60% in Serbia) and advertising (55% in Macedonia and 30% in Serbia).

Although most of the respondents in all four countries consider these types of marketing activities (public relations, advertising and branding) to be important and very important,



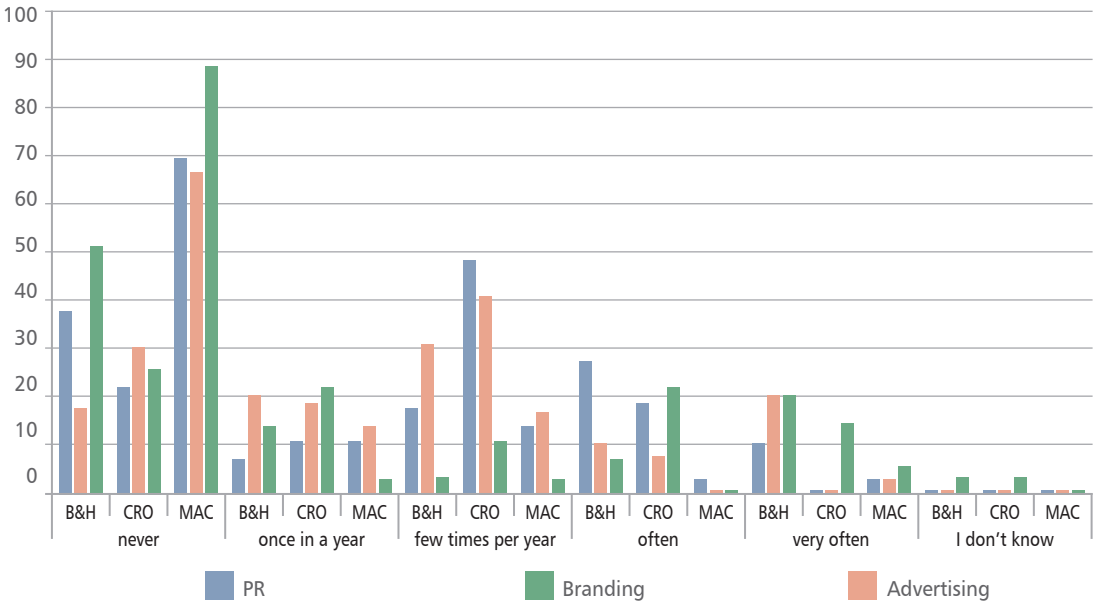
**FIGURE 2.** The importance of marketing tools in % by country (sample size: 30 companies in B&H; 71 companies in Croatia; 36 companies in Macedonia; 91 in Serbia)

they do not use them in their daily businesses, or they use them only once a year or few times per year (Figure 3).

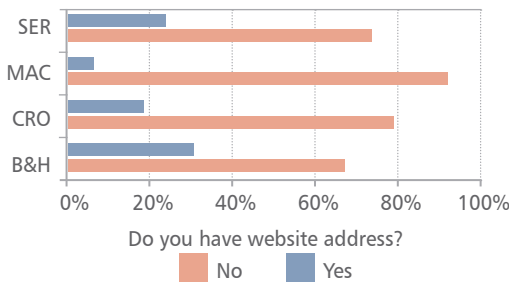
The results have shown general non-use of marketing tools in observed region, with some differences in observed countries. The most obvious are the results from Macedonia where the highest percentage of respondents answered that they had never used marketing tools (69.4 % PR; 66.7% advertising; 88.9% branding). The situation regarding branding is

opposite in B&H (31%) and Croatia (25.9%). However, in Croatia most of the respondents stated that they use PR and advertising “few times per year”. Most of the respondents (57%) in Serbia stated that they currently use “other” marketing tools, such as international and local fairs, social media, etc. Around 1/4 of them (24%) stated that they currently use PR, 28% advertising and 19% branding.

Many companies in all four countries have websites and email addresses (Figure 4). It



**FIGURE 3.** The use of marketing tools in % by country (sample size: 30 companies in B&H; 71 companies in Croatia; 36 companies in Macedonia). Note: Because of the minor differences in questionnaires used in analyzed countries, data for Serbia are not shown in the figure, but only explained in the text.



**FIGURE 4.** The use of websites and email addresses (sample size: 30 companies in B&H; 71 companies in Croatia; 36 companies in Macedonia; 91 in Serbia)

is most visible in Macedonia where 94% of the respondents have positively answered the question, while more than 60% of the respondents in B&H stated that they have websites and e-mail addresses.

Advertising through different type of media (broadcast, newspapers, Internet), branding and fairs are the most successful marketing tools in B&H, Croatia and Macedonia. Respondents from Serbia stated that advertising, branding, and fairs are the most successful marketing tools. An

interviewee from Macedonia explained that “the marketing tools are very useful for my business, I am aware, but my financial resources allow me to go once per year to agriculture fairs where I present my products. In the future I will start using other tools in order to improve my business”. On the other hand, “family brand or branding” was pointed out as a core element in marketing in Croatia, not excluding others as useful marketing tools.

Also, according to the statements of the interviewees from all countries, a conclusion can be drawn that “marketing tools are mostly used to successfully promote companies, products and the quality of the products. At the same time, marketing tools lead to better sale, communication and cooperation between companies and with consumers at the same time, attract reliable buyers and increase the number of consumers”. However, the appliance of these marketing tools is restrained due to a lack of financial resources and a final product.

During the survey the interviewees were asked in the case they had financial resources, where they would invest and why in that concrete activity. Most of them stated that they would invest in equipment, in promotion (branding, advertising), in the improvement of quality, in the cultivation and the plantation of NWFPs, in the increase of the number of purchase points and in business expansion.

Reason for investing in this segment is to secure their businesses on a long-term basis. Higher demand means higher profit, satisfying the market needs, the possibility to sell the products at higher price, etc. It is important to highlight that interviewees from Croatia and Macedonia placed promotion on the top of the list because their opinion is that it will enable more people to see their products, which means higher sale and higher profit. It is an open opportunity for expanding production and for a faster turnover. In Serbia and B&H the respondents stated that their priority was investment in improving the technology and buying new equipment, while in B&H marketing was not a priority at all.

## DISCUSSION AND CONCLUSIONS

The respondents from the analysed countries have a positive opinion on the importance of developed channels of distribution, branding and advertising. In other words, they point out developed channels of distribution and advertising as the most important marketing tool compared to other marketing tools. Still, their business contacts rely on their previous acquaintances, friendships, relatives as it was stated by one interviewee in Macedonia. Building personal networks is vital to the company’s success and they invest considerable time and effort in maintaining good relations with regular clients [17]. Attracting more customers, and thereby increasing business turnaround, is the reason why advertising is important to businesses leading to the increased awareness about the potential of NWFPs [25].

Public Relations, advertising and branding as marketing tools are not often used in daily businesses in NWFPs sector. They are used only once a year or few times per year. According to the results of this research, the uses of such marketing tools for promotion of companies are very rare in the sector of NWFPs in the SEE region. The most common reason is the lack of the final product along with lacking efficient and effective selling, as it was pointed out by an interviewer from Serbia. The overall quantities of NWFPs are exported either raw or semi raw, but in this state they are not ready for direct distribution to end users/consumers [25].

Even though marketing tools are very rarely used by companies in NWFPs sector in the SEE region [6, 25], it can be concluded that in all four countries different marketing tools are often and very often used. According to respondents from B&H, public relation is a tool used often and very often, while Croatian SMEs often use branding. In Macedonia, equal percentage of often and very often used are PR, while branding and advertising is often and very often used marketing tool in Serbia.

Due to oversight during the design of this question, as demonstrated by the research itself, the research team noted that confirmative responses referred to existence of an e-mail address, but the answer was ambiguous on the existence of a website. Therefore several confirmative answers referred only to having an e-mail address. Research conducted abroad shows that e-marketing can be useful for the improvement of SMEs' activities related to promotion [7].

As a conclusion and as the data shows, the most successful marketing tools for NWFPs sector in B&H, Croatia, Serbia and Macedonia are advertising, branding of the products, media, internet and fairs in circumstances when there would be a final product in this sector.

The reasons can be in the fact that enterprises focus on promoting its products and the product's quality. All this leads to achievement of a higher profit, as well as a more economical and efficient manner of working.

The respondents from all countries found that the advanced stage of processing, retail, branded products, a better promotion of companies, the informing of the public and appropriate equipment for the companies are the tools for development of SMEs in NWFPs sector in the SEE region. Additionally, the regulation of the issue of permissions, subsidies, grants, greater use of Instrument for Pre-Accession Assistance funds and soft loans are legislative and supportive measures that would enhance the development of the sector.

According to the respondents, the marketing activities such as advertising, PR and branding were not considered to contribute to SME business at this stage of development due to the fact that the quantities of raw material they collect are not sufficient or they will increase their expenses and decrease their profit. However, several enterprise owners stressed that their personal influence, as well as dialogue and communication with the buyers (the existing and the new ones)

could be defined as self-marketing. This perception could be used as a branding tool contributing to a successful business and increased profit. In addition, one owner of a company that purchases and exports NWFPs from Macedonia states that they have "quality raw materials, think of NWFPs, but without a final product and brand name we cannot make anything more. In this way, we will only export semi-final NWFPs, and others who have purchased and have the final costumers will collect the profits and successes". Intensive use of promotion tools should be seen as an opportunity for NWFPs based enterprises in the SEE region to overcome current challenges and improve their business.

The use of conventional marketing tools, particularly those used by large companies (for example branding or the use of PR) in the SEE countries is still challenging, especially for the small and medium enterprises in the non-wood forest products sector, mostly perceived as expensive and non-profit oriented. The research presented the use of marketing tools as very context dependent and not well developed in the daily businesses of small and medium enterprises. However, promoting the establishment of NWFPs SMEs in the developing SEE region by use of marketing tools can be seen as an opportunity for sustainable use of natural resources, for the improvements of national economies and for eradicating poverty in rural areas.

## Acknowledgments

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# Can Structural Timber Foster Short Procurement Chains within Mediterranean Forests? A Research Case in Sardinia

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## Abstract

**Background and Purpose:** The aim of this paper is to present the idea of a timber short procurement chain as a means to provide an increased value to Mediterranean forests. It is based on the evidence that timber buildings are increasingly used for a number of reasons including sustainability, the speed of erection, and excellent structural and seismic performance. However, most of the timber currently used around the Mediterranean is imported from outside this area.

**Materials and Methods:** The idea is to use the best part of the tree to produce timber boards, while all the remaining part of the tree including the production waste is used for energy production. Important issues to address are the generally low mechanical properties of locally-grown timber such as maritime pine in Sardinia, which would make some wood-based products such as glue-laminated timber not technically viable. Cross-laminated timber panels are a possible solution to this problem because this wood-based product is manufactured in such a way that even with low-quality timber boards it is possible to obtain a medium quality panel. The panel is made of layers of timber boards with the adjacent layers glued under pressure at a right angle. Another issue is the need to grade the local timber, for which a number of specimens must be tested to destruction in order to identify a visual or a machine-stress grading procedure. Last but not least, the panels must be tested to destruction to correlate their mechanical properties to the properties of the boards.

