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Session A: **Natural resource management and potential of rural area**

PARTICIPATION OF STAKEHOLDERS ON PROTECTED AREA MANAGEMENT IN TURKEY: CASE OF KÜRE MOUNTAINS NATIONAL PARK

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Introduction

In the recent years, participation of stakeholders in planning and management processes of natural resources is one of the principles of good governance. Stakeholders have different expectations from natural resource management. Participation of stakeholders in natural resource planning and management depends on their interest and their level of knowledge on these resources [1].

Küre Mountains are internationally important due to richness of animal species, geological structures, and natural ancient forests it accommodates, and are declared as a national park in 2000. Since then, various research studies have been done about the management of the national park. It is hard to say that the efforts to raise the awareness of the local community, as part of these studies, have been adequate. Moreover, in addition to raising the awareness of local community, research and studies on involvement of locals in the national park administration are needed.

This study is conducted in Bartın-Turkey, one of two provinces over which Küre Mountains National Park (KMNP) spreads. In order to understand their perceptions on the park and its management, the survey was conducted and included different questionnaires for four different stakeholder groups in this province. These stakeholders are local government officials, private enterprises, non-governmental organizations (NGOs), and village headmen.

In “Material and Methods” section; detailed information was given about the chosen study area, Küre Mountains National Park, and the conducted surveys. In “Results and Discussion” section, findings derived from the results of the surveys have been presented. In the last section, “Results and Recommendations”, results derived from the data produced throughout the study have been evaluated and various recommendations on managing the park have been developed.

Materials and Methods

Located on the Western Black Sea region of Turkey, KMNP covers an area of 37,753 hectares, spreading between two provinces, Bartın and Kastamonu (Figure 1). The buffer zone around the national park covers 134,366 hectares [2].

Along with its national importance, KMNP holds an international significance due to its rich ecosystem presence, unspoiled nature, social structure, and historic and cultural background. The park has been characterized as one of 122 Important Plant Zones in Turkey by WWF [3]. KMNP also made the list of "100 Forest Hot Spots" to be preserved in Europe, by WWF in 1999 -one of nine protected areas from Turkey [4]. The area of Küre Mountains is also considered to be the second most important karstic zone in Turkey [5].

There exist 123 villages in and around the national park, as part of eight counties and a town. More than 20,000 people live in these villages. Rich cultural backgrounds of these villages suggest a high potential of folkloric tourism in the area. Local products, organic food markets, historical houses, and waterfalls can be listed as cultural values that can be utilized for tourism. KMNP has 3 visitor centers.

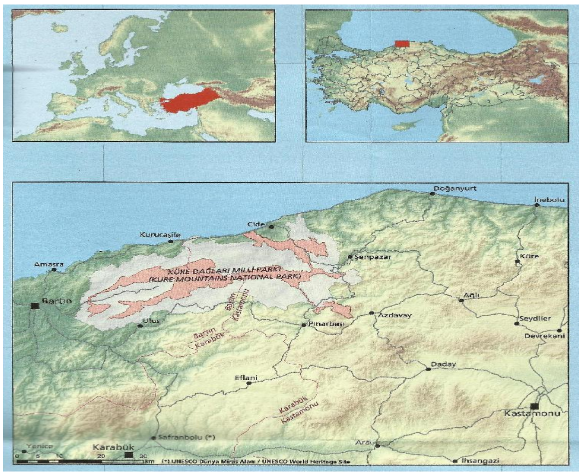

Figure 1. Location of Küre Mountains National Park.

The data used in the study have been compiled from conducted surveys, observations during site visits, and interview notes. Four stakeholders have been identified as most important parties to participate in the management of the national park: government officials, private enterprises, village headmen, and non-governmental organizations. Local residents, tourists etc. have not been included to this study as a stakeholder group. Different questionnaires have been prepared for each stakeholder. In the questionnaires, 25 close-ended questions have been asked. The questions were kept short, simple, and understandable. The survey was conducted by face-to-face interviews with 120 respondents. 91% of participants were male, while only 9% (11 people) were female. Surveys were analyzed by using descriptive statistics (frequencies and percentages). Detailed data set about the study can be found in master thesis, "An Examination of Socio-Cultural Structure in Bartın Section of Küre Mountains National Park, within the Framework of PAN Parks" [6], and the study by Akbulut et al. [7].

Results and Discussion

Perception of National Park

While 83% of the respondents were pleased with the establishment of KMNP, only 5% were not happy (Table 1). On the other hand, only 50% of village headmen expressed their satisfaction with the declaration of national park. This is also the only stakeholder group that contains the largest share of respondents who are not satisfied with the setting-up of the national park (20%). Those village headmen, whose villages are in close proximity to the national park, explained that they are not satisfied with imposed restrictions on the utilization of the park area. The complete prohibition on utilization of absolute protection zone is considered to be an important restriction for the villagers living in the area.

Most of the respondents (57%) described the recreational potential of the national park as high. When asked about effects of the national park on employment, 59% sees a high potential of job opportunities. The stakeholder that expressed the highest belief on job potential was government representatives (67%).

Table 1. Stakeholder satisfaction with the establishment of the Küre Mountains National Park.

Satisfaction	**Private enterprises**		**Village headmen**		**Local governments officials**		**NGO's**		**Total**	
	Freq.	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**
Very satisfied	4	13	1	3	8	27	21	70	34	28
Satisfied	24	80	14	47	21	70	7	23	66	55
Undecided	2	7	9	30	1	3	2	7	14	12
Not satisfied	0	0	6	20	0	0	0	0	6	5
Not at all satisfied	0	0	0	0	0	0	0	0	0	0
Total	**30**	**100**	**30**	**100**	**30**	**100**	**30**	**100**	**120**	**100**

It was stated by 64% of the respondents that the national park possesses a high potential of income for the people of the region. NGOs were those with the most positive opinion (73%). Half of village headmen (50%) interviewed thinks that the potential of income for the people of the region is high. A significant majority of respondents views the national park as having a high potential for an attractive and beautiful landscape, and for preserving the air, the water and the soil.

Public Relations

Most of the respondents (82%) stated that, after the declaration of the KMNP in 2000, efforts to promote the national park to the people of the region were inadequate. While 93% of the people reviewed in the private sector also found the efforts inadequate, a marginal number of respondents, who believed that the activities were quite sufficient, were only from governmental organizations' representatives (Table 2).

Table 2. Promotional Efforts Aimed at the People of the Region.

	Private enterprises		**Village headmen**		**Local governments officials**		**NGO's**		**Total**	
	Freq.	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**
Very adequate	0	0	0	0	2	7	0	0	2	2
Adequate	1	3	0	0	4	13	0	0	5	4
Normal	1	3	6	20	5	17	3	10	15	13
Inadequate	21	70	13	43	16	53	21	70	71	59
Never inadequate	7	23	11	37	3	10	6	20	27	23
Total	**30**	**100**	**30**	**100**	**30**	**100**	**30**	**100**	**120**	**100**

Most of the people interviewed (86%) finds efforts to promote the national park nationwide insufficient. Highest percentage of stakeholder group members that finds the nationwide promotional efforts adequate are governmental organizations' representatives, while most of the members of non-governmental organizations finds these efforts least sufficient (90%). 83% of respondents are aware that the national park is governed by Nature Protection and National Parks Directorate General (NPNPDG). While 90% of governmental organization representatives and 77% of village headmen are aware of NPNPDG's authority, 20% of village headmen thought the national park was managed by forest management directorates.

According to 59% of the respondents, KMNP does not attract the attention of people living in Bartın. The stakeholder with the highest rate (70%) that thinks that the national park does not draw the attention of people living in Bartın is private sector representatives. Village headmen are the stakeholder with the highest rate of belief that residents of Bartın are indeed interested in the national park. While that percentage is 47% among village headmen, it falls to as low as to 10% among other stakeholders. 63% of government organizations' representatives think that KMNP does not attract the attention of the people of Bartın. According to 43% of respondents, KMNP does not attract the attention of tourists (people from out of the province). 47% of village headmen stated that the national park does not draw tourists' attention. The highest percentage of the opinion, which the national park does not attract tourists, belongs to government representatives, by 53%.

Expectations

Among the things that the stakeholders want to see developing the most in and around the national park is ecotourism facilities, by 58%; the second most is an increase in the number of tourists (49%); and the third is the development of roads and other infrastructure services, by 43%. Most of people interviewed in the private sector (83%) wishes to see an increase in the number of tourists, while 73% wants development of ecotourism facilities, and 40% wishes for an expansion of natural and wild life regions. Most of village headmen (63%) want the development of roads and other infrastructure services, 47% wishes for an increase in the number of tourists, and 40% wants to see organic agriculture developing.

Government representatives list what they wanted to see the most as, ecotourism facilities (70%), an expansion of natural and wild life regions (53%), and the development of roads and other infrastructure services (50%). Most of respondents from NGOs (63%) want to see the development of ecotourism facilities, 60% of respondents wants development of organic agriculture, and 50% wishes for an expansion of natural and wild life regions.

Of all the 120 respondents, village headmen take the lead among those who wants an intensive agricultural production. Such response can be interpreted as an expectation from agriculture to provide job opportunities and consequently economic income in the region. Similarly, 26% of village headmen interviewed wants job opportunities to improve. In general, village headmen support activities that generate economic income.

When asked about which facilities to be built in the different regions that will increase the number of visitors to the national park, 90% answered as accommodation facilities, and 64% responded as local product shops. Mostly observed among private sector representatives, the respondents stated that most of the tourism oriented visitors come and leave on the same day, thus solving the accommodation problem will vitalize the region's economy. Bear museum in PAN Park certified Majella National Park in Italy is attracting the attention of the tourists that come to the area [8]. Considering that example, the idea of a museum that introduces indigenous animals has been positively interpreted by 17% of the respondents. On this subject, percentage of positive views from village headmen and non-governmental organizations is the same (20%).

Relationship between park administration and stakeholders

About the relationship between national park administration and governmental organizations, private enterprises, and NGOs, 76% of respondents think it is not good enough, while 6% thinks it is. The highest share of those who perceive relationship between the administration and other stakeholders is found among NGOs, by 10% (Table 3).

Most of private sector representatives (86%) state that relationship between national park administration and the stakeholders is not good. Some private sector representatives say that the national park administration invites them to the meetings, but only talks about their activities, without asking them about their opinions. They believe that this is not an effective way of communication.

Table 3. The Relationship between National Park Administration and Stakeholders.

Level of relations	**Private enterprises**		**Village headmen**		**Local governments officials**		**NGO's**		**Total**	
	Freq.	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**	**Freq.**	**%**
Very efficient	0	0	0	0	0	0	0	0	0	0
Efficient	1	3	2	7	1	3	3	10	7	6
Normal	3	10	10	33	6	20	3	10	22	18
Inefficient	19	63	12	40	19	63	19	63	69	58
Never inefficient	7	23	6	20	4	13	5	17	22	18

Total	**30**	**100**	**30**	**100**	**30**	**100**	**30**	**100**	**120**	**100**

While, after being declared as a national park, 77% of respondents find the investments the government made and the services it brought to the park are insufficient, those who are the most positive are from governmental organizations (26%). It is worth noting that only 10% of respondents find government's investments in the park sufficient. Among all stakeholders, a response of "highly sufficient" was only given by governmental organizations' representatives (13%).

Conclusions

It is possible to make following conclusions, as a result of the field observations, interviews, and survey interpretations;

Most of the respondents have positive opinions about the establishment of the national park. Those village headmen who are not happy about the establishment of the national park claim that they are restrained from utilizing the national park. Despite that, most of the village headmen interviewed is willing to join the activities in the national park.

A vast majority of respondents knows that KMNP is overseen by Nature Protection and National Parks Directorate General (NPNPDG). National park administration has succeeded in establishing an institutional recognition among stakeholders.

According to most of the people interviewed, KMNP is not drawing the attention of people living in the provinces around it. It is thought that people are not interested in the national park, due to its inadequate publicity and, as a result, its limited recognition. Percentage of those who think KMNP draws tourists' attention is the same as those who don't (43%).

KMNP administration is primarily expected to increase facilities and activities for ecotourism, increase the number of tourists visiting, and improve roads and other infrastructure services.

According to respondents, in order to increase the number of people visiting the national park, accommodation facilities, local product shops, and food and beverage facilities must be constructed on different parts of the region.

Most respondents think that an effective communication does not exist between the national park administration and the stakeholders.

Most of the people interviewed find government's investment in the national park and the services it brought inadequate, since the declaration of KMNP.

According to most of the respondents, activities to introduce KMNP to the people of the region and the nation are not sufficient. Especially people interviewed in the private sector agree upon the lack of promotional activities.

In light of the aforementioned conclusions, following recommendations have been developed to ensure the involvement of stakeholders in the management of the national park:

New practices should be adopted to remove negative views developing especially among village headmen against the national park. Public relations activities should be carried out to promote the national park in the region and across the nation.

Lack of technical staff in the national park administration should be dealt with, and healthy relationships should be developed between the administration and the stakeholders. Representation of stakeholders should be ensured in the national park management model.

Amount of investments in the national park should be increased, and these investments should be allocated according to the priorities of the stakeholders' expectations.

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FOREST CERTIFICATION IN BOSNIA-HERZEGOVINA AND SLOVENIA: OBSTACLES AND EFFECTS

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Introduction

Forest Certification is a relatively new, voluntary and market-based concept for improving the quality of forest management. The main purpose of any form of certification is in providing a formal proof that subject of certification (a product, service, resource or process) is managed or conducted in a proper way. When it comes to certification according to the Forest Stewardship Council (hereinafter FSC) it is a process in which an independent institution/body asseses the compliance of forest company activities with FSC principles, criteria and indicators. FSC sets the standards for sustainable forest management (SFM) and chain of custody (CoC) certification, and defines the procedures that certification bodies should follow in their certification assessments [1]. There are two key outcomes of any forest certification schemes claiming to be a credible: (1) Improvement of business performances, with an ultimate goal to achieve sustainable forest management and (2) Increasing of the competitiveness of forest companies through the maintaining a stable position on existing markets and greater access to new and attractive ones. In addition to these effects, forest certification improves transparency and facilitates interactions between stakeholders in day-to-day forest management activities.

On the global scale, there are more than 184 millions of hectares of forests certified by FSC scheme with increasing trend for the future period [1]. In the Western Balkans, studies focusing on forest certification at the national and regional level are still at initial phase. Some authors have explored various issues dealing with forest governance in wider context. These studies are ranging from developing forest certification standards [2, 3, 4] to analysis of relationships between different stakeholders [5, 6, 7]. Still, the obstacles at management level and particularly effects of forest certification remain rather unclear. As the countries where forestry and wood-processing industry play important role in national economy, Bosnia-Herzegovina and Slovenia follow globally increasing trends in forest certification. More than a half of all public forests in Bosnia-Herzegovina are certified according to the FSC scheme. In Slovenia all public forests and some biggest private forests are also FSC certified. Besides, some private forest owners in Slovenia started with PEFC (Program for the Endorsement of Forest Certification) just recently. These two countries were selected for the analysis because they may represent the wide range of economic, social and forest conditions of the former Yugoslavian countries and illustrate a number of changes that occurred after the breakup of former Yugoslavia. Based on the analysis of available reports, prepared by accredited certification bodies, this paper deals with various obstacles forest companies are facing during the assessment process and also critically analyzes effects of forest certification process in Slovenia and Bosnia-Herzegovina.

Materials and Methods

For the purpose of this paper, four official reports on certification process in Bosnia-Herzegovina [8, 9, 10, 11] prepared by audit team of "Qualifor SGS group's forest certification programme" and one public report on certification in Slovenia [12] prepared by audit team of "Soil Association Woodmark" were analyzed. These reports were analyzed in order to identify corrective actions for 4 forest companies in Bosnia-Herzegovina ("Šume RS", "Hercegbosanske šume", "Unsko-sanske šume" and "Šume TK") and 1 in Slovenia "Sklad kmetijskih zemljišč in gozdov Republike Slovenije" (Farmland and Forest Fund of the Republic of Slovenia).

Content analysis was used for the extraction of identified corrective actions which represent an output of the first main assessment of compliance with 10 FSC principles (indicated in the Table 1).

Table 1. FSC principles [1].

Principle 1	Compliance with laws and FSC Principles
Principle 2	Tenure and use rights and responsibilities
Principle 3	Indigenous peoples' rights
Principle 4	Community relations and worker's rights
Principle 5	Benefits from the forest
Principle 6	Environmental impact
Principle 7	Management plan
Principle 8	Monitoring and assessment
Principle 9	Maintenance of high conservation value forests
Principle 10	Plantations

In the analyzed official reports, any non compliances with the FSC principles were coded as one of the two following types of Corrective Action Requests (hereinafter CARs):

- Major CARs (must be addressed and re-assessed before certification can proceed) and

- Minor CARs (do not threaten the certification process. Yet, they need to be addressed within an agreed time frame and checked at the first surveillance visit).

Identified CARs were further processed in order to get frequency analysis and distribution of CARs per each FSC principle.

Results and Discussion

Analysis of four official reports on certification in Bosnia-Herzegovina revealed that 23 non-compliances with FSC standards were identified as minor CARs. Distribution of minor CARs per forest companies are presented in the Figure 1.

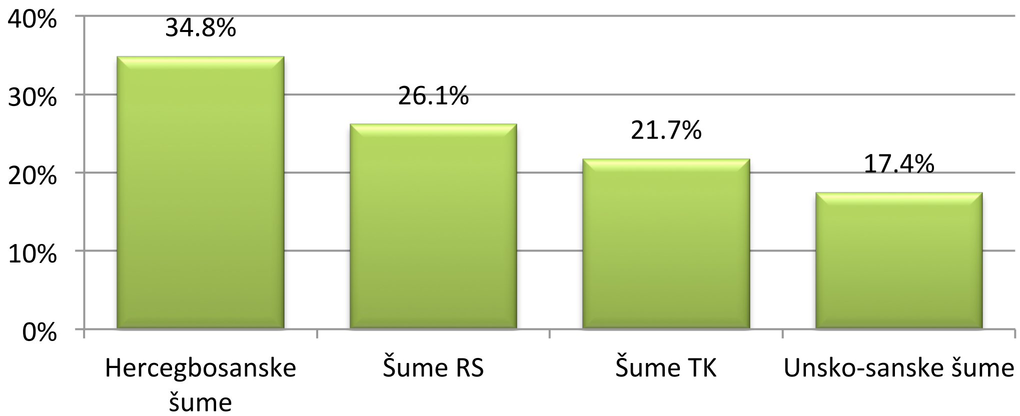

Figure 1. Distribution of minor CARs per forest companies in Bosnia-Herzegovina

As it is shown in the Figure 1, public forest company “Hercegbosanske šume“ had more than one third (8) of identified minor CARs in Bosnia-Herzegovina, public forest company “Šume RS” had more than one quarter (6) of minor CARs while public forest company “Unsko-sanske šume” had only 4 minor CARs during the first main assessment.

When it comes to Slovenia, 19 CARs were identified by the analysis of the report, out of which 18 were minor CARs while 1 was mayor CAR. Besides that 2 recommendations have been proposed. Distribution of CARs per FSC principles and per country is presented on Figure 2 and Figure 3.

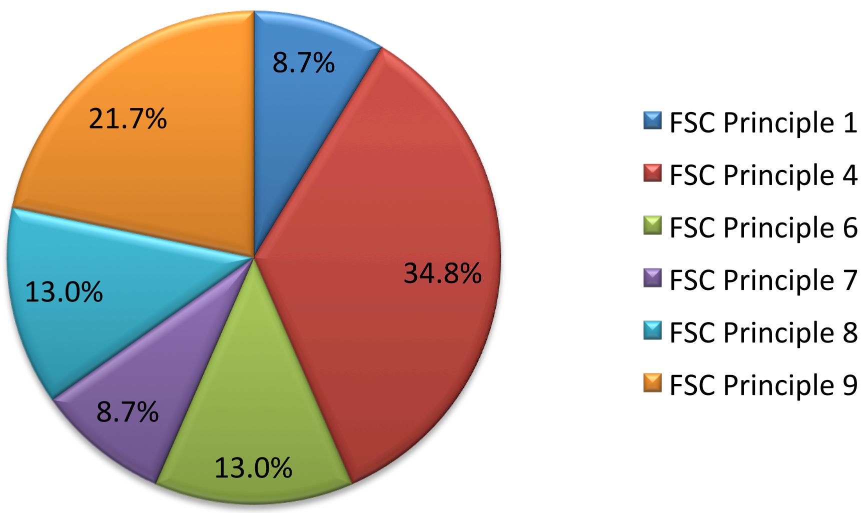

Figure 2. Distribution of CARs per FSC principles in Bosnia-Herzegovina

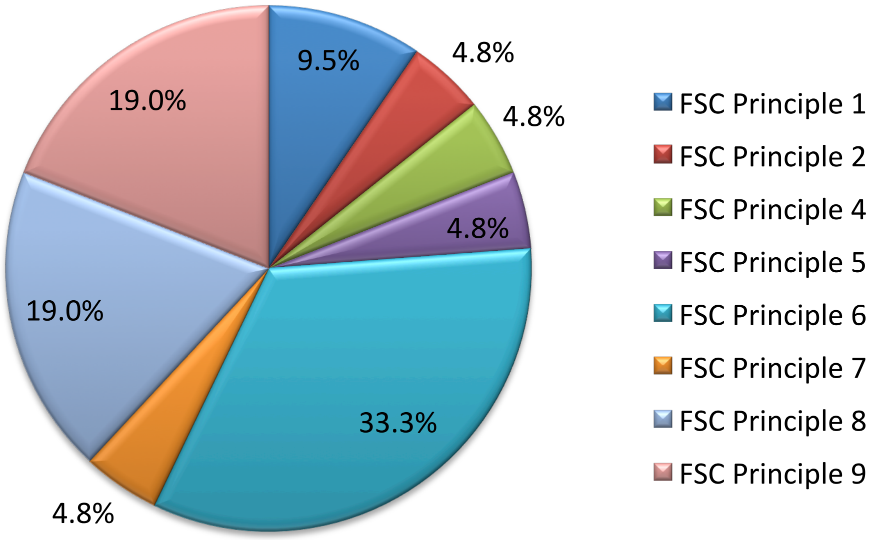

Figure 3. Distribution of CARs per FSC principles in Slovenia

According to the distribution of CARs per FSC principles in Bosnia-Herzegovina (Figure 2), one can conclude that FSC principle 4 (Community relations and worker's rights) is the FSC principle with biggest number of CARs (34.8%). FSC principle 9 (Maintenance of high conservation value forests) is in the second place (21.7%), while FSC principle 8 (Monitoring and assessment) and principle 6 (Environmental impact) have equal number of CARs (13% each). Distribution of CARS per FSC principle in Slovenia (Figure 3) revealed that FSC principle 6 (Environmental impact) has one third of CARs, FSC principle 8 (Monitoring and assessment) and FSC principle 9 (Maintenance of high conservation value forests) share second place with 19% of all CARs while the FSC principle 1 (Compliance with laws and FSC principles) is in the third place with 9.5%. All four public forest companies in Bosnia-Herzegovina had CARs from the principle 4 (Community relations and workers rights). "Hercegbosanske šume" had three minor CARs related to principle 4. The first CAR was related to indicator 4.2.5 indicating that all necessary tools, machines, substances and equipment were not available at work site. Second CAR was related to indicator 4.4.3 indicating that forest company did not maintain and keep updated the list of stakeholders while the third CAR was related to indicator 4.4.7 meaning that sites of special cultural, historical, ecological, economic or religious significance were not identified, described and mapped in cooperation with affected or interested stakeholders. "Šume RS" had two CARs from the principle 4 related to the indicator 4.1.7 indicating that all employees were not paid a fair wage and other benefits, which meet or exceed all legal requirements and those provided in comparable occupations in the same region and second CAR was related to the indicator 4.5.1 indicating that not every effort was made to resolve disputes through fair consultation aimed at achieving agreement and consent. "Šume TK" had also two CARs in the principle 4 related to the indicator 4.4.7 meaning that sites of special cultural, historical, ecological, economic or religious significance were not identified, described and mapped in cooperation with affected or interested stakeholders and indicator 4.5.2 where dispute resolution was not clearly defined. "Unsko-sanske šume" had only one CAR from the principle 4 related to indicator 4.2.5 indicating that all necessary tools, machines, substances and equipment were not available at work site. Farmland and Forest Fund of the Republic of Slovenia had one CAR from principle 4 related to criteria 4.5 - Complaints seem to be solved locally.

In case of Slovenia, the FSC principle 6 (Environmental impact) was the one with the 5 CARs. The first CAR was related to the indicator 6.5.b. where no buffer zones were seen (despite efforts to locate internal streams) and any streamside trees were said to be ‘managed’ rather than treated as non-intervention. Second CAR was related to indicator 6.5.c. - harvesting plans were designed to avoid damages but concessionaires were not sanctioned for damaging trees. Debarking trackside trees is inevitable when skidding long and heavy loads. The harvesting plan includes a map that defines the extraction routes. The contractor did not always give a copy of the map as a part of a formal contract or job description. The third CAR was related to indicator 6.6.b. where Pesticide FASTAC that contains alpha-cypermethrin was used although it has been classified as “highly hazardous” by the FSC. The fourth CAR was related to the indicator 6.6.c. - according to the procedures and interviews with operators, machines were fuelled outside the forest and/or on forest roads while working places/tractors were not provided with spillage kits or equivalent. The fifth CAR was related to criteria 6.7 where no chemical waste or fuel/oil containers seen discarded in the forest. However, contractors waste drink bottles while dumps of household rubbish were evident although not on an excessive scale. In Bosnia-Herzegovina, “Šume RS” had 2 CARs from the FSC principle 6 related to indicators 6.2.2 indicating the absence of an appropriate co-operation with acknowledged experts in identifying rare, threatened and endangered species and indicator 6.4.2 indicating that forest professionals did not create a map and mark in the field at least 5% of the totally managed forest land as a reference sites within 5 years after certification process had been finalized. Public Forest Company “Hercegbosanske šume” had one CAR from the FSC principle 6 related to indicator 6.5.4 indicating that operators were not aware of and able to implement adequate emergency procedures for cleaning up the accidental oil and chemical spillages.

Majority of CARs in Bosnia-Herzegovina refer to the community relations and workers rights (principle 4), maintenance of high conservation value forests (principle 9), monitoring and assessment (principle 8) and environmental impact (principle 6). In general, forest companies in Bosnia-Herzegovina were facing difficulties with social and ecological aspects of forest management during the certification process. For the Farmland and Forest Fund of the Republic of Slovenia, majority of CARs refer to environmental impact (principle 6), monitoring and assessment (FSC principle 8) and high conservation value forests (principle 9). When it comes to certification of public forests in Slovenia, ecological aspects of forest management were mostly expressed as obstacles of the certification process.

Conclusions

This paper is focusing on identification of CARs in Bosnia-Herzegovina and Slovenia and their non-compliances with FSC principles. In the case of Bosnia-Herzegovina, 23 minor CARs were identified. In case of Slovenia, 19 CARs were identified out of which 18 were minor CARs and 1 was mayor. Majority of CARs in both countries refer to different ecological and social aspects of certification that forest companies are dealing with in their everyday activities. Fulfilments of CARs during the FSC certificates revision-period, lead to improvement of traditional and routine forest management practices both in Bosnia-Herzegovina and Slovenia. As a globally accepted approach, forest certification has significant positive effects on forest management in Western Balkan countries. It improves forest management practices in public forest companies through the transparency increasing, building close relations with other stakeholders, establishing high conservation value forests and protection of specific natural values.

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STAND GROWTH CHARACTERISTICS OF COMMON BEECH (*Fagus sylvatica* L.) – PROJECTION OF EVEN-AGED TO MULTI-AGED STAND STRUCTURE

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Introduction

In Croatia, Common beech (*Fagus sylvatica* L.) is a dominant tree species. With 901,848 ha, beech forests (comprising mono-dominant beech forests as well as mixed forests with other tree species) cover 34.9 % of the total forest area (1). Because it is so widespread in Europe it plays an important role in the multiple forest function framework and in fulfilling ecological, social and economic requirements (2, 3). According to Čavlović (1), beech forests in Croatia can be divided into four main management (silvicultural) systems. The largest proportion (42.5 %) is taken by non-mixed and mixed even-aged beech forests based on 100-year rotation length and shelterwood regeneration system. Beech forests with multi-aged and irregular stand structures follow with 26.8 %. Mixed beech-fir stands managed by selection silvicultural system cover an additional 20.0 %, while the remaining 10.7 % of the beech forests are coppice even-aged forests. Beech forest stands in Croatia are mainly established and renewed through natural regeneration.

High importance of multi-aged stand structure for protective functions (4), as well as aesthetic and social functions, has been recognized by Regulations of forest management in Croatia, which prescribed multi-aged forest management in forests exposed to soil erosion, in forests within nature protection areas and in small-scale private forests. However, this forest management system still needs to be accepted and established in operative forest management.

Stand growth characteristics and multi-aged stand structure of beech stands is recognized as a significant research task. Thus, the main aim of this study was to develop stand growth models to provide projection of even-aged to the theoretical stand structure for multi-aged management of beech in Croatia, using indicators of competition (current annual increment in dbh, basal area and crown area).

Materials and Methods

The Common beech was investigated in the hilly region between Drava and Sava rivers in northern part of Croatia (Zagreb, Bjelovar and Našice Forest Administrations). Over the researched area within the pure and well stocked beech stands, in total, 25 circle plots were sampled (radius of 3.5, 7, 13 and 20 m depending on stand age) covering all developmental stages (young, middle-aged, pre-mature and mature pure beech stands). On each plot, on average, 16 trees (between 11 and 26 trees) were measured. Diameter at breast height, total height, two crown diameters were measured on each tree larger than 10 cm of dbh, while half of the sampled trees were randomly selected and cored to estimate current annual increment of tree diameter (CAI_{dbh}). Multivariate regression analysis of influence of tree diameter (dh), tree height (h), age (t) and site quality (sq) as independent variables on crown diameter (CD) as dependent variable, was performed on the sample of 403 beech trees. The modeling of the growth scenarios was based on a fitted curve (growth function according to Mihailov: $y = b_0 \times \exp(-(b_1)/x)$) for the relationship of diameter at breast height and total height with age (for observed data and GYT data (5)).

Following the methodology used by Alder (6) and Perez and Kanninen (7), the competition factor (CF) which shows reduction of current annual increment of dbh as a function of basal area ($CF = 1 - (0.00164 \times 10^{0.05784 \times BA})$) and crown competition factor (*CCF*) as the function of crown area occupation ($CCF_t = \left(\frac{((CD_t^2 \times \pi)/4) \times N_t}{10000} \right) \times f_{qs}$) based on crown diameter (CD)/dbh relationship ($CD = 0.96188 + 12.89926 \times dbh$), were used in growth projection as indicators of competition.

The simulation of dbh growth was based on the fitted model of dbh growth and *CF* as shown in the Equation (1):

$$dbh_{S(t)} = dbh_{S(t-1)} + (dbh_{F(t)} - dbh_{F(t-1)}) \times CF_t \quad (1)$$

where $dbh_{S(t)}$ is simulated dbh in a certain time; $dbh_{S(t-1)}$ is simulated dbh a year before; $dbh_{F(t)}$ is fitted dbh in a certain year; $dbh_{F(t-1)}$ is fitted dbh a year before; CF_t is competition factor in a certain time.

Stand growth projection of each stand (cohort) is based on thinning interventions (intensities and intervals), assumed rotation (100 years) and regeneration with final harvest. Applying the above mentioned models on actual mature beech stand (115 years, 10.5 ha, 28.1 m^2 ha^{-1} of BA, 480 m^3 ha^{-1} of standing volume, 9.6 m^3 ha^{-1} of volume increment), transition of even-aged to multi-aged stand structure was obtained using MS Excel.

Results and Discussion

Results of multivariate regression analysis show that tree diameter at breast height almost completely explain variability of crown diameters (Fig 2a). Including tree height, stand age and site quality in model does not improve explained variability (for only 0.0036). Influences of tree height and stand age are expressed indirectly through dbh, while site quality has no influence on crown diameter.

Regarding the fact that has no obtained influence of site quality on crown diameter, model of dependency of crown diameter versus dbh was obtained by regression analysis of the whole sampled trees (Fig. 1b)

Independent variables in model	R-Square	Adjust. R-Sq.	C(p)	Root MSE
dbh	0.9187	0.9185	17.587	0.6014
h	0.7057	0.7049	1109.3	11.443
t	0.4722	0.4709	2305.8	15.322
sq	0.0036	0.0011	4706.9	21.063
$d_{bh;}$ *sq*	0.9202	0.9198	12.185	0.5971
$d_{bh;}$ *t*	0.9192	0.9188	17.259	0.6002
$d_{bh;}$ *h*	0.9188	0.9183	19.359	0.6029
h; t	0.7145	0.7131	1066.0	11.287
h; sq	0.7091	0.7077	1093.6	1.139
t; sq	0.4936	0.4911	2197.9	15.031
dbh; t; sq	0.9218	0.9212	59.759	0.5915
dbh; h; sq	0.9202	0.9196	13.847	0.5973
dbh; h; t	0.9194	0.9188	18.249	0.6005
h; t; sq	0.7149	0.7128	1065.9	11.291
dbh; h; t; sq	0.9223	0.9216	50.000	0.5901

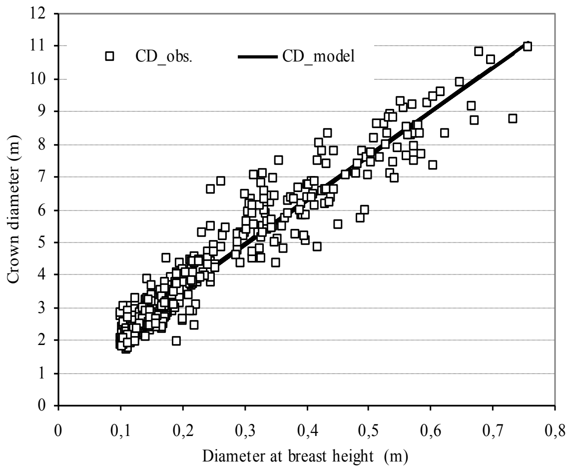

Figure 1. a) Statistics of multivariate regression analysis of influence of tree diameter (dbh), tree height (h), age (t) and site quality (sq) on crown diameter (CD), b) Observed and modeled crown diameter versus tree diameter at breast height for sampled beech trees.

In consideration of the fact that tree sizes (tree diameter, tree height, crown diameter) are influenced by management regimes, stand and site characteristics (8), obtained absence of site quality influence on crown diameter (Fig. 1a) is surprising. Small sample of plots and trees, different status of observed stands regarding intensity, time span after last thinning intervention and absence of stands on the worst site quality class can be used as an explanation. The question is, whether influence of site on crown diameter would be obtained in case of larger sample of plots and presence of worst site quality class.

The mature even-aged beech stand, used as a case stand for projection and transition, was divided into 10 cohorts, each of 1 ha average area. In consideration of structure diversity as to growing volume, basal area, average tree diameter, stand density and spatial distribution, schedule of regeneration and resulted dynamics of thinnings and stand growth before and after regeneration of each several cohort was defined. This dynamics is shown with projection of growing volume per ha of each cohort during 100-year projection period (Fig 2). Cohort with largest growing volume would be regenerated at the beginning and at the end of 100-year period, relatively, all cohorts would be regenerated in the period and balanced composition of developmental stages (theoretical structure of multi-aged beech stand) would be established.

The obtained growth models and growth modifiers (current annual increment of dbh and basal area) as indices for stand density competition (9) usually used to define intensities and the stand age at thinning, provide possibilities to simulate different scenarios regarding management objectives (e.g. maximal stand growth, maximal tree growth (7, 10)). Projection of the actual mature even-aged beech stand to the theoretical multi-aged beech stand (Fig. 2) which was based on equal (10-year) cutting cycles is a simple and applicable scenario. Simultaneous entry (thinning operations and regeneration) in all cohorts every 10 years, minimal basal area of 25 m^2 ha^{-1} after thinning within several cohorts (about 10 m^2 ha^{-1} of removed basal area), 1.3 of maximal crown competition factor before thinning, reduction of increment before thinning up to 80 %, are the main characteristics of the approach. In cases of growth maximization, different intervals between the two thinnings in each cohort would be needed (shorter in the first and longer in the last developmental stages, e.g. (10)). Consequently, interventions/operations/cuts in several parts of multi-aged stand (cohorts) would be needed every 1-2 years, which would be unacceptable in practical and economic sense.

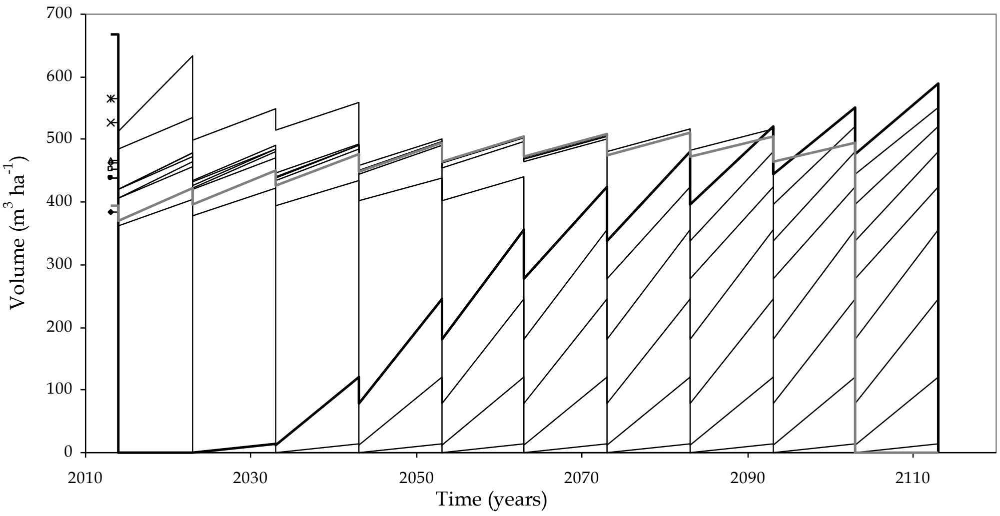

Figure 2. Projection of management (simultaneous regeneration and thinning of several stand parts (cohorts)) and transition of the mature even-aged beech stand to multi-aged stand (black bold line presents first regenerated cohort and grey bold line presents last regenerated cohort).