**Results:** The preliminary mechanical tests carried out on Sardinia maritime pine confirm that the material is low-grade because it is characterized by large knots and a significant grain deviation. Nevertheless, when used in the cross-laminated panels, the properties are significantly improved due to the layout of the panel which reduces the influence of defects in the boards on the mechanical properties of the panel.

**Conclusions:** A timber short procurement chain is a possible means to create job opportunities and reduce depopulation, which is particularly important in several regions of the Mediterranean. By adding value to the forests by means of timber production used in prefabricated components employed in low-rise timber buildings, it is also possible to improve forest management and even extend forested areas which have all positive effects on the environment, the landscape and the reduction of hydrogeological hazard.

**Keywords:** biomass for energy production, cross-laminated panels, forestry, short procurement chain, sustainability, timber, wood

## INTRODUCTION

In Sardinia, a typical Mediterranean island, the National Forest Inventory (INSC) [1] states that approximately one fourth of the land is covered by woods, considering as such the areas larger than 0.5 ha with tree density above 10% and potential height above 5 m. The main species are oaks, followed by other broadleaves. Nonetheless, conifers play a relevant role in the island's history and landscape. The average growth of the trees is limited, being estimated in the range of 2 (+/-5%) cubic meters per hectare per year, which is half of the average value in whole Italy. The potential for firewood production is relatively large and traditionally exploited, whereas viable timber production is possible only on very limited land tracts. As a consequence, this production is not really considered relevant in the local silvicultural tradition and all timber used in Sardinia is currently imported (from Austria, Germany, Russia, etc.).

The use of wood as energy source is increasingly important but it is certainly not the best way to exploit the material since it is a low added value product and it terminates the carbon stocking process. Hence the question in the title: could the activation of structural timber short procurement chains, though quantitatively limited, help improve forestry economy in Sardinia and what is its contribution to sustainability? The paper addresses a broad range of different issues that have to be considered and solved in order to make such a development process possible. The activation of a timber short procurement chain is a relevant topic for the whole Mediterranean region (e.g. Greece, Andalusia, Corsica, etc.) as a means to bring some economical development through forestry good practice and wood

processing industry.

To address the topic, a joint research partnership involving foresters, wood scientists and engineers has been initiated some years ago in Sardinia. Currently the work is being developed within the "EDENSO" research project ("Sustainable use of Sardinia forests in construction and energy production"). The aim of this project is to ensure the possibility of using locally-sourced timber to construct the structural skeleton of residential buildings. One of the target of the research is in fact the construction of a small buildings using locally grown timber.

The first question to answer was which tree species should be considered for timber production. Conifers, for many reasons, are the most used species, whereas there is no large scale exploitation of broadleaves in the production of wood-based timber products such as glued and cross-laminated timber panels. Some trials have been conducted using eucalyptus in mixed species panels but none with really autochthonous Sardinian species. In this research project, a widely spread and relatively fast growing conifer, maritime pine (*Pinus pinaster* Ait.), was chosen as the first focus species.

The paper describes the long and complex procedure that has to be successfully completed before making it possible to use a new tree species in the production of structural timber.

## SILVICULTURAL ISSUES IN SARDINIA FORESTRY

The island has witnessed a century long season of pines plantation forestry [2]. The interventions concerned areas of very diverse extensions and were differently motivated

over the course of time. Large works started in the 1950s and included sand dunes fixation interventions. The need to address the hydrogeological hazard issues motivated the interventions at higher altitudes, covering very large areas. On the other hand, the reforestation served also as a way to provide an income for people living in rural areas where agriculture had suddenly become marginal. Furthermore, the attempt to activate a forestry based productive chain has been cyclically encouraged [3, 4]. Within this 'productive reforestation' strategy, the opportunity to feed the Arbatax paper mill encouraged private owners and public managers to plant large extensions with radiata pine.

According to the National Forest Inventory (INFC), the area covered by conifers in Sardinia was about 50000 ha [1]. The current extension of conifers is almost entirely a result of artificial introduction, initially achieved by seeding and later, since late 1970s, by plantation. Seeds and plants were imported in great quantity and it is quite difficult to trace back the geographic origin of the genetic resources used in most stands. The species used, in order of decreasing surface, are: stone pine (*Pinus pinea* L.), Aleppo pine (*P. halepensis* Mill.), Corsican pine (*P. nigra* Arn.), maritime pine (*P. pinaster* Ait.), radiata pine (*P. pinaster* D. Don) and other species, also including *Cedrus* spp. The precise assessment of the relative importance of the species is not straightforward, given the variety of mixed stands that currently exist. Moreover, different species reflect different historical motivations and concern different social and environmental conditions.

The presence of conifers, namely stone, maritime and Aleppo pines, as a part of the autochthonous flora of the island is not easily documented since its heritage is confined to very limited spots. During the artificial expansion phase, almost nothing was done to exploit this genetic reserve and to preserve the pre-existing populations. Beyond the detailed species distinction, from the silvicultural point of view, conifers in Sardinia are divided into two broader groups, the Mediterranean pines (stone, maritime, Aleppo and Corsican pines) and the

others, considered exotic species.

Most of these artificial stands share a common problem: they did not receive the care they would have required while developing from young to mature stands. The public financial support considered only the work needed to have the small trees surviving initial competition, which is very labour-intensive. No forest management plan was drawn and very little of the required thinning and pruning was carried out, mainly because it is generally true that the financial value of the wood collected during thinning does not pay for operation costs. Negative consequences of the neglected care are now evident in various different situations: the ecological development is slowed down, excessive amounts of deadwood foster pathogens and fires, stems are underdeveloped and badly shaped, and the stands are fragile.

In 2007 the Sardinia Region adopted the 'Regional forests and environmental plan' (PFAR [5]) with provisions to activate, over the whole island, district by district, participatory large-scale planning initiatives. A further focus is on the land properties where, with an appropriate management plan, forests can positively contribute to the possibility of living in the countryside. Main forest management problems of Sardinia are very well tackled, at regional level, in the PFAR initial document.

By connecting PFAR work with INFC results, Campus *et al.* [6] extended the analysis, framing the island's silvicultural issues in broad macro-categories. For the macro-category 'Forests originated as conifers plantations', the work points out that these stands have to face a quite diversified range of management objectives. Tourism-needs and tourist-related use limitations are the main issues in many coastal/suburban areas. Relatively young stands could still profit of late thinning. Older stands are faced with contrasting options, particularly if local productivity levels are above average. With limited productivity, it is generally a question of how to favour the development of a more autochthonous stand, possibly using the conifer component that is removed as biomass for energy production and deciding whether

some memory of the plantation should survive or not. If productivity is not severely limited, the regeneration of the pine stand (generally by plantation, though natural regeneration may be a local option) can be taken into consideration. The hypothesis of sustained timber production is proposed with reference to site conditions of this type for maritime pine. This species is in fact quite widespread also in several Mediterranean regions, and has the advantage of being relatively fast growing.

## ADVANTAGES OF TIMBER AS A CONSTRUCTION MATERIAL

Since the construction sector is currently based on environmentally costly processes, there is a great potential for sustainability recovery by reducing the environmental impact of building materials [7]. In addition, the sector is important for achieving the objectives defined in the Kyoto Protocol since the energy required for the management of the built environment is now the main part of the energy costs of the European countries [8]. The ecological balance of a material describes its life-cycle: from extraction of the raw material, through production and processing, until use and disposal. Wood is the only material that requires just water, air and sun to grow. In addition, wood has the ability to incorporate CO<sub>2</sub>, i.e. wood absorbs the harmful CO<sub>2</sub> from the air and returns it to the environment only after combustion. As shown by the European Union in the Sixth Environmental

Action Programme, each cubic meter of wood used in construction corresponds to almost 1 tonne of CO<sub>2</sub> that is stored during the entire life of the building, which in this way plays an important role in mitigating climate change.

The total energy consumption for the production of structural timber compared to other building materials such as brick or concrete blocks is 75% less [7]. This difference derives from the fact that bricks and concrete block production require very high temperatures for a long period of time, which is usually generated by fossil fuels. By contrast, timber production generally implies relatively little emissions and its processing does not require any demanding or expensive technical processes. The disposal of rubble minerals is very expensive while timber waste can be processed and used in wood-based panels or simply burned to produce energy [9]. Last but not least, wood is one of the few renewable building materials which can be produced within a life cycle of 30 to 60 years.

Timber has also the advantage of being aesthetically pleasing, and therefore it is often chosen by the architects both for finishing and for structural members such as columns, beams and ceilings of floors to create a warm and pleasant living environment (Figure 1). In terms of mechanical properties, it is characterized by an excellent strength-to-unit weight ratio, which is approximately the same as steel and almost five times larger than concrete. These results in lightweight structures, like steel structures, that are approximately five times lighter than reinforced concrete structures. The



**FIGURE 1.** Interior of a house where timber has been used for the structural skeleton (walls and roof), for the flooring and for the doors (courtesy of Rubner Holzbau Spa – Bressanone (BZ)).

advantages of a lightweight structure include reduced seismic actions (which are roughly proportional to the mass of the structure) and hence excellent earthquake resistance, reduced cost of foundations, and the ease of transport and erection.

The reduced weight makes it possible to prefabricate large timber components off-site which can then be easily transported to the building site, craned to a position, and then connected to the other members using simple connections (Figure 2). The connections are usually made of steelwork and metal fasteners such as nails, screws and bolts. The prefabrication and the ease of connection results in a significantly reduced erection time as compared for example to reinforced concrete construction where the concrete is usually cast in situ and therefore a formwork is needed for 28 days to support the fresh concrete in order for it to cure and harden. For these reasons, in a number of cases the investors have decided to build with timber as structural material instead of concrete in order to reduce the construction time [10].

Due to all the advantages listed above, timber is nowadays more and more used not only in central Europe and Scandinavia, but also in several countries of the Mediterranean basin such as Italy, France, Spain, Portugal and Greece where there was no strong tradition in the use of wood in construction. However, in most of

these countries timber is not grown locally, but it is imported, often from countries which are very far (Austria, Germany, Russia). The aim of the research project undertaken in Sardinia is to demonstrate the feasibility of using locally grown timber to manufacture modern prefabricated structural components.