Changes of average structure elements as to decrease of average growing volume, age, tree diameter, tree height and stand basal area, as well as increasing of tree numbers until achieving theoretical values at the end of 100-year period, indicate the transition dynamics of even-aged into multi-aged beech stand. In the stand during the 100-year period regeneration fellings would yield 586 m^3 and thinnings would yield 402 m^3 of volume per ha. With achieving of theoretical multi-aged stand structure at the and of 100-year period, growing volume would decrease by 200 m^3 per ha in relation with the initial structure, and diameter structure would be moved to the left. The theoretical diameter structure and growing volume of 280 m^3 per ha would sustain equal rate of mature and un-mature harvests in total amount of 110 m^3 per ha every 10 years.

The various stocking control approaches have been used to implement and maintain multi-aged management (11, 12). In this study presented approach (continuous and simultaneous thinning and regeneration) as a kind of area control method would lead to conversion of even-aged to multi-aged stand, balanced “uneven-aged” diameter structure, and sustainable wood production. The wood production would be on same level as well as in even-aged management, with a higher protective (4), social and aesthetic potentials.

Conclusions

An irregular and multi-aged stand structure of beech forests in Croatia comprise more than one quarter of total beech forests, while stand structures of the forests potentially related to multi-aged forest management, considerably deviate from the theoretical structure. Due to high potential of the forests, environmental and social reasons, this close-to-nature type of forestry should be supported with an appropriate research and management approaches on forward.

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FIRST RESULTS OF THE STUDY ON THE STRUCTURE OF STANDING DEAD WOOD IN MANAGED FORESTS OF CROATIA

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Introduction

Dead wood (DW) plays important role in forest ecosystems, providing habitat for numerous species (fungi, invertebrates, lichens, bryophytes) as well as nesting and/or foraging grounds for numerous birds (1). Recognition of these facts is visible *e.g.* in the EU legal framework in Habitats Directive (2) and Birds Directive (3). Dead wood is also recognized as an important pool of carbon whose changes countries listed in the Annex 1 to the UNFCCC and parties to the Kyoto protocol have to report in their National inventory reports (4). Forest management for timber has resulted in significantly lower stocks of dead wood in comparison to the primeval forests (5) or forest reserves (6). Additional pressure to dead wood related part of the forest ecosystem is coming from a bigger demand for energy wood, resulting with increased use of smaller diameter tree parts previously left in forest after harvesting, or even stumps, stimulating the loss of naturalness and species diversity (7). Thus, the information on the stocks and structure of dead wood in forests is becoming increasingly important in order to assess and implement most appropriate measures and forest policies aimed at preservation of biodiversity and stability of forest ecosystems.

Stocks, structure and diversity of dead wood have not been extensively investigated in Croatia. The only extensive survey related to dead wood was performed within the First Croatian National Forest Inventory - CRONFI (8), where stocks of dead wood, downed and standing (snags), were reported according to the bioclimate zones (Table 1), administrative division (counties), forest ownership (state and private), and three degrees of decay.

Unfortunately, analysis of dead wood distribution, e.g. according to tree species, dimension, etc., were not reported in CRONFI (8). Several other studies on stocks of dead wood as part of forest carbon balance study have been reported (9,10) and most recently a study on the decomposition of dead wood (11), but all of them are of local character and/or dealing with a single forest type. Therefore, it was important to investigate all possible sources of data that could increase our knowledge on the dead wood in Croatian forests.

The goal of this study is to provide preliminary overview of the structure of standing dead wood (DW_s) in managed forest in Croatia by using data from past surveys with information on dead wood which have not been processed until now. More precisely, the aim is to estimate the share of DW_s with respect to the number of stems and basal area according to: tree species, diameter at breast height (dbh), and stand age (only even-aged forests).

Table 1. Compiled data from CRONFI (8) for living and dead wood stocks in Croatian forests.

Bioclimate zone	Stock (m^3 ha^{-1})			Share of snags	
	Living	Downed DW	Snags	in stock	in DW
Lowland	318.00	6.89	5.01	1.52%	42.1%
Colline	275.81	8.24	6.73	2.31%	45.0%
Alpine	346.85	18.85	12.82	3.39%	40.5%
Sub/epi-mediterranean	463.21	1.95	2.29	0.49%	54.0%
Eu/steno-mediterranean	46.28	4.98	1.52	2.88%	23.4%
Total	232.22	7.95	6.00	2.44%	43.0%

Materials and Methods

We collected data from the field measurements performed by Croatian Forests Ltd., a national forestry company in charge for the management of all state forests. Our study was possible because in the period from 2003 to 2008, at first as a pilot (from 2003 to 2006) and later (2007 and 2008) in accordance with the forest management regulation, Croatian forests Ltd. performed field campaigns with a new methodology. The new methodology had been described and implemented in the Croatian legislation within Forest management rulebook (12). One of the novelties in the Rulebook was the instruction that in the field campaigns a diameter at breast height and tree species should be recorded for both alive *and* dead trees on the plot. Existing raw field data were archived by Croatian Forest Ltd. and have not been used in analysis of DW_s until now. Unfortunately, the regulation was changed in December 2008 and although measurement of DW_s was still foreseen in the field manual, in practice it is rarely done (13). Hence, data on DW_s from 2003-2008 carry great value.

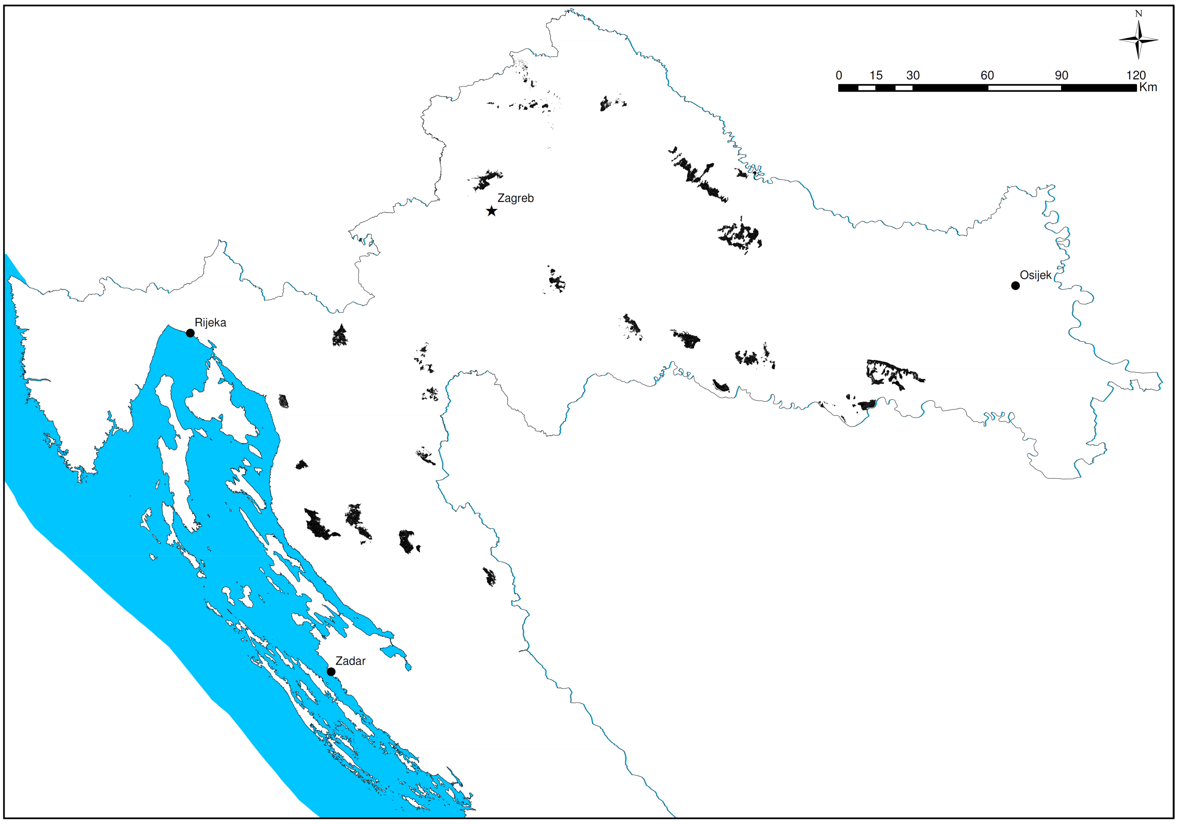

Figure 1. Locations of forest stands in the survey.

List of variables and measurement methodology is described in detail in (12) and we will not elaborate it here. For the purpose of this study only several variables were of importance, namely: tree species, diameter at breast and status of the tree (alive or dead).
Field measurements were conducted on circular plots of variable radius set in a fixed rectangular 100 m x 100 m grid as part of the survey performed for the preparation of standard forest management plans for a given forest management unit (FMU).
Data from 32 FMUs, located all over continental part of Croatia (fig. 1), were included in our analysis. Dataset included data from 37,510 plots with over 332,000 trees in total (living and dead). We compared the share of dead wood according to species, selecting only those tree species that were represented with more than 1,000 trees. Share of snags per species was calculated based on the total number of tree stems and based on the total basal area.
All trees were also categorized according to dbh in size classes of 5 cm (0-5 cm, 5-10 cm, etc.) to evaluate distribution of the share of DW_s. Changes in the share of DW_s with respect to stand age was assessed only for even-aged stands

Results and Discussion

Average share of DW_s in the number of trees and in basal area in the survey was 2.14% and 1.34%, respectively. According to tree species (fig. 2), share of DW_s had the highest values for *Ostrya carpinifolia* (15.8%, 14.2%) and for *Quercus pubescens* (15.4%, 8.9%).

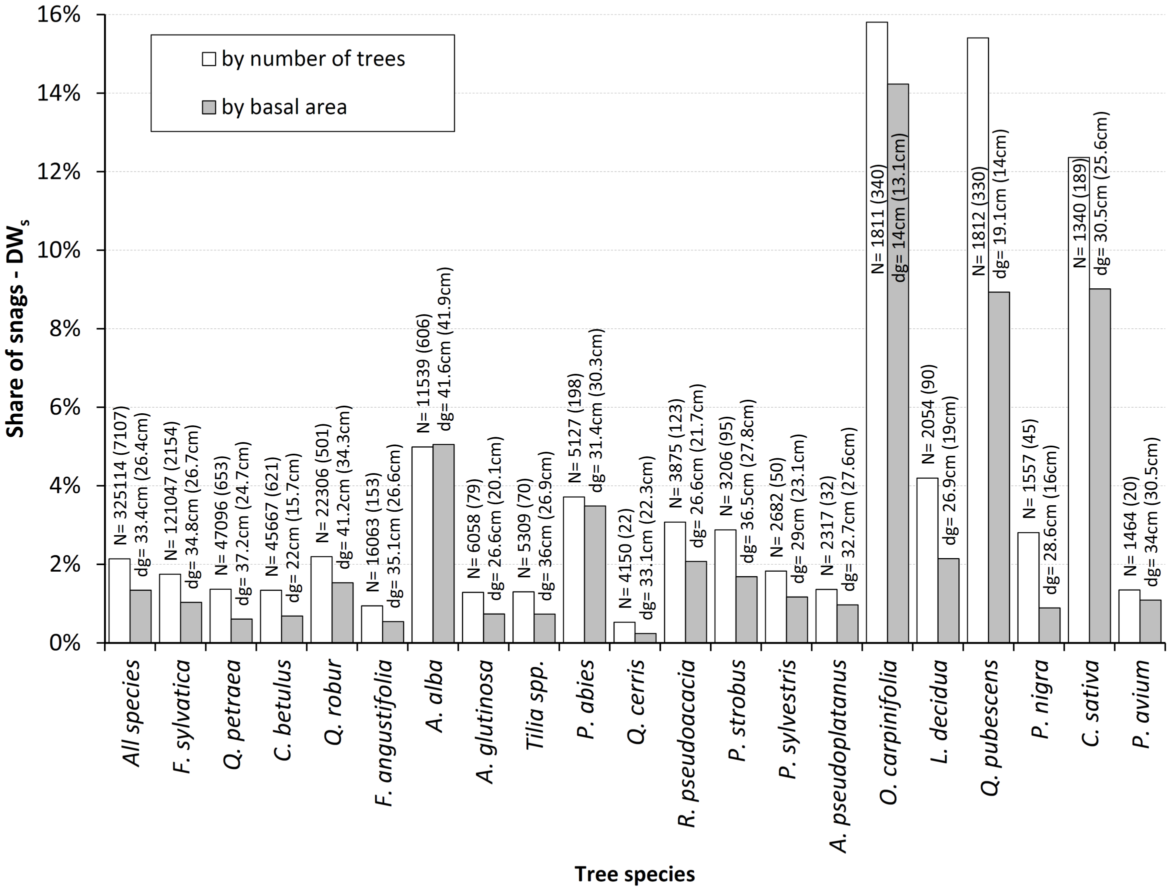

Figure 2. Share of DW_s (snags) in the number of trees and in basal area by tree species (N - number of trees measured; dg - quadratic mean dbh for a given species; values outside brackets – live trees; values inside brackets - snags).

This is not a surprise because those species, in Croatia, are typically related to the degraded forests (shrubs) where management intensity (thinning) is low due to economic reasons. Other species showing high share of DW_s are *C. sativa* (12.4%, 9.0%), *A. alba* (5.0%, 5.1%) and *P. abies* (3.7%, 3.5%). While somewhat higher values for *P. abies* are probably related in part to lower management intensity in areas with difficult terrain, relatively high DW_s share for *C. sativa* is probably result of increased problems caused by biotic factors (chestnut blight fungus *Cryphonectria parasitica*, chestnut gall wasp *Dryocosmus kuriphilus*). With *A. alba* it is most likely a combination of abiotic (drought spells, deposition of pollutants) and biotic factors (e.g. silver fir bark beetle *Pityokteines curvidens*), and past management (note that only for *A. abies* average snag is thicker than average living tree, *i.e.* $dg_{snags} > dg_{alive}$). Other species had values for DW_s share close to average, and *Q. cerris* had the smallest one (0.5%, 0.2%).

Distribution of the share of DW_s, with respect to tree size (fig. 3), shows highest values (up to 5.5%) for thin trees (dbh<25 cm). This is probably a reflection of higher mortality due to self-thinning in young and in degraded stands. For the dbh range of 25 to 65 cm a remarkably stable values (≈1.1%) for share of DW_s are clear indicator that these are managed forests. Further, it could be speculated that gradual rise of the DW_s share in thicker trees (dbh>65 cm) is related with increased mortality rate of older trees. However, such conclusion might not be evident from the results shown in figure 4, where share of snags in older (>80 y.) stands does not exhibit any trend with stand age. Nevertheless, it must be noted that in figure 4 only data from even-aged forests are presented while in figure 3 all data have been used, regardless of forest management type (even- and uneven-aged management). Other possible explanation for this could lay in the fact that in older (even-aged) stands a second generation of trees is emerging as a result of thinning, increasing the overall stands density thus compensating for the snags from older trees.

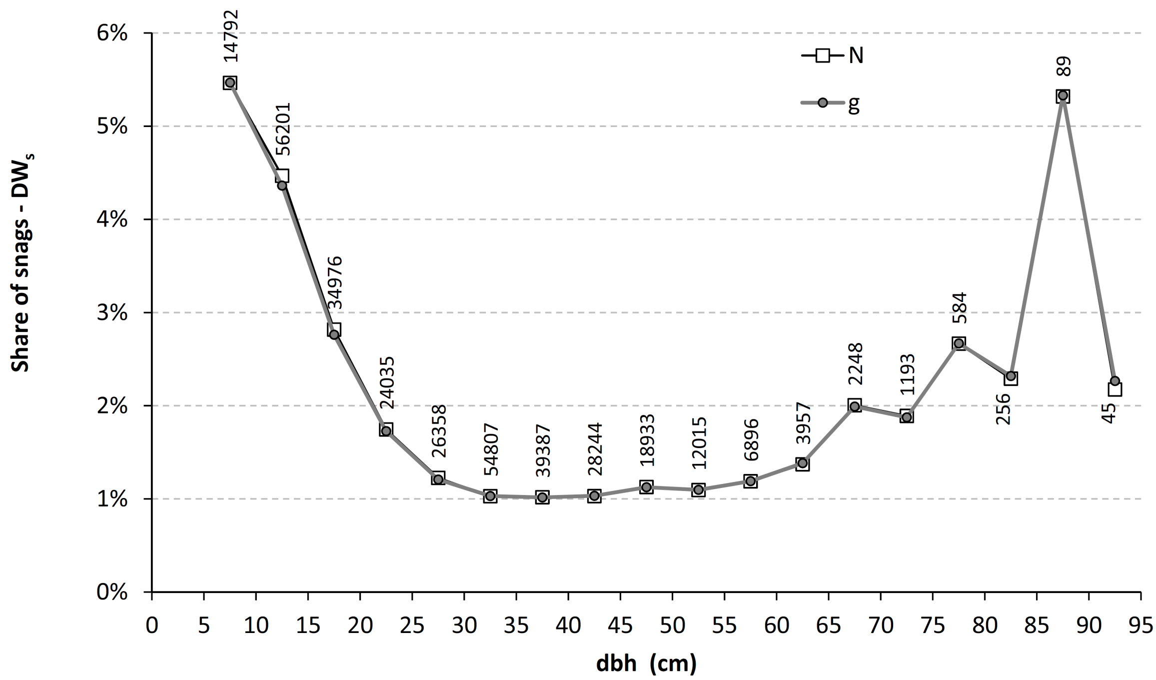

Figure 3. Share of DW_s (snags) in the number of trees (N) and in basal area (g) by dbh class (values represent number of trees in dbh class).

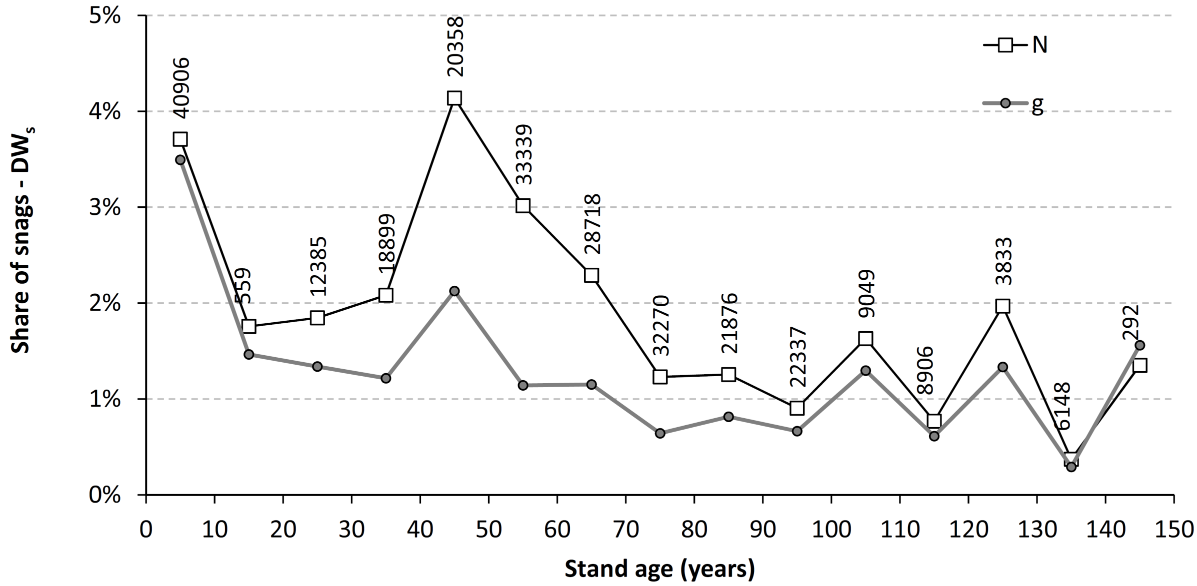

Figure 4. Share of DW_s (snags) in the number of trees (N) and in basal area (g) by according to age class (values represent number of trees in an age class).

For a given subgroup of trees in a stand, their share in total basal area of the stand roughly equals to their share in total volume of the stand. Therefore, it is permissible to compare such shares when they are obtained from different sources, although the shares are not of the same kind. If we compare the average share on of DW_s in basal area (1.34%, fig. 1) with the average share of DW_s in volume (2.44%, tab. 1) we notice a considerable difference.
One of the reasons might be due to the mentioned methodological difference in calculation of the share, but in our opinion it could not justify such a big difference. The issue that has to be taken into account is how representative the data analysed and presented in this work are for the whole Croata? While CRONFI can be considered as representative, FMUs in our study cover mostly forests in lowland and colline bioclimate zones. Those zones have lower than average share in DW_s (tab. 1) which could explain some of the observed differences between CRONFI and our study (*e.g.* DW_s share in lowland forest from CRONFI is only 13% higher than the average in our study). In addition, it is possible that some of the people working on actual field measurement considered dead trees as "less important" or "irrelevant" and have sometimes skipped to measure and record a snag on the plot. This is a problem that we are aware of, but have difficulty in resolving without danger of introducing additional error with the "correction". Therefore, we decided to use data, as they were, without an attempt to compensate for possible bias. Nevertheless, we think that distributions of DW_s with respect to tree species, dbh and stand age have not been affected with this potential error. Values for the share of DW_s presented in this work are probably on the lower limit of the actual DWs share in Croatian forests.

Conclusions

Dead wood stock per ha in Croatia is near the European average (14, p. 79), but demand for energy wood might negatively reflect on that. As our study indicates, current management practice reflects on stocks of DW_s with relatively low share of DW_s in mid-aged and older stands even-age stands, but at the same time relatively high share for *A.* alba (a species typically under uneven-age management). Further research of stocks and patterns of DW, in particularly lying DW, are necessary in order to determine optimal stocks that would secure the protection of biodiversity, but also took into account economic aspects.

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We express our gratitude to Croatian Forests Ltd. for the permission to use data used in this study. We thank Mr.sc. Dubravko Janeš, Dr. sc. Dalibor Štorga and especially Mr. Ivica Milković for their help in obtaining data. This study has been supported in part by Croatian Science Foundation under the project 2492.

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DOES MOUNTAIN FOREST CHARACTERISTICS INFLUENCE VISUAL APPEAL? A STUDY CASE IN AN ALPINE VALLEY IN ITALY

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Introduction

In the last decade, the interest of decision makers and scientific community on the study of public preferences related to the forest planning and management is growing rapidly[4,9]. Public preferences can be defined as the degree to which an individual or a group (e.g. group of interests, association or organization) prefers a situation or feature over other situations or features[12]. The inclusion of public preferences in the participatory decision making process has the advantage of reducing conflicts between users and hostility towards decision makers[11] and increasing the social sustainability[6]. Besides, local knowledge of people could potentially support the decision makers in the definition of management strategies, and in the preservation of resource and of ecosystem services supply by forests [2].

In this context the amenity values, such as scenic beauty and recreational activities, have a particular importance linked to the system of values of post-modern society [3]. People's preferences on the forest landscape features vary according to the scenic beauty (attractiveness), visual impacts of land-use and management strategies[13]. The individual preferences are also linked to the system of personal values and they are influenced by territorial, social and cultural context[1, 10]. The knowledge of socio-cultural context is a pre-requisite before starting any study on public preferences related to scenic beauty and forest management strategies. Besides, often the decision makers have no perception of the meanings and values that individuals attribute to the ecosystem services supply by forests or to the visual aspects related to the management, e.g. forest structure and canopy cover[8]. This information gap could lead to a distorted perception of management decisions by local community. Understanding the preferences that individuals attribute to the forest and considering their needs and opinions, represents a relevant information to improve the capacity of decision makers to communicate with local communities or tourists in an effective way and to involve the population in the forest planning and management[13].

Starting from these considerations, the main aims of this paper are: (1) to define a survey method useful to evaluate the preferences attributed by the individuals to different forest stand features; (2) to test an index for evaluating the visual appeal of forest. The survey method was applied in a case study located in the Italian Alps and characterized by a high forest multifunctionality and tourist attraction.

Materials and Methods

Study area

The study area is a valley called "Val Genova" (46°10′00″ N, 10°46′00″ E). The valley is located in North-East of Italian Alps (Trentino-Alto Adige Region). Val Genova is part of the Adamello Brenta Nature Park and covers about 15,000 ha (approximately 60% of valley is covered by forest). The main forest types are Norway spruce (*Picea abies* (L.) Karst.) forests pure or mixed with silver fir (*Abies alba* Mill.) and European larch (*Larix decidua* Mill.) forests. Val Genova covers a high range of altitudes starting from about 800 m a.s.l. to more than 3,000 m a.s.l. Due to its characteristics the wilderness of the area is very high with a few scattered settlements in lower part of the valley. The landscape is the results from the combination of different elements such as water bodies (river, waterfalls), glaciers, rocks, forests (both evergreen and deciduous forests), mountain shrubs, grasslands and settlements.

Research framework

The people's perception on forest stand features were collected through the administration of a structured questionnaire to a sample of tourists. The questionnaire was structured in 32 closed-form questions decided in advance from the researchers. The advantages of the structured questionnaire are due to the fact that it is relatively quick and easy to administer and analyse. Besides, the questionnaire was subdivided in three thematic sections ("personal information", "visit of Val di Genova" and "visual appeal"). The questionnaire was subdivided in thematic sections because such subdivision reduces the risk the person being interviewed from getting tired or bored[7]. The first thematic section focuses on the personal information of respondents such as age, gender, level of education, job, income and place of residence. The second thematic section considers the information related to the current visit of the Val Genoa (e.g. visit duration, type of accommodation, mode of transportation). The third thematic section considers the visual landscape and forest stand perception: a first set of questions focuses on the visual preferences on the landscape features (i.e. land-use components, landscape density and trees outside forests) and a second set of questions focuses on the visual preferences on the forest stand characteristics. In the present paper we show the results of this last set of questions strictly linked to the local forest management strategies.

The structured questionnaire was administered face-to-face to 215 tourists of Val Genova. The tourists surveyed were selected in a systematic way (one of every two has been interviewed) in three locations of the valley (*Nardis*, *Ponte Maria* and *Bedole*). The locations have been selected to three different elevations, taking into account the different types of visitors (Figure 1): (1) *Nardis* is located in the lower valley, easily accessible with just a few minutes' walk from the car park and close to an important waterfall (*Nardis* waterfall); (2)

Ponte Maria is located in the middle valley in the last point accessible by car; (3) *Bedole* is located in the high valley that is accessible by public bus.

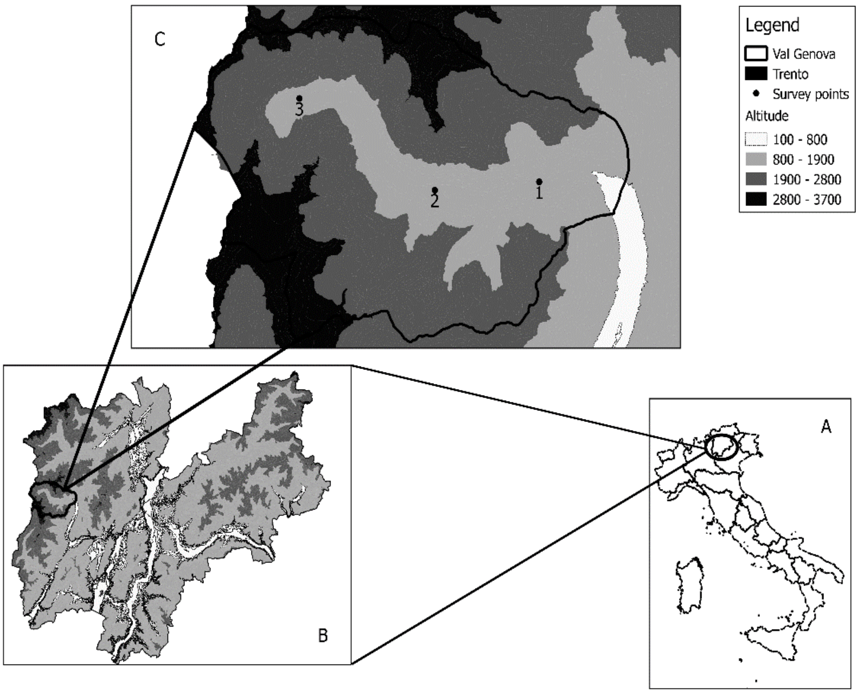


Figure 1. Location of study area with evidenced *Nardis (1)*, *Ponte Maria* (2) and *Bedol*e (3). Capital letters indicate: Trentino in Italy (A), Val Genova in Trentino (B), Survey points in Val Genova (C).

The tourists' preferences on the different types of forests was evaluated using the Forest Perception Index (FMP index) based on the main forest stand characteristics (Table 1).

Stand structure includes diametric and height differentiation of the trees in a forest[5]. Stand structure can be uniform when the trees have similar height and diameter or differentiated when the trees have dissimilar height and/or diameter.

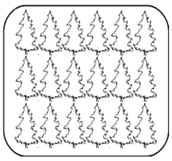
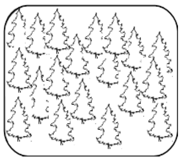
Canopy cover can be defined as the proportion of area covered by the horizontal projection of tree crown. With regards to the canopy cover the forest can be with dense and continuous foliage, with diffuse light, with discontinuous foliage and open.

Trees spatial distribution describe the horizontal distribution of the trees in a forest stand. Regular, random and groups of trees distribution were considered.

Forest system are strictly linked to the past management strategies and it can be subdivided in: high forests, coppices and coppices under high forests.

The visual appeal of each class for every variable was assessed by single tourist using a 5-point Likert scale (from 5 = maximal visual preference to 1 = minimal visual preference).

Table 1. Variables used in the FMP index.

Stand structure (S_s)	Trees of similar size (diameter and height)		Trees with different size (diameter and height)	
				
Canopy cover (C_c)	Forest with dense and continuous foliage (dark)	Forest with diffuse light	Forest with discontinuous foliage (high and low light alternate)	Open forest (high light density)
				
Trees spatial distribution (S_d)	Trees regularly distributed	Trees randomly distributed	Trees growing in groups	
				
Forest system (F_s)	High forest	Coppice	Coppice under high forest	
				

Combining the above mentioned variables 60 type of forest have been found. FMP index for each type of forest was calculated as products of four variables using the following Equation (1):

(1)

Where:

FMP_i index = Forest Management Perception index of the type of forest *i*;

S_s = score of the stand structure for the type of forest *i* (from 1 to 5);

C_c = score of the canopy cover for the type of forest *i* (from 1 to 5);

S_d = score of the spatial distribution of trees for the type of forest *i* (from 1 to 5);

F_s = score of the forest system for the type of forest *i* (from 1 to 5);

n = the *n-th* visitor interviewed;

N = the number of visitors interviewed.

The index is the average score for the entire group of interviewed visitors. The index value is the weighted score of the sum of the analysed variables. Values are weighted dividing per 4 in order to obtain an index with 1 to 5 values.

Results and Discussion

The response rate is 73% (156 on 215 tourists). The response rates in the different locations are highly variable: response rate is 56% in *Nardis*, 82% in *Ponte Maria* and 77% in *Bedole*. These differences are due to two main reasons: intrinsic characteristics of the location and types of tourists. For example in *Ponte Maria* there are no interesting natural features and the tourists are forced to wait the public bus if they want to visit the high valley, while *Nardis* is characterized by the presence of the waterfall mountain.

The results of socio-demographic characteristics show that 57.7% of respondents are male and 42.3% females. 46.8% of respondents have the high school degree, while 36.5% of the respondents have a university degree or higher (MSc, PhD). Considering the age the sample is distributed in as follow: 13.5% of respondents were younger than 30 years, 11.0% of the respondents were between 31-40 years of age, 27.1% of the respondents were between 41-50 years of age, 22.6% of the respondents were between 51-60 years of age, and the remaining 25.8% of the respondents were older than 60.

The analysis of single variable shows that the most appreciated stand structure is characterized by trees with different size (4.55) and with a discontinuous canopy covers (4.12). With regards to the spatial distribution of trees the tourists prefer the randomly distributed trees (4.29), followed by the distribution of trees in groups (3.61). Considering the forest system there were small difference in the tourists' perception inasmuch all classes have close mean values (high forest 3.85, coppice 3.95, and coppice under high forest 3.81). Statistical significance of the observed differences have been tested from the statistical point of view by the Mann-Whitney and Kruskal-Wallis non-parametric tests (Table 2).

The results of FMP index show values between 2.75 and 3.35. The main differences that emerge grouping the forest types per characteristics are:

1. High forest and coppice under high forest have close values (2.62 and 2.69 respectively) while coppices show a lower value of 2.2;
2. The highest value is reached by the forest type characterized by a coppice with trees of different sizes, randomly distributed and with discontinuous foliage (3.35);
3. The lowest value is reached by high forests with trees of the similar size, regular distribution and a canopy cover with dense and continuous foliage (2.75);
4. Combining forest system and stand structure in the formula of FMP index gives the average values which are very close ranging from 2.9 to 3.1;
5. Combining the first three variables (stand structure, canopy cover and trees spatial distribution) in the formula of FMP index gives the highest values which are reached by

forest with random trees distribution, different sizes of trees and a discontinuous foliage cover (3.20), while the lowest value is reached by high forests with trees of similar size, regular trees distribution, with dense and continuous foliage (2.83).

Table 2. Tourists' preferences on the forest stand characteristics.

Classes	N	Mean	St.dev.[1]	Median	Non-parametric test
Stand structure					
Trees of similar size (dbh[2] and total height)	155	3.57	1.06	4	Mann-Whitney test: U=5556.0 p-value<0.0001
Trees different in size (dbh[2] and total height)	155	4.55	0.77	5	
Canopy cover					
Forest with dense and continuous foliage (dark forest)	155	3.25	1.27	3	Kruskal-Wallis test: K observed value=42.976 K critical value=7.815 p-value<0.0001
Forest with diffuse light	154	3.93	1.01	4	
Forest with discontinuous foliage (dark and low light alternate)	155	4.12	0.90	4	
Open forest (high light density)	154	3.57	1.31	4	
Trees spatial distribution					
Trees regularly distributed	152	3.27	1.23	3	Kruskal-Wallis test: K observed value=64.724 K critical value=5.991 p-value<0.0001
Trees randomly distributed	154	4.29	0.96	5	
Trees distributed in groups (of different size)	155	3.61	1.07	4	
Forest system					
High forest	153	3.85	1.12	4	Kruskal-Wallis test: K observed value=0.649 K critical value=5.991 p-value=0.723
Coppice	153	3.95	0.90	4	
Coppice under high forest	153	3.81	1.10	4	

[1] Standard deviation; [2]diameter at breast height.

Conclusions

The present study was focused on the analysis of the visual perception of forest stand characteristics by visitors in a high value natural area. The variables usually used by forest managers in order to define the forest management strategies were assessed by tourists. The order tourists' visual preference was synthesized in an index (FMP index) able to highlight the most appreciated types of forest. The results show that forest system (high forest or coppice) is not a visual variable perceived by tourists. While among the other variables a differentiated structure, discontinuous foliage and irregular distribution of trees over the forest are the stand characteristics more appreciated by the visitors. Consequently, it is important to highlight that the forest characteristics more appreciated by tourists are usually descriptive of a high functional and structural diversity of forests. Finally, the information provided by the FMP index could provide useful guidance to the forest managers when the management purpose is the valorisation of the recreational function considering the visitors' preferences.

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Green Care FOREST – diversification as an opportunity for forestry

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Introduction

The Austrian Research Centre for Forests (BFW) addresses all aspects of forests – from an economic, ecological and social point of view. Based on research, monitoring and training BFW provides its findings and knowledge to different groups of society. Since 2014 Green Care FOREST is a new research project of BFW designed to broaden our scope of activities.

Green Care FOREST – diversification as an opportunity for forestry is based on the Vienna Resolution which was developed in 2003 by the Ministerial Conference on the Protection of Forests in Europe (MCPFE) [1]. Next to half of the Austrian territory is covered with forests. They dominate our natural scenery and fulfil a variety of functions - the economic function, the protective function, the beneficial function and the recreational function - by law [2]. The opportunity for recreation in nature or the services rendered by forests in terms of the conservation of nature and landscape as well as the protection of soil, climate and potable water are often taken for granted. Thanks to sustainable forest management each tap provides potable water in Austria. Forests and their managers are not fully appreciated and valued for those services.

In times of great socio-political challenges we want to support the Austrian social system by using the inclusive potential of forests for the benefits of the socially vulnerable. Green Care FOREST involves a bundling of all theoretical and practical initiatives and activities to use forests for improving the well-being in society by creating additional sources of income through diversification in cooperation with social facilities and by supporting rural development. The products of Green Care comprise the areas pedagogy and social work.

The study aims to provide an overview of the scientific literature on the benefits of woodlands on people's health, well-being, and quality of life. Its intention is to highlight the advantages of the forest as a place for leisure activities, restoration, recovery from stress and further social and therapeutic interventions. Its aim is to take stock of the current state of the scientific knowledge and provide a knowledge base for projects relating to Green Care in woodlands and Green Public Health. Both scientific and practical projects have been taken into account.

Methods

Overall, the study includes 149 peer-reviewed articles as well as 31 landmark publications, originating mainly from Western countries and East Asia, specifically from Korea and Japan. We used the criteria used for systematic reviews of scientific literature in medical research.

The focus was on scientific journals between 1993 and 2013 [3]. MEDLINE, PsycInfo, Psyndex plus, SCOPUS and Web of Science were the used databases for the systematic research. Terms used in the two runs of the literature survey: 1st run (forest* AND; tree* AND; wood*AND; activit; adverse effect; "Green Care"; healing*; health*; intervention*; negative aspect*; recreat*; relax*; restorat*; *therapy; "therapeutic landscapes"; well-being; "well being") and 2nd run (forest* AND; tree* AND; wood* AND; inclusion; mood; pedagogic*; "quality of life"; risk; social).

Results

Generally, the findings of the scientific work suggest that the time spent in woodlands can have positive effects on physical, psychological and social health and on well-being. These effects may come about due to the promotion of physical activity or simply on account of enjoying the atmosphere in the woods. More specifically, spending time in forests increases positive emotions, decreases negative emotions and helps in coping with subjectively experienced stressors. This is how woodlands contribute to mental health. With respect to physiological stress indicators, most of the studies reported positive effects and prevention of stress-related diseases. Social forestry projects make use of the forest's inclusive potential for the benefit of the socially vulnerable.