## ISSUES TO ADDRESS WHEN USING LOCALLY GROWN TIMBER FROM THE MEDITERRANEAN REGIONS

An important issue to address when using locally grown timber in the Mediterranean regions is the generally low quality of the wood. In Sardinia, for example, most of the plantations have not been subjected to any forest management plan or silvicultural care, resulting in low quality timber, namely timber characterized by large knots, grain deviations, and consequently low mechanical properties (strength and the modulus of elasticity). It is necessary to identify a wood-based product that (i) is of medium quality starting from a low quality timber, and that (ii) can be easily produced in low-cost fabrication plants. Glue-laminated timber is an example of wood-based material that complies with criterion (ii) but not with criterion (i); conversely, Laminated Veneer Lumber (LVL) is an example of a wood-based product complying with criterion (i) but not with criterion (ii).



a)



b)

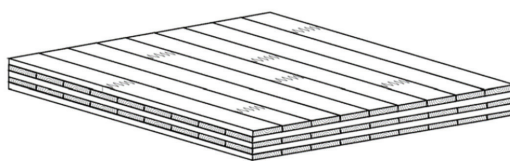
**FIGURE 2.** (a) Crosslaminated timber wall prefabricated off-site (courtesy of Prof. Ario Ceccotti) and, (b) Metal connectors used to connect the prefabricated panel on-site (courtesy of dedaLEGNO).

Cross-laminated timber (CLT) was identified as a wood-based product complying with both criteria. CLT is formed in a similar fashion to the more familiar glue-laminated timber beams (glulam), using small sections of timber boards finger jointed and glued together. Unlike glulam, CLT is a bidimensional (plate-like) member, and the adjacent layers of boards are arranged at a right angle (Figure 3). In this way, the effects of a board defect, such as a knot, on the strength of the panel are markedly reduced and therefore a significant improvement in strength with respect to the boards can be attained [11]. CLT has also comparable mechanical properties in its plane, unlike glulam which has excellent mechanical properties in direction of the boards, and poor mechanical properties in the other directions. Last but not least, CLT does not suffer from significant dimensional variations (shrinkage/swelling) in its plane.

CLT is produced with a hydraulic or pneumatic press which applies the right pressure across the cross layer of boards to ensure an effective gluing process. The presses can have different sizes (and costs) depending on the size of the production plant. Once produced, the panels are cut to size using CNC machines. The entire wall can be prefabricated in the fabrication plant including the openings, and then be transported to the building site and quickly connected to the adjacent panels.

Another issue to address when using timber from new tree species is the need to develop some grading rules.

Due to natural differences in density and the presence of defects such as knots and grain deviation, there is a significant variation in the strength of structural timber, with some pieces eight or more times stronger than others of the same size and species. The way to overcome this drawback is to sort timber into grades based on strength. The pieces which qualify for the better grades are assigned higher working stresses and can therefore be used in smaller sections or over longer spans. The process of sorting timber on the basis of strength is known as strength grading or stress grading. Two methods of strength grading are possible: one is based on



**FIGURE 3.** Crosslaminated timber panel (CLT)

visual inspection and the other one on the use of a grading machine.

The rules relating to the grading indicate the typical characteristics and defects in different strength classes, for a given tree species or group of species, and geographical origin. Both methods of grading, if properly taken, allow a more rational use of solid wood through a more favourable dimensioning of the structure. For the requirements of design, the classification according to strength is expressed by a series of performance profiles for the tree species and quality classes more frequently used.

All building materials have to be legally certified in order to be used as a construction element. Therefore also timber must be classified and certified. When a new wood species (not yet included in the current legislation: i.e. EN338 in EU [12]) is used as structural timber, classification rules have to be verified and possibly recalibrated. This entails a long and expensive process that includes a large number of laboratory destructive tests. The cost of these tests is an investment that has to be done at the beginning, but it will be repaid over the time from the economical, environmental and social benefits brought by development of a timber short procurement chain.

Construction materials, used in Europe, must be graded and certified by applying specific "Harmonised European Standards". For CLT, the first draft of such a standard has been released but not yet approved [13]. For that reason, the suppliers offer CLT that has been given European Technical Approval, i.e. the approval based on a testing carried out according to agreed European levels and relying on the use of certified boards. Therefore, when using a new tree species to produce CLT panels, a number of destructive tests

will also have to be carried out on the panels to determine their mechanical properties.

As an example, since there is no grading rule for Sardinia maritime pine, within the EDENSO research projects several destructive tests are being and will be carried out on the boards and on the CLT made of maritime pine to provide, respectively, the missing grading rules and the properties of the corresponding CLT. These tests are a prerequisite to start a new timber short procurement chain using maritime pine in Sardinia.

## THE IDEA OF A TIMBER SHORT PROCUREMENT CHAIN

The idea of a timber short procurement chain is based on the possibility to use locally grown timber for structural purposes, and corresponding production waste for energy production. By an appropriate forest management and planning, it is possible to grow some selected tree species such as maritime pine, eucalyptus, etc. extensively used in the Mediterranean region. The trees are harvested and the best logs are transported to local sawmills and sawn into boards. After kiln drying, the boards are either visually- or machine-graded to assign each of them to a strength class. The boards are then transported into a workshop and there used within a press to produce cross-laminated timber panels. The panels are cut to size using CNC machines and transformed into prefabricated wall and floor components. The components are then used to construct low-rise residential and office buildings. The parts of trees such as branches, bark, etc. that cannot be used for structural purposes, as well as the planks too weak and all the production waste (sawdust, etc.) are used as biomass for energy production.

The benefits of developing a timber short procurement chain are listed below.

1) By using trees for timber production it is possible to obtain a significant added value with respect to e.g. firewood. This source of revenue can then be re-invested in forest

management, with all the corresponding benefits of (i) an increase in CO<sub>2</sub> sequestration, (ii) the improvement of landscape, and (iii) the reduction of hydrogeological hazard.

2) With a better forest management and with all activities related to timber production, processing and construction, new job opportunities can be created in regions, particularly those in the countryside, often in marginal economic conditions and therefore in strong need of measures to prevent depopulation.

Some possible issues of a timber short procurement chain are listed below.

1) Accurate forest inventory and planning are needed to ensure a continuous flow of wood from the forest to the production plant. Implementation at the district level of the forest planning scheme approved by the Region [5] is vital to this aim.

2) The cost of the locally-produced CLT panels may be comparable or even higher than the material imported from abroad, due to the different scale of the production plants in countries such as Austria where the production volume is significantly higher than in less productive Mediterranean regions.

## THE EDENSO RESEARCH PROJECT

As previously mentioned, the research project entitled 'Sustainable use of Sardinia forests in construction and energy production' investigates the possibility of fostering local short procurement chains in Sardinia, by exploiting locally grown maritime pine in the construction of residential buildings and for energy production. An important aim of the project is to obtain a grading rule for Sardinia maritime pine, which is a prerequisite to use this timber as a structural material. The hope is, in the future, to extend the grading rules of maritime pine also to Italy and to Europe in general, so as to foster maritime pine from all Mediterranean for structural purposes. The second aim of the research is to produce some first prototypes of CLT panels made of Sardinia maritime pine and to test the panels to destruction to investigate



their mechanical properties. This is a potentially interesting outcome of the project as maritime pine is a tree species widely available in Italy and in the Mediterranean region and not particularly expensive. Thirdly, the project also aims to investigate the potential for using the production waste and the wood not suitable for structural purposes as energy source.

The project is funded by Sardegna Ricerche, a public research association of Sardinia, within the Cluster Research Programme "Materials for Sustainable Construction". It involves as the Department of Architecture, Design and Urban Planning of the University of Sassari; the Nuoro Forestry School of the Department of Agriculture of the University of Sassari; the Department of Civil and Environmental Engineering and Architecture of the University of Cagliari; and the IVALSA Trees and Timber Institute, a research institute of the CNR, the National Research Council of Italy, in Florence. There are also some industrial partners as part of the project. The research has been developed through the following steps.

1. With the support of the local council of Pattada, a mountain township in central Sardinia, two maritime pine plantations with stands suitable for logging and processing were

identified, extensively surveyed and sampled (Figures 4, 5). On selected standing trees, based on measurements taken at different heights (Figure 6), the first preliminary grading was applied by sorting for structural and energy use.

2. Trees were harvested by a local co-operative and the logs were finally assorted based on their size and their external defects (Figure 7).

3. The logs were then transported to the local Carcangiu sawmill, where different boards required to build the grading rules and to produce the CLT panels were cut. Each board was then subjected to a non-destructive measurement of the Modulus of Elasticity using acoustic tool for measuring stress wave velocity (Viscan-Microtec).

4. After kiln drying, the required boards (approximately 950) were subjected to non-destructive measurements of their physical properties (density, humidity, defects etc.) using the machine purposely developed by Microtec. The aim was the calibration of this machine in order to enable the machine strength grading of Sardinia maritime pine.

5. The required boards were visually characterized and then tested to destruction in order to measure their strength and correlate this values to the presence of defects, knot diameters and positions, grain deviation, etc.

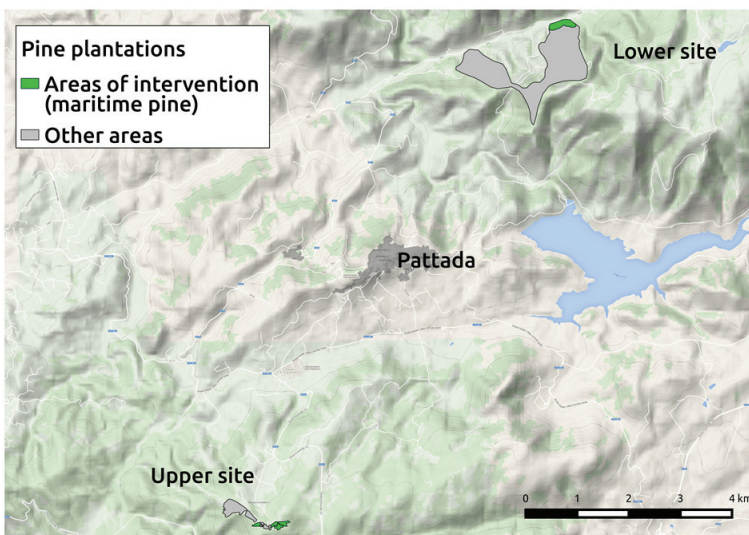


FIGURE 4. Pine stands owned by the Pattada local council



FIGURE 5. Inside the pine plantation



FIGURE 6. The assortment of a standing tree

6. Based on the results of phases #4 and #5, the visual and machine based grading rule for Sardinia maritime pine will be developed and proposed for approval.

7. By applying the newly developed grading rules, suitable boards will be selected for the production of some prototypes of CLT panels by Area Legno, a CLT producer. These panels will then be tested to destruction to determine their mechanical properties and to correlate them to the properties of the single boards.



FIGURE 7. The final assortment of the bole into logs

8. At this stage it will be possible to estimate the distribution of the wood production among the different value classes: structural timber, other boards, firewood, energy resource (chips, sawdust).

9. A small one-storey public building will finally be constructed in Sardinia out of Sardinia maritime pine by a local constructor, as a demonstration building.

## CONCLUSIONS

This paper investigates the possibility to develop a timber short procurement chain. Reference is made to Sardinia, where currently no timber is produced from the local forests, but it could be easily applied to other Mediterranean regions. The idea is to start from an appropriate forest management to develop a production of timber boards that, even if low-grade, can be effectively incorporated into medium quality cross-laminated panels. These panels are prefabricated off-site into local production plants and then used as structural components in low-rise timber buildings. These buildings are easy and very quick to construct, aesthetically pleasing, seismically resistant, and sustainable. The construction waste is then used for energy production. Issues to address are (i) the need to develop appropriate grading rules for new timber species (e.g. Sardinia maritime pine), and (ii) the mechanical characterization of the cross-laminated panels made of new timber species. These issues are currently being addressed in Sardinia by a specific research project named 'EDENSO'.

The reference, in the title of the paper, to "short procurement chains" implicitly frames the work in the context of sustainability. Why is procurement chain length a relevant topic? Not only because of the environmental costs of the transportation of goods, for example, when wood pellets are imported from the other side of the Atlantic, but also because of all the ecological, social and cultural aspects that are involved. Rural areas of Sardinia are

dramatically losing inhabitants, which means that living there appears no longer sustainable. Making an income is a relevant problem but not the only one. Perceiving the choice of remaining in the home town as a meaningful perspective is equally relevant. If local timber acquires some value, the total value of the production from the forest is positively influenced. Even though the direct effect on the incomes might be quite limited, what makes the difference is the positive change in the cultural and social perspective. This change can represent a good reason for local inhabitants to decide to live at their home instead of migrating elsewhere.

The limitation of the proposed solution is that an industrial plant has to be set up and maintained to produce CLT panels, meaning that a production flow has to be maintained, year after year, above given minimum levels.

In the worst case scenario, considering all aspects, due to the limited potential production, one could conclude that such an industrial plant is not economically sustainable. In this case, having established a grading rule for the use of maritime pine grown in Sardinia as structural timber will nonetheless contribute to giving the opportunity to a more valuable production from existing plantations. When the possibility of combining this with other species might eventually appear, the option of CLT production in Sardinia might be reconsidered.

In the best case scenario, obviously, it may be concluded that the establishment and the management of the production plant is economically sustainable. In this case, by collecting and organizing in a coordinated system all the scattered productive patches of plantations (or forests) that exist throughout Sardinia, the minimum annual supply of timber required to keep the business running is estimated to be sufficient. CLT production and processing plant(s) are then established. In this scenario, great care will be required in order to limit and manage the environmental impacts that timber plantation, growing and exploitation can cause, but, on a slightly

wider scale, this can ensure that forests will be looked after and even that living in rural or mountainous areas could develop a new appeal.

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# Economic Valuation of Urban Trees: Ribnjak Park Case Study, Zagreb

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## Abstract

**Background and Purpose:** Population growth, urbanisation and technological development are creating a growing need for urban forests and parks, which are becoming green oases for recreation and relaxation. Apart from the sociological and economic components, urban forest valuation is presented through tourism, the market value of main and secondary forest products, and the growing value of real estate in the vicinity of green areas. Environmental economics explores the optimal ratio between the costs and the benefits received from the investment in the environment. The aim of this research is monetary valuation of urban trees.

**Materials and Methods:** A Danish model for tree value determination was applied in Ribnjak Park as a case study. The model is based on tree growing costs and the present value. It is limited by the subjective aesthetic tree value estimation, but it is used in Europe because of its practicality. Individual tree value estimation is used because of the tree damage from vehicles or new residential buildings. The method is suitable for individual trees or groups of trees, but it is not appropriate for forest stands. Twenty random selected trees from nine different tree species have been analysed in the park. Diameter at breast height, tree height, expected age, aesthetic value and location were recorded for each tree. Furthermore, ecological, social and health tree values were taken into account separately with the calculation of points.

**Results:** According to the evaluation, the average monetary value of one tree in Ribnjak Park is 542 EUR. The average diameter at breast height is 57.86 cm with the average age of 96.14 years. Plane trees have the highest value in comparison to other sampled species.

**Conclusions:** Tree values vary depending on age, dimension or aesthetic values. The disadvantage of this method is in the estimation of very old tree value and in high involvement of personal estimation, which creates an opportunity for future development of the model and for its modification. The calculation of urban forest values is a complex process because of its impossibility to do a market evaluation of all of the benefits that could be considered as public wealth.

**Keywords:** urban tree, urban forest, evaluation, compensation price

## INTRODUCTION

The importance of trees and forests in industrial cities has always been related to health, recreation and aesthetic benefits. Forests and forest lands are especially important from a local, social and cultural point of view. Individual trees and other forest complexes have not been sufficiently recognised in the planning of the urban environment development. The purpose of the valuation is to provide information necessary for decision making because available resources are scarce and it is important to manage those resources in a way that contributes to human well [1]. There is a need for research on the role of urban forests and trees in improving the environment and on their relation to social functions, such as mental and physical benefits [1, 2].

One of the most common purposes of urban forests and parks is to provide an opportunity for recreational activities. Walking is the most common type of recreation with a 81% share in Finland [3] and 74% in the Netherlands [4]. The average visit rate in Italy is only four times a year, but one visit lasts almost four hours. The average visit rate is much higher in Finland, with 72-110 visits a year, but the visits last usually from 30 minutes to one hour [5]. Other common activities include cycling, jogging, picnics or picking berries and mushrooms. Forests closer to big cities are the most frequented ones. The Dutch State Forest Service has estimated for city forests approximately 1000 visitors per hectare a year. The majority of visitors are aware of the importance of ecological management over the past ten years [6].

In the survey on the City of Salo (Finland) half of the participants pointed out remoteness as the main reason why urban recreational areas are underused [7]. Stress reduction, measured through the muscle tension, blood pressure and the brain's electric activity can be found in the human body within few minutes of being exposed to a green area [8]. The key factor for safety in forests is visibility, which presupposes the active management of the ground floor, giving the impression of a controlled area [6].

Social interaction and cohesion is another way of linking nature and health. While European research on this subject matter is still scarce, several researches conducted in Chicago (USA) show that green surfaces, especially trees, contribute to social interaction between neighbours [9] since they reduce the sense of social isolation, one of the factors which cause depression.

The traditional methods for evaluating the forests' value include the opportunity costs, the estimated maintenance costs and the forest production value. These methods are based on assessing the market value of forest goods, so their use in city forests is limited [10]. The methods used in evaluating the benefits (services) of forests without including the market value are: Contingent Valuation Method (CVM), Hedonic Price Method (HPM) and Travel Cost Method (TCM). Additional methods for evaluating the benefits of city forests include the tree value evaluation and the ecological benefit evaluation [4, 10]. Putting value on urban forest benefits helps the decision makers to make informed decisions about urban forests, ideally based on cost-benefit analysis. This is in line with the concept of usable science, where scientific results can serve as valuable information to the political actors in the process of deliberation [11]. The objective of this research is to determine monetary values of urban trees in Ribnjak Park using Danish method.

## MATERIALS AND METHODS

### Research Object

Ribnjak Park is located in the centre of Zagreb and stretches along the eastern walls of Kaptol. It was named after the bishop's fishponds that existed there until the 19<sup>th</sup> century. Afterwards the entire area was transformed into an English type park with waterfalls, exotic plants, a decorative statue "Stid" (eng. shame) by Antun Augustinčić and a monument to the Croatian poet Ivan Goran Kovačić. The present park has the area of 40000 m<sup>2</sup>; it has a 30 year old *Taxus baccata* L., an old specimen of *Cedrus atlantica*

Manetti, many magnolias and a liquidambar. The park was fully open to public in middle 1940 and it was extended northward to include the former canonical gardens. Landscape architecture plans, which were made in 1946, are still actual today and they include some modern Croatian sculptors [12].

Ribnjak Park, as a green area in the old town, is surrounded by a frequent traffic road with tram lines and thousands of cars per day. During the day, there is an active children playground in the park and during the night it is a local club circuit. In the meantime there are tourists visiting the park and citizens walking their pets. It is clear that such a frequently used green area needs improvements in services and management.

## Research Method

The benefits of trees and forests in the city are: i) social (recreation), ii) aesthetic and architectural, iii) climate and physical, iv)

economic (the market value of forest products such as timber, mushrooms and berries, increased property value, tourism), and v) ecological. The selected method for determining the price of trees presupposes the value of wood based on the following factors: size (diameter at breast height - DBH, tree height), expected age, aesthetics, location, form and other special features (Table 1).

The method is based on the depletion principle; it is spread widely and used in Europe on account of its practicality [2]. It is necessary to determine the price of individual trees to be able to be compensated in case of damages caused by cars or construction. Notwithstanding, this is based on subjective estimation to a certain extent. It is usually used for individual trees or groups of trees, and it is not appropriate for woody areas. Moreover, it does not directly calculate the values offered by the trees (shade, pollution absorption).

**TABLE 1.** Example of determining the price of trees using the Danish formula [17]

VAT 03 – Tree evaluation – Danish model developed in 2003			
Locality: Zagreb, Ribnjak Park Species: Oak Date: May 12 <sup>th</sup> 2014 Registration No: 17		Pn - price of new tree Cn - new tree circumference Cd - damaged tree circumference E - setting up costs a- real age (years) b- expected age (years) Average with two decimals Result in 100 of monetary units If a < b/2, than A=1	
Basic value (B) = E + (Pn /Cn) * (Cd - Cn)		B=629.53	
Health (H)	Rating (0-5, 5 max.)	Location (L)	Rating (0-5, 5 max.)
Roots	5	Adaptation	3
Trunk	4	Architecture	3.5
Limbs	1	Aesthetics	4
Smaller branches	3	Visibility	3.5
Twigs, leaves, buds	4	Surroundings	4
Total / 25	H= 0.68	Total / 12.5	L= 1.44
$\text{Age (A)} = \sqrt{\frac{(b-a) \times 2}{b}}$		A= 0.87	
Tree value = B * H * L * A			

The evaluation of the trees' ecological benefits tries to determine how much trees influence the city climate (for example, in terms of shade, evapotranspiration and air circulation changes). These benefits were valued as an alternative cost of the environment control, and they include the people's willingness to pay for air pollution control or for noise reduction. A concrete example would be energy saving for heating and cooling [13, 14]. In areas with relatively dense woods it is possible to improve the quality of air by 5-10% in a limited area [15, 16]. In 1991, researchers in Chicago determined that the value of the removed air pollution totalled \$1 million. The trees' potential for reducing energy consumption for residential cooling and heating was also researched. The tree coverage of 10% (roughly three trees per building) decreases energy consumption by 5-10%. Even though this approach is suitable for evaluating all green surfaces, many countries still lack information on the influence of trees on the city climate.