In woodlands, well-being can be affected by the duration of the stay, the activities undertaken and the physical exercise performed as well as the social context. Even short visits can have recreational effects [4]. Sports activities and exercises can enhance these positive effects [5]. Especially for mental health, woodlands seem to provide more benefits than other environments and settings [6]. A sense of safety, supported way-finding, accessibility, easy legibility of the terrain and walkability, but also a certain degree of natural diversity and alternation are important aspects of the beneficial effects. Both crowding and the complete absence of other people are considered negative, with perceived safety being an important aspect of well-being [7]. A well-kept but natural-looking impression, open tree stands and lots of light at the site as well as the absence of noise are further advantages that get people to feel at ease in woodlands [8].

As an example Shinrin-Yoku[i], which translates as "forest bathing", is currently considered a preeminent and hot topic in restoration research. Shinrin-Yoku strives at connecting the positive effects of forests for physical and mental well-being.

Discussion

We found there to be an ongoing social trend towards visiting wooded areas more frequently. This trend is reflected in the growing number of scientific publications all over the world. Empirical evidence for the positive effects of natural landscapes in general on health and well-being appears to be better researched than that for woodlands in specific. Similarly, empirical evidence for the restorative power and health benefits of woods appears to be better evaluated than therapeutic interventions. As a special natural area, woodland can have numerous positive effects on physical, psychological and social health as well as human well-being.

Projects in practice could benefit from the reported findings in three ways. Firstly, from the theoretical and empirical background. Secondly, from the detailed information relating to the planning, development and evaluation of an intervention. Thirdly, from the special layout requirements that wooded areas need to meet in order to satisfy the needs of the respective user group. The study “Green Public Health – Benefits of Woodlands on Human Health and Well-being” does not include any cost-benefit calculations and does not cover any issues relating to the legal framework. These should be taken into account in future work, however. Future research should further strive to undertake systematic reviews as well as meta-analyses and be committed to evidence-based practice. Common standards and guidelines on how to evaluate forest-based programmes and interventions are needed to ensure comparability of the results and warrant the quality of the programmes [9].

The results of the study make clear that the implementation of Green Care activities requires close collaboration between the forest and the health sector. Therefore, BFW in its current activities works together with experts from these fields to identify potential forest-related products and services for Green Care. Examples include social work with permanently unemployed people and handicapped people. Further examples are Forest-kindergarten, Forest-youth project weeks and educational programs for different age groups.

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Impact of the Actual Structure and Management on the Future Development and Sustainable Management of the Pedunculate Oak Forests in Eastern Croatia

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Introduction

Pedunculate oak forests are the most common forest type in eastern Croatian with a 50% share of total pedunculate oak forests and area of 129 thousand hectares [1]. Therefore, pedunculate oak forests in eastern Croatia present a significant and valuable natural resource whose sustainable exploitation should be based on appropriate and sustainable forest planning and management. The area represents a favourable spatial framework for considering strategic and long-term management planning of pedunculate oak forests. The main starting points of forest management planning are: existing age structure and forest development directing; structural and development stand characteristics; forest regeneration intensity and dynamics.

The age of stand, that is, its relation to theoretical age is crucial for future management planning [2,3]. As oppose to the balanced forest structure and stand, in poorer conditions the relation and range of the stands' felling age can vary considerably in rotation period. However, the balance is required between the stands with early and postponed regeneration [4]. Therefore, the deterioration level of the stand structure and the value of wood stock are becoming more important criteria for planning the regeneration fellings [5, 6]. The issue of planning suitable regeneration dynamics and selecting the stands for regeneration is especially highlighted in low-land forest management, primarily pedunculate oak forests, where drying of trees and parts of the stands presents a huge ecological and economic problem [7,8]. The presumption is to use the approach of priority regeneration of the (understocked stands) more deteriorated structure independently of their age, at the same time regenerating the deteriorated habitat by oak trees dieback [5,9,10].

Using SIMPLAG computer programme, the study's aim is to conduct a projection of the structure development on the level of each 6538 stands of the pedunculate oak management class in eastern Croatia according to several different scenarios of spatiotemporal dynamics of forest regeneration; and to analyse the development of the forest age structure and achieved potential revenues from the value of wood. Furthermore, the aim is to discuss and evaluate the applied management scenarios on the basis of several criteria and suggest optimal scenario on the basis of which the evaluated forest could be subject to long-term sustainable management with improving the existing state and reaching potential productiveness.

Materials and Methods

This study covers the area of the pedunculate oak management class in eastern Croatia, that is, the area divided in five Forest Administrations on the basis of forest and management criteria: Osijek, Vinkovci, Našice, Požega and Nova Gradiška. In total forest area of eastern Croatia (357.986 ha), the forests structurally classified as pedunculate oak forests cover the area of 103.440 ha or 29%. The age structure of pedunculate oak forests of eastern Croatia has features similar to the age structure of all pedunculate forests in Croatia. Concerning the regional spatial management level (a total of 79 management units) which includes the discussed age structure, considerable deviations from theoretical values exist. The characteristics of the age structure are: excessive 100 years old stand coverings (46,0%), small coverings of young (7,5%) and middle-aged stands (19,4%), but also mature stands (7,4%), with intensive regeneration over the past 20 years (19,7%).

In total, 6538 stands of the pedunculate oak management class on the research area have been selected based on the inventory of the Croatian Forest Fund (database of Croatian Forests Ltd.). On the basis of data on the related management unit (year of measurement, age of stand, area, number of trees, basal area, volume and volume increment per hectare and per tree species) a database was created for each subcompartment, and logic analysis and control was performed. SIMPLAG application for simulating the development of the pedunculate oak forest was used for projecting the development of the existing stands up to the time of their regeneration and determining the main felling as well as the development of the newly regenerated stands [10]. The application simulates various spatiotemporal regeneration dynamics, that is, predictions according to space and intensity of different 10 year main felling, which defines specific management scenarios. Different management approaches are defined according to four management scenarios:

- Scenario 1 – Intensity of the main fellings are continuously applied on 60% area of theoretical 20-year old age class. The evaluation includes stands that were determined to be over 100 years old in the moment the felling was classified.
- Scenario 2 – During the projection period, the main fellings are continuously applied on the basis of stands over 130 years old.
- Scenario 3 – Dynamic area of the main fellings are determined according to the area of stands determined to be over 100 years old
- Scenario 4 - Dynamic area cut of the main fellings is determined according to the area of stands determined to be over 120 years old in the moment of determining the main revenue cut.

The deterioration level of the stand structure was the selection criteria chosen for scenarios 1, 3 and 4. The cut volume of the main fellings for all scenarios results from the planned main felling area and total area of the included stands which makes the determined scenarios significantly different one from another.

Besides projecting the development model of the stands, on the basis of the embedded models of assortment structure and wood price, SIMPLAG also calculates values of achieved cuts which enables cross-analysis and evaluation of each scenario's applicability [11]. The criteria of the scenario management valuation and the approach for determining the regeneration stands are based on the relation between the development elements obtained from real forests and theoretical forests. The elements of the theoretical forest are determined on the basis of appropriate equations for defining a model of theoretical regulated even-aged forest [12].

The valuation of total achieved volume cuts and revenues is based on achieved total, that is, average amounts and compared individual indicators that deviated from the defined theoretical indicators [2]. Comprehensive evaluation and the scenarios' success in achieving the management goals were conducted according to the method of mutual pair comparison [13]. Structural and economic indicators of the applicability of each management scenario were compared as well as the volume cut sustainability during a certain projection period. Furthermore, the successfulness of determining a theoretical age structure, forest eco-stability, balances and short-term and also long-term management sustainability were analysed according to the determined scenarios. The calculation of the required variables, database formation and graphic overviews were performed in *Microsoft Excel 2010*. SIMPLAG application was used for forest development projections [10].

Results and Discussion

By analysing the development of an even-aged forest on the basis of seven indicators and their deviation from the theoretical ones (based on the results presented in Table 1), the management approach based on Scenario 1 stands out since 128% area of the pedunculate oak forests would be regenerated using this scenario's intensive regeneration approach. This would result in reducing the stands' average age and the stands' regeneration age; it would also reduce growing forest stock, with increasing the main regeneration cut by 15-16.5 million m^3, decreasing the intermediate cut (5-5.8 million m^3) and achieving value of total fellings which would be HRK1 billion higher in relation to other management approaches. From the point of view of revenues sustainability, Scenario 3 shows the smallest deviations of forest status and management indicators in relation to the theoretical criteria, which is most preferable solution.

Table 1. Management approach (scenarios) valuation by comparison of total achieved amounts over the projection period and deviations over the projection period for the appropriate management criteria. PDS – average stand age, PSEGD – average stand regeneration age, DZš – growing forest stock (average amount), UPO – total regenerated forest area, EG – regeneration cuts, EM – intermediate cuts, EUK – total fellings, BEGP – gross value of regeneration fellings, BEMP – gross value of intermediate fellings, BUKP – value of total fellings.

Criteria		Scenario							
		1		2		3		4	
		∑	I_{ODS}	∑	I_{ODS}	∑	I_{ODS}	∑	I_{ODS}
PDS	*year*	63	0.1583	68	0.1137	72	0.039*	70	0.0762
PDSEG	*year*	123	0.1291	135	0.033*	134	0.1369	135	0.0843
$DZ_š$	10^6	33.81	0.1126	36.95	0.1369	37.35	0.110*	37.31	0.1518
UPO	10^3	132.94	0.1995	107.14	0.4621	114.29	0.157*	108,79	0.3673
EG	10^6	75.02	0.1927	58.52	0.4451	60.41	0.178*	58.73	0.3187
EM	10^6	70.46	0.2127	77.30	0.2291	76.03	0.178*	78,43	0.2320
EUK	10^6	145.48	0.1448	135.82	0.2478	136.43	0.170*	137.16	0.1877
BEGP	10^9	30.74	0.289*	26.46	0.4512	25.56	0.3868	25.65	0.3805
BEMP	10^9	16.41	0.3313	19.19	0.2855	20.17	0.239*	20.23	0.2907
BUKP	10^9	47.14	0.292*	45.65	0.3494	45.73	0.3120	45.88	0.3086

* scenario with smallest criteria deviations from the theoretical model

Besides considering the development of the even-aged forests' main features per each scenario and their mutual comparison, their total valuation and selection of the most favourable one for applying in real forests is what the end-user considers important. The valuation has to encompass all long-term advantages and disadvantages of each scenario [14]. Furthermore, it is important to analyse is it possible and in which period it is possible to achieve optimal age structure, optimal forest spatial structure, forest eco-stability and management balance during the projected period.

Quantification of the acceptability of each management scenario, that is whether their application is justified, was performed for six criteria (Table 3) which encompass the most important forest management goals (Table 2). Each scenario was rated according to the criteria and the result was a rank list of scenarios per each criteria. These results point to the economic superiority of Scenario 4 and relatively most acceptable age structure movements through the projected period of Scenario 3 (Table 2).

Table 2. Evaluation matrixes for selected feasibility criteria of applying the determined management scenarios (example for two criteria).

Development of forest age structure through the projected period	1	2	3	4	Total	Transformed	Relative
1		5	-4	6	7	**37**	46.84
2	-5		-7	-3	-15	**15**	18.99
3	4	7		4	15	**45**	56.96
4	-6	3	-4		-7	**23**	29.11
Total	-7	15	-15	7	0	83	100.00

Economic profit	1	2	3	4	Total	Transformed	Relative
1		5	4	3	12	**42**	53.16
2	-5		-2	-1	-8	**22**	27.85
3	-4	2		-2	-4	**26**	32.91
4	-3	1	2		0	**30**	37.97
Total	-12	8	4	0	0	78	100.00

On the basis of the achieved points for each scenario, it is possible to perform the final acceptability evaluation of each scenario and form the rank list according to acceptability of each scenario which would be based on all important requirements of contemporary forest management planning. On the basis of total points, Scenario 3 stands out as the most acceptable since its implementation during the future period of one rotation period could achieve the set management goals or come as close as possible, uniting the advantages in economic and environmental sense (Table 3). Scenario 1 is applicable only in the circumstances of extremely excessive numbers of mature stands, and only for a short-term during several economic half-period. Generally speaking, static methods for determining the main fellings are not applicable in the long-term sense, except in theory, since they do not correspond to multi-criteria and overall planning approach and management implementation.

Table 3. Total table of determined points per each management scenario.

Evaluation criteria of management scenarios	Management scenario			
	1	2	3	4
	points*	points *	points *	points *
Level of establishing even-aged structure at the end of the projected period	27	16	37	26
Development of forest age structure during the projected period	37	15	45	23
Forest spatial structure (area and spatial layout of the stands)	22	44	28	26
Forest eco-stability during the projected period	29	26	40	29
Revenues sustainability and management balance	30	18	50	22
Economic profit	42	22	26	30
Total	187	141	**226**	156

*points per each scenario relate to transformed values of pair comparison of individual scenarios from Table 3

Concerning the highly considered theoretical area (over 50% surface of the pedunculate oak management class in Croatia) and the starting forest age structure, the scenario based on 50% area of the theoretical area of a 20-year old age class and main fellings formation based on the deterioration degree of the stands' structure requires further research. It is assumed that it would correspond to the analysed Scenario 3 based on the projected results or it would possibly exceed it with somewhat more balanced revenues.

The fact is that long-term projections can be very helpful when selecting management models or reducing mistakes which are long-term in forestry and thus very expensive. On the other hand, in the circumstances of intensive and hardly predictable changes in the habitat, long-term projections of forest development are very ungrateful. In this study the projections are burdened with several key assumptions: unconditional successful regeneration, absolute realisation of management regulations and structural development of newly regenerated stands equal to theoretical model. In this sense, future research should include risk assessment and feasibility of individual processes. Including spatial criteria (over-felling, surface cut layout in space, possible stand fluctuation between management classes and changes in stand surfaces) could provide options for improving long-term projections of forest planning and management.

Conclusions

Forest habitats of the pedunculate oak in eastern Croatia with an area of 130.000 ha, that is, over one third forests in Slavonia and around a half of all pedunculate forests in the Republic of Croatia, are extremely valuable and important natural resource. Based on the structure of forests and stands, there are considerable options and requirements for gradual improvement of the existing state and achievement of potential productivity in the sense of forming an even-aged and spatial forest structure; shaping and quality promotion of the existing and newly regenerated stands as well as increasing the value of the fellings. Since the negative influences on forest eco-systems are not easily predicted, the goal of achieving the mentioned management criteria and potential 10 year gross revenue of around HRK4.4 billion could be supported by the management approach according to scena rio 3. According to given scenario cutting intensity is based on stand area dynamics of stands older than 100 years, as well as by objective criteria (economic, structural and habitat features of the stands) for selecting the stands for regeneration. Such approach has proven to be the most acceptable for sustainable management in the long-term and short-term sense.

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Session B: Functional food and useful products from natural source

PROBIOTIC FERMENTED MILK WITH FREEZE DRIED IMMOBILIZED *LACTOBACILLUS CASEI* ATCC 393 CELLS ON APPLE PIECES

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Introduction

The development of novel functional foods containing probiotic is a highly growing area of the food industry and attracts special interest from the field of nutrition, due to their beneficial properties to human health [1]. However, to deliver their health benefits, probiotics must be present in food products above a threshold level (>6 logcfu/g) at the time of consumption, in order to survive the passage through the upper and lower parts of the gastrointestinal tract [2]. *Lactobacillus casei* ATCC 393 strain has been reported to have beneficial effects for cholesterol removal [3], activity against cancer cell proliferation [4], survived the passage through the gastrointestinal tract in a rat model and potential regulates intestinal microbiota [5]. In order to be of practical use, immobilized biocatalysts must be formulated as products capable of storage, distribution and application in agricultural and food marketplace [6]. Freeze drying is the most convenient and successful method of preserving yeasts, sporulating fungi and bacteria [7]. The aim of the present study was to evaluate the effect of freeze dried immobilized *Lactobacillus casei* ATCC 393 cells on apple pieces, on physicochemical and microbiological characteristics of probiotic fermented milk during 4 weeks storage at 4^{o}C and to monitor its survival.

Materials and Methods

Strain

L. casei (DSMZ, ATCC393) and the thermophilic yogurt starter, CH-1 (Chr. Hansen, Hørsholm, Denmark) *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* were used.

Immobilization procedure and freeze-drying

Apples were cut in small pieces (cubes of ≈ 1 cm acme) and placed in a conical flask containing *L. casei* cells in MRS broth. The mixture was left undistributed at 37 ^{o}C for 24 h. When the immobilization was completed, the fermented liquid was decanted and the immobilized cells were washed twice with sterilized ¼ Ringer solution. The immobilized biocatalyst was transferred in sterile bottles and was covered with the solution of protective agent (10% sodium glutamate) for 1 hour at 20°C. Then the solution was decanted, the biocatalyst was frozen to −45°C (cooling rate 5°C/min) and finally freeze-dried. The freeze dried biocatalyst was rehydrated with deionized water.

Fermented milk manufacture

Pasteurized bovine milk (3.7 % fat) was heated at 37°C and the immobilized cells (10% w/v wet) were added. After 30 min, all samples were inoculated with the activated CH-1 culture. Milks were fermented at 37°C until pH value of 4.6. Thereafter, the fermented milks cooled to 15°C in ice water and stored at 4°C for 28 days (sample AP). For comparison reasons control fermented milk with only the CH-1 culture (sample C), and fermented milk with free cells of *L. casei* (sample FC) were also produced.

Determination of culture viability

Fermented milk samples were added to Ringer solution and the appropriate serial dilutions were prepared. *S. thermophilus* was enumerated in M-17 agar supplemented with lactose, at 45°C. *L. bulgaricus* in MRS agar, adjusted to pH 5.2 at 45°C. *L. casei* in lithium propionate MRS (LP-MRS) agar (lithium chloride 0.2% w/v and sodium propionate 0.3% w/v) at 37°C.

pH: Using a digital pH meter. *Titratable acidity*: By titrating with 0.1N NaOH solution.

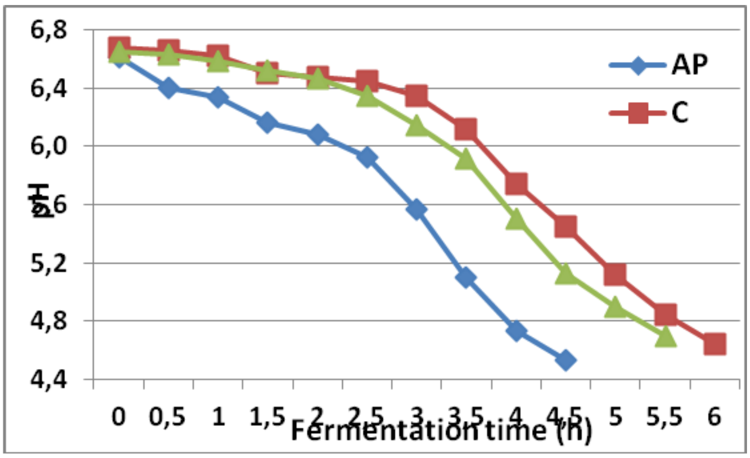
Sensory evaluation

20 panelists, familiar with the consumption of fermented milk, were used to evaluate the produced fermented milks (after storage for 21 days at 4 °C) for color, sweet odor, smoothness, sweetness, viscosity and overall acceptability, using a 10-point hedonic scale ranging from 1 (“dislike extremely”) to 10 (“like extremely”).

Results and Discussion

Fermentation process

Figure 1 shows the decrease of pH in fermented milks after the addition of cultures. The use of *L. casei* either as free cells or freeze dried immobilized on apple pieces led to faster fermentation. More specifically the use of free cells led to 8.5% reduction in fermentation time, while the use of freeze-dried immobilized cells to 25% reduction. This result is very important from technological point of view for the dairy industry, since the reduction of fermentation time may lead to increase in productivity.



(C: Fermented milk without *L. casei*; FC: Fermented milk with free cells of *L. casei*; AP: Fermented milk with freeze dried immobilized *L. casei*)

Figure 1. Acidification of milk during fermented milk production.

The effect of culture type and storage time on pH value of fermented milks is presented in table 1. The initial pH value in all cases range between 4.65 – 4.70, however during the storage time a decrease was observed in all cases. This decrease was more rapid in the case of fermented milks with *L. casei* cells. More specifically after 28 days of storage the pH value of fermented milks were 4.07 in control, 4.05 in free cells and 3.85 in freeze-dried immobilized cells of *L. casei* on apple pieces.

Titratable acidity

Table 1 shows the effect of culture type and storage time on titratable acidity of fermented milks. The use of immobilized and free *L. casei* cells led to higher titratable acidity of fermented milks during storage at 4^{o}C. The cultures continued to produce acid until the end of the storage period, resulting in increased titratable acidity, from day 0 to day 28. The use of freeze-dried immobilized *L. casei* cells on apple pieces led to higher acidities (0.95% w/v), compared to free cells of *L. casei* (0.90% w/v) and control (0.88% w/v).

Table 1. Acidity and pH of fermented milks during refrigerated storage at 4°C.

Storage days	**Acidity (lactic acid %w/v)**			**pH**		
	C	**FC**	**AP**	**C**	**FC**	**AP**
0	0.69±0.02	0.70±0.03	0.70±0.01	4.70±0.05	4.69±0.06	4.65±0.06
7	0.76±0.01	0.77±0.01	0.78±0.01	4.51±0.06	4.49±0.03	4.25±0.03
14	0.79±0.01	0.80±0.01	0.82±0.03	4.36±0.04	4.30±0.04	4.18±0.03
21	0.82±0.01	0.83±0.03	0.85±0.02	4.16±0.03	4.13±0.02	4.03±0.02
28	0.88±0.02	0.90±0.01	0.95±0.01	4.07±0.02	4.05±0.02	3.85±0.01

C: Fermented milk without *L. casei*; FC: Fermented milk with free cells of *L. casei*; AP: Fermented milk with freeze dried immobilized *L. casei*

Determination of culture viability

Starter bacteria *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* produce inhibitory substances such as acid and hydrogen peroxide during fermentation which could potentially reduce the viability of probiotic bacteria [8]. For this reason two-step fermentation process was used. At the first step of fermentation only the free or immobilized cells of *L. casei* was added and after 30 min, in the second step, the CH-1 culture was added. This method gives time to the probiotic culture to increase in numbers prior the addition of CH-1 culture. *S. thermophilus* retained high numbers of viable cells throughout storage (Table 2). Our findings are in agreement with those of previous studies, that *S. thermophilus* generally survives well (>10^{8}cfu/mL) in fermented milks stored under refrigeration for 3 to 6 weeks [9, 10]. In all samples and at any point of storage the numbers of *S. thermophilus* were higher than those of *L. delbrueckii* ssp. *bulgaricus*. Furthermore, the sum of *S. thermophilus* and *L. bulgaricus* strains was above the minimum requirement of 10^{7} viable microorganisms per gram [11]. In the case of fermented milks produced using also cells of *L. casei* the numbers of these bacterial strains were lower compared to control fermented milks. This may be attributed to the competitive action of *L. casei* cells. By the end of the storage period, the sum of the viable counts of *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, in all cases, had declined to ≈2 x 10^8 - 4 x 10^7 cfu/g, which is in accordance to other studies [12].

An increase in the numbers of *L. casei* cells was observed during the fermentation process. After that period the viable cells declined. However this decline was faster in the case of free cells. This may be attributed to the protective role of apple pieces. It must be noted that during fermented milk production and storage the numbers of *L. casei* were above the minimum requirement (6 logcfu/g) for conferring a probiotic effect.

Table 2. Survival of *Streptococcus thermophilus, Lactobacillus bulgaricus* and *Lactobacillus casei* (logcfu/g) in fermented milks during refrigerated storage at 4°C.

Storage days	***Streptococcus thermophilus* (logcfu/g)**			***Lactobacillus bulgaricus* (logcfu/g)**			***Lactobacillus casei* (logcfu/g)**	
	C	**FC**	**AP**	**C**	**FC**	**AP**	**FC**	**AP**
0	8.60±0.05	8.56±0.06	8.50±0.06	8.48±0.08	8.58±0.06	8.40±0.07	8.63±0.08	8.72±0.06
7	8.40±0.04	8.34±0.03	8.28±0.03	8.39±0.07	8.31±0.06	8.05±0.05	8.25±0.03	8.51±0.06
14	8.35±0.03	8.22±0.04	7.91±0.03	8.11±0.04	8.15±0.04	7.67±0.03	8.05±0.04	8.35±0.02
21	8.29±0.03	8.20±0.03	7.72±0.02	7.97±0.03	7.91±0.03	7.37±0.04	7.80±0.02	8.25±0.03
28	8.12±0.04	8.02±0.02	7.57±0.03	7.75±0.02	7.78±0.02	7.01±0.02	7.61±0.03	8.06±0.02

C: Fermented milk without *L. casei*; FC: Fermented milk with free cells of *L. casei*; AP: Fermented milk with freeze dried immobilized *L. casei*

Sensory evaluation

The sensory evaluation ascertained the overall quality of the probiotic fermented milks that scored similar values with the commercial sample, apart from the control fermented milk which scored lower values (Figure 2). In general fermented milk with freeze dried immobilized *L. casei* cells on apple pieces scored higher values in the case of sweetness, and smoothness than all the other samples and similar values with the commercial fermented milk in the other categories. The overall acceptability was higher for the fermented milk with freeze dried immobilized *L. casei* on apple pieces and for the commercial sample followed by fermented milk with free cells of *L. casei* and control.

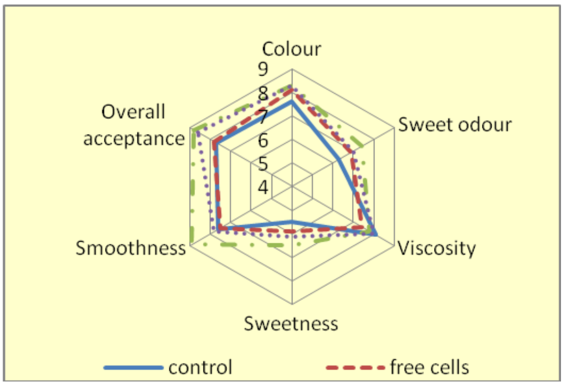


Figure 2. Sensory evaluation of fermented milks after storage at 4 °C for 21 days.

Conclusions

Freeze-dried immobilized *L. casei* ATCC 393 on apple pieces proved suitable biocatalyst for the manufacture of fermented dairy products potentially capable of producing a beneficial effect on human metabolism and health even after 4 weeks of refrigerated storage.

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TURKISH CACİK AND LABNEH PRODUCTION METHODS AND SOME PROPERTIES

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Introduction

Traditional Turkish dairy products, are commonly made in all region of Turkey. Traditional producing method is not standardized, but generally cow's milk is used in the production and when sheep and buffalo milk are available, these milks are mixed with cow's milk. Production time is generally in summer season (1).

Cacik, produced in the eastern and southeastern parts of Turkey, is a dairy byproduct produced by heating of defatted yogurt, which is called "*ayran*" in Turkish. Some water is added to the yogurt during butter production; so ayran contains some additional water (probably 1:1 ratios, yogurt and water). The manufacturing steps are similar to those for Cokelek or Tomas cheeses, but some herbs are added to Cacik. Firstly, yoghurt is churned for butter production by adding some water. Cacik has an aroma of wild garlic and other herbs (2).

Labneh (concentrated yoghurt), which is popularly known as labneh' in the Middle East and as strained yoghurt' in Greece and the rest of Europe, is consumed as a main dish at breakfast in many middle eastern countries, such as Iraq, Iran and the Lebanon, but it can also be served as a dip with garlic, dried herbs (usually mint and parsley) and red peppers, or with cucumber and olive oil. Traditionally, concentrated yoghurt was made by pouring normal yoghurt. Although technology has provided new equipment for preparation of labneh, the conventional method is still in common use at the domestic level as well as in many dairy plants. Generally, this method simply consists of hanging the cold full-fat or plain yogurt in a cloth bag to remove some of the whey and achieve the desired body having a semisolid consistency (3).

Production of cacik (herb added cokelek) and properties

Traditional producing method is not standardized, but generally cow's milk is used in the production and when sheep and buffalo milk are available, these milks are mixed with cow's milk. Production time is generally in summer season. For production of Cokelek, milk is first filtered through a cheese-cloth prior to heat treatment to remove unwanted materials.

For production of Cokelek, milk is first filtered through a cheese-cloth prior to heat treatment to remove unwanted materials. After clarification, it is cooled to 40-45°C and inoculated with yoghurt culture (2-3%). The inoculated milk is filled into big containers and incubated. The yoghurt is then churned into the butter and Ayran (buttermilk). Ayran is heated to 90-100°C for about 10 min until a white coagulum floats on the surface; the curd is transferred into a cheese cloth and some weight is placed on it to drain water off until the desired solids level is

reached. The curd is salted 2-3% with dry salt and filled into cotton bags. The bags are hung up in a warm room for 2-3 days. The bags are occasionally turned over for homogeneous dryness. The cheeses reached up 60 to 70% total solids content after storing in a cool place for 4-5 months can be marketed (4). Kes cheese is commonly produced in small family businesses for their needs and also by small dairy processing plants for commercial purposes.

Cacik, produced in the eastern and southeastern parts of Turkey, is a dairy byproduct produced by heating of defatted yogurt, which is called "*ayran*" in Turkish. Some water is added to the yogurt during butter production; so ayran contains some additional water (probably 1:1 ratios, yogurt and water). The manufacturing steps are similar to those for Cokelek or Tomas cheeses, but some herbs are added to Cacik. Firstly, yoghurt is churned for butter production by adding some water. After the removal of milk fat, ayran is boiled for 5-10 min or until a white coagulum floats on the surface. The coagulum is collected and transferred to a cloth for draining off the excess serum. It has a low fat content; the chemical composition of Cacik are: total solids, 16.5-20.8%; fat, 1.5-4.3%; protein, 8.1-13.9%; salt, 0.3-3.2%; pH, 3.2-4.2 (2).

Herby cacik is a traditional food specially produced from mixer of yoghurt, ayran and sometime small amount of whey. Milk should be clarified prior to heat treatment to remove unwanted materials. After clarification, the milk is boiled for 20-30 min to increase its solid content. It is then inoculated with yoghurt culture. The yoghurt is then churned and butter and ayran produced. Yoghurt or ayran is heated about 10min until a white coagulum floats on the surface: the çökelek is formed by placing this in a clot bag and putting weights on it to drain water off until the desired solids level is reached. Then prepared herbs are added at a level of 1-2% and mixed well. The cacik can be eaten fresh or it can be used with herby cheese to fill the cheese container (6).
Lactic acid bacteria (LAB) counts were the dominant bacteria during the storage period and may contribute to Kes cheese production. Levels of lactic acid bacteria on MRS agar were found 4.46 log cfu/g. Kucukoner et al. (2) found similar results in Cacik samples. *Lactic bacterial* produces lactic acid from lactose and is able to grow at high acid levels. However, during storage, lactobacilli increased while *lactococci* decreased, because lactobacilli are more resistant to high acid levels than *lactococci*. It is known that the proteolytic activity of *lactobacilli* is higher than that of *lactococci*. Thus, depending upon the increase in lactobacilli numbers, TCA (trichloroacetic acid) values increased. Proteolitic microorganism counts of Kes cheeses (4) were similar to lactic acid bacteria counts. The proteolytic enzymes of yeast and mould may contribute to a small increase in water soluble nitrogen and tricloro-acetic acid soluble nitrogen values.

The herbs are commonly used in cacik as follows; *Allium* sp., *Chaerophyllum macropodum, Ferula* sp., *Prangos* sp., *Silene vulgaris, Tymus* sp. and *Mentha* sp. and sometimes cucumber, green pepper and other additives, they are added to cacik at different levels. Some researchers reported that the herbs had antimicrobial activity (7). These additives give the cacik its flavor, as well as aiding in the preservation of cacik.

Kucukoner and Tarakci (8), worked on chemical and microbiological properties of 15 cacik samples. According to their analysis results, the dry matter 22.07%, fat 2.69%, protein 14.51%, total ash 3.31%, salt 1.97% and titratable acidity 1.93 were found averagely. Tarakci et al. (4) analyzed a dairy product kes (dried concentrated yogurt, kurut) and concluded that average dry matter 68.03%, fat 11.35%, protein 42.34%, total ash 8.33%, salt 7.08% of samples were found.

Production of labneh (concentrated yogurt) and properties

Labneh, concentrated yoghurt, popularly known as labneh in the Middle East and as strained yoghurt in Greece and the rest of Europe, is consumed as a main dish at breakfast in many Middle Eastern countries, such as Iraq, Iran and Lebanon. Labneh can also be served as a dip with garlic, dried herbs (usually mint and parsley) and red peppers, or with cucumber and olive oil. Traditionally, concentrated yoghurt is made by pouring plain labneh (120-140 g⁄kg total solids) into a cloth or animal-skin bag and letting it to drain away until the residual product had a composition of some 230-250 g⁄kg total solids, 80-100 g⁄kg fat and an acidity of 1.8-2.0 g⁄100 g lactic acid (9). Labneh, traditional fermented milk foods made from the milk of cows, sheep, and goats are still competitive with newer products. In the Middle East region, concentrated yogurt (labneh) is highly appreciated and consumed with bread all year around. Labneh is an important supplement to the local diet and provides vital elements for growth and good health. According to Lebanese standards, labneh is defined as a semisolid food derived from yogurt by draining away part of its water and water-soluble compounds. Usually, labneh is prepared with two solids concentration ranges either around 22 wt % or around 40 wt % (labneh). The former is prepared to be consumed within two weeks and usually stored in refrigerators; the other one is stored in vegetable oil at room temperature and can be consumed within two years (10, 11). It is a semi-solid product derived from yogurt by draining away part of its water and water-soluble components. The total solids and fat contents of Labneh are typically 23 to 25 g/100 g and 10 g/100 g, respectively, and the product is characterized by a cream or white color, a soft and smooth body, good spread ability, and a flavor that is clean and slightly acidic (12).

Effects of adding herbs; purslane, dill, parsley, cress, coriander, rocket, diplotaxis and mint on physicochemical and sensorial properties of labneh were investigated by Tarakci et al. (13). It was found that labneh made with the adding of herbs differed from each other depending on the herb strain and storage time. The addition of herbs to labneh were lead to a decrease in the L^* and a^* values. During storage, labneh pH decreased, but the titratable acidity, L^* and b^* values increased. Adding herbs to labneh influenced the mineral contents to different degrees. There were significant differences in Ca, P, Na, Mg, Zn, Cu, Co and Mo content of the samples. At the end of sensory evaluations, appearance and colour, body and texture were not significantly affected by storage time while flavor decreased. Moreover, higher awareness of the perceived health risks associated with the consumption of diets high in fat has fueled the growing consumer demand for low-fat products and led to an increase in the production of reduced-fat varieties of Labneh (3).

In the sensorial evaluations, labneh samples containing mint, parsley and diplotaxis added labneh were preferred to other labneh samples. Dill, mint, parsley, coriander, diplotaxis, purslane would be recommended for the production of labneh. Different types of milk can be used in the production of Labneh; namely, cow, sheep, and goat milks, although cow and, to a lesser extent, goat are more common (13). Labneh is traditionally produced by straining natural or plain full-fat yogurt in cloth bags until the desired level of total solids is achieved. However, the cloth bag method has many disadvantages compared with large- or factory-scale operations. Modern Labneh manufacturing methods include the use of centrifugation, recombination technology, and ultrafiltration (12).

Conclusion

The level of essential minerals and trace elements that occur in cow milk depend on a number of factors such as genetic characteristics, stage of lactation, environmental conditions, and types of pasture. The levels, in which they are present in dairy products, depend also on the technological treatment of the products. Cacik a traditional Turkish dairy products, is commonly made in Eastern Anatolia in Turkey. Cacik has an aroma of wild garlic and other herbs. It is widely produced and consumed in eastern and southeastern parts of Turkey. Therefore it is also produced and consumed other parts of country.

Labneh is a traditional dairy product with high nutritional value, unique taste and aroma. Chemical composition and there for sensorial properties of labneh samples are significantly different due to ingredients used such as the ayran, skim milk and cheese added, different manufacturing, packaging, and ripening conditions. In order to have a standard product, all process steps should be standardized.

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THE EFFECTS OF STARTER CULTURE ON CHEMICAL COMPOSITION, TEXTURAL AND SENSORY CHARACTERISTICS OF TURKISH TULUM CHEESE WITH HALF FAT DURING RIPENING

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Introduction

The excess fat that is taken with the diet is known to lead to serious health problems such as obesity, cardiovascular diseases and high blood pressure. However, in recent years along with increasing health awareness the demand for low-fat and fat-free food products has increased. The biggest market share in the reduced-fat segment pertains to dairy products, cheeses having the largest among. A research by Borg [1] indicates that in 1998 the low-fat and reduced-fat cheeses accounted for the 20% of the total cheeses sold in the United States. According to International Dairy Products Association in US low-fat and fat-free cheese consumption increased by 134 million pounds while full-fat cheese consumption decreased by 54 million pounds between 1999 and 2005. In the same period Mozzarella cheese, which has lower fat content, took over the throne of the Cheddar cheese and became the most preferred cheese in the US. In addition to the current products in the market, the food industry launches novel reduced-fat products every year to respond to this demand. Although the volume is not high, reduced- or low-fat cheese manufacture is available in our country and its share increases with the passing years.

Milk fat is not only an energy source but also a contributor to the flavor and texture of cheese while adding some functional properties. Reducing the fat content of cheese would result in physical and organoleptic defects. Therefore, the manufacturers have been using different ingredients and production methods for producing low fat cheeses giving similar mouth feel and taste with the full-fat cheese. Use of starter cultures is one of the preferred methods to improve the quality and remedy these defects. Characteristics of low-fat (~ 7%) and full-fat (~ 22%) Feta cheeses those were made using commercial adjunct cultures were investigated during the 120-days ripening period by a group from Greece. In regards to ripening time the culture added low-fat cheeses had no significant differences in compositional and textural characteristics in comparison with the low-fat control cheese. Although the experimental low-fat cheeses did not reach the total score of full-fat cheeses they received significantly higher total scores than the low-fat control cheese [2] suggesting that starters can contribute the perception and likeness of low-fat cheeses.