The values of individual trees were calculated based on the formula developed by Danish professor Thomas B. Randrup [17]. According to Randrup *et al.* [17], the model has been developed to be used under four different circumstances: (i) a tree is damaged to a degree to which the tree will have to be felled, (ii) an existing tree needs to be protected during a construction period, and a penalty value is calculated in order to prevent possible damage, (iii) one or several trees need economic valuation due to expropriation, (iv) a tree is damaged to an extent where it loses value but does not need to be removed. In this research randomly selected samples of 20 trees from all park areas were chosen and the first approach (i) was used. According to this method, the value of trees was determined based on the following indicators: size, expected age, aesthetics, location, shape and other special features. The method does not take into consideration the influence the trees have on the climate. The value of trees was obtained from several parameters: basic values, location coefficient, health and age. The basic value is the initial parameter expressing the value of 1 cm of the tree's circumference; it is multiplied with grades that either decrease or

increase the value. The estimated parameters of location and health are in correlation and could significantly change the estimated economic value. Real tree age could be measured on site but expected tree age is a rough estimation of biological tree parameters. The parameters like Roots, Adaptation, Trunk, Architecture, Limbs, Aesthetics, Smaller branches, Visibility, Twigs, Leaves, Buds and Surroundings are ranked according to the scale from 1 to 5. It means that the best quality is attributed with grade 5. This process could be very subjective and it is a weak part of the methodology.

## RESULTS

According to the selected methodology, the basic physic and aesthetic characteristics of the trees were measured or estimated (Table 2). Some variables were measured, like diameter at breast height (DBH) and tree height, while others were estimated. Those variables depend on researcher's judgment and have significant influence on the tree economic value.

In the calculation of the prices, the circumference of the new seedling for all species totals 19 cm, and the cost of setting up all new seedlings is 132.44 EUR. The wholesale price of the new seedling is based on the price list of Zagrebački Holding d.o.o., Zrinjevac Affiliate (Table 3).

According to the evaluation of trees in Ribnjak Park, the average monetary value of one tree is 542 EUR. The average tree diameter at breast height is 57.86 cm with the average age of 96.14 years (Table 3). According to the used formula (Tree value = B·H·L·A), the total value of 11 measured trees is 81914.01 HRK (10849.53 EUR).

Plane trees have the highest monetary value in comparison to all other species which have been sampled in this research. Spruce trees have the lowest monetary value (Figure 1).

The economic value calculated in this research represents the utility and the aesthetic value as one value. In urban forests and parks the share of aesthetic value has the highest share in the total economic value (TEV).



TABLE 2. Dimensions of trees and the aesthetic features assessment

Species	Circumference (cm)	Height (m)	Health condition					Location attractiveness				
			Root	Trunk	Limbs	Small branches	Leaves	Adaptation	Architecture	Aesthetics	Visibility	Environment
Horse chestnut	183	20.4	4	2	3	3	3	3	3.5	4	3.5	4
Northern red oak	255	24.3	3	5	4	3	5	3	3.5	4	3.5	4
Plane tree	300	23.5	5	2	3	4	5	3	3.5	4	3.5	4
Large-leaved linden	160	20.4	4	3	3	4	4	3	3.5	4	3.5	4
Ash	131	23	3	3	4	3	4	3	3.5	4	3.5	4
Ash	153	31.6	3	5	4	3	3	3	3.5	4	3.5	4
Ash	165	27.2	3	4	3	2	3	3	3.5	4	3.5	4
Ash	122	21.4	4	3	4	2	2	3	3.5	4	3.5	4
Maple	105	19.9	3	4	5	3	4	3	3.5	4	3.5	4
Beech	200	21.4	4	2	3	4	5	3	3.5	4	3.5	4
Ash	230	26.8	3	5	4	3	4	3	3.5	4	3.5	4
Ash	160	20.5	4	3	4	3	3	3	3.5	4	3.5	4
Ash	145	16.1	5	4	4	3	3	3	3.5	4	3.5	4
Ash	164	19.8	3	3	3	3	2	3	3.5	4	3.5	4
Ash	190	25.2	3	5	4	3	3	3	3.5	4	3.5	4
Spruce	125	22.3	4	4	2	3	5	3	3.5	4	3.5	4
Oak	233	24.1	5	4	1	3	4	3	3.5	4	3.5	4
Ash	248	36	3	5	4	4	4	3	3.5	4	3.5	4
Ash	180	23	3	5	4	3	5	3	3.5	4	3.5	4
Ash	185	24	4	5	4	4	5	3	3.5	4	3.5	4

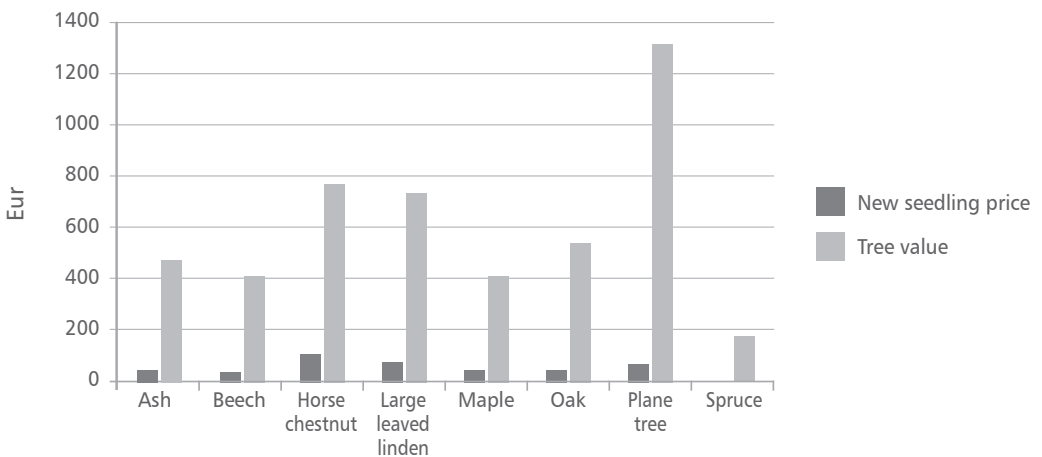


FIGURE 1. The comparison of average tree prices

**TABLE 3.** Economic value results

Species	Diameter (cm)	a		b		A	New seedling price wholesale (EUR)	L	H	B (EUR)	Price (EUR)
		Age (years)	Expected age (years)								
Horse chestnut	58.28	97	150	0.84	108.08	1.44	0.6	1058.34	772.79		
Northern red oak	81.21	135	200	0.80	52.98	1.44	0.80	785.32	732.23		
Plane tree	95.54	159	300	0.97	75.23	1.44	0.76	1236.90	1320.01		
Large leaved linden	50.95	84	150	0.93	84.77	1.44	0.72	756.51	735.45		
Ash	41.71	69	150	1.00	52.98	1.44	0.68	441.83	435.50		
Ash	48.72	81	150	0.96	52.98	1.44	0.72	502.77	502.53		
Ash	52.54	87	150	0.91	52.98	1.44	0.6	536.01	425.29		
Ash	38.85	64	150	1.00	52.98	1.44	0.6	416.90	362.58		
Maple	33.43	55	150	1.00	52.98	1.44	0.76	369.81	407.40		
Beech	63.69	106	150	0.76	39.74	1.44	0.72	507.62	405.06		
Ash	73.24	122	150	0.61	52.98	1.44	0.76	716.07	481.30		
Ash	50.95	84	150	0.93	52.98	1.44	0.68	522.16	479.42		
Ash	46.17	76.9	150	0.99	52.98	1.44	0.76	480.61	522.48		
Ash	52.22	87	150	0.92	52.98	1.44	0.56	533.24	396.56		
Ash	60.5	100.8	150	0.81	52.98	1.44	0.72	605.26	511.37		
Spruce	39.8	66.3	200	1.00	7.42	1.44	0.72	172.69	180.23		
Oak	74.2	123.6	200	0.87	44.50	1.44	0.68	629.53	542.12		
Ash	78.98	131.6	150	0.49	52.98	1.44	0.8	765.93	439.52		
Ash	57.32	95.5	150	0.85	52.98	1.44	0.8	577.56	570.72		
Ash	58.91	98.1	150	0.83	52.98	1.44	0.88	591.41	626.98		

## DISCUSSION AND CONCLUSION

Constant social changes and an increased pressure on different types of green surfaces are a challenge to the traditional maintenance method. Due to insufficient information about the social needs and the expectations of different user groups, problems related to the maintenance and various conflicts have been occurring during the past couple of decades.

Green urban projects are often designed according to architectural and aesthetic standards which are not directed enough to the local public and its specific needs.

The use of methods for urban tree valuation has been recorded in Croatia. Historically the oldest attempt is the tree pricing method by Marinković [18] and Piškorić [19] which uses equation to calculate the value of an individual tree in the park and other areas based on the

material value of timber which is then multiplied based on species' significance, condition and importance, as well as on the location of the tree. The most comprehensive research regarding the aesthetic and the recreational value of Croatian coastal forests was carried out in period from 1995 to 2001 [20, 21]. The economic impact of the aesthetical/ambient value of forests in relation to tourism and the local population was estimated only for the coastal part.

In the region, the forestry sector, mostly due to its long tradition, has not fully adapted to the changing of the paradigm of forestry from a "timber-gaining" part of the economy to a service that manages forest in a multifunctional way, providing equally important timber, non-wood products and services to a large number of user groups. This is even more apparent when it comes to urban forests. In the last few decades, the urbanization was growing in the main cities of South East Europe region. In these cities, the density of the population has increased dramatically, which also changed the borders of the cities. This is connected both to the economic growth and to the process of industrialization. The behaviour of urban residents, being a part of the social system, influences urban forests, and, vice versa, the amount and the accessibility of these critical resources shape human behaviour. Knowing the behaviour of urban residents could provide a valuable input to the management and the decision making related to urban forests. The demand for green surfaces with their related social and cultural services will make them more appealing, and increase their number in the future. The applied Danish method is based

on the depletion principle, according to which the value of the trees is reduced according to the higher age. The method uses the biological predefined maximal DBH according to the predefined expected tree age, and calculates the economic tree value which has the DBH smaller than the predefined maximum. The main obstacle in applying this method is that it cannot be successfully used when the tree exceeds the expected age.

When developing or applying a model for plant appraisal, it is important that it could be used by the entire green industry and accepted by the law framework [17, 22]. A variety of approaches have been used to estimate the value of urban trees. Depending on the objective of the valuation, the existing tree valuation methods can be divided into four groups [23]. Indirect methods such as the Contingent Valuation Method (CVM), the Hedonic Price Method (HPM) and the Travel Cost Method (TCM) are most widely used for valuation of all green areas [24]. These methods are not considered to be the official valuation methods for urban trees [25]. Among direct methods, the formula method like the Danish one [17] is an appropriate method for the individual tree assessment [23].

The social development and the increasing number of urban forest users will also increase the need to determine the economic value of the urban green infrastructure and trees. Since a unique evaluation method does not exist, future benefits and values of the urban forests will change depending on the need and the trends of the people who use them, and they should be included in calculation.

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# Mapping and Assessment of Ethno-Medicinal Trees in Built Up Areas - University of Port Harcourt, Nigeria

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## Abstract

**Background and Purpose:** Several urban tree species are important in ethno-medicine, especially in the developing tropical regions. Their assessment in urban landscapes is becoming an important issue. The study assessed and mapped the ethno-medicinal trees in the built up area land use type of the University of Port Harcourt, Nigeria, with a view to examining their spatial variation in terms of composition and diversity between the residential and non-residential areas of the University Park.

**Materials and Methods:** The study employed the use of geographic information system (ArcGIS 9.3) for the mapping. Built up area land use was subdivided into residential and non-residential where the ethno-medicinal trees were recorded, identified and enumerated. Global positioning system was used to determine the coordinates of each tree. The species composition and diversity were calculated and a comparison was made between the residential and non-residential land use types. The pattern of spread of the ethno-medicinal trees was determined by the nearest neighbour analysis.

**Results:** A total of 37 ethno-medicinal trees species were found in the study area, while the species composition was 499 in the residential area and 438 in the non-residential area. *Azadirachta indica* was the highest (233) in composition. Ethno-medicinal tree species in the study area consist of 19 families of which Anacardiaceae, Rutaceae, Moraceae and Combretaceae were the highest. Species diversity was higher in the non-residential land use (2.698) than in the residential land use (2.222).

**Conclusion:** The nearest neighbour analysis reveals that the z-score value was higher in the non-residential area (-23.06) than in the residential area (-0.30), but the pattern of distribution in both areas were clustered. The study recommended periodic monitoring and the assessment of ethno-medicinal trees in the study area for conservation purposes.

**Keywords:** geographic information systems, nearest neighbour analysis, urban forestry, species diversity, species richness, species evenness

## INTRODUCTION

The assessment of ethno-medicinal plants in urban landscapes is becoming an important issue, especially in the developing world, where many of these plant species are being used as avenue [1] and ornamental trees. Urban forestry integrating ethno-medicinal plants is particularly beneficial to mankind and its importance cannot be underestimated, especially in this era, when the developing countries are witnessing tremendous changes [2, 3]. However, Ajewole [4] explained that urban forestry is a planned, integrated and systematic approach to the management of trees and woodland/forest resources in urban and peri-urban areas for their contributions to the physiological, sociological, psychological and economic well-being of the urban society.

Since the first earth summit in Rio de Janeiro, there has been a sustained global awareness of the importance of the superfluity of biodiversity and natural resources from tropical forests for several purposes, which included the potent ethno-botanical uses of the plants in these forests [5]. World Health Organization (WHO) [6] reported that about 80% of the population in the developing countries depends on medicinal plants in the treatment of diseases, and that medicinal plants represented a primary health source for the pharmaceutical industry. Hence, WHO produced guidelines for the global use of traditional medicine. According to Dambatta and Aliyu [7], the use of herbal medicine in Nigeria represents a long history of human interaction with the environment, and the plants used in traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Nwauzoma and Dappa [8] noted that herbal or traditional medicine has been a major aspect of the socio-cultural heritage in Africa for hundreds of years even before the advent of conventional medicine. In addition, Wahab *et al.* [9] reported that the use of medicinal plants as a source of relief from illness is as old as mankind. As a result, the ethno-medicinal plants require adequate and periodic monitoring and assessment which can help to understand their structures (the diameter

at breast height - dbh, species composition, tree height, crown spread, biomass and tree location) [10] and aid their environmental sustainability for use and human survival in such societies.

The application of geographic information system (GIS) in phenomenon location has been well spelt in various spatial-related studies and this can be extended to urban forestry. According to Wood [11], GIS in urban forestry has long been recognized as a useful tool in the management of natural resource development, land use planning, wildlife management, environmental planning and forestry planning. Miller [12] also noted that urban tree mapping and inventories are key areas that can be greatly enhanced by GIS. GIS is therefore a tool that gives urban foresters and planners the ability to manage and predict the future growth of the urban forests in a better way [11, 13].

There are several studies on ethno-medicinal trees in Nigeria [8, 9, 14-16]. The majority of these studies used a questionnaire to carry out the inventory on ethno-medicinal trees. The inventories were not carried out within an institution, except in the work of Jimoh *et al.* [17] in the University of Agriculture Campus, Makurdi, Nigeria. The mapping of ethno-medicinal trees with the use of geo-information technologies such as GIS in natural forest and agro-ecosystem was carried out in Pakistan and Spain [18, 19], but such work is rare, especially in institutional-based environment, such as universities, research Institutes and teaching hospitals globally. This study therefore focused on mapping and assessing the ethno-medicinal trees within the University Park of the University of Port-Harcourt with a view to examining their spatial variation in terms of composition and diversity between the residential and non-residential areas of the Park.

## MATERIALS AND METHODS

### Study Area

The research was conducted in the University Park, University of Port Harcourt, Port Harcourt, Nigeria. Covering the total area of 461 ha the University Park extends from 04°52'30" to

04°55'00" north latitude and from 06°54'40" to 06°55'40" east longitude (Figure 1). Port Harcourt is situated in subequatorial region. A moist south-west wind and northeast trade winds are responsible for the variations in weather conditions experienced in Port Harcourt City. The moist south-east air stream blows over the region between February and November and the region receives its rains, while the northeast trade wind blows over Port Harcourt in from November up to February, which ushers in the dry season. Port Harcourt records a mean annual temperature of 28°C; relative humidity is generally high over Port Harcourt with a mean annual figure of 85%. The peak of rainy season usually occurs from June to October, with the total annual rainfall of more than 2500 mm. The soil of Port Harcourt city is of the recent alluvial soil. Port Harcourt is dominated by low-lying coastal plains which structurally belong to the sedimentary formation of recent Niger delta, with an elevation less than 15.24 m.

## Land Use Map Generation

SPOT imagery of the study area of 2.5 m x 2.5 m spatial resolution was acquired from Google Earth, 2013 version to generate the land use map. The imagery was geo-referenced in ArcGIS 9.3 to world geographic coordinate system (WGS 84). Land use types were captured in polygons from which built up area land use was dissolved. The built up area land use for this study was further subdivided into the residential area and the non-residential area.

## Tree Mapping Generation

The coordinates (i.e. latitudes and longitudes) of all ethno-medicinal trees in the built up area of the University Park were recorded by global positioning system (GPS) of Garmin eTrex 30 with the precision level of  $\pm 7$  m. An experienced traditional healer was assisted in identifying the ethno-medicinal uses of the recorded tree species. Tree specimens were collected, identified, pressed and deposited in the herbarium

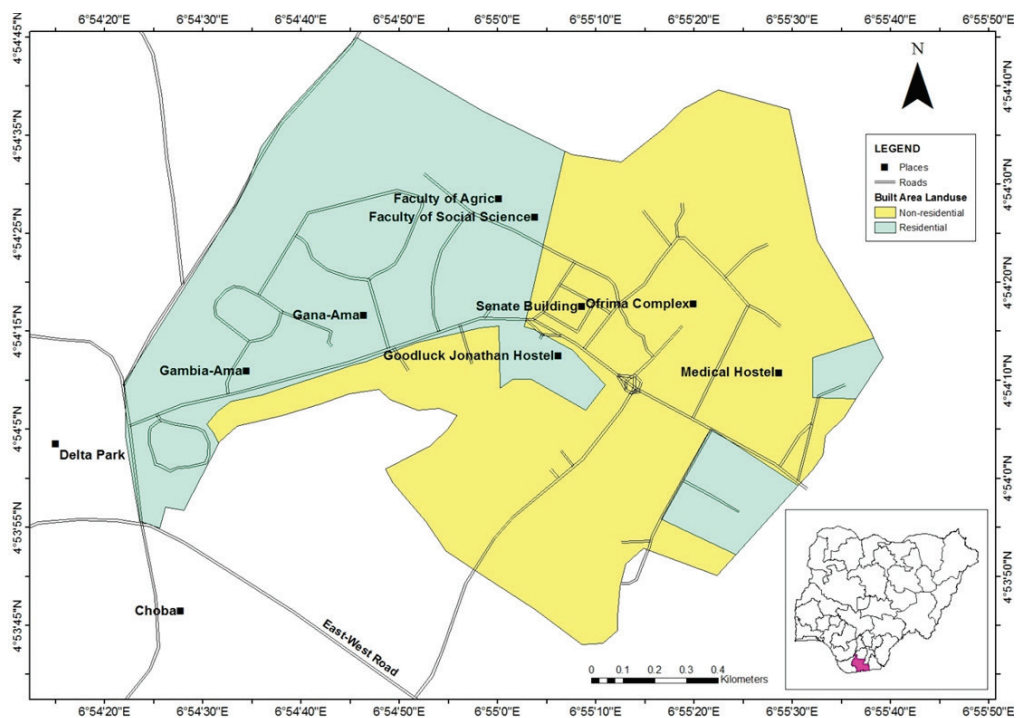


FIGURE 1. Map of University Park, University of Port Harcourt



of the Department of Forestry and Wildlife Management, University of Port Harcourt, for referencing. The recorded coordinates were imported to ArcGIS 9.3 for mapping.

### The Determination of Ethno-Medicinal Tree Diversity, Richness and Evenness

Tree species diversity index ( $H'$ ) was computed using Shannon and Wiener's diversity index, a commonly used index in the literature on biological diversity and ecological monitoring [20, 21]:

$$H' = -\sum_{i=1}^s p_i \ln(p_i)$$

where:  $p_i$  is the proportion of individuals belonging to the  $i$ -th species calculated as  $p_i = S/N$ ;  $\ln$  is logarithms (base  $e$ ),  $S$  is the number of individuals of one species, and  $N$  is the total number of all individuals in the sample.

The species richness was determined using Margalef's index [22] expressed as:

$$D_{mg} = S-1/\ln N$$

where:  $D_{mg}$  is Margalef's index,  $S$  is the number of species,  $N$  is the total number of individuals encountered, and  $\ln$  is the natural logarithm (base  $e$ ).

Species evenness of ethno-medicinal trees was calculated using Pieolu's index [23] modified by Magurran [24]:

$$E' = H'/\ln S$$

where:  $H'$  is the Shannon-Wiener diversity index and  $S$  is the number of species.  $E'$  is constrained between 0 and 1.

### Statistical Analysis

Descriptive analysis was used to describe the composition and diversity in both residential and non-residential areas of the built up areas. The nearest neighbour analysis was used to determine and compare the pattern of distribution of ethno-medicinal trees in both residential and non-residential land use types using the z-score value. This analysis was performed in ArcGIS 9.3 version.