In another study by Oner et. al. [3] Tulum cheeses made with two different starter compositions were compared with starter-free cheeses. Sensory evaluation results indicated that both starter added Tulum cheeses received higher scores compared to the cheese with no starter culture addition. A similar study on Tulum cheeses over different types of starter cultures showed that only one out of three starters had better taste, texture and odor than the cheeses without starter culture [4]. A study investigated the effects of adding adjunct cultures to low-fat Kefalograviera cheese to improve its sensory quality. They found that the addition of the adjunct cultures did not significantly change the compositional and textural properties. However, the low-fat cheeses made with the adjunct cultures received significantly higher scores for flavour intensity, body and texture than the control low-fat cheese after 90 and 180 days of ripening. Although the low-fat cheeses (9.7%) made with adjunct cultures received significantly lower body and texture scores, their flavour scores were found to be similar to those of the full-fat (30.6%) cheese [5].

Lactobacillus heveticus ve Lactobacillus delbrueckii subsp*. bulgaricus* were used as adjunct cultures in white cheese manufacture and they were found to not to affect the composition but accelerate the ripening process [6]. Tarakçı and Tunçtürk [7] showed that degradation of αs1 and β-caseins was slightly higher in the adjunct culture containing cheeses than the control and high amounts of casein degradation products were observed in the experimental cheeses than the control cheese produced with standard culture based on the urea-PAGE electrophoretogram. Tulum cheese is one of the traditional cheeses of Turkey produced and consumed all over the country especially in northern region. In this study, selected starter cultures were added to pasteurized 1.7% fat milk to obtain Tulum cheeses and a non-cultured cheese was used as a control. After dry salting the cheeses were packed in glass jars and ripened at refrigerator temperature. Chemical and sensory analyses of the cheeses were conducted at certain time periods (2, 15 and 60 days) during ripening. Furthermore, the changes in protein fractions which are significantly effective in ripening were analyzed.

Materials and Methods

Manufacture of Tulum Cheese

Manufacturing process was carried out at Korsas AS, a local dairy plant in Ordu. Cow milk that was used for the cheese production was about 150 liters and was obtained from surrounding farms. The fat content of milk was adjusted to 1.7% after the milk was pasteurized at 85°C for 10 minutes. Then, reduced fat milk was divided into four batches for starter addition and further steps. Three different starters were added into three separate batches according to manufacturer's instructions while one batch was kept as a control with no culture addition. Fermentation and pH measurements were followed by calcium chloride addition. When the pH reaches approximately 6.0 Ren-Na calf rennet (strength, 1:16000 mcu/ml; Mayasan AS) was added to all four batches to coagulate the milk within 45 minutes.

The coagulum was cut into ~ 1 cm^3 and the curd pieces were transferred into tin pots lined with cheese cloth and pressed for 2 hours. Subsequently cheese curd was broken into 3-4 cm^3 sized pieces. The next day cheese pieces were broken up into smaller pieces manually and dry salted (2.5% w/w). The cheese yield was about 18% before salting. Salted cheeses were blended several times to drain excess water as much as possible during the next two days. Subsequently, cheeses were packed into glass jars without any air holes. They were left to ripen at 4 ± 1°C for 60 days. The jars were turned upside down leaving the caps loose to allow water drainage during ripening and accumulated water was removed away periodically. Analyses were performed on the 2nd, 30^{th} and 60^{th} days of ripening and a new jar of cheese was opened for each analysis period [8].

Starter Cultures

Three types of liyophilized starter cultures used in this study were kindly provided by Turker Teknik Tic. Ltd. Sti. (Istanbul, Turkey) and their compositions are listed below:

Starter Culture 1 (Choozit tm **MA** 11, Danisco, France)*; Lactococcus lactis* subsp. *lactis: Lactococcus lactis* subsp. *cremoris,*

Starter Culture *2* (Choozit tm **BT** 01, Danisco, France)*; Lactococcus lactis* subsp. *lactis: Lactococcus lactis* subsp. *cremoris: Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis,*

Starter Culture *3* (Choozit **Feta** A, Danisco, France)*; Lactococcus lactis* subsp*. lactis: Lactococcus lactis* subsp. *cremoris: Streptococcus thermophilus: Lactobacillus delbrueckii* subsp. *bulgaricus: Lactobacillus helveticus.*

Chemical Analysis

Cheese samples were analyzed for moisture by the gravimetric method [9], for fat by the Gerber Method, for salt according to the Mohr Method by titration with $AgNO_3$ [10]. Titratable acidity was determined as %lactic acid of cheese using the AOAC method [11]. For pH measurement 10 grams of cheese was macerated in 100 ml and readings were obtained using a digital pH meter (model Starter 3100; OHAUS, NJ, USA). Water soluble nitrogen (WSN), 12% TCA-soluble nitrogen (TCA-SN) and 5% phosphotungstic acid-soluble (PTA-SN) fractions were determined by the Kjeldahl Method [12]. Ripening index was calculated as the percentage of WSN to total nitrogen. All cheese samples were analyzed in duplicate.

Urea-Polyacrylamide Gel Electrophoresis (Urea-PAGE).

Urea-PAGE electrophoresis was applied according to Tarakci and Akyuz [13].

Sensory Evaluation

Sensory tests were applied to a panel consisting of 8 untrained people at Ordu University Faculty of Agriculture. They graded the cheeses for four criteria; (1) color and appearance, (2) body and texture, (3) odor and (4) flavor. Based on their preference and likeness panelists were requested to grade the samples out of 5; 1 is being unacceptable and 5 is being very much like it. Samples were served as portions of 15-20 grams with water and whole wheat crackers.

Results and Discussion

Biochemical Changes

In general the BT cheese had the lowest pH values all over the analysis period. Therefore, it would be proper to say this culture has a higher acid production rate comparatively. On the other hand, as expected the control cheese had the highest pH values since it did not include any additional culture. The titratable acidity levels increased with the ripening time similar to the results obtained by Tarakci et. al. [8]. Salt and fat levels did not change significantly with the ripening time and among different types of cheeses. The slight changes are likely related with the moisture content (Table 1). Although there are fluctuations the total solid content increased for all cheeses when the beginning and final moisture contents were compared. The total solid contents were found to be similar to the values of Oner et al. [3].

Table 1. Chemical compositions and pH values of Tulum cheeses at 2nd, 30th and 60th days.

		Cheeses			
Variables	Days	BT	MA	Feta	Control
pH	2	4.91 ± 0.01	5.12 ± 0.00	5.24 ± 0.01	5.42 ± 0.00
	30	4.91 ± 0.02	5.03 ± 0.01	5.12 ± 0.00	5.19 ± 0.02
	60	5.03 ± 0.01	4.90 ± 0.01	5.15 ± 0.01	5.18 ± 0.01
Titratable acidity (%)	2	1.53 ± 0.04	1.17 ± 0.04	1.14 ± 0.00	1.20 ± 0.00
	30	1.74 ± 0.08	1.32 ± 0.08	1.38 ± 0.00	1.35 ± 0.04
	60	1.59 ± 0.08	1.62 ± 0.08	1.47 ± 0.00	1.65 ± 0.04
Salt-in-moisture (%)	2	2.16 ± 0.00	2.22 ± 0.00	2.46 ± 0.00	2.31 ± 0.04
	30	2.72 ± 0.04	2.57 ± 0.00	2.54 ± 0.04	2.43 ± 0.12
	60	2.49 ± 0.04	2.31 ± 0.29	2.40 ± 0.00	2.34 ± 0.00
Fat-in-DM (%)	2	26.03 ± 1.27	29.66 ± 1.88	27.89 ± 2.47	26.54 ± 1.21
	30	29.86 ± 0.00	30.09 ± 0.00	29.36 ± 0.60	29.60 ± 0.60
	60	31.04 ± 0.00	30.31 ± 0.60	27.25 ± 0.00	27.68 ± 0.00
Total protein (%)	2	15.00 ± 6.33	17.77 ± 0.19	11.15 ± 0.12	17.38 ± 3.47
	30	12.76 ± 2.28	17.46 ± 7.30	5.04 ± 3.74	3.92 ± 1.58
	60	19.32 ± 0.12	17.87 ± 0.53	12.60 ± 4.38	16.43 ± 4.67
Total solids (%)	2	55.71 ± 1.23	56.47 ± 0.15	57.36 ± 0.87	58.41 ± 0.67
	30	58.60 ± 0.22	58.16 ± 0.38	58.76 ± 0.05	58.28 ± 0.24
	60	57.99 ± 0.55	58.57 ± 0.19	60.54 ± 0.27	59.61 ± 0.45

Soluble nitrogen fractions readings and ripening index did not give a clear idea about the ripening progress due to the unstable results. The only conclusion that can be drawn from the ripening index is the Feta culture had visibly higher ripening index. The total nitrogen values were found to be similar for all cheeses except the Tulum cheese that was cultured with Feta (Table 2).

Table 2. Soluble nitrogen fractions of Tulum cheeses at 2nd, 30th and 60th days.

		Cheeses			
Variables	Days	BT	MA	Feta	Control
Total N (%)	2	2.35 ± 0.99	2.79 ± 003	1.75 ± 0.02	2.72 ± 0.54
	30	2.00 ± 0.36	2.74 ± 1.14	0.79 ± 0.59	0.61 ± 0.25
	60	3.03 ± 0.02	2.80 ± 0.08	1.97± 0.69	2.58 ± 0.73
Ripening Index	2	13.74 ± 0.41	6.67 ± 0.35	11.02 ± 0.00	7.83 ± 1.07
	30	24.92 ± 0.46	17.97 ± 0.67	59.02 ± 0.32	69.47 ± 11.91
	60	13.68 ± 3.63	14.32 ± 0.00	37.70 ± 0.46	27.64 ± 2.84
TCA (% of Total N)	2	1.75 ± 0.00	1.73 ± 0.35	3.15 ± 0.00	1.26 ± 0.36
	30	10.03 ± 0.46	4.96 ± 0.33	9.84 ± 2.32	27.37 ± 0.00
	60	6.68 ± 0.38	5.54 ± 0.65	7.87 ± 0.98	4.27 ± 0.36
PTA (% of Total N)	2	1.75 ± 0.83	0.99 ± 0.00	0.79± 0.00	0.50 ± 0.00
	30	1.29 ± 0.00	1.35 ± 0.00	6.83 ± 0.93	4.51 ± 2.13
	60	0.64 ± 0.30	1.15 ± 0.33	1.64 ± 0.46	0.50 ± 0.00

Sensory Evaluation

Regarding odor, body & texture and color & appearance the BT culture had the highest score predominantly until the last measurement suggesting consumers look for the buttery flavor, crumbled texture and whitish color in Tulum cheeses. Although control cheese received the lowest overall sensory scores for the 2nd and 30th days, only one out of three cultured cheeses had higher score than the control cheese at the end of 60 days period which is probably due to over proteolysis and lipolysis and their products contributing to the overall sensory characteristics in cultured cheeses. When taste & flavor and odor were considered control cheese received the highest score suggesting cultures were effective on the bitter and sour taste as well as the strong flavor by the 60th day of ripening (Table 3).

Table 3. Sensory scores of Tulum cheeses at 2nd, 30th and 60th days.

		Cheeses			
Variables	Days	BT	MA	Feta	Control
Color & Appearance	2	4.63 ± 0.74	3.75 ± 1.28	4.13 ± 0.64	4.00 ± 1.41
	30	4.13 ± 1.13	3.75 ± 0.46	3.88 ± 0.64	3.13 ± 0.83
	60	3.86 ± 0.90	4.14 ± 1.07	3.14 ± 0.90	3.14 ± 1.07
Body & Texture	2	4.38 ± 0.52	3.88 ± 1.13	4.00 ± 1.07	3.34 ± 1.13
	30	3.5 ± 1.07	3.38 ± 1.06	3.38 ± 0.92	3.44 ± 1.40
	60	3.14 ± 0.69	3.50 ± 1.04	2.93 ± 0.93	3.29 ± 1.25
Taste & Flavor	2	4.33 ± 0.47	3.50 ± 1.41	3.96 ± 0.88	3.48 ± 1.28
	30	2.94 ± 1.57	3.50 ± 1.51	4.13 ± 0.83	2.75 ± 1.39
	60	3.07 ± 1.24	3.29 ± 0.95	3.14 ± 0.90	3.86 ± 1.07
Odor	2	4.43 ± 0.79	3.57 ± 1.13	3.14 ± 1.21	3.86 ± 0.69
	30	4.25 ± 0.71	3.38 ± 0.92	3.63 ± 1.30	3.88 ± 1.36
	60	3.29 ± 0.76	3.57 ± 0.79	3.14 ± 0.90	3.86 ± 0.90

Conclusions

As a conclusion, while the total solids, pH and titratable acidity increase the fat and salt (2.16-2.72) concentrations did not change considerably during the 60 days ripening period at refrigerator temperature for Tulum cheeses that were produced with reduced fat cow milk. Total protein concentrations were found to be similar for all cheeses except the Tulum cheese with Feta culture. Cultured cheeses had lower pH but similar acidity compared to control cheese. As an indicator of proteolysis; PTA and TCA values increased during the ripening period for all cheeses and had lower values in the control cheese suggesting starter cultures played an important role in the proteolysis.

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EFFECTS OF ADDING CHERRY LAUREL (*Laurocerasus officinalis*) ON MICRO MINERAL CONCENTRATIONS OF TARHANA

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Introduction

Tarhana, a traditional fermented food produced from a mixture of wheat flour and yoghurt, is widely consumed in Turkey. It is prepared by mixing tarhana, cereal flours and a variety of cooked vegetables, including red and green peppers, onions and tomatoes flavouring agents are also added, including salt, mint, paprika and various herbs [1]. The amount and type of ingredients, as well as the processing techniques, might vary from place to place in Turkey. The mixture is then fermented for 1-7 days [2].

Cherry laurel (*Laurocerasus officinalis*) belongs to the *Rosaceae* family and is a popular fruit (dark purple or black when mature), mainly distributed in the coasts of the Black Sea region of Turkey and is locally called ''*Karayemis*'' [3]. In Turkey, approximately 5000 tons of Cherry laurel fruit is produced per annum. The composition of Cherry laurel marmalade is: 80.32% moisture, 15.31% soluble solids, 112.6 g/kg total sugar, 108.7 g/kg invert sugar, 3.7 g/kg sucrose, 4.69 pH, 0.47% crude fiber, 0.23% pectin, 2139.6 mg/kg total phenolic matter, 0.53% ash, 90.19 mg/100 g potassium [4]. It is mostly consumed as fresh fruit in local markets although it may also be dried, pickled, and processed into pekmez, jam, marmalade, and fruit juice products [5]. Besides its use for food, both fruit and seeds of cherry laurel are well known as traditional medicines in Turkey and have been used for many years for the treatment of stomach ulcers, digestive system complaints, bronchitis, eczemas, hemorrhoids, and as a diuretic agent, among others [6]. The aim of this study was to investigate the effect of cherry laurel pulp on the some micro mineral characteristics of Tarhana.

Materials and Methods

Sample Obtaining

Tarhana samples used in this study were obtained from the research carried out by Tarakci et al. [7]. As mentioned in that study, white commercial wheat flour with the protein content of 12% on dry basis was used. Tomato paste was double concentrated to a content of 30. Cow milk with a fat content of 3% was added to the Tarhana. Compressed bakers' yeast in wet form, yogurt, wheat flour, onion, tomato, green peppers and were purchased from local markets in Ordu, Turkey.

Production of the experimental tarhana

The tarhana samples are composed of 300 g flour, 300 g yogurt, 30 g tomato paste, 60 g green pepper, 75 g onion, 3 g yeast. Tomato, green pepper, and onion were chopped into pieces prior addition to the tarhana mixture. The cherry laurel pulp concentrations (0%

(control), 5%, 10%, 15% and 20%) were added to the tarhana, and samples were coded as TC, T1, T2, T3 and T4, respectively. The resulting dough was fermented at 30°C for 24 h. At the end of fermentation, the dough was dried in an air oven at 55±2°C. The drying process was continued until the moisture content of 10% was reached. The samples were milled in a hammer mill up to the particle size of 1 mm to standardize the sizes and then were stored in glass jars at 22±2°C in a dark cupboard.

Micro Mineral elements analysis

5 g of each samples were ashed in a porcelain crucible, solubilized with 10 ml of 6 N HCl, quantitatively transferred into 50 ml volumetric flasks, and diluted to volume with double-deionized water and filtered after 5-6 h with blue-band filter paper and again regulated to 50 ml [8]. Concentrations of cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), zinc (Zn), and Selenium (Sn) were measured by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). All the analyses were performed in triple and the results reported as mean values.

Statistical analysis

Statistical analysis was performed using MINITAB software program [9]. Micro mineral contents of Tarhana were analyzed using one-way analysis of variance (ANOVA) and Fischer's multiple range tests.

Results and Discussion

Data on the micro mineral content of Tarhana samples are given in Table 1. The highest Cd, Cr, Co, Cu, Fe, Mo, Ni and Zn contents in tarhana samples were obtained in control tarhana. Tarhana samples that include 10% of fruit pulp have the lowest mineral contents of Cr, Co, Cu, Fe, Mn, Mo, Ni and Zn. Tarhana samples made by using 20% fruit pulp have the highest content of Mn and Sn. The control tarhana includes highest levels of all kinds of minerals except the minerals of Pb, Mn and Sn. In our study the differences are not statistically important among the tarhana samples for the minerals of Pb, Mo and Sn. It is demonstrated that increasing fruit pulp levels decreased the Cd amount markedly, probably, due to diluting effect of high pulp supplementation with poor Cd content. On the contrary, the Mn content was increased in a small quantity by fruit pulp additions; due to use of the higher amount Zn containing pulp in tarhana making.

Ertas et al. [10] studied the mineral content of tarhana. Their results for Cu (2 ppm), Mn (5 ppm), Fe (30-50 ppm) and Zn (50-60 ppm) were lower than in the present study. This difference probably, due to diluting effect of pulp utilization and amount of yogurt used in the preparation of the tarhana samples in this study. Tarakci et al. [11] studied the mineral content of corn flour and whey added tarhana. Their results for Fe (13.7 ppm) and Zn (17.5 ppm) were lower than in the present study. Bilgicli et al. [12] studied mineral contents of wheat germ and wheat bran added tarhana. They found Cu (0 ppm), Fe (19.8 ppm), Mn (5.9 ppm) and Zn (9.8 ppm) contents lower than the values in our study. Ozdemir et al. [13] mentioned that tarhana includes Fe and Zn with a concentration of 36 ppm and 18 ppm, respectively which were lower whereas Cu (4500 ppm) and Mn (6120 ppm) values were higher than in the present study. Bilgicli et al. [14] reported the Zn content in tarhana as 12 ppm that was lower than in this study.

Table 1. The micro mineral contents of tarhana samples.

Element (ppm)	**Tarhana Type**				
	TC	T1	T2	T3	T4
Cd	5.92±0.09[a]	5.56±0.16[ab]	5.69±0.26[ab]	5.24±0.07b	5.21±0.15b
Cr	5.49±0.09[a]	4.87±0.40[a]	3.88±0.32[b]	4.94±0.34[a]	5.23±0.14[a]
Co	1.72±0.09[a]	1.60±0.04[ab]	1.56±0.05[b]	1.67±0.02[ab]	1.65±0.03[ab]
Cu	182.66±6.17[a]	138.60±4.77[bc]	111.18±4.26[c]	179.04±11.1[a]	154.62±14.4[ab]
Fe	700.70±7.06a	687.30±44.10[a]	570.55±9.84[b]	665.76±38.00[a]	706.35±4.87a
Pb	0.16±0.2[a]	0.17±0.5[a]	0.14±0.04[a]	0.17±0.02[a]	0.12±0.03[a]
Mn	2002.99±249.0[ab]	1736.56±97.10[bc]	1408.53±82.60[c]	2294.33±238.0[a]	2316.87±129.0[a]
Mo	1.86±0.31[a]	1.57±0.23[a]	1.43±0.20[a]	1.53±0.06[a]	1.50±0.08[a]
Ni	4.66±1.05[a]	2.72±0.32[ab]	2.46±0.17[b]	3.73±0.44[b]	3.57±0.16[b]
Sn	0.41±0.01[a]	0.35±0.06[a]	0.36±0.07[a]	0.40±0.01[a]	0.42±0.02[a]
Zn	177.97±16.60[a]	148.86±9.52[ab]	126.56±6.32[b]	143.93±7.88[b]	143.21±4.75[b]

TC; Control, with no fruit pulp, T1; 5% fruit pulp, T2 (10% fruit pulp, T3; 15% fruit pulp and T4; 20% fruit pulp,

[ab]Letters indicate significant differences among tarhana samples with added fruit pulp, $P<0.05$.

Fe is a component of hemoglobin, which transports oxygen to body tissues and Fe is also a component of numerous other proteins and enzymes. Similarly Zn is an essential component of nearly 100 enzymes in human body. The recommended dietary allowances (RDA) for adult males are 8 mg of Fe, 11 mg of Zn [15]. When an amount of 100 ml tarhana soup (includes 6.25 g of tarhana) of tarhana samples containing different fruit blends was consumed, 44.5-54.7%, 7.21-10.11%, of RDA for Fe and Zn, respectively, could be provided. For comparison, RDA percentage of 54.7% for Fe and 10.11% for Zn would be provided by control tarhana samples. So it is possible to state that tarhana is a good source of Fe and Zn minerals.

Conclusions

Tarhana is one of the most important traditional fermented foods in Turkey. It is made from cereal flours, yoghurt, different vegetables, salt, herbs, and spices and is easily digested. Tarhana is produced by Lactic acid bacteria and yeast fermentation. Cherry laurel pulp was successfully incorporated into tarhana formulation. Cherry laurel pulp addition decreased the mineral contents of Zn, Ni, Cu, Co, Cd and Cr. It can be argued that pulp addition decreased the micro mineral contents in tarhana samples those have adverse effects on human health.

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Session C: **Implementation of green technology**

GREEN SOLVENTS FOR GREEN TECHNOLOGIES

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Introduction

In many industrial processes, large quantities of volatile and flammable organic solvents, based on non-sustainable resources such as oil, are used in various reaction systems and separation steps defining a major part of the environmental and economic performance of a process. Since Directive 2010/75/EU on industrial emissions requires plants to limit emissions for certain volatile organic compounds as well as other important air pollutants, a growing area of research in the development of green technologies is devoted to designing new, more environmentally friendly solvents which use would meet technological and economical demands.

Over the few years, among neoteric solvents (neoteric = new, recent, modern) deep eutectic solvents (DESs), promising alternatives to traditional organic solvents from both the environmental and technological perspectives, have been dramatically expanding in popularity as a new generation of chemicals with potential uses in various industrial fields [1]. DESs present a new generation of liquid salts and are generally based on mixtures of cheap and readily available components: nontoxic quaternary ammonium salts (e.g. cholinium chloride) and a naturally-derived uncharged hydrogen-bond donor (e.g. vitamins, amines, sugars, alcohols and carboxylic acids) [2]. DESs have unique physicochemical properties and thanks to possibility of designing their properties for particular purpose, their low ecological footprint and attractive price, have become of growing interest both for academia and industry. Since their emergence, these solvents have attracted attention in synthesis, electrochemistry, nanomaterials, biochemistry, separation, and analysis, whereby the number of related references has increased rapidly from 2009 to 2013 (more than 300 papers) [2,3].

Herein, we present the results of our study on DESs application in lipase-catalyzed esterification and extraction of biologically active compounds from grape skin.

Materials and Methods

Preparation of DESs

All chemicals for preparation were dried in the vacuum concentrator (Savant SPD131DDA SpeedVac Concentrator) at 60°C for 24 hours before use. DESs were synthesized in certain ratios of cholin chloride (ChCl) to hydrogen donor (glucose, sorbose, glycerol, ethylene glycol, malic acid and oxalic acid) to obtain liquids at room temperature, as shown in Table 1. The mixture of ChCl and hydrogen donor was stirred in a flask at 80 °C for 2-6 h until a homogeneous transparent colourless liquid was formed. DES samples were vacuum dried prior to further use. Additionally, tertiary mixtures ChCl :EG: water in molar ratios 1:2:0.5, 1:2:1, 1:2:1.5 and 1:2:2 were prepared.

Table 1. List of the DESs used in this study.

Combination	**Abbreviation**	**Molar Ratio**
choline chloride : glucose	ChCl:Glc	2:1
choline chloride : sorbose	ChCl:Sor	1:1
choline chloride : glycerol	ChCl:Gly	1:2
choline chloride : ethylene glycol	ChCl:EG	1:2
choline chloride : malic acid	ChCl:MA	1:1
choline chloride : oxalic acid	ChCl:OA	1:1

Lipase-catalyzed esterification

The reaction started by adding 5 mg of Novozym 435 to 2 mL of DES (ChCl:EG), tertiary mixture ChCl:EG:water or *n*-heptane containing 0.05 mol L^{-1} acetic anhydride and 0.1 mol L^{-1} *n*-butanol. Reactions without the enzyme were also performed. When monitoring reactions in DES, butyl acetate was recovered at specified time intervals *via* liquid extraction using *n*-heptane until no further raise in yield was detected. The biphasic mixture containing 50 µL of reaction aliquots and 50 µL of *n*-heptane was strongly shaken for 3 min and analyzed by a gas chromatograph. Butyl acetate concentration was calculated by taking into account the partitioning coefficient for butyl acetate in the n-heptane/reaction solvent. For reactions performed in *n*-heptane, aliquots of 20 µL were directly analyzed by a gas chromatograph. All experiments were carried out in duplicates and the average values were calculated from the results.

Butyl acetate, *n*-butanol and acetic anhydride concentrations in the *n*-heptane phase were determined by a gas chromatograph equipped with electrospray ionization mass spectrometry (Shimadzu, Japan) equipped with a Rxi_5Si/MS column (30 m x 0.25 mm i.d. x 0.25 mm). Helium was used as a carrier gas at a flow rate of 109.6 mL min^{-1}. The temperature of the oven at the injection was 55°C and was kept constant during whole analysis time (3 min). Injector and detector temperatures were set at 250°C. The ionization of the samples was achieved at 70 eV using the SCAN mode.

Extraction and analyzes of phenolic compound from grape skin with DES

Amount of 0.2 g of freeze-dried and grained Plavac mali grape skin powder was weighed in extraction tube and 2 mL of solvent of interest (DES or conventional organic solvent) was added and placed in shaker for 3 hours, at room temperature. The supernatant obtained was filtered and submitted to further analysis. Conventional extraction procedure of phenolic compounds was performed using water, aqueous methanol (MEOH; 70:30, v/v) and acidified aqueous solution of methanol (MEOH12MHCl; 70% methanol/12 M HCl, 99:1, v/v, with pH=1.25) and water, under the same conditions as described above. The content of phenolic compounds was analyzed by HPLC according to the previously reported methods [4]. For simplicity, we showed data as Total phenolics which was calculated as sum of all quantified compounds: total phenolic acids (sum of gallic acid, gallic acid derivate and *p*-coumaric acid), catechin and total anthocyanin (sum of delphinidin, cyanidin, petunidin, peonidin and malvidin -3-*O*-monoglucosides; peonidin and malvidin -3-*O*-acetylmonoglucosides, peonidin and malvidin-3-(6-*O*-*p*-coumaroyl)monoglucoside). All extraction procedures using DES or conventional organic solvents were conducted in triplicate.

Results and Discussion

Lipase- catalyzed esterification within DES

Within this work ChCl:EG and tertiary mixtures ChCl:EG:water in different molar ratios have been evaluated as media for the *C. antarctica* lipase B-catalyzed butyl acetate synthesis accomplished by acylation of *n*-butanol with acetic anhydride. Blank experiments without the enzyme were also performed and no conversion was observed within the reaction times used in this study. The time-courses of esterification performed in different solvents are presented in Figure 1. To compare esterification efficiency, experiment was also performed within *n*-heptane, well known as excellent medium for lipase-catalyzed esterification [5].

The profile of flavour ester production at various time intervals within different solvents indicate that during the esterification within ChCl:EG no product was formed, while by addition of water to eutectic solvent (establishing tertiary mixtures ChCl:EG:water in different molar ratios) drastic increase in esterification yield was observed (up to 80% for $ChCl:EG_{1,5molH2O}$), which is higher than the yield observed in *n*-heptane (50%). This significant improvement in esterification efficiency within tertiary mixtures ChCl:EG:water versus esterification in the anhydrous eutectic solvent could be explained by the role of water in the 3D organization of enzyme molecule, which directly affects the active site and thermodynamic equilibrium [6], but also by creating hydrogen bonds between the substrates and components of eutectic solvents (substrates unavailable for reaction). Namely, adding a protic co-solvent to the system, which creates hydrogen bonding more easily than the substrates, probably lessen the interaction between the DES and the substrates, making the latter more available for reacting. However, if the water content exceeds the optimum, negative impact on reaction yield was observed (yield for $ChCl:EG_{1,5molH2O}$ was 65%), which could be explained by the fact that excess of water molecules near the enzyme active site generate a competitive hydrolysis reaction, resulting in lower yields.

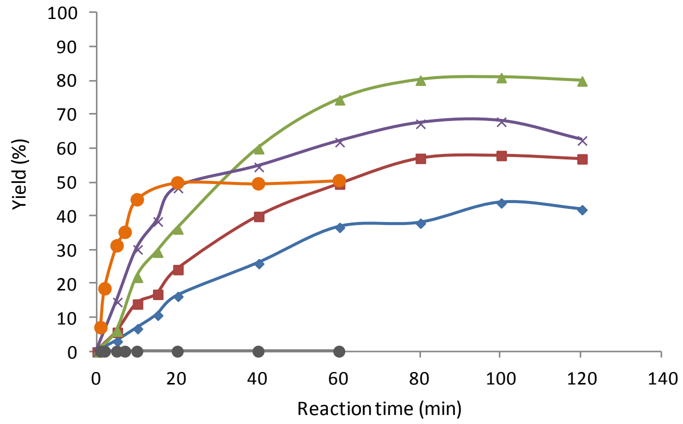

Figure 1. Time courses of lipase-catalyzed butyl acetate syntheses within *n*-heptane, eutectic solvent ChCl:EG and tertiary mixtures of cholin chloride ChCl (1mol):ethylene glycol EG (2 mol) as a function of water content (0,5-2 mol). Reaction conditions: 0.05 mol L^{-1} acetic anhydride; 0.1 mol L^{-1}*n*-butanol; 5 mg Novozym 435; 25 °C. Each point represents the average of two experiments

•=*n*-heptane; •=ChCl:EG; ♦=ChCl:$EG_{0,5molH2O}$; ■=ChCl:$EG_{1molH2O}$; ▲=ChCl:$EG_{1,5molH2O}$; x=ChCl:$EG_{2molH2O}$

Extraction of phenolic compounds from grape skin with DES

Among various plant biologically active compounds, phenolics have drawn increasing attention due to their potent antioxidant properties and their marked effects in the prevention of various oxidative stress associated diseases such as cancer [7]. Due to their complex structure there is no universal extraction method suitable for extraction of all plant phenolic and conventional extraction techniques are usually associated with high organic solvent consumption and long extraction times. On the other hand, a few authors have reported DESs application in extraction of bioactive compounds, showing that many compounds are dissolved by 10-100 times better than in water or lipids [8, 9]. Therefore, we studied exaction efficiency of 4 different DESs (choline chloride in combination with glycerol, oxalic, malic acid and sorbose) for phenolic compounds extraction form grapes skin of Croatian native red grape cultivar Plavac mali. Extraction of phenolic compounds was performed with DES containing different water content. Also, in order to compare extraction efficiency with conventional ones, extractions were performed by using water, aqueous methanol and acidified aqueous solution of methanol (Figure 2).

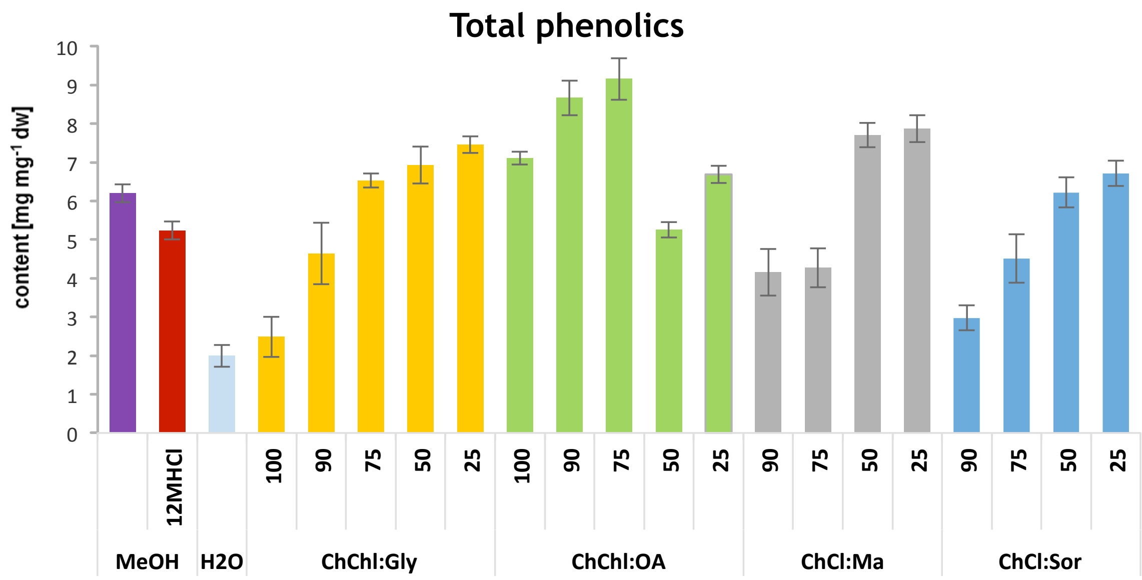

Figure 2. Total phenolic content in Plavac mali grape skin extracted with different DES and conventional extraction solvents. The numbers on the graph (100-25) indicate content of DES in water (%).

In general, DES capacity to extract phenolic compounds showed to be dependent on its composition and water content varying considerably according to the target phenolic groups. For example, the best performance for total phenolic compound extraction showed 75% of ChCl:OA in water. Other DESs showed better performance with higher content of water. Our data are similar to literature where a few studies showed that DESs have a high ability to extract phenolic compounds, which is related with the H-bond interactions that are established between the phenolic compounds and the DES components. Namely, DESs have the ability of donating and accepting protons and electrons, which confers their ability to form hydrogen bonds, increasing therefore their dissolution capability [8,9]. Additionally, in comparison to conventional solvents, all tested DESs at certain concentration in water showed better performance.

Conclusions

In this paper, it is shown that the water has a crucial role in enzyme-catalyzed esterification in DESs. Optimization of water content in water in DES tertiary mixtures enables achieving of esterification yields higher than in classical organic solvents.

Furthermore, DESs have proved to be effective in the extraction of phenolic compounds compared to conventional extraction with methanol and acidified methanol, which indicates the possibility of a potential replacement of conventional organic solvents with eutectic solvents in the extraction of phenolic compounds from the grape skin and other plant material.

The use of eutectic solvents from natural resource in biocatalytic reactions as well as in extraction processes represents a contribution to the development of new green process.

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PROPOLIS BY SUPERCRITICAL CO_2 EXTRACTION

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Introduction

Recently, extraction with supercritical (SC) CO_2 became increasingly important separation method, particularly when dealing with natural products[1]. Supercritical CO_2 exhibits liquid-like densities and therefore corresponding solvation power at pressures above 200 bar and at temperatures above critical (31.04 °C); moreover, it is kinetically favorable, with gas-like diffusion coefficients. Low separation temperatures are particularly suitable for separation of thermally unstable natural products. However, most of them are polar while CO_2 is a nonpolar substance and therefore a poor solvent. This may be partially overcome by adding small quantities of cosolvents, such as ethanol, to increase their solubility. Downstream separation of dissolved product is easily achieved, by reducing the pressure. However, recycling of solvent is done by repressurization, and this is rather expensive, and so is the equipment. Therefore, the method is limited to valuable products. A typical example of the process in use is the decaffeination of coffee.

In this paper the method is tested for the extraction of valuable natural compounds – flavonoids – from the equally valuable substrate – propolis, following the previous efforts reported in literature[2–4]. Flavonoids are believed to act favorably onto human health condition, they might prevent coronary diseases and they are notorious antioxidants. They originate from plants, where they serve as pigments and protecting agents against parasites, pathogens etc. Nevertheless, they are digested by bees and transferred into propolis – a perfect waxy sealant for beehives with antipathogenic properties. Propolis is an effective antiinflammatory agent in human medicine as well, presumably due to its flavonoids content. The preparation of propolis commonly includes extraction with ethanol to remove waxy compounds – the product is the so-called propolis tincture. Alternative methods of extraction, such as supercritical CO_2 extraction, are always worth investigating. The aim is to find suitable extraction conditions (temperature and pressure) to maximize the flavonoids content in the product, either in the extract or in the remains (raffinate).

Materials and Methods

Raw propolis of Croatian origin was used for the investigation. Propolis balls were firstly finely ground into powder which was subsequently transferred into conventional Soxhlet extractor. The material was extracted using pure ethanol as a boiling solvent. After six hours of extraction, the extract solution was dried in a rotational vacuum evaporator to obtain dry Soxhlet extract of propolis (SEP). Another, very similar extraction procedure was used to remove all the waxy compounds from the main batch of propolis. Pure ethanol was stirred with raw propolis (10 ml per 1 g) using magnetic stirrer at ambient temperature for 24 hours. The ethanol solution was then cooled in a refrigerator and filtered three times using

Büchner's funnel. After final filtering, the extract solution was dried in a rotational vacuum evaporator to obtain dry ethanol extract of propolis (EEP).

The samples of EEP were subjected to supercritical CO_2 extraction (SCE) using a homemade semi-batch apparatus located at the University of Maribor, Faculty of Chemistry and Chemical Engineering, figure 1. The samples (5 or 10 g) were exposed to a constant flow of CO_2 of approximately 1.886×10^{-3} kg/min at preset values of temperature and pressure. The mass of extracted material was recorded as a function of time. The extracts (SCE 1–9) were collected in test tubes for further analysis.