## RESULTS

### Species Composition of Ethno-Medicinal Trees

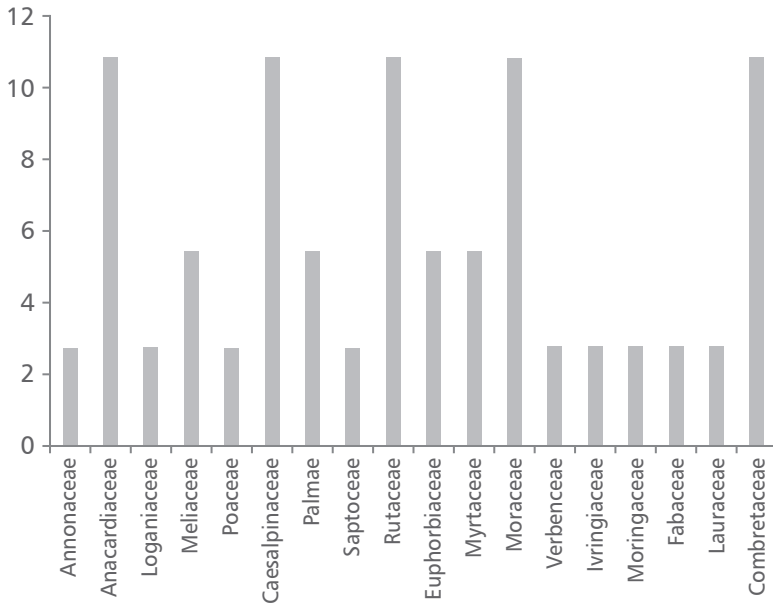
The species composition of ethno-medicinal trees in the residential and non-residential areas of the built up area land use is shown in Table 1. A total of 37 tree species of ethno-medicinal importance were found in the study area. However, the total frequencies of ethno-medicinal trees were 936 of which 499 were in residential and 437 in non-residential areas. Margalef's index showed the ethno-medicinal tree species richness in residential and non-residential areas of the built up areas in the University Park as 4.0240 and 4.6035 respectively (Table 2). According to the results of the family diversity of ethno-medicinal tree species in the study area (Figure 2), Combretaceae, Anacardiaceae, Caesalpinaceae, Moraceae and Rutaceae families had the highest occurrence of four ethno-medicinal tree species, each in the University Park built up areas. Species representing the four (4) families above possess the ability to perform dual functions of local medicines and edible fruit or shade provision, and therefore they are planted or protected in the built up areas of the university.

### Species Diversity of Ethno-Medicinal Trees in the Residential Areas (RAs) and Non-Residential areas (NRAs)

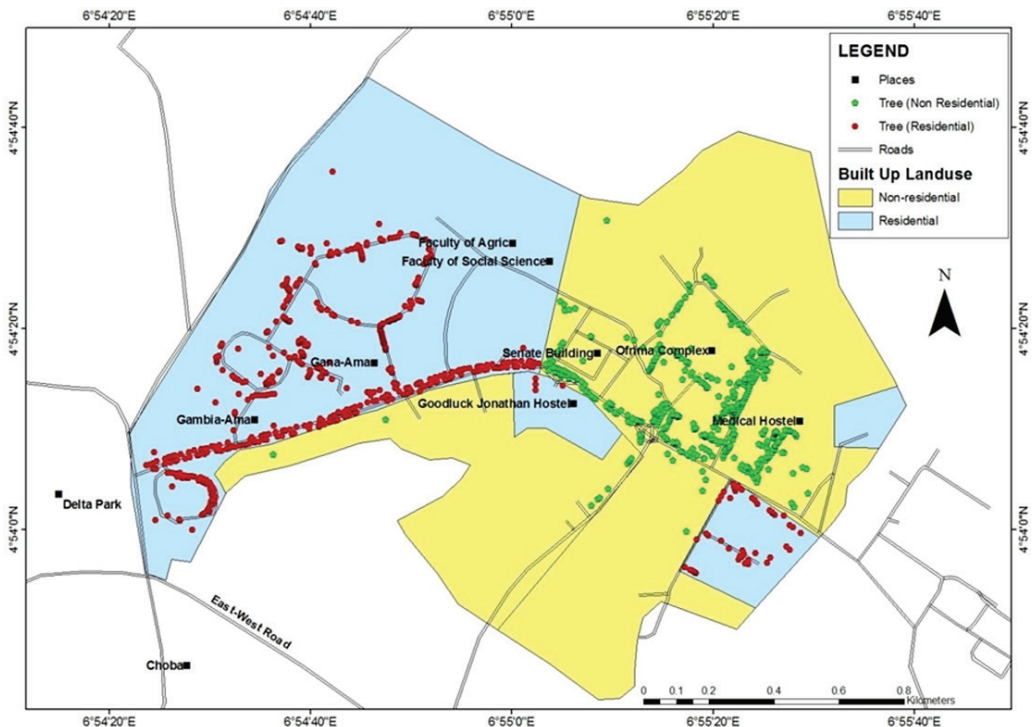
Species diversity of ethno medicinal trees in the study area is shown in Table 3. Ethno-medicinal tree species were more diversified in NRA (2.6981) than RAs (2.222) due to the fact that NRAs mostly include ornamental trees which may not be given priority in the RAs. Plants used for medicine and food are usually cultivated in home gardens.

### Spatial Distribution of Ethno-Medicinal Trees

Spatial distribution of ethno-medicinal trees (Figure 3) revealed that most of the ethno-medicinal trees were found along the roads within the University Park, especially *Azadirachta indica* A.Juss, which was prominent along the



**FIGURE 2.** Diversity of ethno-medicinal trees in built up areas of University Park, University of Port Harcourt, Nigeria



**FIGURE 3.** Distribution of ethno-medicinal trees in residential and non-residential area

**TABLE 1.** Species composition and richness of ethno-medicinal tree

S/N	Species	Common name	Local medicinal uses	RA	NRA
1	<i>Annona muricata</i> L. (Annonaceae)	Sour sop	Leaves used to lessen effect of cancer, fruits eaten as snacks	0	2
2	<i>Anacardium occidentale</i> L. (Anacardiaceae)	Cashew	Diarrhea, diabetes, fruits eaten as snacks	19	14
3	<i>Anthocleista vogelii</i> Planchon. (Loganiaceae)	Cabbage tree	Root used to treat gonorrhoea	1	0
4	<i>Azadirachta indica</i> A.Juss (Meliaceae)	Neem tree	Decoction of leaves and stem barks as antimalarial	172	61
5	<i>Bambusa vulgaris</i> Schrader ex Wendl. (Gramineae)	Bamboo	Abortifacents, leaves used as male contraceptives	2	0
6	<i>Bauhinia tomentosa</i> L. (Caesalpinaceae)	Yellow Bauhinia	Diarrhea, dysentery and diabetes	1	0
7	<i>Borassus aethiopicum</i> Mart. (Palmae)	African fan palm	Aphrodisiac, boiled tender roots as snacks and condiments	3	12
8	<i>Chrysophyllum albidum</i> L. (Sapotaceae)	Cherry/African star apple	Fruits as snacks	2	9
9	<i>Citrus limon</i> (L.) Burm.f. (Rutaceae)	Lemon	Fruits and leaves used for cough and malaria	7	3
10	<i>Citrus reticulata</i> Blanco (Rutaceae)	Tangerine	Fruits and leaves used for cough and malaria	1	0
11	<i>Citrus sinensis</i> (L) Osbeck (Rutaceae)	Sweet orange	Leaves used for cough, ringworm and malaria	25	19
12	<i>Citrus paradisi</i> Macfad. (Rutaceae)	Grape fruit	Fruits and leaves used for cough and malaria	0	5
13	<i>Crotom zambesicus</i> Mull-Arg. (Euphorbiaceae)	Bushveld	Root as antidiabetic and antimalarial	1	13
14	<i>Delonix regia</i> (Boj. Ex Hook) Raf.(Papilionaceae)	Flame of the forest	Anthelminthic/ornamental	17	14
15	<i>Elaeis guineensis</i> Jacq. (Palmae)	Oil Palm tree	Malaria, fruits used for palm oil	79	88
16	<i>Eucalyptus camaldulensis</i> Dehnh (Myrtaceae)	Eucalyptus	Sore throat	34	27
17	<i>Ficus exasperata</i> Vahl. (Moraceae)	Sand paper tree	Wound healing	1	0
18	<i>Ficus mucoso</i> Welw. (Moraceae)	Fig tree	Snake bite	0	2
19	<i>Ficus sur</i> Forssk. (Moraceae)	Wild fig	Wound healing	0	1
20	<i>Gmelina arborea</i> L. Roxb. (Verbanaceae)	Gmelina	Wound healing	10	8
21	<i>Hura crepitans</i> L. (Euphorbiaceae)	Sandbox tree	Purgatives	12	32
22	<i>Irvingia gabonensis</i> (Aubry- Lecomte ex O'Rorke) Baill. (Irvingaceae)	Wild mango	Food condiment	2	0
23	<i>Mangifera indica</i> L. (Anacardiaceae)	Mango	Leaves and stem bark as antimalarial	60	45
24	<i>Milicia excelsa</i> (Welw.) CC Berg (Moraceae)	Iroko	Malaria, body pain and antiaging	0	4

**TABLE 1.** Species composition and richness of ethno-medicinal tree - continuation

S/N	Species	Common name	Local medicinal uses	RA	NRA
25	<i>Moringa olivera</i> Lam. (Moringaceae)	Moringa, miracle tree	Diabetes and hypertension	1	0
26	<i>Musanga cecropioides</i> R.Br. ex Tedlie (Cecropiaceae)	Umbrella tree	Stomach ache, hypertension	1	0
26	<i>Peltophorum pterocarpum</i> (DC.) Backer ex Heyne (Fabaceae - Calsalpinaceae)	Copperpod	Leaves used to treat pain at child birth	0	2
28	<i>Persia americana</i> Mill. (Lauraceae)	Avocado pear	Hypertension	1	7
29	<i>Psidium guajava</i> L. (Myrtaceae)	Guava	Leaves used as antimalarial	8	28
30	<i>Spondias mombin</i> L. (Anacardiaceae)	Hug plum	Chest pain, antiaging	1	1
31	<i>Spondias cytherea</i> L. (Anacardiaceae)	Golden apple	Antiaging	0	2
32	<i>Senna fistula</i> L. (Caesalpinaceae)	Indian laburnum	Skin infections and laxatives	0	4
33	<i>Senna</i> spp. (Caesalpinaceae)	-	Laxatives	0	1
34	<i>Terminalia superba</i> Engl. & Diels (Combretaceae)	Limba, Afara	Stem bark used for treatment of malaria	0	1
35	<i>Terminalia mantaly</i> H. Perrier (Combretaceae)	Button tree	Ornamental	8	1
36	<i>Terminalia catappa</i> L. (Combretaceae)	Almond fruit	Fruits edible and leaves boiled for malaria	30	30
37	<i>Terminalia ivorensis</i> A. Chev. (Combretaceae)	Black afara	Stem bark used for treatment of malaria	0	2
<b>Total</b>				<b>499</b>	<b>438</b>

RA - residential area; NRA - non-residential area

Source: Field survey, 2014

**TABLE 2.** Species richness of ethno-medicinal trees (Margalef's index -  $D_{mg}$ )

Land use type	S	S-1	N	lnN	$D_{mg} = (S-1)/lnN$
Residential area	26	25	499	6.2126	4.0240
Non-residential area	29	28	438	6.0822	4.6035

S - number of species; N - total number of individuals encountered; ln - natural logarithms (base e);

 $D_{mg}$  - Margalef's index

Source: Field survey, 2014

major roads from Delta Park entrance to the University Teaching Hospital (UPTH) road. *A. indica* was used as an avenue tree in the study area, while *Mangifera indica* L. was prominent within the residential area. *Terminalia catappa* L. spread across the two areas because of its role in shade provision during hot weather, while

*Psidium guajava* L. was more frequent in the non-residential area.