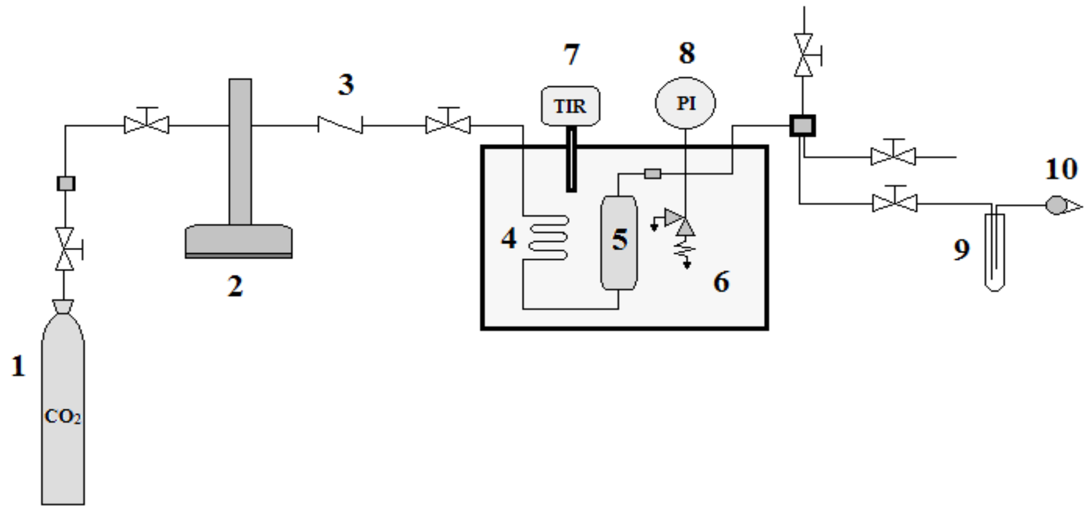

Figure 1. Scheme of the apparatus used for supercritical CO_2 extraction. 1 – liquid CO_2 tank, 2 – high-pressure pump, 3 – check valve, 4 – heating coil, 5 – autoclave (extractor), 6 – hot water bath, 7 – heater and temperature regulator, 8 – pressure gauge, 9 – glass test tube for collecting extract, 10 – gas flow meter.

The total flavonoids content, expressed as quercetin equivalent, was determined spectrophotometrically using a variation of the Dowd method[5], by measuring the absorbance at 415 nm. The total phenolics content, expressed as gallic acid equivalent, was determined spectrophotometrically as well, using the Folin–Ciocalteu method (see *e.g.*[6]), by measuring absorbance at 760 nm. The extract samples SCE 1–9, EEP and SEP were analyzed, the flavonoids and phenolics content in raffinate were calculated using the mass balance equation.

Results and Discussion

The yields of SCE extraction (mass of extract per mass of sample) are given in table 1. No strict regularity was observed, contrary to literature data[2–4]. However, higher yields were generally found with increasing temperature and pressure, as expected. The possible reason for the observed deviations may be found by plotting yield (y) *vs.* mass of CO_2, figure 2, which is equivalent to yield *vs.* time. It seems that the extraction yield in the apparatus used is – at least partially – kinetically governed. Discontinuities in the kinetic extraction curve are clearly observed, indicating a stepwise extraction pattern, where classes of compounds are presumably extracted in distinct extraction periods. However, this was not proven so far, and it requires more elaborate detection and quantification methods. Discontinuities are more clearly visible in differential plots, an example of which is given in figure 2.

Table 1. The final yields of extraction using supercritical CO_2. Experiments are coded as well.

Final yield / %		Temperature/ °C 40	50	60
Pressure/ bar	200	6.422 (SCE-1)	3.066 (SCE-4)	4.774 (SCE-7)
	250	2.35 (SCE-2)	2.834 (SCE-5)	13.58 (SCE-8)
	300	1.814 (SCE-3)	4.414 (SCE-6)	9.78 (SCE-9)

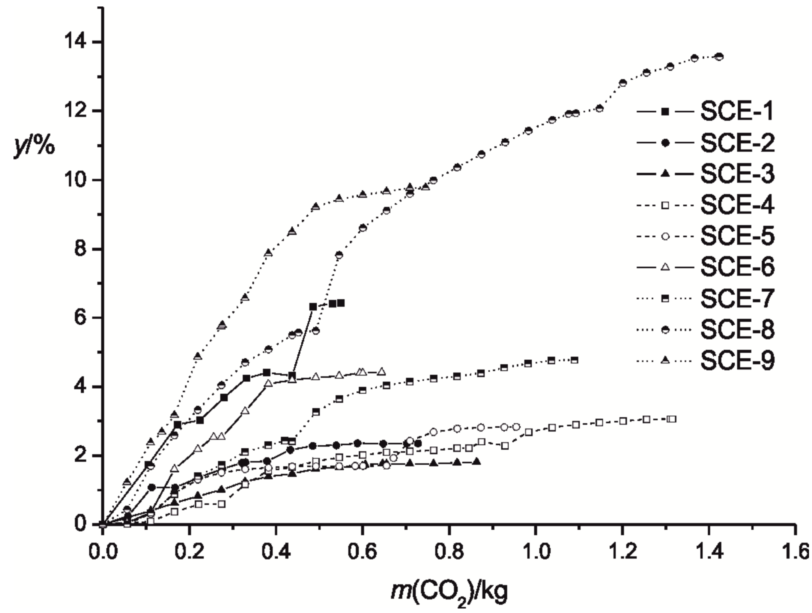

Figure 2. The yield of extraction *vs.* mass of SC CO_2 solvent for all nine SCE experiments.

In table 2 the concentrations of flavonoids and phenolics in various extract and raffinate samples are given. It may be observed that Soxhlet extraction may serve as a tool for concentrating flavonoids since their total concentration is increased from 9.664 to 11.390 mg/g. However, ordinary ethanol extraction acts even better giving 12.939 mg/g. SC CO_2 extraction, on the other hand, concentrate flavonoids in the raffinate, due to their poor solubility in nonpolar CO_2. However, the further increase to the maximum value of 13.638 mg/g is not very pronounced, due to the rather low overall extract yield. When discussing total phenolics concentrations, they are generally much higher in comparison to total flavonoids (of course, the latter form only a small subgroup of the former). Soxhlet extraction was found to increase significantly the total phenolics content from the value of 48.563 mg/g in raw propolis to the value of 114.694 mg/g; the raffinate remains poor in phenolics (12.854 mg/g). Ordinary ethanol extraction also gives high phenolics content in the extract of 113.333 mg/g. SC CO_2 does not produce large difference between phenolics content in extract and raffinate. This means that the selectivity of CO_2 towards total phenolics is much lower than towards total flavonoids which might be, in an ingenious extraction design, used to separate the former from the latter. In general, one may conclude that Soxhlet extraction succeeds to harvest most of the phenolics, including flavonoids, which gives it clear advantage over the SC CO_2 extraction in the first step of isolating those valuable components from propolis.

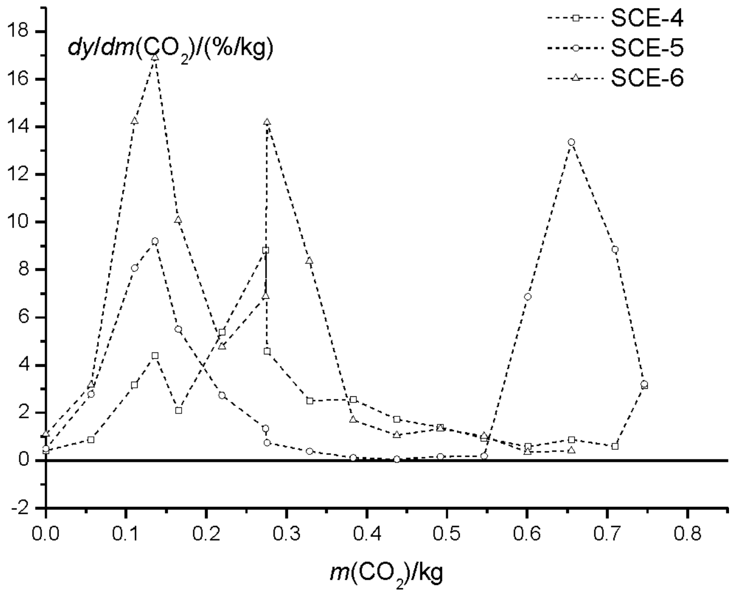

Figure 3. Derivative of the yield of extraction *vs.* mass of SC CO_2 solvent for all nine SCE experiments. Peaks of maximum extraction rate are clearly visible.

Table 2. The concentrations of flavonoids (quercetin equivalent, QU) and phenolics (gallic acid equivalent, GA) in various extracts and raffinates.

Samples	$w_{QU.extract}$ / (mg/g)	$w_{QU.raffinate}$ / (mg/g)	$w_{GA.extract}$ / (mg/g)	$w_{GA.raffinate}$ / (mg/g)
SCE-1	4.102	13.546*	60.139	116.983
SCE-2	5.446	13.119*	109.510	113.425
SCE-3	7.261	13.044*	108.047	113.430
SCE-4	6.979	13.127*	112.357	113.363
SCE-5	7.203	13.106*	105.158	113.571
SCE-6	8.595	13.140*	105.152	113.710
SCE-7	7.071	13.233*	109.868	113.506
SCE-8	8.490	13.638*	104.557	114.712
SCE-9	7.568	13.521*	108.742	113.830
SEP	11.390	8.733	114.694	12.854
EEP	12.939	–	113.333	–
Raw propolis	9.664*	–	48.563*	–

*Values calculated using mass balance equations

Conclusions

The most important conclusions of the investigation presented here are as follows.

The kinetics of supercritical CO_2 extraction shows that it might be regarded as the stepwise process. Therefore, the measured extraction yields are not necessarily the maximum ones. In addition, such a stepwise character opens a possibility for kinetically driven separation of particular components. However, definite conclusions can be drawn only after elaborate additional experimentation.

The maximum extraction yield of 35.064 is found with Soxhlet extraction which utilizes ethanol as a suitable polar solvent for polar phenolics and flavonoids. CO_2 is nonpolar and therefore cannot produce such high yields. The low concentration of phenolics in raffinate after Soxhlet extraction proves that it can be used as a definite first step in isolating the phenolics from propolis.

Soxhlet extract rich in overall phenolics might be – in a future experiment –subjected to SC CO_2 extraction in an attempt to separate flavonoids from common phenolics. Flavonoids are mostly kept in the raffinate, while common phenolics are distributed between extract and raffinate with much less selectivity.

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GREEN APPROACH TO PREVENTING CORROSION OF METALS AND ALLOYS

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Introduction

Corrosion of metals and alloys results in a large economic losses caused by shortening the duration of the industrial equipment, plant shutdowns, loss or contamination of products and efficiency reduction [1]. Aluminium due to its excellent physical and chemical properties is the second most used metal in the world. Good corrosion resistance aluminium owes to a compact layer of oxide that is formed on its surface in contact with air and water [2]. This protective oxide layer on aluminium may be destroyed by action of strong acids, bases, or due to contact with the halide ions, especially chloride ions. One of the most widely used methods to protect metals from corrosion in aqueous solutions is the addition of corrosion inhibitors, substances that significantly reduce the corrosion rate. Previous investigations have shown that organic compounds containing oxygen, nitrogen and sulphur atoms in its structure, have very good corrosion inhibition properties for many metals [3,4], unfortunately, most of them can be highly toxic and dangerous to the environment.

These known hazardous effects of corrosion inhibitors have now urged researchers to focus on the use of natural products such as plant extracts and essential oils [5-7]. This work focuses on the studying essential oils of lavender (*Lavandula angustifolia* L.), bay laurel (*Laurus nobilis* L.), dill (*Anethum graveolens* L.) and basil (*Ocimum basilicum* L., *ct.* linalool) as inhibitors for corrosion protection of aluminium and its alloy in HCl and NaCl solutions. These plants are widespread in the Mediterranean region and they are used as a spice and cure in traditional medicine. Moreover, their essential oils are used in many industries such as food, cosmetics and pharmaceuticals. The investigation of corrosion inhibition parameters was performed by weight loss, potentiodynamic polarization and electrochemical impedance spectroscopy (EIS).

Materials and Methods

Corrosion tests were performed using coupons prepared from 99.85% pure aluminium and AA5754 aluminium alloy. The chemical composition (wt%) of the AA5754 alloy sample is Mg 3.10, Si 0.25, Fe 0.40, Cu 0.10, Mn 0.10, Cr 0.15, Zn 0.20, Ti 0.20 and balanced Al. Pure 100% *Lavandula angustifolia* L., *Laurus nobilis* L. and *Anethum graveolens* L. essential oils were provided by "Aromara, Zagreb", while pure 100% *Ocimum basilicum* L., *ct.* linalool, essential oil was provided by "Oshadhi". The stock solution of essential oils was prepared by dissolving it in 96% ethanol as 30% (v/v) solution which was used as inhibitor. A specific volume was taken from the stock solution and added directly to 1 M HCl and 3% NaCl solution to prepare the desired concentrations.

All the surfaces of the specimens were abraded successively with fine grade emery papers (up to 1200 grids), then the metal surface was rinsed with distilled water and ultrasonically degreased in absolute ethanol. In each experiment, the cleaned aluminium and AA5754 alloy coupons were weighed and suspended with the aid of glass rod and hook in a beaker containing 150 mL of test solution. Electrochemical measurements were carried out in a conventional three electrode cylindrical glass cell using Potentiostat type VersaSTAT 3 (Princeton Applied Research), controlled by a personal computer. A saturated calomel electrode (SCE) and graphite electrode were used as reference and auxiliary electrodes, respectively. The all electrochemical measurements were performed in the test solution after reaching the open-circuit potential (E_{ocp}). Potentiodynamic polarization studies were performed at scan rate of 0.5 mV s^{-1} in the potential range from ±150 mV with respect to the E_{ocp}. All reported potentials refer to SCE. Electrochemical impedance spectroscopy (EIS) measurements were performed in the frequency range from 100 kHz to 10 mHz with an a.c. voltage amplitude perturbation of 5 mV. Chemical composition of oils was obtained from the suppliers and the main constituents are shown in Table 1.

Table 1. Chemical composition of studied essential oils (main constituents).

Essential oil	Chemical composition
Lavandula angustifolia L.	linalool (35.0%), linalyl acetate (34.0%), 1,8-cineole (11.0%),camphor (5-12%).
Laurus nobilis L.	1,8- cineol (46.0%), methyl eugenol (10.0%), *α*-terpinyl acetate (9.1%), linalool (8.5%), sabinene (5.7%).
Anethum graveolens L.	*S*-(+)-carvone (76 %) and limonene (18 %)
Ocimum basilicum L., *ct.* linalool	linalool (65%), eugenol (4.5%), 1,8-cineole (4.4 %), geraniol (3.5%).

Results and Discussion

The values of aluminium corrosion parameters obtained from weight loss measurement with the addition of 200 ppm of essential oils are summarized in Table 1. The corrosion rate (W) and the surface coverage (Θ) were calculated from Equations (1) and (2):

$$W = \Delta m / S \cdot t \tag{1}$$

$$\theta = (W_{corr} - W'_{corr}) / W_{corr} \tag{2}$$

where Δm is average weight loss of three parallel coupons (mg), S the total area of the specimen (cm^2), t is immersion time (h), W'_{corr} and W_{corr} are the corrosion rates of coupons with and without inhibitor, respectively. While the inhibition efficiency η_w (%) can be calculated as: $\eta_w(\%) = \theta \times 100$ (3)

Table 2. Corrosion rate (W), surface coverage (Θ) and inhibition efficiency η_w%, obtained from weight loss measurements for aluminium in 1 M HCl solution in absence and with addition of 200 ppm of essential oils at 25 °C, after 3 h immersion time.

System	W (mg cm^{-2} h^{-1})	Θ	η_W (%)
1 M HCl	0.466	-	-
Laurel	0.165	0.646	64.6
Basil	0.102	0.781	78.1
Lavander	0.053	0.886	88.6
Dill	0.028	0.938	93.8

It can be seen from Table 2 that the addition of 200 ppm of essential oils in 1 M HCl solution have significant influence on the aluminium corrosion reduction. The corrosion rate values (mg cm^{-2} h^{-1}) decrease, while surface coverage and inhibition efficiency values increase in essential oils presence. The maximum η_w% is reached with the addition of dill oil (93.8 %), while the addition of laurel oil causes the lower inhibition efficiency (64.6 %). These results indicate that all investigated essential oils can act as a very good corrosion inhibitors for aluminium in 1 M HCl solution.

Potentiodynamic polarization curves (a) and Nyquist plots (b) obtained for aluminium in 1 M HCl solution in the absence and presence of investigated essential oils are shown in Figure 1, while the obtained electrochemical parameters such as corrosion potential (E_{corr}), corrosion current density (I_{corr}) and inhibition efficiency η_{PP} (%) are summarized in Table 3. It is well known that in high acidic solution, the main anodic reaction of aluminium is the dissolution of aluminium in the form of Al^{3+}, while the main cathodic reaction is a reduction of hydrogen ions and evolution of hydrogen gas [6]. From Figure 1 (a) it can be seen that the addition of all investigated essential oils have almost no influence on the anodic polarization curves and only a decrease of cathodic current densities were obtained (hydrogen evolution reaction is suppressed), while E_{corr} values are almost constant in the presence of inhibitor (around -800 mV). These results indicate that active molecules present in oils acting by adsorption on the aluminium surface and form a barrier film, without changing the aluminium corrosion mechanism. The EIS results of corrosion behaviour of aluminium in 1M HCl solution in absence and presence of investigated oils are shown by Nyquist plots (Figure 1 (b)). The EIS parameters (Table 3) were estimated using Zsimp Win programme and appropriate electrical circuits (Figure 1b).

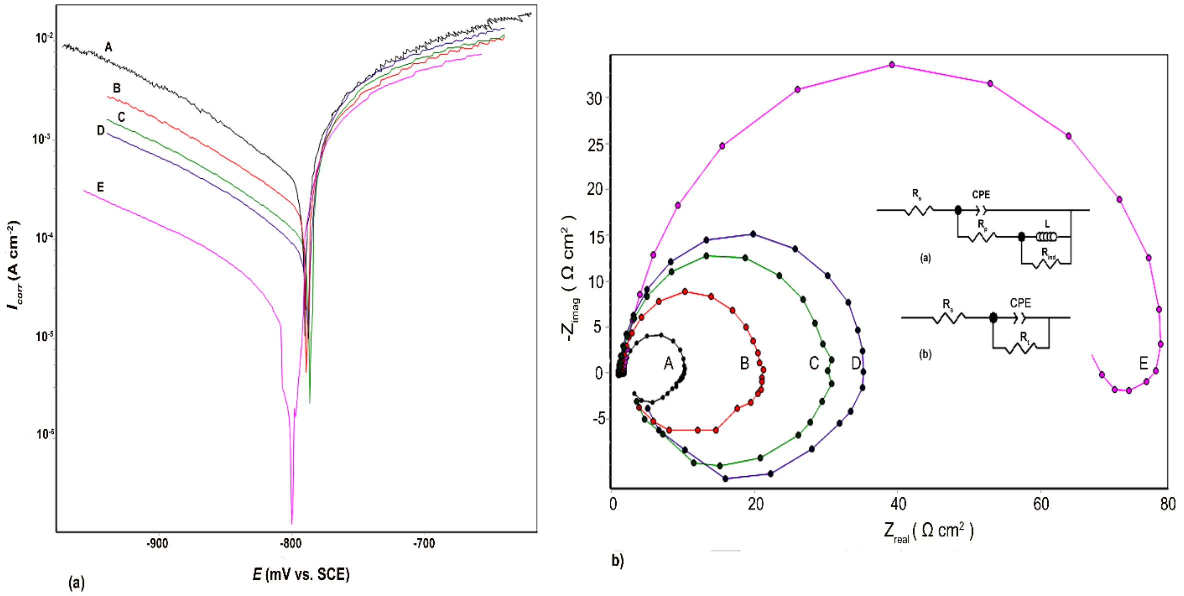

Figure 1. Potentiodynamic polarization curves (a) and Nyquist plots (b) obtained for aluminium in 1 M HCl solution in the absence (A) and presence of 200 ppm of laurel (B), basil (C), lavender (D) and dill (E) oils at 25 °C.

Table 3. Electrochemical parameters obtained for aluminium in 1 M HCl solution in absence and presence of 200 ppm of essential oils at 25 °C.

Inhibitor	E_{corr} (mV_{SCE})	I_{corr} (mA cm^{-2})	η_{PP} (%)	R_p (Ω cm^2)	R_{ind} (Ω cm^2)	R_{ct} (Ω cm^2)	η_{EIS} (%)
0	-790	8.48	-	1.35	6.80	8.15	-
Laurel	-789	2.40	71.7	3.90	15.8	19.7	58.6
Basil	-786	1.57	81.5	3.13	27.7	30.8	73.5
Lavender	-787	1.25	85.3	6.50	30.2	36.7	77.8
Dill	-800	0.34	96.0	-	-	74.7	89.0

The impedance behaviour of aluminium in HCl solution without addition of inhibitors, and with the addition of laurel, basil and lavender oils solution can be expressed by two time constants. The time constant in the high frequency region is described by the capacitive loop and the time constant of the low-frequency field is described by inductive loop. On the other hand, the addition of dill oil in HCl solution caused the disappearing the inductive loop, indicating the formation of thicker and more compact adsorbed film on the aluminium surface. The addition of all essential oils increases the diameter and size of the capacitive loop (increased the values of charge transfer resistance R_{ct}), indicating a physical blocking of electrode surface. R_{ct} is the resistance of the charge transport, while for inductive loop R_{ct} values are calculated by summing R_p (polarization resistance) and R_{ind} (inductive resistance).

On the basis of previous results it can be concluded that the active components of each tested essential oil significantly influence on its inhibitory efficiency. In high acidic solution these components can exist either as neutral molecules or in the form of protonated species (cations), therefore two modes of adsorption can be possible, physisorption and chemisorption. Inspection of Table 1. reveals that major components containing mainly oxygen atoms and double bonds in their structure, so they can be adsorbed on the metal surface *via* the lone pairs of electrons of an oxygen atom or on the basis of interactions between π-electrons of double bonds in aromatic ring and vacant p-orbitals of Al. Moreover, the presence of various adsorption active centres being able to act synergistically.

Aluminium and its alloys are generally passive and corrosion resistant in neutral aqueous solutions except for pitting corrosion due to some reactive species, such as chloride, which may attack the oxide layer [5]. The effects of laurel and lavender oil solutions on the corrosion of aluminium and AA5754 alloy in 3% NaCl were determined by polarization measurements and results are shown in Table 4. Inhibition efficiency in the case of pitting corrosion can be evaluated from the difference in the amount of separation between pitting potential, E_{pit}, and the corrosion potential E_{corr} obtained without and in the presence of studied inhibitor. The pitting potential, E_{pit} can be defined as the potential below which the metal surface remains passive and above which pitting corrosion starts to grow on the metal surface. At the pitting potential, dramatic rise in current densities can be observed, so if the corrosion potential is close to the pitting potential, only a small increase in potential would lead to pitting attack. Therefore, if these differences are larger, it is more difficult for pitting corrosion to occur. As can be seen, from the data for differences between E_{cor} and E_{pitt} mentioned in Table 4, it is clear that with addition of studied oils these differences increase, what indicates that the added oils (especially lavender) acted as a good pitting inhibitors for corrosion of pure aluminium and AA5754 alloy in 3% NaCl solution. Comparison of the current densities values, determined for the investigated materials in 3% NaCl solution, shows that aluminium alloy is more corrosion resistant than aluminium itself.

Table 4. Electrochemical parameters of corrosion obtained from potentiodynamic polarization for aluminium and AA5754 alloy in 3% NaCl solution in absence and presence of 20 ppm of lavender and laurel oils at 25°C

Material	Inhibitor 20 ppm	E_{corr} (mV_{SCE})	E_{pitt} (mV_{SCE})	E_{corr} - E_{pitt} (mV_{SCE})	i_{corr} (μA cm^{-2})	η_p (%)
Al	0	-770	-758	12	100.8	-
	Lavender	-887	-780	107	8.33	91.7
	Laurel	-823	-772	51	23.3	76.8
AA5754 alloy	0	-775	-748	27	26.2	-
	Lavender	-971	-780	190	0.57	97.8
	Laurel	-782	-761	21	10.6	59.5

Conlusions

Electrochemical and gravimetric measurements confirmed the inhibitory action of laurel, lavender, basil and dill essential oils as 30% (v/v) solutions on aluminium corrosion in 1 M HCl and the best corrosion protection was performed with addition of 200 ppm of dill oil. Moreover, laurel and lavender oils drastically reduced the pitting corrosion of aluminium and AA5754 alloy in 3% NaCl solution. Good inhibition efficiency of these oils can be attributed to their major components. In high acidic solution these components can exist either as neutral molecules or in the form of protonated species (cations), therefore two modes of adsorption can be possible, physisorption and chemisorption. In neutral solution they can be adsorbed on the metal surface *via* the lone pairs of electrons of an oxygen atom or on the basis of interactions between π-electrons of double bonds in aromatic ring and vacant p-orbitals of Al. The presence of different adsorption centers indicates on possible synergistic effect of these compounds.

Essential oils are natural and environmentally benign products and they can be used instead of toxic chemical inhibitors in preventing corrosion of aluminium and its alloys in acidic and neutral solution.

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THE POTENTIAL OF POPLAR (*Populus nigra* var. *italica*) IN THE PHYTOREMEDIATION OF LEAD

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Introduction

Lead (Pb) is one of the most common heavy metal present in the soil. Its increased concentrations have the genotoxic effect and cause loss of vegetation [1]. The biggest challenge today is finding adequate methods for the restoration of contaminated sites. Especially demanding is remediation of the areas contaminated with heavy metals [2]. Ecologically and environmentally acceptable solution provides a new biotechnological process called phytoremediation, which is based on the ability of plants to clean and restore contaminated area. Since numerous studies indicate that plants have a large genetic potential for the removal of toxic metals from the soil, they play a major role in the process of phytoremediation [2]. Phytoremediation is environmentally friendly and visually acceptable, inexpensive and offers the possibility of soil bioremediation. Over the past decade special attention in phytoremediation processes development is focused on the potential use of trees in phytoremediation of heavy metals from soil, due to a number of positive characteristics, such as fast growth accompanied with high biomass yield, a deep root system, aesthetically acceptable and cheap cultivation [3].

Aim of this study was to determine the potential of poplar in the phytoremediation of soil contaminated with Pb. In order to investigate the effect of Pb on the poplar morphology and physiology, biomass of the whole plant and its different parts (root, stem and leaf) and the distribution of Pb in different plant parts (leaves, stems, roots) were determined. Furthermore, in order to estimate the extent of the oxidative stress caused by exposure to increased Pb concentrations, the levels of lipid peroxidation and H_2O_2 content in the leaves and roots of poplar were determined as well as the activity of antioxidative enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APOX), glutathione-*S*-transferase (GST) and glutathione reductase (GR) in response to oxidative stress. Obtained results will be used for determination of the plant's response to stress caused by heavy metals present in the soil which will ultimately lead to evaluation of the poplar potential in phytoremediation of the soil contaminated with different amounts of Pb.

Materials and Methods

Plant material and experimental design

Cuttings of Poplar (*Populus nigra* var. *italica*) were collected and grown at the *Croatian Forest Research Institute.* For experiment purposes, one-year cuttings were used. To stimulate field conditions, the pot experiment was conducted outdoors from 15 June 2011 to 1 September 2012 at the experimental station of the Croatian Forest Research Institute, Jastrebarsko, Croatia (N 45°40'18.71", E 15°39'4.27"). The soil was placed in containers and spiked with an appropriate solution of $Pb(NO_3)_2$ (Sigma-Aldrich, Germany) in order to obtain Pb concentrations of 0, 400, 800 and 1200 mg Pb kg^{-1} soil and then left to equilibrate outdoors under a waterproof tarpaulin for two months. Soil samples were placed into 10 L plastic pots and single cuttings of Poplar in 10 pots per treatment group, i.e. control group with 0, 400, 800 and 1200 mg Pb kg^{-1}, were planted. For analysis, six plants were harvested from each treatment group after 77 day of plant growth and plant biomass was recorded.

Chemical analysis of plant material and soil

Harvested plants were separated into roots, stems and leaves. They were washed twice, first with tap water to remove soil, and then with deionised water. Plant samples were oven-dried at 80°C for 24 h [4]. Dry samples were ground to obtain a homogeneous powder in a metal-free mill (Ika-Werke, M20, Germany). Collected soil samples were air-dried, ground using a mortar and pestle and passed through a mesh sieve (fractions < 2 mm) [5]. Approximately 0.3 g of sub-samples were digested using a mixture of 30% H_2O_2 (1 mL) and 65% HNO_3 (5 mL) for plants and a mixture of 65% HNO_3 (3 mL) and 37% HCl (9 mL) for soil in a closed high-pressure microwave system (Anton-Paar, Multiwave 3000, Germany). Sub-samples were analyzed for Pb using Flame Atomic Absorption Spectroscopy (Perkin-Elmer, Aanalyst 600, USA).

Evaluation of lipid peroxidation and H_2O_2 content

The levels of lipid peroxidation in the leaves and roots of Poplar were evaluated by measuring malondialdehyde (MDA) using the thiobarbituric acid method [6]. The MDA content was calculated according to its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as nmol g^{-1} FW. H_2O_2 content, were evaluated by method described in Singh et al. [7]. The content of H_2O_2 was calculated according to its extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µmol g^{-1} FW.

Antioxidant enzyme extraction and assay

For enzyme analysis, fresh leaves and roots were homogenized with 0.1 g hydrated PVPP (polyvinylpolypyrrolidone) in 100 mM potassium phosphate (K_2HPO_4/KH_2PO_4) buffer pH 7.0 solution that included 1 mM EDTA using a pre-chilled mortar and pestle. The homogenates were centrifuged at 15 000 *g* for 30 min at 4°C. The supernatant was used for enzyme activity and protein content assays. Total soluble protein contents of the enzyme extracts were estimated according to Lowry (1951) [8] using bovine serum albumin (BSA, Sigma Aldrich) as a standard. The activity of following enzyme SOD, POD, APOX, CAT, GST and GR [9] were determined.

Results and Discussion

After 77 days long growing period in soil poplars (*Populus nigra* var. *italica*) treated with different concentrations of Pb (w = 400, 800, 1200 mg kg^{-1} soil) showed morphological, physiological and biochemical changes. During the growing period visual symptoms of phytotoxicity and partial reduction of biomass yield were observed (Table 1). The Pb tolerance of plants can be measured by their variation in biomass production in response to

Pb toxicity as compared to the control [10]. As shown in table 1, measured plant biomass values after 77 days long growing period indicate statistically significant growth reduction ($p<0,05$) in poplar treated with 400 mg Pb kg^{-1} soil and 1200 mg Pb kg^{-1} soil in comparison with control plants. Exception was found for poplar treated with 800 mg Pb kg^{-1} soil where increased values of biomass compared to the control were measured. The higher decrease of the aboveground biomass in comparison to the roots was observed. Allocation of biomass to the root might be indicative of plant Pb tolerance, since plants translocate their resources in the roots as a first line of defence against heavy metals contamination [11].

Table 1. Growth of poplar (*Populus nigra* var. *Italica*) and Pb accumulation in different plant parts after 77 days of treatment with different lead concentrations *

Parameters		Pb treatment (mg kg^{-1} soil)			
		0	400	800	1200
dry weight (g)	Entire plant	38.3^{b}±0.9	34.6^{b}±5.0	48.5^{a}±2.0	26.6^{c}±3.3
	Leaves	13.5^{a}±1.1	11.1^{a}±2.1	13.2^{a}±0.9	6.6^{b}±0.9
	Stem	13.2^{b}±0.9	12.0^{b}±1.6	17.6^{a}±0.9	11.1^{a}±1.9
	Roots	11.6^{b}±1.0	11.5^{b}±1.6	17.8^{a}±1.6	8.9^{b}±0.8
Pb content (mg g^{-1} d.w.)	Leaves	0	1.2^{b}±0.07	1.5^{a}±0.04	0.8^{c}±0.02
	Stem	0	0.7^{c}±0.02	0.9^{a}±0.01	0.9^{b}±0.03
	Roots	0	37.2^{b}±7.71	141.3^{a}±7.07	155.5^{a}±7.07

*Mean values (n = 6) ± SD in each row followed by different lower-case letters are not significantly different ($P < 0.05$) within the specific plant organ for each treatment as measured by Tukey's HSD test

Furthermore, Pb accumulation varies in different parts of the plant. Most of the accumulated Pb was measured in the root while in stem and leaves very low Pb concentration was measured. The concentration of accumulated Pb in the root increases with increasing Pb concentration in the soil. Thus the lowest accumulated Pb concentration in root was found in poplar treated with 400 mg Pb kg^{-1} soil in comparison with poplar treated with 1200 mg Pb kg^{-1} soil where the highest Pb concentration in root was found. These results correspond to the results of other studies conducted on different tree species. The highest Pb concentrations are recorded in root, from 85 % to 90 % of total apsorbed Pb [11, 12, 13]. These results indicate that lead is relatively difficult to translocate to the aboveground parts of the plant, so the focus should be on the phytostabilization processes of Pb contaminated areas. Phytostabilization or phytoimmobilization is the use of certain plants for stabilization of contaminants in contaminated soils. This technique is used to reduce the mobility and bioavailability of pollutants in the environment, thus preventing their migration to groundwater or their entry into the food chain [14].

The maintaining the balance between ROS and the antioxidative system is crucial for plant adaptation and survival under stressful condition such as heavy metals. In order to determine the potential role of the antioxidative enzymes in defense to oxidative stress caused by increased Pb concentrations in soil, activity of the antioxidative enzymes SOD, POD, APOX, CAT, GST and GR was measured. Furthermore, the levels of lipid peroxidation and H_2O_2 content were measured in order to determine the potential growth adjusment of poplar in contaminated area (Table 2).

Table 2. Changes in MDA and H_2O_2 content and antioxidative enzyme activities in leaves and roots of poplar after 77 days of treatment with different lead concentrations

Plant part	Pb treatment (mg kg^{-1} soil)			
	0	400	800	1200
H_2O_2 / µmol g^{-1} fw				
Leaves	84.8^{b}±7.0	84.7^{b}±0.4	137.2^{a}±1.5	127.5^{a}±4.4
Roots	13.8^{c}±0.5	18.2^{a}±1.2	17.4^{ab}±0.2	15.4^{bc}±0.2
MDA /nmol g^{-1} fw				
Leaves	2551.3^{b}±41.5	2875.9^{a}±163.0	1423.3^{c}±60.8	385.3^{d}±35.7
Roots	1186.7^{b}±19.3	1068.0^{b}±17.4	1424.0^{a}±23.1	1186.7^{b}±19.3
SOD / U mg^{-1} P				
Leaves	130.14^{c}±9.8	115.61^{c}±8.0	269.24^{b}±36.3	1124.87^{a}±119.7
Roots	234.56^{b}±18.4	228.52^{b}±29.9	825.69^{a}±78.4	980.22^{a}±29.2
POD / nmol mg^{-1}min^{-1} P				
Leaves	30.21^{d}±1.0	198.88^{b}±31.0	472.24^{a}±19.7	107.83^{c}±19.3
Roots	57.69^{c}±3.2	203.88^{a}±33.8	82.03^{b}±71.8	193.78^{a}±5.7
CAT / nmol mg^{-1}min^{-1} P				
Leaves	609.95^{b}±57.2	715.0^{a}±24.7	417.56^{c}±29.2	255.78^{d}±0.9
Roots	635.85^{b}±34.0	266.09^{c}±34.5	610.43^{b}±86.6	722.17^{a}±15.3
APOX /nmol mg^{-1}min^{-1} P				
Leaves	364.2^{b}±68.5	453.6^{a}±24.9	420.78^{a}±62.4	497.30^{a}±35.1
Roots	629.42^{b}±88.8	586.71^{b}±61.7	1439.03^{a}±48.6	1339.06^{a}±240.2
GR / µmol min^{-1} mg^{-1} P				
Leaves	10.68^{a}±0.1	4.23^{b}±0.1	3.17^{c}±0.06	2.26^{d}±0.1
Roots	3.49^{c}±0.05	6.41^{b}±0.08	5.90^{b}±0.5	13.56^{a}±0.4
GST / µmol min^{-1} mg^{-1} P				
Leaves	993.26^{d}±14.0	3146.54^{c}±108.8	6466.38^{b}±139.4	7949.68^{a}±536.4
Roots	1241.76^{c}±20.0	2269.81^{a}±29.6	1883.23^{b}±178.9	2344.26^{a}±702.3

*Mean values (n = 3) ± SD in each row followed by different lower-case letters are not significantly different ($P < 0.05$) within the specific plant organ for each treatment as measured by Tukey's HSD test

Based on obtained results, it can be concluded that the Pb undoubtedly affects the level of lipid peroxidation and the amount of H_2O_2 and thus the activity of antioxidant enzymes SOD, POD, APOX, CAT, GST and GR. Significant enzyme inhibition was determined for CAT and GR enzymes most commonly in the leaf samples. This is probably due to the impact of increased concentrations of heavy metals. Heavy metals probably change the conformation of the enzymes by nonspecific binding and thus inhibit them [15]. The highest enzyme activity, in the majority of the extracts, was determined in samples treated with higher Pb concentrations. Increased concentrations of metals in environment represent stressful conditions for a plant and lead to increased formation of ROS. The primary line of defense against ROS is activity of antioxidant enzymes.

Conclusions

The accumulation of Pb and biomass results were the best for poplar growing in soil treated with 800 mg Pb kg^{-1} soil. At this concentration tested poplar showed excellent properties for use in phytostabilization. Increased Pb concentration (1200 mg Pb kg^{-1} soil) had visually negative growth effects of the tested poplars. According to obtained results it can be concluded that further increasing of Pb concentration in the soil cause significant changes in the plant. Furthermore, Pb concentration of about 800 mg kg^{-1} soil is optimal for successful phytostabilization process of Pb by poplars. Also, it is necessary to conduct further studies in order to increase the accumulation of lead in the roots and to improve biomass growth under increased Pb concentrations in soil by specific agronomic techniques such as fertilization, water availability and chelates addition. This techniques can improve the efficiency of the phytostabilization process.

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PROPERTIES OF STARCHES IN MIXTURES WITH SUPERCRITICAL CO_2 AND THEIR USE AS CARRIERS IN PGSS MICRONIZATION PROCESS

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Introduction

Starch is the most abundant storage reserve carbohydrate in plants. It is a polymer of glucose which is linked by glycoside bonds. Starch especially corn or wheat starch, is a common food ingredient, available in large quantities and at a low cost, which has found uses beyond just human nutrition, including in paper, textile, cosmetics and adhesive products and as a substrate for ethanol production and viscosity modifier in oil drilling (1). Solubility and diffusivity data are important when designing technologies for processing natural or synthetic polymers. The application of supercritical carbon dioxide (SC CO_2) in polymerization reactions and polymer processing is rapidly expanding. SC CO_2 has also shown to be an attractive solvent and processing aid for the chemical and physical modification of polysaccharides such as starch, cellulose and chitin (2).

Materials and Methods

CO_2 (purity 2.5) was obtained from Messer (Ruše, Slovenia). Pure starch and glycerol tristearate were provided by Sigma-Aldrich (Maribor, Slovenia).

Magnetic suspension balance (MSB)

The solubility and diffusion coefficients of CO_2 in starches were measured using a magnetic suspension balance (MSB – RUBOTHERM, Germany). The MSB allows the gravimetric measurements of the quantity of gas dissolved in the polymer, with a resolution and accuracy of the microbalance of 10 µg and ± 0.002 % respectively. The measurements can be performed over a wide range of temperature and pressure, due to the location of the balance outside the measuring cell, in normal conditions of pressure and temperature. A detailed description of the device and of the working procedure can be found in literature (3). The measuring cell of MSB is also equipped with a window, which allows observation of the sample and estimation of volume changes during the sorption measurements. The polymers were shaped into discs and placed in a glass sample container inside the measuring cell of MSB, so that only one surface was in contact with the gas. The solubility and diffusivity of CO_2 in starches were measured at three different temperatures (313, 333 and 353) K in the pressure range (0 to 30) MPa (with an uncertainty u_c = 0.005 MPa). The measurements were performed using a step by step method: increasing the pressure for approximately 2 MPa, allowing the system to reach equilibrium, while recording the sorption curve, before applying the next pressure step (3).

Formulation with PGSS™

In this paper, the micronization of dyes from different varieties of raspberry juice (samples A, B and C) and carriers (pure starch and glycerol tristearate) with supercritical CO_2 were performed. For the micronization process, the PGSS™ technique was used. The concentration of the raspberry juice was 35 % (water solution). The melted glycerol tristearate was mixed with the emulsifier using a homogenizer. Pure starch was added into the raspberry juice. The solution was mixed for 1 min. Raspberry juice mixture was then added to the glycerol tristearate emulsion and everything was mixed using a homogenizer.

The autoclave was filled with the mixture and the liquid CO_2 from the gas cylinder was compressed into the system by means of a high-pressure pump. When the desired pressure and temperature were reached, the mixing of the system began. The system was shaken at constant temperature and pressure (328 K, 15.5 MPa) until the equilibrium was reached (approximately 100 min). When the system is equilibrated, the CO_2 is solubilized in the mixture of substances. The gas saturated solution was then rapidly expanded through the nozzle (diameter of the nozzle was 0.6 mm) in a spray tower (expansion unit) and the gas (CO_2) evaporated. Owing to the Joule-Thomson effect and/or the evaporation and the volume-expansion of the gas, the solution was cooled down below the solidification temperature of the solute, and fine particles were formed (4).

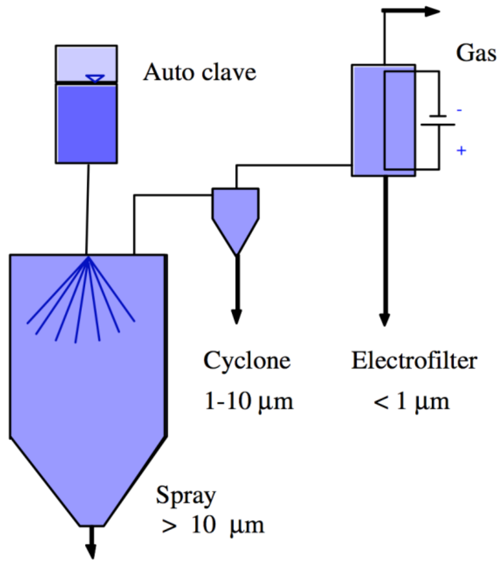

Figure 1. Basic scheme of batch operated apparatus for PGSS™ micronization (5).

Results and Discussion

Results of MSB

The solubility isotherms for starch are presented in Figure 2. The solubility of CO_2 in starch increases with increasing pressure and decreasing temperature. This phenomenon is generally observed during experiments concerning the absorption of CO_2 in polymers. It can be explained by the plasticization effect of CO_2. By raising the pressure, the gas molecules are forced between polymer chains, expanding the space between molecules, and thus increasing their mobility and allowing more gas molecules to be absorbed once the pressure is further raised (6). At the same time, an increase in temperature accounts for a lower gas density, which can be correlated with the lower solubility. The maximum solubility of CO_2 in starch is 0.31 wt %, at 313 K and pressure 30 MPa.

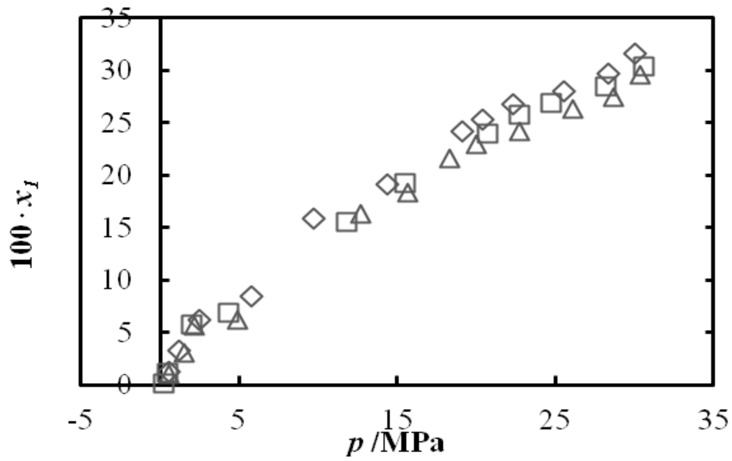

Figure 2. Solubility of CO_2 (1) in starch (2): ◊, 313 K; □, 333 K; Δ, 353 K.

For the design of processes involving polymer-gas systems it is necessary to know the mechanism of diffusion and how the rate of diffusion is affected by variables such as temperature or gas concentration. Diffusion of gasses into nonporous polymers is a two-step process. In the first step the gas has to dissolve in the polymer at the interface and then it has to diffuse as a solute through the polymer matrix (7). Some gasses tend to interact with polymers and cause them to swell and as a result the diffusion coefficient becomes concentration dependent. Other parameters which can affect the rate of diffusion are polymer characteristics. Small changes in crystallinity, polymer chain orientation, sample morphology, molecular weight distribution and degree of branching can alter the diffusion path and influence diffusion coefficients (8). The binary interdiffusion coefficients of the system CO_2/polysaccharide derivative were measured at three different temperatures 313, 333 and 353 K) for a pressure range from 0.5 to 30 MPa. The diffusion coefficients as a function of the CO_2 solubility are presented in Figure 3 for the starch.

At lower pressure (from 0.1 to approximately 15) MPa the diffusion coefficients increase with increasing pressure for all studied systems, afterwards they stay constant over a small range (15 – 16) MPa and finally they decrease with further increasing the pressure. The rate of diffusion depends on the number and size distribution of existing holes and the ease of whole formation which are influenced by chain mobility (9).

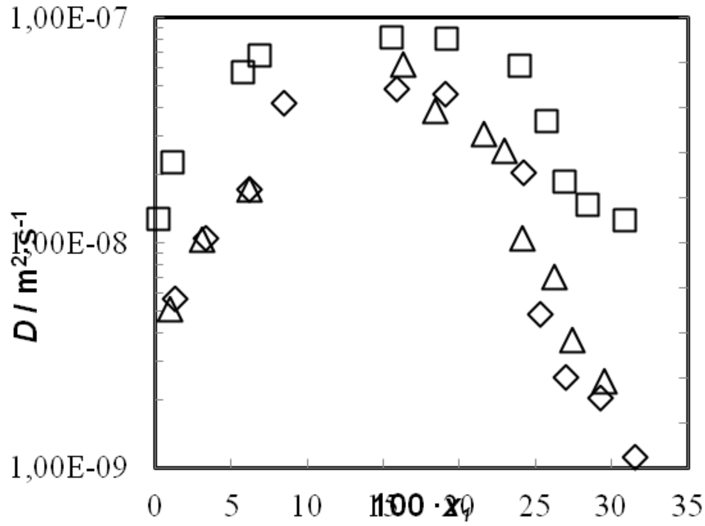

Figure 3. Binary interdiffusion coefficients for the system CO_2 (1) / starch (2): ◊, 313 K; □, 333 K; Δ, 353 K.

Results of micronization

Table 1 shows the composition of samples A, B and C and the operating parameters applied for the particles formulations.

Table 1. Results of formulation of raspberry juice particles

Samples	**Different varieties of raspberry juice** (%)	**Emulsifier** (% of total mass)	**Glyc. Trist.** (%)	**Pure starch** (%)	***p*** (MPa)	***T*** (K)	**Yield** (%)
A	40	2	50	10	15.5	328	92
B	40	2	50	10	15.5	328	92
C	40	2	50	10	15.5	328	94

Figure 4. Micronized samples A, B, C.

The efficiency of the micronization was above 90 % for all three samples. The highest efficiency was obtained for the sample C and amounts to 94 %. From the picture 4 it can be observed homogenously colored fine free-flowing powders with different shades of red color (dark red, purple, bright red). The shade of color depends on anthocyanin's (pigments) that may appear red, purple, or blue depending on the pH of the raspberry juice.

Conclusions

The high values obtained in this study for the solubility and diffusivity of CO_2 in starch derivative suggests that supercritical fluids represent a viable »green« alternative to the traditional methods of processing biopolymers. The data presented above represent an insight in the phenomena that may occur during SC CO_2 processing technologies of polysaccharides, with potential applications in a large number of fields and industries. Micronized powder of raspberry juice could be used in food industry as a food additive, especially as a natural dye.

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DEEP EUTECTIC SOLVENT CHOLINE CHLORIDE: GLYCEROL AS SELECTIVE SOLVENT FOR EXTRACTION OF PYRIDINE and THIOPHENE FROM *n*-HEXANE

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Introduction

Demands concerning liquid fuel purity as well as green chemistry principles consequently influence trends in the field of research and development of new technologies [1]. Besides production of fuels with ultra low sulfur content, demands for low nitrogen content must also be fulfilled. Alternative desulfurization processes were widely investigated and numerous solutions have been published. On the other hand, only few articles about denitrification can be found. Liquid-liquid extraction with environmentally friendly solvents, as one of the possible solution for denitrification, became interesting since different types of ionic liquids dissolve nitrogen compounds together with sulfur compounds [2-4]. As a new type of solvent, ionic liquid analogues, deep eutectic solvents (DESs), have attracted great attention because of their excellent physical and chemical properties [5]. DESs are advantageous because they can be easily prepared in high purity at low cost, and their components are biodegradable and possess low toxicity. Their properties can be altered by change of hydrogen bond donor or acceptor, so similarly to ionic liquids, DESs can be designed for a specific task. DESs have been widely used in catalysis, organic synthesis, materials preparation, electrochemistry, substance dissolution and separation processes. The key advantages of DESs in liquid-liquid extraction are their high selectivity, non-volatility and their capability to dissolve different types of organic, inorganic and polymeric compounds. The use of DESs for denitrification of model fuels have not been published jet while only one article dealing with desulfurization can be found in the available literature [6]. Despite the advantages of these environmentally friendly solvents, there are not a lot of data on their thermophysical properties and phase equilibrium data [7]. Knowledge of this data is fundamental for the ionic liquids, its mixtures and DESs to be used as selective solvent in liquid-liquid extraction. It is also essential for the development of new separation technologies, applications as well as for possible industrial implementation.

In this article, separation of pyridine and thiophene from its mixture with *n*-hexane by means of liquid-liquid extraction with cholinium-based DES (choline chloride:glycerol (1:2,n)l) was analyzed.

Extraction efficiency was calculated from the following Equation:

$$\varepsilon = \frac{x_F - x_R}{x_F} \qquad (1)$$

Materials and Methods

Preparation of cholinium-based DES choline chloride:glycerol (ChCl:Gly)

Choline chloride and glycerol were dried in the vacuum concentrator (Savant SPD131DDA SpeedVac Concentrator) at 60°C for 24 hours before use. Cholinium salt and glycerol (molar ratio 1:2) were directly weighed in a flask and the mixture was placed on the rotary vacuum evaporator (250 mbar and 60°C) until a colourless liquid was formed. Prepared DES was dried in a vacuum concentrator at 60°C for 24 hours before use. Viscosity of prepared DES was measured on the Brookfield DV-III Ultra Programmable Rheometer. Density was measured with Mettler Toledo densitometer Densito 30PX.

Liquid-liquid equilibria

The binodal curve points were determined by the titration method [8]. The refractive indices of hydrocarbon/thiophene mixtures were measured at 25 °C as a function of composition (Abbe refractometer Model RMI, Exacta Optech) to prepare calibration curves for the determination of tie lines composition. Calibration curves are given below:

for thiophene:

$$w = -22.27 \cdot n_{D,25}^2 + 70.94 \cdot n_{D,25} - 55.41 \qquad (2)$$

for pyridine:

$$w = 46.77 \cdot n_{D,25}^2 - 119.54 \cdot n_{D,25} + 75.97 \qquad (3)$$

Two-phase three-component solutions were prepared by weighing the components. The solutions were shaken well for 24 h and left in a thermostated air bath at 25 °C to settle and reach equilibrium separation. The refractive indices of the hydrocarbon-rich phase were determined using the above mentioned instrument at 25 °C. These were converted into compositions using the calibration curves.

Liquid-liquid extraction

The binodal initial concentration of thiophene and pyridine in the binary mixture with *n*-hexane was 14.0 % and 20.0 % (by weight). Liquid-liquid extraction experiments were carried out in a laboratory batch extractor. Quantities of solvent and feed solution were mixed together at three solvent ratios (0.25, 0.50 and 1.00 kg/kg) for a given period of time. Based on the literature [6], the extraction time was set to 30 minutes. During this time period the phase equilibrium was achieved. Afterward, the heavier and lighter phases are separated and the concentration of the raffinate was determined by measuring the refractive indices.

Results and Discussion

Measured viscosity and density of ChCl:Gly are 0,3027 Pa s and 1192 kg/m^3 respectively. Relatively high viscosity can affect the interphase surface area, and consequently the rate of mass transfer. Both thiophene and pyridine have higher density, viscosity and surface tension than n-hexane, so binary mixtures of solute (thiophene or pyridine) and *n*-hexane will have higher values than *n*-hexane. The density difference between the feed and ChCl:Gly is high enough for fast phase separation.

Experimentally obtained phase equilibria for the systems *n*-hexane-pyridine or thiophene – DES are shown on figure 1. *n*-hexane and ChCl:Gly are immiscible in the whole range of concentrations, so ChCl:Gly is highly selective solvent for both solutes (thiophene and pyridine). At high solute (thiophene or pyridine) concentration the slope of tie lines become positive leading to more favorable separation of solute and *n*-hexane. Based on the tie lines slopes, investigated solvent is better for the extraction of pyridine than thiophene.

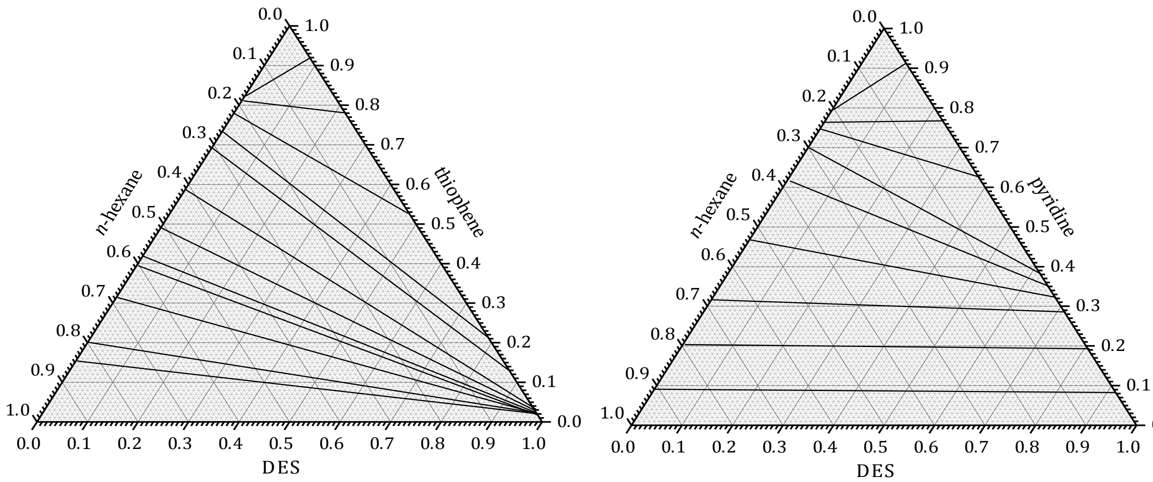

Figure 1. Liquid-liquid equilibra for systems *n*-hexane – thiophene or pyridine - ChCl:Gly.

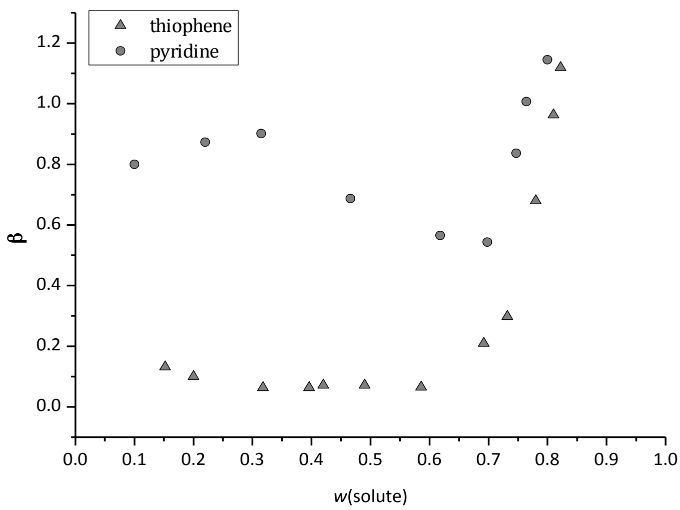

Figure 2. The influence of the solute (thiophene or pyridine) concentration in the raffinate on the solute distribution coefficients

The feasibility of using ChCl:Gly as a solvent for extraction of solute (thiophene and pyridine) from a mixture with hydrocarbon was evaluated by the distribution coefficient. Distribution coefficient is defined by following equation:

$$\beta_1 = \frac{w_1^{II}}{w_1^{I}} \tag{4}$$

where w is the mass fraction, superscripts I and II refer to the hydrocarbon-rich and ChCl:Gly -rich phases and subscript 1 denote solute. The influence of the solute mass fraction in the raffinate phase on the distribution coefficients for thiophene and pyridine are given on figure 2. Their values are in concordance with the slopes of tie lines. Distribution coefficients for pyridine are higher than for thiophene. Selectivity of ChCl:Gly cannot be numerically expressed because *n*-hexane is insoluble in it.

During the extraction experiments low degree of dispersion was observed. This can be explained with relatively high viscosity and surface tension of choline chloride:glycerol.

Since the initial solute concentration in the feed solution is an important parameter, the influence of thiophene and pyridine concentration in their mixture with *n*-hexane on the extraction efficiency was analyzed. The obtained results are shown in table 1. Extraction efficiency increases with increase of the solute concentration due to the high capacity of ChCl:Gly. This means that solvent was not saturated by the solute, so ChCl:Gly can be reused before regeneration. Higher values can be observed for pyridine than for thiophene, so ChCl:Gly is better for denitrification than for desulfurization. This also means that larger amount of solvent should be used for desulfurization in order to achieve the same extraction efficiency as for denitrification. The solvent ratio positively influences the extraction efficiency. At higher solvent ratio, higher amount of solvent is used for extraction and consequently higher amount of solute is transferred between two phases. For the investigated range of feed concentration and solvent ratio, the distribution coefficient for pyridine increases with the pyridine concentration. Quite opposite trend can be observed for thiophene. Even though the numerical values of distribution coefficients are lower than 1, due to the high selectivity and capacity to dissolve relatively large quantities of solute, ChCl:Gly can be used for extraction of thiophene and pyridine from their mixtures with *n*-hexane.

Table 1. Extraction efficiency of ChCl:Gly for pyridine and thiophene.

w_F	S, kg/kg	ε, %	
		pyridine	thiophene
0.14	0.25	14.04	4.14
	0.50	28.42	14.53
	1.00	53.48	18.71
0.20	0.25	19.98	11.12
	0.50	38.12	17.20
	1.00	58.69	22.98

Conclusions

Liquid–liquid equilibria for the three-component systems with *n*-hexane – thiophene or pyrinine – ChCl:Gly were determined at 298.15 K and atmospheric pressure. ChCl:Gly is highly selective solvent for the separation of thiophene and pyridine from *n*-hexane. Extraction efficiency increases with increase of the solute concentration in the feed solution and the solvent ratio. Due to the higher numerical values of distribution coefficients and extraction efficiencies, it can be concluded that ChCl:Gly is better for denitrification than for desulfurization.

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LIQUID–LIQUID EQUILIBRIUM FOR THE SYSTEMS HYDROCARBON – THIOPHENE – 1-HEXYL-3,5-DIMETHYLPYRIDINIUM BIS(TRIFLUOROMETHYLSULFONYL)IMIDE

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Introduction

Diesel and gasoline rich in sulfur will produce exhaust gases containing SO_x, a major contributor to air pollution and acid rain. Today, commonly used industrial process for desulfurization of diesel and gasoline is hydrodesulfurization (HDS), in which organic sulfur compounds are converted to H_2S and the corresponding hydrocarbons. The process requires high temperatures and pressures, as well as large amounts of hydrogen, making HDS an expensive and relatively environmentally unfriendly process. Moreover, this process requires even more expensive process conditions for removal of compounds such as dibenzothiophene derivatives [1]. For that reason scientists explore new environmentally friendly and energy saving processes [2]. During the past decade, an increasing trend of investigations concerning the possibility of replacing the existing desulfurization process with the extraction of fuels by ecologically acceptable ionic liquids can be observed. Ionic liquids are very good solvents for a wide range of organic, inorganic and polymeric compounds. They are non-volatile and easy to regenerate by simple vacuum evaporation and therefore may be considered as selective solvents in separation processes. The extraction is based on a better solubility of sulfur compounds in an appropriate solvent with respect to non-aromatics. The process efficiency, for the most part, depends on the proper solvent selection, which means that a compromise must be reached between solvent selectivity and capacity. Other considerations, such as environmental and toxicological issues have to be included in solvent selection as well.

In this article liquid–liquid equilibria have been determined experimentally for four systems, at 298.15 K and 101325 Pa, with thiophene as the key component to be distributed between a hydrocarbon (toluene, *n*-hexane, *n*-heptane or *i*-octane) and ionic liquid component. The equilibria have been modeled by NRTL and UNIQUAC models, following the procedure that was described previously [3]. The applicability of [C_6mmpy][Tf_2N] for desulfurization of model solutions by means of liquid–liquid extraction was tested experimentally, on several three- and seven-component systems.

Materials and Methods

In this work, the equilibrium compositions for the four systems comprising a common ionic liquid component [C_6mmpy][Tf_2N] (IL, 1-hexyl-3,5-dimethylpyridinium bis(trifluoromethyl-sulfonyl)imide) as component (3) were experimentally determined. Component (1) was selected among four hydrocarbons (*n*-hexane, *n*-heptane, *i*-octane, toluene) and component (2) was thiophene The measurements were performed at 101325 Pa and at temperature of 25 °C. All the composition determinations were done gravimetrically according to the procedure described below. Every weighing involved in the experimental work was carried out on a Kern ALJ 220-4 NM balance with a precision of ± 0.0001 g.

In addition, several liquid–liquid extraction experiments were performed with a model solution to investigate the performance of [C_6mmpy][Tf_2N] in extracting sulfur components from hydrocarbon mixtures.

Ionic liquid, [C_6mmpy][Tf_2N] was synthesized according to the procedure described in details in [4]. After synthesis, IL was dried under high vacuum at 90 °C for 8 h prior to use and characterized by ^{1}H NMR.

Binodal curve measurements

All the measurements were performed at 25 °C, in a thermostated air bath. The binodal curve points were determined by the titration method [5]. The concentrations in the mixture were expressed in mass fractions.

Tie lines

The refractive indices of hydrocarbon/thiophene mixtures were measured at 25 °C as a function of composition (Abbe refractometer Model RMI, Exacta Optech) to prepare calibration curves for the determination of tie lines composition. Two-phase three-component solutions were prepared by weighing the components. The solutions were shaken well for 24 h and left in a thermostated air bath at 25 °C to settle and reach equilibrium separation. The refractive indices of the hydrocarbon-rich phase were determined using the above mentioned instrument at 25 °C. These were converted into compositions using the calibration curves. The compositions of the IL-rich phase were then calculated by the mass balance line and binodal curve data.

Extraction experiments

The two-component model solution consisted of 3–6 % of thiophene (by mass) in the mixture with a hydrocarbon. The six-component model solution was prepared according to the literature [6]. The composition of model solution was: 25.26 % of *n*-hexane, 29.11 % of *n*-heptane, 24.02 % of isooctane, 9.62 % of toluene, 6.08 % of thiophene and 5.91 % of pyridine. The concentrations of all components of the model solution after extraction were determined by means of gas chromatography (GC-2014-Shimadzu). ^{1}H NMR spectra were recorded in deuterated chloroform on a Bruker Avance 300 spectrometer with 0.03 % tetramethylsilane (TMS) as an internal standard. Liquid–liquid extraction experiments were carried out in a laboratory batch extractor (internal diameter of 0.04 m) equipped with a magnetic stirrer. Desired amounts of ionic liquid and model solution were mixed together at the defined mass ratio (ionic liquid/model solution = 0.25 kg/kg) for 20 minutes. The mixing rate was set to the value at which complete dispersion is achieved. This state was visually observed. Afterwards, the heavier and lighter phases were separated in a settling unit.

Results and Discussion

Type II ternary equilibria were obtained for all the investigated ternary systems. Due to space limitations, we present graphically only the equilibria for systems with *n*-hexane and toluene, Figure 1. Calculated interaction parameters of the NRTL and UNIQUAC activity coefficient models, together with corresponding root-mean-square deviations of experimental and calculated equilibrium mass fractions are given in Table 1 for all the systems. Geometric parameters of UNIQUAC were calculated using the approach by Lei [7]. The agreement seems to be very good, particularly with UNIQUAC.

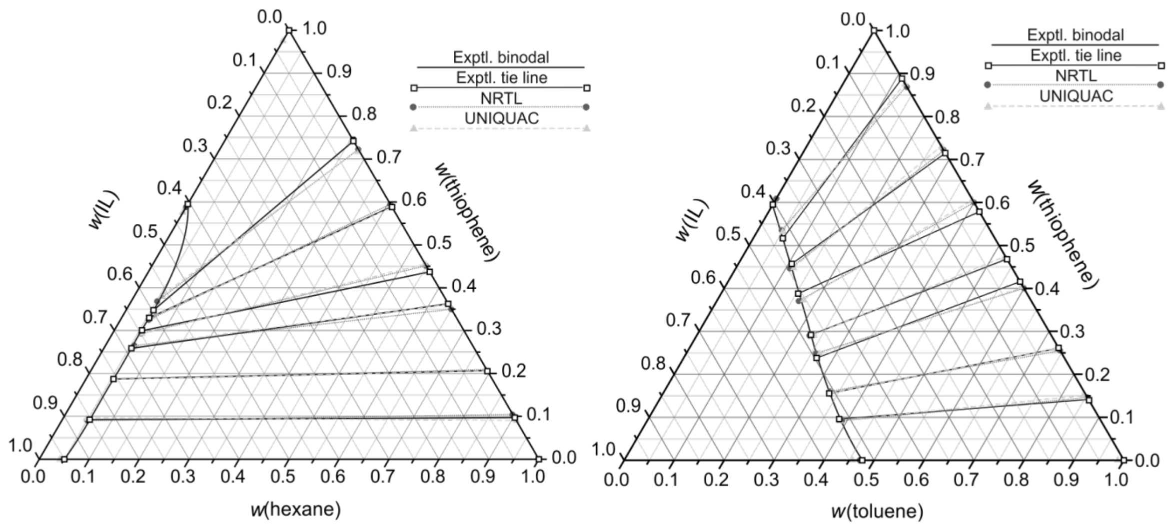

Figure 1. Liquid–liquid equilibria

All the systems exhibit tie-lines with negative slope. The feasibility of using [C_6mmpy][Tf_2N] as a solvent for extraction of thiophene (2) from a mixture with hydrocarbon (1) was evaluated by the distribution ratio and selectivity calculated from experimental tie-lines [8]. The results are given in Table 2.

Table 1. Interaction parameters of NRTL and UNIQUAC models, τ_{ij}, as well as corresponding root-mean-square deviations of experimental and calculated equilibrium mass fractions, *A*.

NRTL, α_{12}. α_{13}. α_{23} = 0.3; 0.3; 0.3	τ_{12}	τ_{13}	τ_{21}	τ_{23}	τ_{31}	τ_{32}	*A*
n-hexane (1) – pyridine (2) – IL (3)	1.2504	10.2784	0.9255	4.7923	2.7222	-2.8898	0.0040
n-heptane (1) – pyridine (2) – IL (3)	1.0673	13.1706	1.1736	5.1122	3.6390	-2.7404	0.0161
i-octane (1) – pyridine (2) – IL (3)	0.4507	12.0771	0.9196	4.9368	3.4115	-3.8991	0.0092
toluene (1) – pyridine (2) – IL (3)	1.8554	14.0654	1.1988	1.3897	0.5684	-1.2534	0.0014

UNIQUAC	τ_{12}	τ_{13}	τ_{21}	τ_{23}	τ_{31}	τ_{32}	*A*
n-hexane (1) – pyridine (2) – IL (3)	0.5109	0.3081	1.1539	1.6341	1.1468	1.1227	0.0028
n-heptane (1) – pyridine (2) – IL (3)	0.6907	0.3530	0.9648	1.5724	1.2140	1.2576	0.0042
i-octane (1) – pyridine (2) – IL (3)	0.4855	0.3406	1.1954	1.4842	1.2399	1.0932	0.0031
toluene (1) – pyridine (2) – IL (3)	1.3556	0.0260	2.3717	1.0000	0.2671	2.8438	0.0013

Distribution ratios of thiophene are higher than distribution ratios of the corresponding hydrocarbon for all the investigated systems. The thiophene distribution ratio generally decreases with increasing solute mass fraction in the hydrocarbon rich phase. The opposite trend can be observed with the hydrocarbon distribution ratio. The selectivity decreases with the increasing solute mass fraction in the hydrocarbon rich phase, as well. High values of the selectivity, except for the systems with toluene, imply favorable separation of aliphatic hydrocarbons and thiophene by means of liquid–liquid extraction with the selected IL. Desulfurization is expected to be accompanied with dearomatization according to the low values of selectivity with respect to toluene.

Table 2. Distribution coefficients and selectivity of ionic liquid

hydrocarbon (1) – thiophene (2) – IL (3)											
n-hexane			*n*-heptane			*i*-octane			toluene		
β_1	β_2	S	β_1	β_2	S	β_1	β_2	S	β_1	β_2	S
0.0609	0.9549	15.68	0.0468	0.8699	18.58	0.0665	0.9881	14.85	0.4439	0.6883	1.55
0.0693	0.9088	13.12	0.0527	0.7738	14.67	0.0787	0.9724	12.36	0.4498	0.5957	1.32
0.0863	0.7143	8.28	0.0640	0.6818	10.65	0.0900	0.9257	10.28	0.4552	0.5726	1.26
0.0977	0.6885	7.05	0.0757	0.6307	8.33	0.1028	0.8938	8.69	0.4286	0.6239	1.46
0.1334	0.5614	4.21	0.1013	0.5590	5.52	0.1449	0.7675	5.29	0.3653	0.6708	1.84
0.2132	0.4863	2.19	0.1427	0.5922	4.15	0.1822	0.6689	3.67	0.3754	0.6392	1.70
						0.1668	0.6251	3.75	0.5527	0.5603	1.01
						0.1491	0.6154	4.13	0.5308	0.5812	1.09

In order to investigate the influence of the composition of model solution on the extraction efficiency, extraction experiments with three and seven component mixtures were performed. Results obtained for two component model solution are presented in table 3. Based on the results obtained for six-component model solution (GC of model solution and ^{1}H NMR of ionic liquid), it can be concluded that only thiophene, pyridine and toluene are involved in mass transfer between model solution and ionic liquid. Extraction efficiencies for thiophene, pyridine and toluene are: 12.05 %, 39.11 % and 8.97 %, respectively. Calculated values of distribution coefficients for thiophene and toluene are 0.6683 and 0.5403, respectively, so the selectivity of IL (for thiophene with respect to toluene) is 1.24 which is lower than for the three component systems.

Table 3. Extraction efficiency of ionic liquid for separation of thiophene from its mixtures with hydrocarbons

hydrocarbon	w_2^F, %	β_1	β_2	S	Extraction efficiency, %	
					thiophene	hydrocarbon
n-hexane	6.22	0.0468	0.7427	15.84	35.09	1.31
n-heptane	5.23	0.0416	0.7777	18.69	26.24	1.85
i-octane	4.29	0.0563	0.8427	14.96	44.74	1.59
toluene	3.08	0.4607	0.8631	1.87	24.79	22.65

Conclusions

Liquid–liquid equilibria for the three-component systems with hydrocarbon (toluene or *n*-hexane or *n*-heptane or *i*-octane) – thiophene – ionic liquid [C_6mmPy][Tf_2N] were determined at 298.15 K and atmospheric pressure The agreement between experimental and calculated data seems to be very good, both for NRTL and UNIQUAC activity coefficient model, with UNIQUAC giving somewhat better results for all the systems. The applicability of [C_6mmPy][Tf_2N] as selective solvent for separation of pyridine and hydrocarbons has been evaluated from the distribution coefficients and selectivity and validated with the extraction experiments. The composition of model solution affects both the pyridine distribution ratio and selectivity of ionic liquid.

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SEPARATION OF TIOPHENE FROM *n*-HEXANE BY THE MIXTURE OF IONIC LIQUIDS

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Introduction

The production of fuels with ultralow sulfur content has become one of the mostly investigated tasks due to the strict regulatory requirements on sulfur content in fuels [1, 2]. Since the commercial desulfurization process, hydrodesulfurization, is conducted at elevated temperatures and pressures and uses large amounts of hydrogen and catalyst, the possibility of replacing such process with some environmentally more friendly technology, has been widely investigated [3]. Among few separation processes, liquid – liquid extraction has drawn attention due to the mild process conditions. In order to make this process as green as possible, replacement of commonly used organic solvents with nonvolatile room temperature ionic liquids seems to be a reasonable idea. Ionic liquids are very good solvents for a wide range of organic, inorganic and polymeric compounds [4]. Properties like density, viscosity and surface tension of solvents are important due to its impact on the specific surface area available for mass transfer. In order to decide if solvent is appropriate for extraction, besides the knowledge of physical properties, phase equilibrium as well as the corresponding distribution coefficients and selectivity should be determined [5]. Properties of ionic liquids can be modified by simple combination of anion and cation as well as by addition of some other solvents. Recently, binary mixtures of ionic liquids have been used as selective solvent for separation of toluene and n-heptane [6, 7].

In this article, the possibility of using a mixture of two ionic liquids at different mass ratios ([C2mim][EtSO4]-[C5mim][Tf2N]; [C2mim][EtSO4]-[C6mmPy] [Tf2N] and [C2mim][EtSO4]-[bzmim][Tf2N]) as selective solvents in the separation of tiophene and n-hexane by liquid-liquid extraction has been investigated. Extraction efficiency was calculated from the following equation:

$$\varepsilon = \frac{x_F - x_R}{x_F} \qquad (1)$$

Materials and Methods

Selected ionic liquids were synthesized and purified according to the procedure described in details in [8]. For the separation of thiophene and n-hexane pure ionic liquids and its mixtures were used. The initial concentration of thiophene in the mixture with n-hexane was 19.5 % (by weight). Liquid-liquid extraction experiments were carried out in a laboratory batch extractor. Quantities of solvent and feed solution were mixed together at the solvent ratio 0.25 kg/kg for a given period of time. Afterward, the heavier and lighter phases are separated and the concentration of the raffinate was determined by measuring the refractive indices. Calibration curve for the calculation of mass fraction of thiophene in the mixture with n-hexane from the measured refractive indices:

$$w = -22{,}27 \cdot n_{D,25}^2 + 70{,}94 \cdot n_{D,25} - 55{,}41 \quad (2)$$

Results and Discussion

A solvent can be evaluated when its properties are known. Properties such as density, viscosity and surface tension can help during preliminary screening for suitable solvent that can be used in liquid-liquid extraction. High density difference, low viscosity and surface tension are favorable. However, for the final decision thermodynamic data (phase equilibrium) should also be available. For the investigated model solution and ionic liquids measured results were presented in table 1. It can be seen that differences in density between the model solutions and ionic liquids were satisfactory. From the viscosity and interfacial tension point of view, the most suitable ionic liquid is [C_5mim][Tf_2N] due to the lowest numerical values. Liquid with low viscosity and interfacial tension will easy be dispersed into the other, and higher rates for mass transfer will be achieved.

Table 1. Density, viscosity and surface tension of model solutions and selected solvents

Solvent	ρ, kg m^{-3}	η, Pa s	σ, mN m^{-1}
model solution	706	$3{,}62 \cdot 10^{-4}$	19,5
[C_2mim][$EtSO_4$]	1236	0,0896	49,3
[C_5mim][Tf_2N]	1404	0,0525	32,6
[C_6mmPy] [Tf_2N]	1332	0,1152	34,7
[bzmim][Tf_2N]	1491	0,1508	41,5

Based on the solubility of thiophene and *n*-hexane in the selected ionic liquids (Figure 1.) it can be concluded that [C_6mmPy] [Tf_2N] is the moist suitable solvent for the separation of thiophene and *n*-hexane, even though the highest selectivity exhibits ionic liquid [C_2mim][$EtSO_4$]. These results are in concordance with the physical properties of ionic liquids, since pyridinium based ionic liquid exhibits relatively low values of viscosity and surface tension.