### The Nearest Neighbour Analysis

The pattern of spread of ethno-medicinal trees through the use of the nearest neighbour analysis is shown in Table 4. The nearest neighbour

**TABLE 3.** Species diversity (Shannon and Wiener's diversity index) of ethno-medicinal tree in University of Port Harcourt, Nigeria

Species	Residential area				Non-residential area			
	S	pi	ln pi	pi ln(pi)	S	pi	ln pi	pi ln(pi)
1 <i>Annona muricata</i>	0	0	0	0	2	0.0045	-5.4036	-0.0243
2 <i>Anacardium occidentale</i>	19	0.0380	-3.2701	-0.1242	14	0.0319	-3.4451	-0.1098
3 <i>Anthocleista vogelii</i>	1	0.0020	-6.2146	-0.0124	0	0	0	0
4 <i>Azadirachta indica</i>	172	0.3446	-0.0653	-0.3671	61	0.1392	-1.9718	-0.2744
5 <i>Bambussa vulgaris</i>	2	0.0040	-5.5214	-0.0220	0	0	0	0
6 <i>Bauhinia tomentosa</i>	1	0.0020	-6.2146	-0.0124	0	0	0	0
7 <i>Borassus aethiopum</i>	3	0.0060	-5.1159	-0.0306	12	0.0273	-3.6008	-0.0983
8 <i>Chrysophyllum albidum</i>	2	0.0040	-5.5214	-0.0220	9	0.0205	-3.8873	-0.0796
9 <i>Citrus limon</i>	7	0.0140	-4.2686	-0.0597	3	0.0068	-4.9908	-0.0339
10 <i>Citrus reticulata</i>	1	0.0020	-6.2146	-0.0124	0	0	0	0
11 <i>Citrus sinensis</i>	25	0.0501	-2.9937	-0.1499	19	0.0433	-3.1396	-0.1359
12 <i>Citrus paradisi</i>	0	0	0	0	5	0.0114	-4.4741	-0.0510
13 <i>Croton zambesicus</i>	1	0.0020	-6.2146	-0.0124	13	0.0296	-3.5199	-0.1041
14 <i>Delonixregia</i>	17	0.0340	-3.3813	-0.1149	14	0.0319	-3.4451	-0.1098
15 <i>Elaeis guineensis</i>	79	0.1583	-1.8432	-0.2917	88	0.2009	-1.6049	-0.3224
16 <i>Eucalyptus camaldulensis</i>	34	0.0681	-2.6867	-0.1829	27	0.0616	-2.7870	-0.1716
17 <i>Ficus exasperata</i>	1	0.0020	-6.2146	-0.0124	0	0	0	0
18 <i>Ficus mucoso</i>	0	0	0	0	2	0.0045	-5.4036	-0.0243
19 <i>Ficus sur</i>	0	0	0	0	1	0.0022	-6.1192	-0.0134
20 <i>Gmelina arborea</i>	10	0.0200	-3.9120	-0.0782	8	0.0182	-4.0063	-0.0792
21 <i>Hura crepitans</i>	12	0.0240	-3.7297	-0.0895	32	0.0730	-2.6172	-0.1910
22 <i>Irvingia gabonensis</i>	2	0.0040	-5.5214	-0.0220	0	0	0	0
23 <i>Mangifera indica</i>	60	0.1202	-2.1185	-0.2546	45	0.1027	-2.2759	-0.2337
24 <i>Milicia excelsa</i>	0	0	0	0	4	0.0091	-4.6994	-0.0427
25 <i>Moringa oleivera</i>	1	0.0020	-6.2146	-0.0124	0	0	0	0
26 <i>Musanga cecropioides</i>	1	0.0020	-6.2146	-0.0124	0	0	0	0
26 <i>Peltrophorum pterocarpum</i>	0	0	0	0	2	0.0045	-5.4036	-0.0243
28 <i>Persia americana</i>	1	0.0020	-6.2146	-0.0124	7	0.0159	-4.1414	-0.0658
29 <i>Psidium guajava</i>	8	0.0160	-4.1351	-0.0661	28	0.0639	-2.7504	-0.1757
30 <i>Spondias mombin</i>	1	0.0020	-6.2146	-0.0124	1	0.0022	-6.1192	-0.0134
31 <i>Spondias cytherea</i>	0	0	0	0	2	0.0045	-5.4036	-0.0243
32 <i>Senna fistula</i>	0	0	0	0	4	0.0091	-4.6994	-0.0427
33 <i>Senna spp.</i>	0	0	0	0	1	0.0022	-6.1192	-0.0134
34 <i>Terminalia superba</i>	0	0	0	0	1	0.0022	-6.1192	-0.0134
35 <i>Terminalia mantaly</i>	8	0.0160	-4.1351	-0.0661	1	0.0022	-6.1192	-0.0134
36 <i>Terminalia catappa</i>	30	0.0601	-2.8117	-0.1689	30	0.0684	-2.6823	-0.1834
37 <i>Terminalia ivorensis</i>	0	0	0	0	2	0.0045	-5.4036	-0.0243
<b>Total</b>	<b>499</b>			<b>2.2220</b>	<b>438</b>			<b>2.6981</b>

S - number of individuals; pi - proportion of individuals belonging to the i-th species calculated as  $pi = S/N$ ; N - total number of all individuals in the sample; ln - natural logarithms (base e)

Source: Field Data analysis, 2014

**TABLE 4.** Nearest neighbour analysis

Land use	Nearest neighbour ratio	z score	Decision
Residential area	0.30	-0.30	Clustered
Non-residential area	0.42	-23.06	Clustered

Source: Field Data analysis, 2014

ratios of ethno-medicinal trees were 0.30 and 0.42 in the residential and non-residential area respectively.

The z-score revealed standard deviation of -0.30 in the residential land use type and -23.06 in the non-residential land use type. It may be inferred that the distribution pattern of ethno-medicinal trees in both residential and non-residential land use was clustered. This analysis shows the plants' locations in Figure 3, while the pattern may be due to the same environmental quality functions (avenue and shading functions) of the ethno-medicinal trees.

## DISCUSSION

Different land use forms have a significant effect on the species composition and diversity of flora resources of an ecosystem. The results from the study indicate high species diversity for the two land use forms with remarkable differences in the composition. Barbour *et al.* [25] noted that a large index value indicates greater species diversity; an index value above 2 is regarded as medium to high species diversity. Ethno-medicinal tree species diversity in the built up areas (RAs – 2.22 and NRAs – 2.69) are higher than 2 in the University Park, the University of Port Harcourt. It can then be rationally regarded high. Higher species composition of ethno medicinal trees in the RAs (499) may be due to the conservation measures of the residents by the cultivation and protecting of the trees. Larinde and Oladele [26] reported that the residents of the University of Port Harcourt plant and protect medicinal and fruit bearing trees in their homesteads. The culture of conserving frequently used medicinal plants in home gardens and traditional healers' premises

were equally observed in south western Nigeria [27] in similar studies. Commonly used plant species are cultivated around residential areas for easy access and utilization. *A. indica* (172) and *M. indica* (60) are frequently employed in the treatment of malaria in tropical West Africa; this may be associated with the abundance of the two tree species in the RAs, since malaria has been reported to be the most prevailing disease among the poor population of the developing countries in West Africa [28]. *A. indica* contained Gedunin (seed oil), Nimbolide (leaves) and Azadirachtin (stem bark) as the active chemical compounds that inhibit the effect of malaria parasite *Plasmodium berghei*. *M. indica* contained 1,2-benzenedicarboxylic acid that has antimalarial properties [29-31], and therefore they are planted in the RAs to meet the local needs as an antimalarial among the low income earners. Of the 499 plant population recorded in the RAs, ethno-medicinal tree species such as *Elais guineensis* Jacq. (79), *M. indica* (60), *Citrus spp.* (33), *T. catappa* (30) and *Anacardium occidentale* L. (19) have multiple uses: in the local medicine, in food provision, in generating additional household cash income and in providing environmental services. Dau and Elisha [32] noted the species abundance of plants with multiple usages in farmlands among farmers in Bauchi state of Nigeria. Abundant species in the NRAs comprise of ornamental and shade providing plants, and the species with massive branching habits observed in the survey include *Hura crepitans* L. (32), *M. indica* (45), *P. guajava* (28), *T. catappa* (30), *Delonix regia* (Boj. ex Hook.) Raf. (14), *A. occidentale* (14), *Crotom zambesicus* Müll.-Arg. (13) and *Terminalia mantaly* H.Perrier (8). Besides the beautification of the university landscape, shade provision is an essential service provided

by the trees during hot tropical weather. People enjoy the cool breeze from the trees in sunny days to ease the tension, while the students are usually clustered around the trees for relaxation during extreme hot weather periods for stress relief. There are a lot of references on positive psychological and physiological effects of urban forests such as the stress reduction, and anxiety and depression management [33, 34].

An adequate understanding of the ecological indices of an ecosystem could provide better management approach for sustainable utilization of tree resources, especially in urban centres. Also, species diversity in a land use form determines the functioning of such area; RAs in the study is richer than NRAs in composition as a result of the functions thereof due to its ability to support livelihood and traditional healthcare. However, the tree diversity in NRAs possesses the potential for ecological restoration, unlike the RAs in the study. The z-score of the nearest neighbour analysis revealed a clustered arrangement of ethno-medicinal trees in the study. This is an indication that the trees were purposely arranged to satisfy a dual function of shade or fruits bearing and of landscape beautification, and therefore they are located close to buildings or as avenue trees, as revealed in Figure 3. The collection of plant parts for local medicine is usually done without permission from the university management, while stem barks and leaves are in most cases harvested

indiscriminately without recourse to the survival of the plants. The total and unsustainable removal of *A. indica* and *M. indica* stem barks around the breast height have sometimes resulted in the death of many stands of these species.

## CONCLUSION

Changes in land use/cover have a direct impact on ecosystem services in many ways, such as on the flora and fauna diversity, the products and services for human survival and the environmental balance. This study has confirmed the capacity of GIS to map ethno-medicinal trees. The species composition was higher in the residential area, while the species diversity was higher in the non-residential area. It is therefore recommended that there is need for periodic monitoring and assessment of ethno-medicinal trees in the study area for effective management and sustainable utilization. The monitoring should include other land use along with a public awareness campaign and the training of the residents on conservation measures for adequate knowledge of ethno-medicinal trees conservation. The training on the identification of ethno-medicinal trees, and the deliberate planting of other ethno-medicinal plants should be encouraged and the existing ones should be maintained by agencies concerned.

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