Initial extraction experiments have been performed with pure ionic liquids and the obtained results are presented on figure 2. Once again, pyridinium based ionic liquid is confirmed as the moist suitable solvent, while [C_2mim][$EtSO_4$] shows the lowest extraction efficiency.

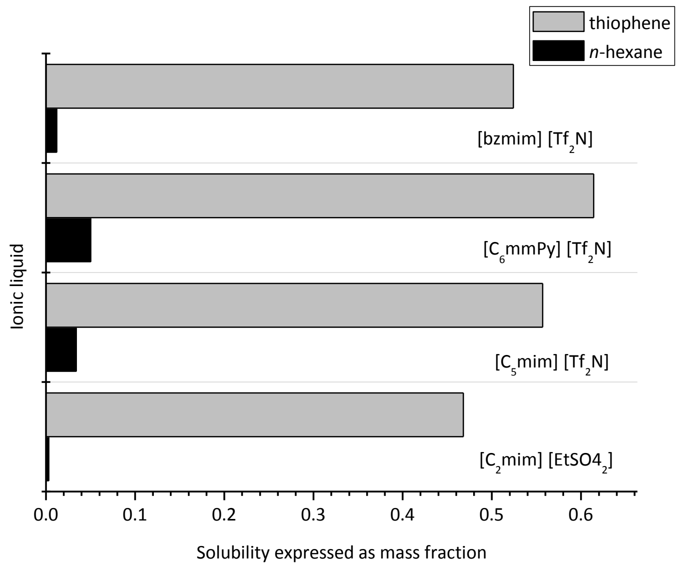

Figure 1. Solubility of thiophene and *n*-hexane in selected ionic liquids.

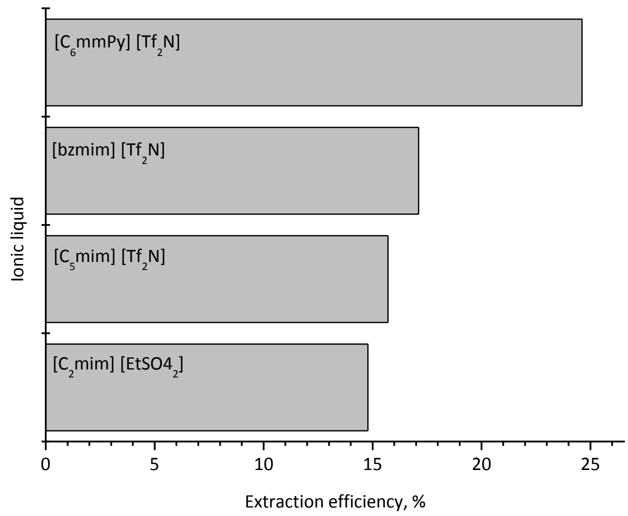

Figure 2. Extraction efficiency of pure ionic liquids for thiophene.

Since ionic liquid [C_2mim][$EtSO_4$] is the least suitable solvent its properties were modified by addition of [C_5mim][Tf_2N], [C_6mmPy] [Tf_2N] and [bzmim][Tf_2N]. The influence of the composition of binary mixtures of ionic liquids on the extraction efficiency is shown on figure 3. It can be seen that addition of all ionic liquids positively influences the extraction efficiency of [C_2mim][$EtSO_4$]. Increase of mass fraction of [C_5mim][Tf_2N], [C_6mmPy] [Tf_2N] or [bzmim][Tf_2N] in the binary mixture increases the extraction efficiency. As expected, the best results were obtained with the mixture [C_2mim][$EtSO_4$]-[C_6mmPy] [Tf_2N]. This can be explained with the highest solubility of thiophene in [C_6mmPy] [Tf_2N] as well as suitable density difference, low viscosity and low surface tension.

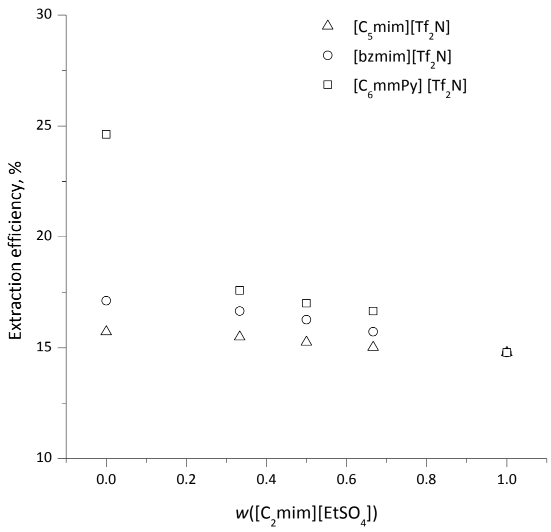

Figure 3. The influence of the mass ratio of ionic liquid [C_2mim][$EtSO_4$] in the mixture of two ionic liquids on the extraction efficiency for thiophene

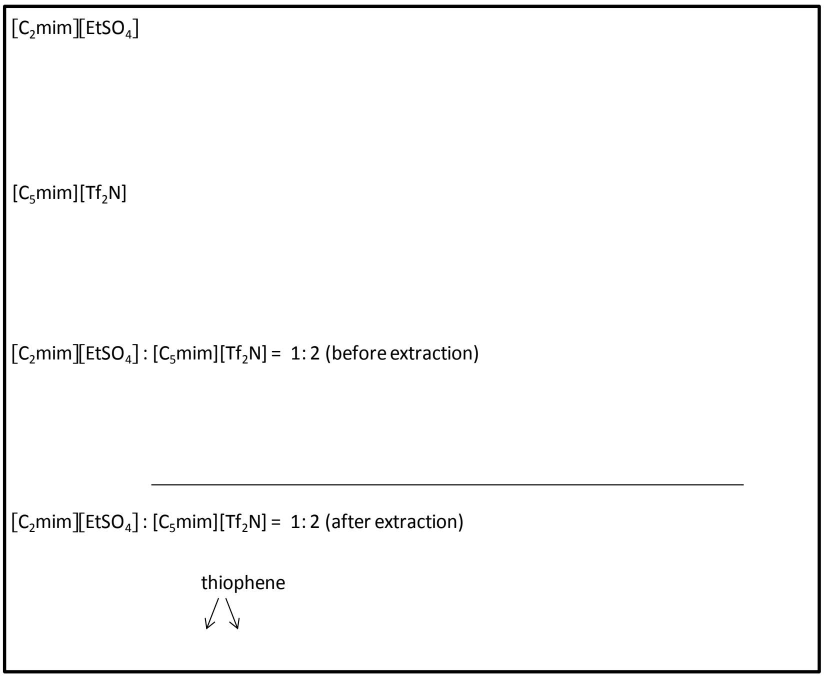

Figure 4. ^{1}H NMR spectras of fresh ionic liquids [C_2mim][$EtSO_4$] and [C_5mim][Tf_2N] and its mixture, before and after extraction.

Purity of ionic liquids and its binary mixtures before and after extraction was determined by means of ^{1}H NMR spectroscopy, figure 4. Mixture of two ionic liquids has peaks that correspond to both ionic liquids. With model solution experiment, the peaks that correspond to thiophene can be clearly observed in the spectrum, and no peaks corresponding to *n*-hexane are observed.

Conclusions

All selected ionic liquids can be used for the separation of thiophene and *n*-hexane due to its suitable selectivity. The less suitable ionic liquid, [C_2mim][$EtSO_4$], achieves better extractive properties in its binary mixtures with [C_5mim][Tf_2N], [C_6mmPy] [Tf_2N] or [bzmim][Tf_2N]. Increase of mass fraction of [C_5mim][Tf_2N], [C_6mmPy] [Tf_2N] or [bzmim][Tf_2N] in the binary mixture increases the extraction efficiency.

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AQUEOUS TWO-PHASE EXTRACION OF POLYPHENOLS FROM RED AND WHITE WINE IN A MICROEXTRACTOR

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Introduction

Polyphenols, a large group of natural antioxidants, are broadly distributed in the plant kingdom and are the most abundant secondary metabolites found in plants. The importance of polyphenols is due to their positive contribution to cellular processes within the body and it is proved that they have antioxidant, anti-inflammatory and anti-carcinogenic activity. They increased longevity and cardiovascular protective effects due to its ability to reduce platelet aggregation, modulate lipid metabolism, and inhibit oxidation of low-density lipoprotein [1]. Extraction is one of the most common methods used for isolation of polyphenolic compounds and usually it is mainly performed with different organic solvents (methanol/hydrochloric acid, hexane/butanol, benzene, ethyl acetate/etoxyethain) [2]. These extraction procedures are efficient but the extracts are not safe for human consumption due to the potentially toxic effect of the residual solvents [3]. Therefore, the need to develop effective, economical, green and rapid purification methods for polyphenol extractions that are not based on different organic solvents is becoming imperative.

Extraction in aqueous two-phase systems (ATPSs) has attracted interest for many years and has been developed as a primary stage unit operation in the downstream processing of different biological products such as cells, viruses, plasmids and proteins [4]. For a long time ATPSs have been used as a preparative and analytical tool in biochemistry and are formed in general when two incompatible polymers like polyethylene glycol (PEG) and dextran are mixed and usually contain 80–90% (w/w) water. Phase systems can also be obtained by mixing certain polymers (e.g. PEG) with lyotropic salts (e.g. Na_2HPO_4) or detergents [5]. The main advantages of ATPSs are that they provide a suitable environment to maintain biological activity and solubility because of high biocompatibility, high water content and low interfacial tension of these systems that minimizes product degradation. Also, good resolution and yields can be obtained simply by varying certain experimental conditions such as pH, ionic strength, and polymer molecular weight [4]. The technique is well suited for handling of both soluble and insoluble matter and is also easy to scale up to large scale or to scale it down to smaller scale.

Grapes contain a large amount of polyphenolic compounds in skins, pulp and seeds, which are partially transferred to wine during wine-making [6]. The objective of this study was to investigate application of ATPSs for continuous polyphenol extraction from white and red wine. As it was mentioned, good resolution and high extraction yields depend on experimental conditions such as pH, ionic strength, and composition of APTSs so the extraction was made under previously optimized experimental conditions [7]. Extraction was performed in macro- and microextractor and the results were compared with extraction performed in batch system.

Materials and Methods

Chemicals: Polyethylene glycol 6000 (PEG_{6000}) and sodium carbonate (Na_2CO_3) were purchased from Merck (Germany). Potassium dihydrogen phosphate (KH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were from Kemika (Croatia). Ammonium sulphate (AMS, $(NH_4)_2SO_4$) and gallic acid ($C_7H_6O_5$) were procured from Acros (Belgium) and methanol (CH_3OH) was procured form Sigma (Germany). The Folin-Ciocalteu reagent was purchased from Fluka (Switzerland).

Total phenol (TP) content: The total phenol (TP) content was determined by the Folin-Ciocalteu method [8]. Briefly, aliquots of 0.1 mL of samples and standards were mixed with 0.1 mL of the Folin-Ciocalteu reagent. 0.1 mL of the saturated sodium carbonate solution was added to the mixture and made up to 2 mL with distilled water. After incubation in the dark (T = 25 °C, t = 2 h), the absorbance of the reaction mixture was measured at 725 nm (double-beam UV spectrophotometer, UV – 1601, Shimadzu, Japan). The calibration curve was prepared using the standard solution of gallic acid (1 – 100 mg/L; R^2 = 0.9984).

Aqueous Two-phase Extraction: Polyphenol extraction from white wine (Graševina, Daruvar 2008), red wine (Dingač, Pelješac 2008) and model solution of gallic acid was carried out in two continuous extracting systems: macroextractor and microextractor at process conditions previously optimized in the batch system (pH = 6.50, w_{AMS} = 0.0925, $w_{PEG6000}$ = 0.1037) [7].

Macroextractor Continuous System: The continuous separation of polyphenols was performed in the mixer-settler that consisted of two sections, cylinder form mixer section with capacity of 103 mL and cone shaped edges settler section with capacity of 261 mL. A settler section was divided in two compartments: bottom phase compartment (raffinate) and top phase compartment (extract). The mixer and the settler sections were placed inside the thermostated cylinder vessel. Stock solutions of PEG_{6000} (w_{PEG}=0.1037 g/g, η_{PEG}=7.496 g/ms), ammonium sulfate (w_{AMS}=0.0925 g/g, η_{AMS}=1.063 g/ms), polyphenols and phosphate buffer (potassium-sodium phosphate buffer 0.2 M, pH 6.5) were fed into the mixer section using peristaltic pumps (FE 411 B. Braun, Biotech International) and mixed. When the mixture approached the edge of the mixer section, it overflowed into the settler section where the phases separate. When mixture approached flooding limit two pumps for draining the raffinate and the extract phase where turned on. The continuous separation was carried out for serious of different flow rates in the range 1.5-17.5 mL/min. All experiments were carried out at 25 °C in triplicate and the average values are reported (at 95 % confidence interval results have no statistical difference).

Microextractor Continuous System: A microextractor system was consisted of a two microchips connected in series (Micronit Microfluidics B.V., Enschede, Netherlands). The first microchip was equipped with micromixers (first configuration swirl micromixers (V = 1 µL) and second configuration teardrop shape micromixers (V = 2 µL)) to enhance mass transfer. Second, tubular microchip (V = 6 µL) was used for phase separation. The first microchip had two "Y" – shaped inlets, so fluids could be injected separately using syringe pumps (PHD 4400 Syringe Pump Series, Harvard Apparatus, USA), and one outlet that was connected by a fused silica connection to the inlet of the second microchip. The aqueous PEG_{6000} solution in the phosphate buffer was fed from one inflow, and AMS and polyphenol solution in the phosphate buffer from another inflow in the first microchip. The inlet streams were fed in the first microchip at different flow rates to ensure equal volume distribution in a microchannel (PEG:AMS=1:3) in the range of 2.5-200 µL/min. Outflows from the second microchip were gathered in separate vials.

The volumes of the collected phases in macro- and microextractor systems were noted and total phenolic content was analyzed according to the method described previously. All experiments were carried out at 25 °C in triplicate and the average values are reported (at 95 % confidence interval results have no statistical difference).

Results and Discussion

As mentioned in the introduction, possibility to use ATPSs for polyphenol extraction was investigated in macro- and microextractor. In one of our previous work [7], process optimization was performed while efficiency of the process strongly depended on process conditions (Azevedo et al., 2007). Optimal process conditions in experiment with gallic acid aqueous solution as model system were pH = 6.5, w_{AMS} = 0.0925 and $w_{PEG6000}$ = 0.1037. Besides the determination of optimal process conditions, applicability of the system was evaluated in experiments with real samples, respectively experiments were performed with white wine (Graševina, Daruvar, Croatia, 2008) in batch macroextractor and continuous microextractor. Obtained results indicated that ATPSs are applicable for the extraction of polyphenols from liquid sources.

In order to extend previous research, experiments were performed in continuous macroextractor and microextractor with teardrop shape micromixers. Additionally real samples of red wine (Pelješac, Dingač 2008) were used as source of polyphenols. Both macro- and microextraction continuous systems were investigated in order to replace the generally applied batch extraction processes with more efficient, continuous one. In comparison with the batch extractor the equal extraction efficiency was obtained for shorter time in continuous systems (Table 1).

Additionally, significantly shorter residence time (τ = 15 s) was necessary for equal extraction efficiency in a microextractor in comparison to macroextractor experiments (τ = 600 s) (Figure 1).

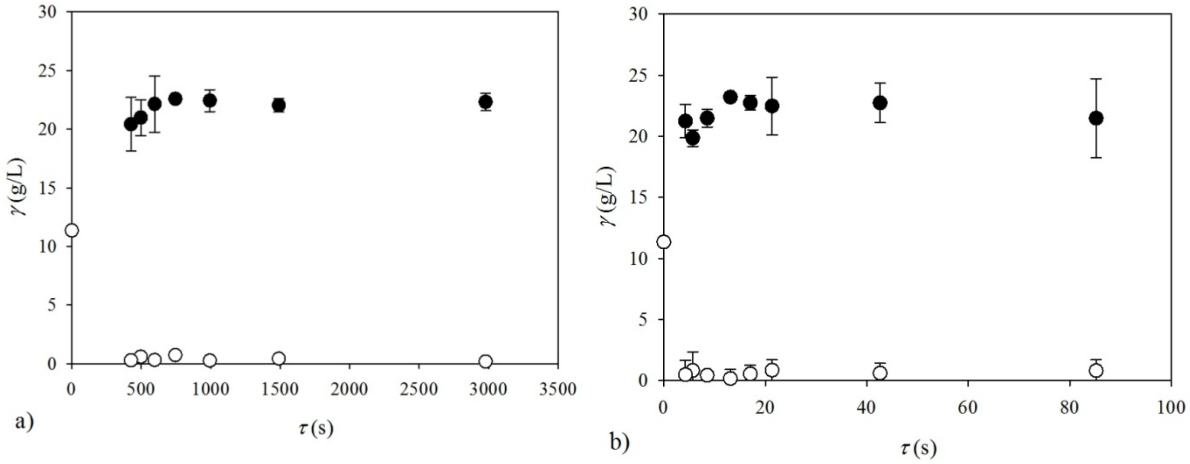
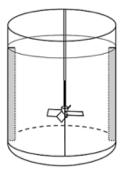
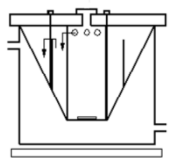
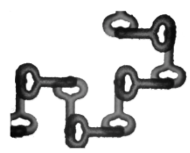
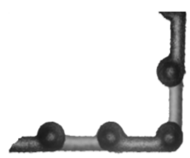


Figure 1. Distribution of total polyphenols in (a) macroextractor and (b) microextractor, ● - extract, ○ - raffinate

Experiments in a microextractor were performed at equal flow rates of both phases in the range q_V = 2.5 - 100 μL/min, which corresponds to the residence time of τ = 2.6 - 108 s. Resulting Reynolds numbers were below 50, indicating laminar flow conditions for all investigated flow rates. Microextractor that was used in our previous research [7], with swirl shaped micromixers, is usually used when working with higher Reynolds number (Re > 50). For lower Reynolds numbers, microextractor with teardrop shape micromixers is more suitable. In this type of microextractor mixing is reached by the splitting and recombining the flow.

Table 1. Extraction efficiency of polyphenols using different types of extractors

	Extractor type							
Solution	Batch extractor		Macroextractor		Microextractor with teardrop shape micromixers		Microextractor with swirl shape micromixers	
								
	t (s)	η (%)	τ (s)	η (%)	τ (s)	η (%)	τ (s)	η (%)
Model solution - gallic acid	3600	89.11*	2000	93.88	97	84.09	21	88.70*
Red wine	3600	96.43	595	95.78	-	-	17	93.62
White wine	3600	95.52*	744	83.00	-	-	42	87.00

*[7]

Comparing results obtained in both types of microextractors, the same extraction efficiency was achieved faster in a microextractor with swirl shape micromixers (Table 1). In this type of microextractor, mixing is achieved by inertial force that is used to fold the flow. Double swirl chamber geometry spirals down the fluid reducing the mixing length. Although the manufacturer (Micronit Microfluidics B.V., Enschede, the Netherlands) claims that expected results should be different, probably, due to viscosity and density of ATPSs solutions, better mixing (mass transfer) is achieved folding the flow by inertial force.

Conclusion

The obtained results indicate that aqueous two-phase system can be successfully used for extraction of polyphenols from red and white wine in macro- and microextractor. In all analysed extraction systems, for all investigated solutions, best extraction efficiency was obtained in microextractor, followed by continuous macroextractor and batch extractor. Going small with the size on extraction process systems could be the next step for fast and efficient polyphenol extraction.

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THE POSSIBILITIES OF WATER RECYCLING IN THE PRODUCTION OF BITUMEN BASED PRODUCTS

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Introduction

Water is essential to humans and other living organisms. Moreover, it is used in most process industries for a wide range of applications such as washing, a solvent, raw material and heating exchange media in industrial cooling systems. Industrial processes and systems using water are being subjected to increasingly stringent environmental regulations relating to discharge of effluents. There is a growing demand for fresh water, which makes it precious in many countries. Moreover, in some parts of the world fresh water presents a crucial commodity. There is a clear correlation between access to safe water and global economic growth [1]. Therefore the need for improved water management and wastewater minimization has increased. The adoption of water minimization techniques can effectively reduce overall fresh water demand in water using processes and subsequently reduce the amount of effluent generated. This can result in reducing cost engaged for the acquisition of fresh water and the cost of the treatment of effluent streams [2]. In this paper, the possibility of water recycling in the production of bitumen based products has been studied on the example of industrial facility located in northern-west part of Croatia. A project of water saving was proposed.

Bitumen is a dark brown to black cement obtained from residuals in the process of distillation of crude oil in petroleum refining. This engineering material is produced to meet a variety of specifications based upon physical properties. There are many bitumen based products [3]. For their production large quantities of water either as heating exchange media in cooling system or a raw material incorporated in final product has been used. In this work only bitumen emulsion and bitumen paint will be discussed. Bitumen emulsion is a mixture of two normally immiscible components (bitumen and water) and emulsifying agent (usually a surfactant). Bitumen emulsions are utilized in paving, roofing and waterproofing operations. Bitumen paint - cut back bitumen made to treat bare metal or concrete or wood surfaces giving a bond between the surface and an enamel or a bituminous membrane or bonding bitumen used as protective coating in waterproofing [3].

The facility studied in this work produces bitumen based products such as bitumen emulsion and bitumen paint used in road construction works. A possible solution in order to minimize use of raw water and costs of water discharge by collecting of water used in the process of cooling, storing it in special container and reused as raw material in production was suggested. Also, rain water collected from roofs of the facility was considered. Taking into account that rain water is going to the sewage system at the moment, by its collection and use in the process, the costs of raw water will be decreased as well as the costs of discharge of water used in the production process.

Materials and Methods

On the example of an industrial facility located in northern-west part of Croatia, a possibility of water recycling in production of bitumen based products such as bitumen emulsion and bitumen paint was studied. Water originated from public water supplying system has been used for cooling of colloid mills seals in the process of emulsion production. After the water has been passed through the seals it goes into the sewage system. For production of 10 m^3 of emulsion, consumption of water is more than 1 m^3 per hour. Besides that, before the start of production it is necessary to warm up pipelines so the seals should be cooled down. After finishing production process the seals should be cooled down until the inner temperature in the mill drops down below 100 °C. So, for cooling process beside production purposes (i.e. incorporation of water in the product), more than 1 m^3 of water from public water supplying system is consumed. [4]

Beside emulsion production, water has been used for the cooling in the process of bitumen solution in organic solvents and in production of bitumen paint. Consumption of water for these processes exceeds more than 2 m^3 per hour or more than 2 m^3 for 1 m^3 of produced bitumen paint.

According to proposed project of water recycling (Fig. 1) after production process the water from public water supplying system which has been used for cooling would be transferred in a special tank and used in emulsion production afterwards. Instead of discharging rainfall water in the sewage system it could also be used for cooling which will bring decrease of costs of the raw water as well as discharge. Collecting of rainfall water could additionally minimize necessity for the use of water from public water supplying system.

In this study, the data from 2010 were analyzed. It was found out that around 1500 m^3 of water was consumed in that year. For production (incorporation of water in new products) 600 m^3 of water was consumed which means that 900 m^3 of water was used for cooling purposes. By reusing of cooling water about 900 m^3 of water from public water system would be preserved.

Besides that, by collecting of rainfall water the necessity for the water from public water supplying system would be decreased. The average amount of rainfalls on the location of facility according to Meteorological and Hydrological Service (MHS) exceeds 850 mm/m^2. The area for water collection is 850 m^2 (50 m×17 m) which would on annual base exceed 720 m^3. So, by solely collection of rainfall the need for water necessary for production on the base of 2010 data would be covered. All rainfall water couldn't be used since a part of rainfall falls down in winter months when the capacity of production process is not maximal. Moreover, the storage of water is not easy due to possibility of freezing but anyway at least one third of water could be used in the production process.

Collected water could be used for cooling of emulsion and the water would be heated at the same time. Such heated water could be then used for production of emulsifying agent which will reduce energy consumption necessary for heating of water with cooling down of finished product - emulsion at the same time. Emulsion would have better quality and could be prepared in shorter period of time for delivery by decanting in barrels.

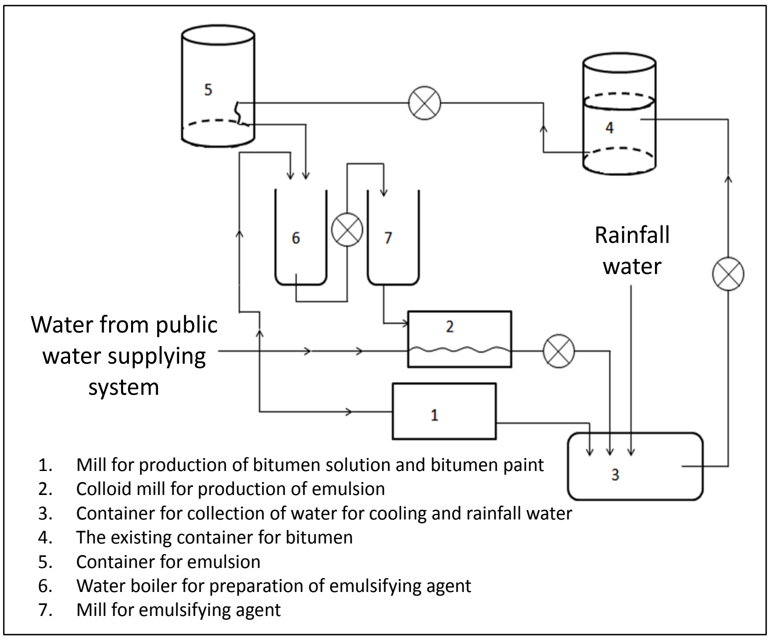

Figure 1. A schematic diagram of proposed project of water saving in the production of bitumen based products.

Results and Discussion

According to suggested project of water savings in the process of production of bitumen based product the water from public water supplying system would be used as a raw material for production of emulsion and for cooling down of colloid mill seals (Fig. 1, No. 2). In the process of production of bitumen solutions and paint (Fig. 1, No. 1), water is used just for cooling purposes. At the moment, water for cooling ends up in the container (Fig. 1, No. 3) and it is transferred to the sewage system by submersible pumps. This water has not been polluted so it could be reused in the production process. Therefore, that water will be collected in the container (Fig. 1, No. 4). In the same container the rainfall water could be collected. Collected water could be than used for cooling down of freshly produced emulsion (Fig. 1, No. 5) and for preparation of emulsifying agent (Fig. 1, No. 7). Water for preparation of emulsifying agent should be heated up to 50 °C in a water boiler (Fig. 1, No. 6).

A schematic diagram of water recirculation project is shown with the Fig. 1. There are three water flows that could be collected and reused: water for cooling down the seals of colloid mill, water for cooling down of mill placed in separated room and rainfall water.

Collection of water for cooling down of colloid mill seals (Fig. 1, No. 2) is easy. The existing discharge of water for cooling down of seals is not far away from the container (Fig. 1, No. 4) so it should be extended up to the inlet of the container with introduction of additional vents for discharge of water from that pipeline in winter months in order to minimize the possibility of breaking pipes caused by freezing.

Firstly, it is necessary to collect water for cooling down of mill in separate room in the buffer container with 1 m^3 of minimal capacity so the water could be transferred automatically from it by special submersible pump into the container (Fig.1, No. 4). For that purpose it is necessary to put cooling discharge pipes of mill out of separate room and connect them on buffer container. The same container would be used for collection of rainfall water and could have spillway into the sewage in order to prevent spillage from the container (Fig.1, No. 4). Before the pump a filter should be placed in order to protect the pump from the larger particles which could cause its malfunction.

Rainfall water should be collected in two buffer containers – from the both side of roof each of them. A buffer container which serves for collection of water from the mill placed in separate room could be used for collection of water at one side. The other buffer container would have the same characteristics but it would be placed on the opposite side of the roof. Pumps in these buffer containers would be switch on automatically by the raise of the level in buffer containers and automatically switched off when the level fall down on certain minimal value. Pumps in these buffer containers would stop working in the case that water level in the container (Fig. 1, No. 4) raise more than the allowed one, i.e. if container (Fig. 1, No. 4) is filled up. The excess of water would go than from buffer container into the sewage system over the spillway.

Water collected in the container (Fig. 1, No. 4) would be at the first place used for preparation of emulsifying agent necessary for making of bitumen emulsions. By using collected water for cooling down of emulsion the water would be heated and emulsion cooled down so the cost of energy for heating of water for preparation of emulsifying agent would be decreased with the increase of emulsion quality at the same time. In order to use collected water it is necessary to construct pipeline from the container (Fig. 1, No. 4) via container for emulsion (Fig. 1, No. 5) up to the boiler (Fig. 1, No. 6) located in the production hall.

In the container for emulsion (Fig. 1, No. 5) it is necessary to build in heat exchanger. Collected water from the container (Fig. 1, No. 4) would be pumped through exchangers in container for emulsion (Fig. 1, No. 5) into the water boiler (Fig. 1, No. 6) in production hall. Boiler in the hall should be exchanged so the intake of water from water supplying system and intake of reused water comes from the above while from the lower side the heated water would be pumped into the mill for the emulsifying agent. So, in that way a possibility of water reuse would be achieved in the case that enough water was not collected (current operation system) with disabling of breakthrough of recirculated water in the water-pipes.

Conclusions

Recycling of water is safe, sustainable and economical way of water management for everyone who takes care about natural resources consumption. Recycling of water could be applied everywhere where a drinking water quality is not required, i.e. cooling in industrial processes. Also, rainfall water can be used as well. In this paper, a possibility of water recycling in the process of cooling during production of bitumen based products was considered. Namely, used water in one cycle of cooling is not polluted so it can be reused without any treatment in the next cooling cycle. Moreover, rainfall water could be collected so the necessity for fresh water is decreased. Implementation of such approach will bring cost decrease for water taken from water supplying systems as well as water discharge.

In this paper, a project of water saving in the process of bitumen based products was proposed. This will bring conservation of 900 m^3 of water taken from water supplying system which with the price of water of 23.21 kn+VAT exceed 20.890 kunas per year. Besides obvious economical savings, suggested project contributes to conservation of natural resources as well. It is extremely important that industries begin to be aware of existence of many possible solutions in their production process, especially those industries where fulfillment of healthy standard for used water is not prerequisite, such as production of bitumen based products. Reuse of water is very important for conservation of natural water reserves which are endangered and its amount on planet Earth decreases every day.

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Session D: Biomass and sustainability

OPTIMISING THE ENVIRONMENTAL SUSTAINABILITY OF SHORT ROTATION COPPICE BIOMASS PRODUCTION FOR ENERGY

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Introduction

Solid biomass from Short Rotation Coppice (SRC) has the potential to significantly contribute to European renewable energy targets and the demand for wood as fuel for energy (heating and electricity production), driven mainly by market forces and supported by the targets of national and European energy policies [**Error! Reference source not found.**]. The term Short Rotation Coppice (SRC) refers to biomass productions systems cultivated for energy purposes using fast-growing tree species (willow, poplars, black locust, eucalyptus…) with the ability to resprout from the stumps after harvest which occurs in short intervals (2-6 years). The management practices for SRC such as soil preparation, weed control, planting, fertilization, harvest, resemble more those of agricultural annual crops than of forestry, despite that the currently used species in commercial SRC plantations in Europe are tree species. Apart from its potential role in EU renewable energy policies, SRC cultivation on larger scale could help meet social and economic targets of other EU policies (e.g. EU Rural Development, CAP reform). This combination of technological and political drivers has stimulated the interest and a rapid large-scale shift from "conventional" agricultural crops to SRC has been predicted [2]. This will have positive and negative implications on a range of environmental issues. An increase of SRC grown on agricultural land is anticipated in areas neighbouring power stations or local producers of heat, using biomass as a fuel. In such areas, SRC might need to be cultivated on a substantial fraction of all available agricultural land to fulfill biomass needs for fuel, being simultaneously economically and energy efficient. This, coupled with the above-mentioned special features of SRC will surely affect the landscape and have potential implications for the local water and soil quality, hydrology, carbon storage in soil, and biodiversity. The aim of this paper is to refer to and analyse above-mentioned aspects of environmental sustainability and to provide with a number of things-to-consider in SRC practice that will enable optimization of SRC cultivation towards an environmentally sustainable local SRC production. Based on the existing knowledge on the impact of SRC on soil quality, water and biodiversity referred in the literature, the management measures for optimized sustainable SRC production are recommend.

Materials and Methods

In order to understand the impact of SRC production on the environment, a detailed review of existing research was undergone with a focus on impacts of SRC on soil and water quality, and biodiversity. The research refers mostly to willow and poplar species as they have been predominantly used in Europe. The review of the existing research in the field will serve as a basis for development of recommendations for sustainable SRC cultivation that will be promoted within SRCplus project. It is important to note that in this paper only a small portion of the overall review is given.

Results and Discussion

The review is divided according to category of the SRC impact on the environment – impact on soil quality, impact on water quality and impact on biodiversity.

Impacts of SRC on soil

The potential effects of SRC on soil quality are usually divided into two large aspects, the first referring to the changes of soil carbon and the second to the changes of the heavy metal concentrations when SRC is cultivated. Concerning changes in carbon storage in the soil of willow and poplar, the results of various studies [3-9] have been conflicting and it has been concluded that the site-specific variability in the effects of SRC on the soil carbon pool is high, that previous studies [3-9] may not have covered a sufficiently long period to detect significant changes in soil carbon stocks, and that the fundamental mechanisms responsible for soil organic carbon accumulation in SRC are not well understood. However, when comparing carbon concentrations in the topsoil between SRC grown for a number of years with the respective concentrations in adjacent to SRC arable fields, increased carbon concentrations are found [10, 11]. Another soil quality parameter that has been broadly connected to the positive impacts of SRC cultivation is the reduced trace element concentrations in the soil, mainly for cadmium (Cd) [12, 13]. Due to the ability of especially willow to take up trace elements from the soil, SRC plantations have been used for phytoremediation of soils and waters containing these hazardous elements. The use of SRC as multi-purpose plantations for phytoremediation of contaminated soils (e.g. extraction of Cd, Zn and other heavy metals, and degradation of organic compounds) can be combined when biomass is produced in such sites and improve the soil quality of moderately contaminated arable land, but also of marginal land that can be returned to agricultural production after SRC cultivation for a number of years [14]. In such systems, the produced biomass with elevated heavy metal amounts is used as a fuel mixed with other biomass sources. The bottom ash is usually recycled back to the fields, but the fly ash, which contains most of the heavy metals and is of smaller volume, is deposited in landfills to avoid contamination [14].

Impact of SRC on water

The two issues that are mostly brought up when discussing the SRC impact on water are SRC impact on water balance and on groundwater quality. Increased biomass accumulation has been linked with high water use, especially in warmer climates. Coupled with the fast-growing feature of SRC, fears for high water use and consequent concerns about the effects on local hydrological balance and flow to neighboring streams/rivers have been expressed in several reports predicting future biomass supply from agriculture [15, 16]. The levels of water consumption of SRC in relation to other crops grown in the same area seem to depend on site-specific factors as soil type, precipitation and others, and might vary from case to case, although SRC seem to have higher evapotranspiration than arable crops in most cases. Since the use of pesticides is limited in SRC, almost all research related to water quality has been focused on nutrients leaching and not determination of chemical compounds in ground water. Close to zero or very low levels of nitrogen leaching from SRC were reported [17, 18]. Due to this ability of utilizing nitrogen in combination with low nitrogen leaching to the groundwater, SRC has been used to treat and utilize nitrogen-rich wastewaters such as municipal wastewater or landfill leachate, but also solid residues such as sewage sludge. All the above show the potential for using SRC in intensively managed agricultural areas to reduce nutrient leaching either by replacing existing crops or by using SRC as buffer zones between intensively managed arable land and water bodies to reduce surface run off and groundwater leaching.

Impacts of SRC on biodiversity

Protection and increase of biodiversity is a political commitment set by the European Union, and therefore it would be of key importance if biodiversity could be increased within the stand and/or in the surroundings when SRC replaces other crops in agricultural areas intensively managed. Several studies reported an increased number of species in SRC compared to neighbouring arable land [19-21]. It has been also mentioned that plantation size and shape seem to be important for biodiversity, with higher species numbers recorded at the edge of a plantation than within it. However, SRC establishment might negatively affect phytodiversity, especially habitats of threatened species such as undisturbed peat land, forest wetlands, or areas adjacent to lakes or rivers. Research studies for animal diversity in SRC have been conducted mostly for birds and ground beetles, and more research for invertebrates is needed [22]. Vertebrate diversity, equated with species richness, differs considerably in SRC in comparison to arable fields; whereas bird diversity in SRC is higher than in agricultural cropland higher diversities of ground beetles have been found in arable fields. For mammals, little research has been conducted, but species observed in SRC plantations in England included 17 mammals [23] suggesting that SRC provided a more attractive habitat for small mammals than arable land, with older coppices being more attractive. The varying results for zoodiversity are explained on the dependency of animal diversity on a number of factors such as the age of the plantation, the tree species/clone, the plantation size, the habitat structures and the location of the plantation (surroundings and other uses).

The sustainable SRC production, at least from the aspect of environment, can be achieved if a number of general recommendations to optimize SRC practice towards sustainable production of SRC can be developed. In an effort to list all these recommendations for SRC management/practice to increase positive impacts and decrease negative impacts on the environment, the most important recommendations considering biodiversity, soil and water issues are presented in Table 1. These are based on the results of review of existing research in the field combined with practical experience and issues that will enable high economic profit. An overview of the series of publications where these recommendations were based upon can be found in Weih and Dimitriou [24].

Table 1. Recommendations for environmentally sustainable SRC production

Impact category	Recommendation
Soil	• SRC could be cultivated in fields with low initial soil organic matter content to increase this content and with this the fertility and carbon storage of the soil. • SRC should be cultivated especially in areas with a high risk of erosion, e.g. with relief, to lower the loss of fertile topsoil and nutrients by water and wind. • Application of municipal residues such as sewage sludge for recycling of nutrients to SRC can be encouraged, since SRC can contribute to prevent nutrient losses and can extract heavy metals efficiently. • SRC should be used to remediate soils with increased Cd concentrations caused e.g. by the long-term use of Cd-containing P-fertilizers or other sources of environmental pollution. • SRC fields should be established at the same location for at least three cutting cycles to achieve soil quality improvements concerning carbon storage and Cd uptake. • SRC should be harvested in winter in countries when soil is frozen to avoid soil compaction.
Water	• SRC could be cultivated in fields located close to N sources (e.g. animal farms, N vulnerable zones, wastewater treatment plants etc) to decrease N outflow to adjacent water bodies. • SRC should be cultivated in areas where low groundwater level is anticipated (potentially flooded areas and areas near water bodies which can potentially flood). • Application of municipal residues such as sewage sludge for recycling of nutrients does not affect water quality, and should therefore be encouraged. • More frequent harvests lead to a higher average groundwater recharge, and therefore should be encouraged to ameliorate possible negative impact of groundwater recharge reductions.
Phytodiversity	• The establishment of SRCs in areas with high ecological status should be avoided (e.g. areas with protection status for nature conservation, areas with rare species, wetlands, peat bogs, swamps). • High structural heterogeneity provides habitats for different plant requirements and thus increases diversity. High structural diversity at one SRC location can be achieved by: i) Planting different tree species and clones ii) Harvesting at different times so that the trees have different rotation ages within one area • Edges of SRCs have great species diversity, and planting several smaller plantations instead of one big SRC is advised because smaller plantations have longer edges for their size than larger ones. If that is not possible, planting long rectangular plantations can provide more benefits considering increased phytodiversity. • An increase in forest ground species can be achieved by reducing the irradiance reaching the ground vegetation. This can be done by long rotation periods, high plant densities and planting willow instead of poplar. Another possibility is aligning planting rows in the east-west direction to reduce radiation reaching ground vegetation by shading the planted crop.
Zoodiversity	• Where possible SRCs should be designed with a large edge to interior ratio. • A mix of varieties and clones should be used. • Rotational harvesting in mixed age-class blocks should be preferred. • Huge blocks of SRC should be separated, e.g. by rides and hedges. • Where possible, and in case of growing willow, planting of willow hybrids (Salix sp.) with a range of flowering times should be preferred. • The use of pesticides should be limited if highest zoodiversity is to be achieved. • A percentage of the SRC area should be reserved for small habitats like strips of grass and stepped wood boundaries. • There should be no SRCs in high wildlife-value habitats like wetlands, wet meadows, set asides, dry fallows, semi-natural grassland.

The balance between maximum environmental benefits and maximum attained biomass production from SRC is a big challenge that all stakeholders involved in SRC cultivation (farmers, decision-makers, researchers, and others) should deal with. Despite all the above-mentioned expected positive environmental impact of SRC, farmers need to be convinced to cultivate the crop, and this is typically achieved when the economic profit from the cultivation of a new crop such as SRC is equal to or higher than that of other "established" or "conventional" crops. To encourage farmers to grow SRC instead of other crops in order to achieve environmental benefits, decision-makers should be prepared to contribute with direct or indirect incentives to the farmers. For instance, a potential economic compensation to the farmers could be a form of "reward" for those helping to fulfill national and European environmental goals already set and simultaneously keeping agricultural land into production. Such issues concerning the added value of SRC cultivation, when at the same time important environmental goals are achieved, should be one of the drivers for sustainable SRC cultivation, besides or in combination with drivers for producing biomass for energy to achieve renewable energy commitments. Social and economic aspects of the sustainability are not addressed within this paper, since they depend greatly on the local conditions and national policies. These will be address in depth in later research within SRCplus project.

Conclusions

The extensive review of the existing research on the impact of SRC production on the environment has shown that a number of benefits could be achieved with SRC. If the presented recommendations within this paper are followed, SRC production can show significant improvement of soil and water quality, enhance biodiversity and diversify the landscape. It is important to note that a balance between the potential maximum profit for the farmer and the maximum environmental advantages for the society need to be achieved at a specific area. Before establishing a SRC plantation, all related aspects and obtained research findings need to be considered, in combination with the site-specific features of the available sites towards a sustainable SRC cultivation.

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EFFECT OF PH AND TEMPERATURE ON ACIDOGENESIS OF SUCROSE USING FREE AND IMMOBILIZED MIXED ANAEROBIC BACTERIA ON MINERAL KISSIRIS

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Introduction

Volatile fatty acids (VFAs), which mainly contain acetic, propionic, iso-bytyric, butyric, iso-valeric and valeric acid, have many applications, including biogas and biodiesel production [1], and mainly produced through chemical synthesis using fossil fuels [2]. Recently their production through anaerobic fermentation of biomass and wastes has gained attention. This alternative has the advantage of a cost-effective and environmentally friendly process. Contino et al., [3] evaluated the use of ethyl esters of low molecular weight fatty acids in a homogenous charge compression ignition engine with promising results.

Anaerobic digestion, i.e. the biological conversion, in the absence of oxygen, of degradable organic compounds into methane and carbon dioxide, consists of four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [4]. Short-chain VFAs such as formic, acetic, propionic, butyric and valeric, are produced mainly in the second step of acidogenesis, however, the production of lactic, succinic and other acids has also been reported. Other fermentation products during acidogenesis are alcohols such as methanol and ethanol, carbonyl compounds, carbon dioxide and hydrogen [5, 6]. Factors such as pH, temperature, C/N ratio and hydraulic retention time play important role, controlling the production of VFAs during fermentation [7].

In addition in previous studies it was shown that methane, alcoholic and acidogenic fermentations can be promoted in the presence of porous materials such as γ-alumina pellets and kissiris [8-11]. The results showed that under selected process conditions (initial sugar concentration, pH, temperature, type of substrate) the anaerobic acidogenesis can lead to products with different compositions of VFAs and ethanol, giving the possibility to produce different types of ester-based new generation biofuels [10, 11]. Mineral kissiris (a porous volcanic rock found in Greece; 70% SiO_2) was also used as a promoter for alcohol production [12]. Based on the above studies, the aim of the present study was to examine the effect of kissiris as immobilization carrier and promoter on the anaerobic acidogenesis of sucrose. In addition the effect of initial pH and fermentation temperature on VFAs composition and concentration was also evaluated.

Materials and Methods

Culture and growth media

Mixed bacterial anaerobic culture was obtained from a UASB bioreactor and inoculated in a medium containing 50 g/L glucose, aqueous NH_3 and 50% H_3PO_4 (to achieve a COD:N:P ratio of 100:5:1), $NaHCO_3$ 4 g/L, yeast extract 4 g/L. Cell growth was carried out at 37 °C. The medium was sterilized by autoclaving at 120 °C for 10 min.

Immobilization on kissiris

The experimental apparatus used for cell immobilization consisted of 0.25 L conical flasks which were filled with 100 g kissiris. Equal volumes of 20 g/L sucrose medium and anaerobic culture were added (75 mL of each). The conical flasks were placed in an incubator set at 37°C (and at 20°C, 30°C, 52°C for the study of temperature effect) and there were left to ferment for two days without feeding in order to achieve cell immobilization. Subsequently, the fermented medium discarded and fresh sucrose medium was added and left to ferment for a few more days in order to succeed a steady state.

Batch fermentation of sucrose

For the experiments on different pH, fermentations were carried out at 37 °C with initial pH of 4-8 and 40 g/L sucrose medium was used. For the experiments on different temperature conditions, fermentations for immobilized cells were carried out at 20 °C, 30 °C, 37 °C and 52 °C with initial pH 8 and 20 g/L sucrose medium was used. All the experiments were also conducted on free cells at pH 7.

Determination of VFAs

VFAs and other organic acids (OAs) were determined by high performance liquid chromatography (HPLC), using a Jasco chromatograph LC-2000 Plus (Jasco Inc., Japan) with a Bio-rad Aminex HPX-87H column (300×7.8 mm i.d., 9 μm particle size), a PU-2089 plus quaternary gradient pump), a CO-2060 Plus oven at 50 °C, a MD-2018 plus photodiode array detector operated at 210 nm) and an AS 2050 PLUS autosampler. H_2SO_4 0.008 N was used as mobile phase with a flow rate of 0.6 mL/min. The samples were filtered through disposable syringe cellulose acetate filters (CHROMAFIL) with 0.20 μm pore size and all the data were processed with ChromNav program. All determinations were done by means of standard curves.

Determination of residual sugar

Residual sugar was determined by HPLC, using a Shimadzu chromatograph with a NUCLEOGEL ION 300 OA column, a LC-9A pump, a CTO-10A oven at 30 °C and a RID-6A refractive index detector. H_2SO_4 0.008 N was used as mobile phase with a flow rate of 0.8 mL/min and propanol-1 was used as an internal standard. A volume of 0.25 mL of sample and 0.625 mL of a 1% (v/v) solution of propanol-1 were diluted to 25 mL. The samples were filtered with a disposable syringe cellulose acetate filter (CHROMAFIL) 0.20 μm pore size and then 60 μL of the final solution were injected directly to the column. Residual sugar concentrations were calculated using standard curves.

Determination of ethanol

Ethanol was determined on a Shimadzu GC-8A system, with a Teknokroma HAYE SEP Q 80/100 column, a C-R6A Chromatopack integrator, He as carrier gas (40 mL/min), and a FID detector. Injection port and detector temperature were 210°C. The column temperature was 130 °C. Samples of 2 μL were injected directly into the column. Determinations were done by means of standard curves.

Results and Discussion

Effect of pH

Table 1 shows the results of sucrose acidogenesis at each pH for free cells and immobilized on kissiris. The fermentations were carried out at batch mode, with 40 g/L sucrose and with initial pH value varying from 4 to 8. During fermentations the pH was not controlled. In all pH values the use of kissiris led to increased OAs concentrations. The OAs concentrations at pH 4, 5, 6, 7 and 8, using kissiris, were 5.9, 7.1, 9.8, 14.8 and 16.2 g/L respectively, indicating that the greatest OAs production was occurred at higher pH, which is in accordance to other studies [13, 14]. The maximum OAs concentration was 23 g/L and obtained using kissiris at pH 8. The OAs yield factor at pH 8 for kissiris was 0.6 g/g of sucrose consumed, which was 2-fold higher than pH 4 with kissiris and 2-fold higher than pH 8 with free cells. In both free and immobilized cells on kissiris the main acid produced was lactic acid (more than 50% of total OAs) followed by succinic acid and acetic acid. A small amount of ethanol was also produced varying from 1.6 (pH 8) to 6.5 mL/L (pH 6) for free cells and from 1.7 (pH 8) to 3.4 mL/L (pH 6) for cells immobilized on kissiris. These results suggest that also ethanol type fermentation took place.

Table 1. Effect of initial pH on acidogenesis of sucrose (40 g/L).

pH	Ethanol (mL/L)	Acetic acid (g/L)	Butyric acid (g/L)	Valeric acid (g/L)	Succinic acid (g/L)	Lactic acid (g/L)	Total OAs (g/L)	Residual sugar (g/L)
				Free cells				
4	2.4±1.3	-	-	-	1.5±0.9	2.0±1.5	3.5±1.3	14.8±1.5
5	4.3±1.7	-	-	-	1.6±0.9	2.2±1.3	3.8±1.1	15.1±1.6
6	6.5±1.8	0.3±0.1	-	0.8±0.4	1.5±0.7	4.2±1.7	6.8±1.9	13.0±1.8
7	1.9±1.2	0.2±0.1	0.3±0.3	-	1.5±1.0	5.9±1.0	7.9±0.9	13.9±1.5
8	1.6±1.0	0.3±0.1	0.5±0.5	1.4±1.0	1.1±0.3	5.8±0.8	9.0±0.9	12.8±2.1
				Kissiris				
4	3.2±1.7	0.2±0.1	-	-	0.9±0.1	4.8±0.5	5.9±0.5	18.7±1.6
5	2.9±1.0	0.3±0.1	-	-	1.0±0.1	5.8±0.7	7.1±0.6	17.3±2.0
6	3.4±1.5	0.7±0.4	-	-	1.2±0.4	7.9±1.0	9.8±0.6	19.5±1.5
7	1.8±1.5	0.9±0.3	0.6±0.5	-	1.0±0.1	12.4±4.9	14.8±5.0	16.1±1.4
8	1.7±1.0	1.0±0.4	0.7±0.3	1.3±0.8	0.9±0.1	12.6±3.6	16.2±6.6	14.3±1.6

Effect of temperature

Table 2 shows the results of sucrose acidogenesis at each temperature for free cells and immobilized on kissiris. The fermentations were conducted at pH 7 for free cells and pH 8 for cells immobilized on kissiris. In all cases the promotional effect of kissiris was observed leading almost two-fold higher OAs concentrations. In the case of kissiris an increase in OAs concentrations was observed with the increase of temperature from 18^{o}C to 37^{o}C. The OAs concentration at 52^{o}C, 10.1 g/L was lower than that at 37^{o}C, 14.9 g/L, indicating a lower acidogenesis at higher temperatures, which is in accordance to other studies [13, 15]. The OAs yield factor at 37^{o}C with kissiris was 0.7 g/g of sucrose consumed, which was 1.4-fold higher than 52 ^{o}C, 2.3-fold higher than 18 ^{o}C and 1.4-fold higher than 37 ^{o}C with free cells. The main acid was lactic acid (more than 50% of total OAs) indicating that lactate-type fermentation occurred as was also observed in other studies [14].

Table 2. Effect of fermentation temperature on acidogenesis of sucrose (20 g/L).

Temp (^{o}C)	Ethanol (mL/L)	Acetic acid (g/L)	Propionic acid (g/L)	Butyric acid (g/L)	Isovaleric acid (g/L)	Valeric acid (g/L)	Succinic acid (g/L)	Lactic acid (g/L)	Total OAs (g/L)
				Free cells					
18	8.1±2.4	1.0±0.1	-	-	-	-	0.8±0.1	1.6±0.3	3.4±0.2
30	0.3±0.2	0.5±0.1	-	0.4±0.1	-	-	0.6±0.4	1.4±0.8	2.8±0.5
37	3.8±2.0	0.4±0.2	-	0.1±0.1	-	-	1.1±0.4	7.9±2.9	9.6±3.1
52	-	1.6±1.0	0.2±0.1	-	1.2±1.0	-	0.6±0.5	3.0±2.2	6.7±1.0
				Kissiris					
18	-	0.3±0.2	-	1.7±0.4	-	1.9±1.2	1.1±0.2	1.1±0.6	5.5±1.6
30	2.4±1.5	0.4±0.3	-	6.7±1.0			0.4±0.4	1.8±0.8	9.1±1.0
37	3.7±1.9	0.6±0.1	-	1.7±0.9	-	-	0.9±0.1	11.6±1.7	14.9±0.8
52	-	0.5±0.3	-	0.1±0.1	-	-	0.6±0.3	8.9±2.9	10.1±2.7

Conclusions

A high OAs production could be obtained during acidogensis at 37 ^{o}C and high initial pH (7-8). OAs production was significantly enhanced by using mineral kissiris as immobilization support, with a concentration of 23.0 g/L and yield of 0.9 g/g of sucrose consumed. Lactic, succinic and acetic acids were the dominant components of OAs. These results will be useful for the optimization of acidogenic processes to control the composition of the produced VFAs, which is crucial for valorization in some applications [16].

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CHEMICAL COMPOSITION OF WHEAT STRAW AS A POTENTIAL RAW MATERIAL IN PAPERMAKING INDUSTRY

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Introduction

Almost all pulp for paper manufacturing is worldwide based on the use of woods from different types and species of trees. Many countries in the world are nowadays struggling with a lack of resources such as forest, but this problem could become even greater for future generations. This lack of raw material is the result of irrational exploitation of the natural resources for different purposes (lumber, heating material, pulpwood). As the demand for the paper products is growing and wood become insufficient raw material for paper production, alternative sources of virgin cellulose fibres are of great importance for the papermaking industry. Non-woods fibres gained from the by-products of agricultural production of annual crops have imposed themselves as an important alternative fibre source in the papermaking industry. Straw is inexpensive and annually renewable source of fibre available in large quantities in many regions of countries across Europe. A good pulp need to provide good paper-machine runnability and appropriate quality in the final product. Some of these properties are result of the raw material quality, other depends on the conversion of raw material to pulp (chipping, cooking, bleaching, blending, etc.), and many are a combination of these two factors influencing the pulp quality [1- 4]. The research presented in this paper focuses on the possibility of obtaining non-wood cellulose fibres from the wheat straw as one of the most abundant species in Croatia. For that purpose chemical composition analysis of wheat straw and isolated fibres were made.

Materials and Methods

Straw of winter wheat grown in the continental Croatia was cut manually into 1 to 3 cm long pieces. Cellulose fibres were isolated from wheat straw by two types of alkaline treatments meeting the conditions presented in Table 1.

Table 1: Process conditions for fibres isolation methods.

		Method 1.	Method 2.
	Used straw	360 g	
Soaking pre-treatment	Chemical NaOH, %		16
	Bath ratio		1:10
	At 25°C		24 h
Decantation		-	+
Cooking treatment	Chemical NaOH, %	16	-
	Tap water	-	10 l
	Bath ratio	1:10	1:5
	At 120°C, 170 kPa	60 min.	60 min.
Decantation and rinsing in tap water		2 × 10 l	
Defibration in Holländer Valley mill	Tap water	23 l	
	At 24°C	40 min.	
	pH	8,5 – 9,0	

Analysis of straw and isolated wheat fibres elements were made by ICP-MS method. According to wet ashing method for organic matter destruction all samples were converted into solution [5]. In that way macroelements (potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P)), microelements (boron (B), iron (Fe), copper (Cu), manganese (Mn), molybdenum (Mo), zinc (Zn)) and metals/metalloids (aluminium (Al), barium (Ba), bismuth (Bi), cadmium (Cd), cobalt (Co), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), silicium (Si), vanadium (V)) were detected.

Organic and inorganic compounds of straw and isolated wheat fibres were determined by standard isolation methods for major plant chemical components (Fig. 1.). Main organic (cellulose, α-cellulose, lignin, accessory material, moisture) and inorganic compounds were determinated according to TAPPI standards.

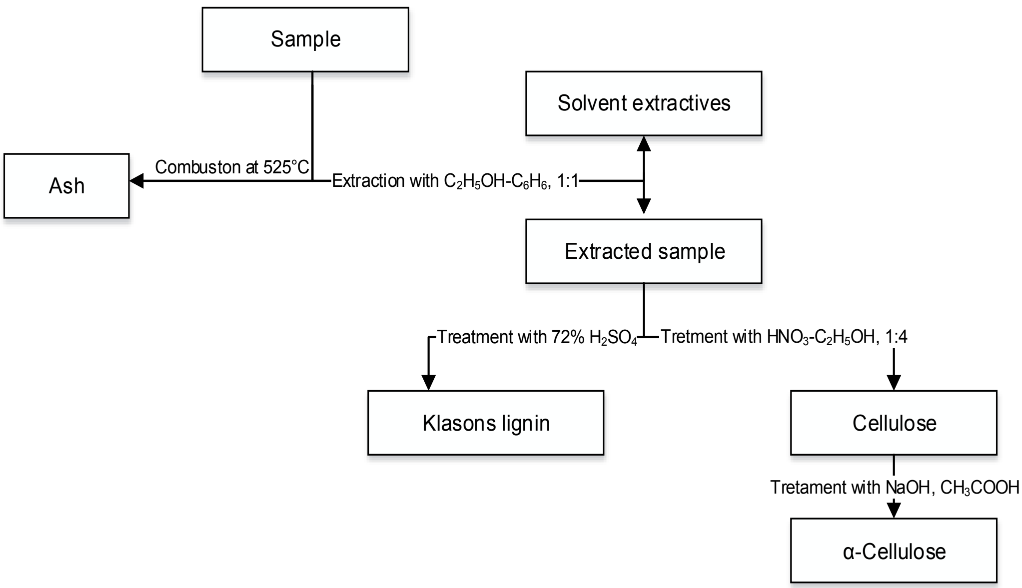

Figure 1. Schematic view of major plant components isolation methods

Results and Discussion

It is well known that the content of organic and inorganic components depends not only on plant species but also on farming condition as climate, land and human influence on growing phase of plant [4, 6]. Results of ICP-MS analysis of wheat straw are presented in Table 2.

Table 2. Mean values of nutrient (macroelements, microelements, metals/metalloids) in wheat straw and isolated fibres

Nutrients			Straw	Isolated fibres	
				Method 1.	**Method 2.**
mg/kg	**Macroelements**	K	10340.50	209.00	189.50
		Ca	3300.00	8328.00	5654.50
		Mg	1051.00	2245.50	1937.50
		P	595.00	147.50	141.00
	Microelements	Zn	53.69	112.04	25.17
		Fe	47.04	145.34	194.31
		Mn	30.74	27.89	39.57
		Cu	3.78	5.02	5.46
		B	3.55	13.72	9.39
		Mo	0.99	0.07	0.00
	Metals/Metalloids	Al	193.24	8.56	51.38
		Bi	117.62	489.80	322.15
		Si	71.08	19.61	22.54
		Ba	53.03	27.90	25.95
		Cr	3.68	32.31	2.98
		Pb	1.76	7.50	3.50
		Ni	1.57	1.01	1.02
		Cd	0.15	0.19	0.15
		Co	0.03	0.07	0.04
		V	0.00	0.00	0.45
		Hg	0.00	0.00	0.00

Wheat straw contain potassium in notable higher concentration than others macroelements. High concentration of potassium is characteristic of all grain straw, like wheat [1-3] and barley [1]. Macroelemenets in wheat straws are in a sequence K>Ca>Mg>P. High content of some nutrients in straw can have negative influence on facilities equipment during raw material conversion to pulp (silicium) and some (copper, iron, cobalt, manganese, lead and zinc) on optical and mechanical properties on paper substrate as a final product. These metals can be introduced into the paper from the fibres source (straw) which may contain trace elements from the ground in which it was grown, or by the equipment, water and chemicals used in paper manufacture. From gained ICP-MS analysis results of wheat straw these potentially problematic elements (except silicium) are detected in a trace (concentration lower than 50 ppm). It is interesting that their concentration in isolated virgin fibres is strongly influenced by the provided isolation method (used chemicals and its chemical composition and process parameters).

Chemical characteristics of the straw as a raw material and fibres which depend on used method for isolation are important for pulp quality. Content of cellulose, especially α-cellulose and lignin in the selected fibre plant is the most important indicator of its usage in paper production. As results presented in Table 3. have shown wheat straw has higher content of cellulose and smaller content of lignin than most wood species [7]. As expected, ash content is relatively high because wheat is non-wood species.

Table 3. Chemical composition of wheat straw.

w, %					
cellulose	**α-cellulose**	**lignin**	**ash 525°C**	**solvent extractives**	**moisture**
48.28 ± 1.01	31.47	24.66 ± 1.63	9.27 ± 0.33	3.00 ±0.57	6.94 ± 0.97

Provided methods for fibres isolation from wheat straw (Table 1.) resulted with virgin fibres of different chemical composition (Fig.2.). Method 1. is more efficient for lignin removal from straw lignocellulose structure, while Method 2. is better for achieving fibres with higher content of cellulose (especially α-cellulose).

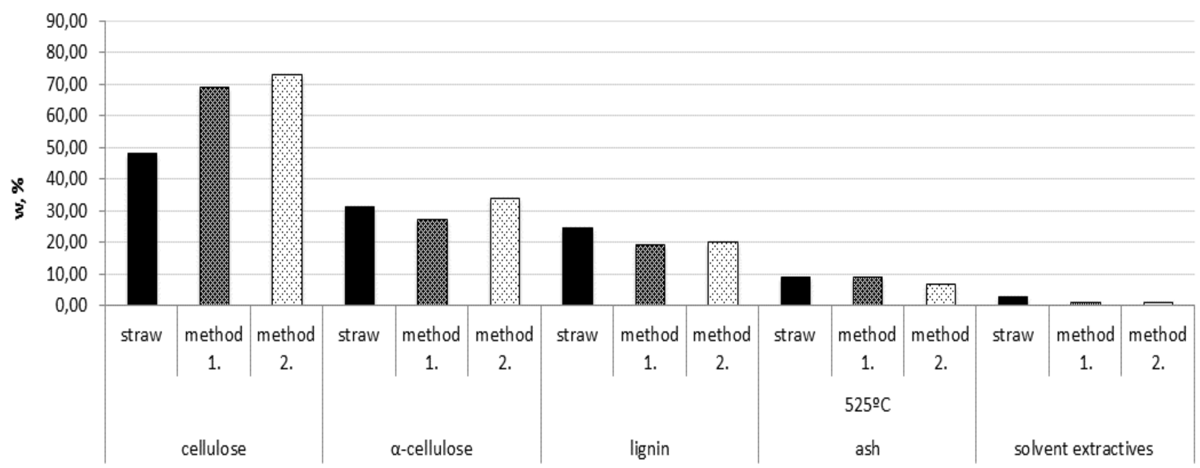

Figure 2. Organic and inorganic compounds in isolated fibres

Lignin removal is more efficient if the delignification process in sodium hydroxide solution of high concentration is supported by high temperature. From gained results it is obvious that Method 1. is not optimized well, because by removing lignin the loss of cellulose (especially the α-cellulose) is noticed. Method 2. has not so good delignification results. Straw was pre-treated by soaking in sodium hydroxide solution for 24 hours at room temperature and then cooked in tap water. Sodium hydroxide influenced on removing hemicellulose from lignocellulose structure which resulted by isolated fibres with higher content of cellulose and α-cellulose. It is evident that major influence on virgin fibres composition has well optimized isolation process conditions.

Conclusion

As the fibre properties of raw materials significantly affect the quality and the use of the paper substrate, it is very important to find quality alternative sources of virgin fibres. The research presented in this paper proved that the wheat straw is a valuable raw material for the papermaking industry. Content of cellulose determinated in wheat straw is relatively higher and the lignin content is smaller than in most wood species. Ash content is high but it is a characteristic of all non-wood species. Important is that in the wheat straw the content of most problematic elements for paper production is in traces (lower than 50 ppm). With this research it was confirmed that the use of proper method for fibres isolation is of great importance in order to avoid loss of cellulose content during separation of non-cellulose components from lignocellulose structure of straw. To obtain good quality virgin fibres the most important is to optimize process conditions during the chemical treatment of straw.

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USE OF FOREST RESIDUES IN A FINAL CUT OF REPRODUCTIVE FELLING

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Introduction

In the technological process of forest utilization part of the wood mass that remains in the forest is a potential raw material for energy production [1]. It has been estimated that the amount of forest residues varies from 50 to 100 odt/ha (oven dry tonnes) depending on species, age, site type and wood assortments harvested [2]. This wood mass is composed of branch wood, wedges, butresses, forks, stump wood etc. The use of this wood mass depends on both the tree species and stand or site conditions. The residues left in the forest impede forest regeneration and increase the risk of forest fire [3]. The share of residue (branch wood) in the forests of the Republic of Serbia, calculated from the ratio of gross and net volumes of wood, ranges from 15 to 18%. It is impossible to use the whole mass of hardwoods for several reasons (biological, technological, economic reasons). Part of the timber, such as thin branches with leaf mass, should be left in the forest to enable the return of a part of nutrients to soil through the process of humification. The rest of the timber can be used, although there are technological limitations to its use. These forestry residues are bulky, difficult and expensive to collect and transport, and currently have little commercial value [4]. The sustainability of woody biomass utilization depends on technical, economic, ecological and socio-institutional factors [5].

The spatial position of the primary (forest truck roads) and secondary (tractor roads and skid trails) forest road infrastructures is crucial for an economically viable use of forest biomass. The constraints for recovering forest biomass can be considered the same as those for conventional logging operations [6]. In addittion to other numerous functions, the main role of forest roads is the transport of wood products, and therefore also of forest residue or chip wood. The use of forest residue is not possible without the use of an appropriate technology of use that provides small costs per unit of output, i.e. the costs that will affect the offer of that wood supply as an energy source to the market [7]. The research related to the selection of an optimal technology of work aimed at the use of forest residues in mountainous regions of Serbia began in 2011, and the starting point was recognizing of the specifics of forestry in this area. The initial step was to evaluate the efficacy of the existing methods of wood assortment production. Bearing in mind the analyses performed and the results obtained, an original method of wood assortment production was developed that is based on the use of total volume of large trees with a diameter of over 3 cm with bark. Wood mass thinner than 3 cm is left in the forest for the return of nutrients to soil.

The aim of this study is to make an assessment of the effectiveness of that new method in regeneration cuts (final cuts) of high even-aged beech stands.

Materials and Methods

The research was carried out in the PE "Srbijašume" Belgrade, FE "Stolovi" from Kraljevo in the territory of FA "Ušće" and the FMU "Željin". In this management unit high (uneven-aged) beech forests cover 47.36% of the area and high (even-aged) forests of beech 15.34%.

Figure 1. Spatial position of Management Unit "Željin"

The entire vegetated area includes high stands (86.17%), coppice stands (1.68%) and artificially established stands (12.15%). The final cut in this management unit was planned on an area of 136.9 ha, with a planned gross felling volume of 19,576.2 m^3. The sample plot and the control plot were positioned in a pure high even-aged stand of beech (*Fagenion moesiacae montanum*) on acid brown soil in compartment 57/a, where the final cut was planned [8].

On the sample and control plots 118 and 83 trees were harvested and processed, respectively, and they were sorted by diameter degrees. The production of wood assortments in the sample plot was performed using the Danilović *et al.* method [7], whereas on the control plot the same operations were performed using the standard assortment method. Felled trees were previously bucked into logs of optimal length for skidding, in accordance with the national quality standards for roundwood and abiding by the principle of maximum financial effect.

The measurement of all branches exceeding 3 cm in diameter without bark was performed on tree parts with reduced crown. In addition, the notching of branches was performed to reduce load width and the degree of damage to the regeneration. After felling a tree, the chainsaw operator performed pruning of the branches thinner than 3 cm in diameter with bark. After pruning the branches with a diameter of up to 3 cm with bark, the chainsaw operator notched the branches (Figure 2) in the zone of branch foot , i.e. at the branching-off spots on the trunk and places where thin branches branch-off from the thicker ones [7]. On the control plot, technical stack wood was produced at the stump in accordance with the SRPS standard. The stack wood was produced as one-meter wood and subsequently stacked (Figure 3).

Figure 2. Notching of branches

Figure 3. Stack wood produced on the control plot

The felling of trees was performed with a Husqvarna 390 chainsaw in the organizational form of work 2MR. During felling, special attention was paid to the felling direction, since it significantly affects the occurrence of damage in the regeneration [9]. The general felling direction was determined by the requirements of Phase I of transport (skidding), and the choice of individual felling direction was guided by the safety of workers and the least possible damage to the regeneration, which was at an advanced stage of development as the final cut was delayed for one whole management period. The transport of trunk parts and technical roundwood was performed using an adapted farm tractor IMT 577 DV with a double drum winch "Bratstvo Travnik" 6 tons (Figure 4), whereas the stack wood was transported using an IMT-577 tractor on a single-axle trailer (Figure 5).

Figure 4. Skidding of trunk parts with an IMT – 577 DV tractor

Figure 5. Transport of stack wood on a single-axle trailer

Technical roundwood and reduced crowns were skidded to a roadside landing, where technical roundwood was sorted into, large tree parts of over 7 cm in diameter and branches of 3-7 cm in diameter. The entire work was focused on the preservation of the regeneration, and the aim was to minimize the damage to the regeneration as the final cut was delayed, thereby maximizing the use of wood mass. The degree of damage to the regeneration is expressed as the number of damaged plants per operation and transport cycle.

The sample size was determined on the basis of variation statistics. The trees were marked with numbers, and the positioning of each tree, as well as its felling direction towards the skid trail and the slope of the terrain, were positioned on a map.

The positioning of each stump was performed using theodolite Zeiss THEO010B. The recording of roads, skid trails, water flows and landings was performed using a GPS device Trimble – Juno SB handheld. The forming of the load was performed with several standing points, for an easier formation of an optimal load and minor damage to the regeneration. An attempt was made not to have more than one piece with branches in a single transport cycle. The number of damages is related to the number of pieces in a load, i.e. its volume. Any resulting damage to the regeneration was marked with a spray.

Standard statistical and mathematical methods were used for data processing.

Results and discussion

A total of 118 trees were felled and processed on the sample plot. The average diameter of felled trees was 41.8 cm. The gross wood volume of felled trees on the sample plot was 169.45 m^3. The volumes of certain categories of wood of over 3 cm in diameter measured from the bark are shown by diameter degrees (Table 1).

Table 1. Volumes of different wood categories on the sample plot

Number of trees	Diameter degrees	Volume of technical roundwood	Volume of firewood of up to 7 cm in diameter	Volume of branches from 3 to 7 cm in diameter	Total volume
	cm	m³	m³	m³	m³
1	12,5	-	0,10	0,00	0,10
2	17,5	-	0,43	0,00	0,43
14	22,5	-	4,85	0,32	5,17
15	27,5	1,27	6,90	0,43	8,60
19	32,5	7,82	9,32	0,68	17,81
23	37,5	20,40	9,96	1,13	31,49
21	42,5	27,40	7,34	1,14	35,87
13	47,5	23,85	6,38	0,60	30,83
6	52,5	13,36	3,14	0,56	17,06
3	57,5	7,98	2,73	0,17	10,88
1	62,5	3,11	1,06	0,05	4,22
118		**105,18**	**52,22**	**5,07**	**162,47**

In the total amount of processed wood the share of technical roundwood amounts to 64.74%, the percentage of firewood and wood residue (buttresses, forks, wedges, etc.) of over 7 cm in diameter with bark amounts to 32.14% and the portion of branches from 3 to 7 cm in diameter to 3.12%. The final processing of wood assortments was performed at a roadside landing, where the assortments were also measured. The measurements showed that the share of wood residue of over 7 cm in diameter with bark (buttresses, forks, wedges, etc.) was 2.03%. Figure 6 shows the portions of processed wood and the summary of the shares of firewood and wood residue of up to 7 cm in diameter as another portion.

The results of these studies indicate that the share of wood residue and branches of from 3 to 7 cm in diameter amounts to 8.361 m^3 or 5.2% of the total volume of processed wood. With the rise in diameter at breast height the volume of certain wood categories also increases (Figure 7). The functions that represent the natural correlation between the variables are shown in Table 2.

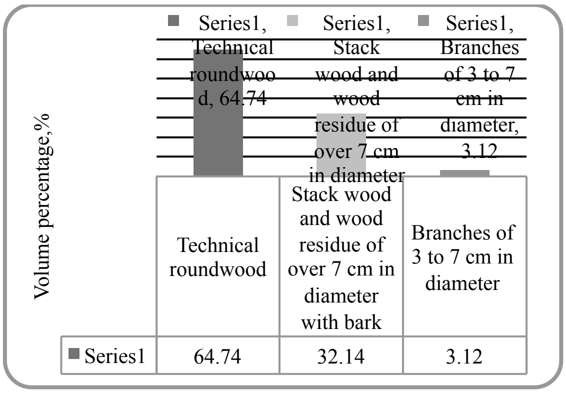

Figure 6. The shares of different wood categories in the total wood volume on the sample plot

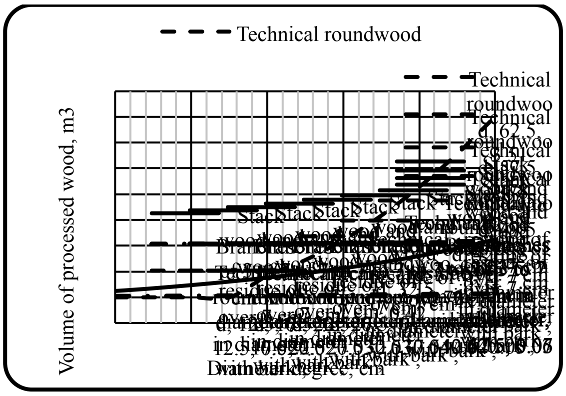

Figure 7. Correlation between diameter at breast height and the volumes of different categories of wood

Table 2. Functions of dependence of the volume of processed wood of certain categories on diameter at breast height

Type of wood assortment	Function	Correlation coefficient	Standard regression error
Technical roundwood	$V_{TO} = (-0.684 + 0.0409 \cdot D_{1.3})^2$	0.982	0.103
Stack wood and wood residue of over 7 cm in diameter with bark	$V_{PR+DO} = \frac{1}{\left(-0.986 + \frac{117.3}{D_{1.3}}\right)}$	0.923	1.01
Branches of 3 to 7 cm in diameter	$V_{G=(0.112+0.0025 \cdot D_{1.3})^2}$	0.760	0.031

A total of 83 trees were felled and processed on the sample plot. The average diameter of felled trees was 43.0 cm, and the gross volume of wood mass was 120.61 m^3. The shares of wood volumes of certain categories of wood in the total wood volume are shown in Table 3 by diameter degrees.

Number of trees	Diameter degree	Volume of technical roundwood	Volume of firewood of over 7 cm in diameter	Total volume
	cm	m³	m³	m³
8	17,5	-	1,17	1,17
7	22,5	-	1,93	1,93
9	27,5	2,06	3,02	5,08
11	32,5	5,61	4,14	9,75
14	37,5	13,00	4,90	17,90
16	42,5	21,65	5,31	26,96
10	47,5	16,51	4,49	21,00
5	52,5	10,85	2,83	13,68
1	57,5	2,47	0,41	2,88
1	62,5	3,52	0,69	4,21
1	67,5	4,33	0,48	4,81
83	**-**	**79,99**	**29,38**	**109,37**

Table 3. Volume of different wood categories on the control plot

The share of technical roundwood amounted to 73.1% and of firewood of up to 7 cm in diameter with bark to 26.9%. The stack wood was produced at the stump, and that amount of wood did not include the wood mass of butresses, forks, small branches, etc.

The analytical form of the function and the dependence of the volume of processed technical roundwood and stack wood on DBH are shown in Table 4.

Table 4. The functions of dependence of the volumes roundwood and stack wood on diameter at breast height on the control plot.

Type of wood assortment	Function	Correlation coefficient	Standard regression error
Technical roundwood	$V_{TO} = (-0.724 + 0.042 \cdot D_{1.3})^2$	0.990	0.103
Stack wood	$V_{PR=(0.373+0.0059 \cdot D_{1.3})^2}$	0.839	0.067

Figure 8 shows correlation between the diameter at breast height and total wood volume of the wood processed using two different methods.

Table 5. Functions of dependence of the total volumes of wood processed using two different methods on diameter at breast height.

Plots	Function	Correlation coefficient	Standard regression error
Sample plot	$V_{OP} = 0.00025 \cdot {D_{1,3}}^{2.36}$	0.999	0.046
Control plot	$V_{KP} = 0,000113 \cdot {D_{1.3}}^{2,54}$	0.996	0,098

The graphic form of the functions is shown in Figure 8.

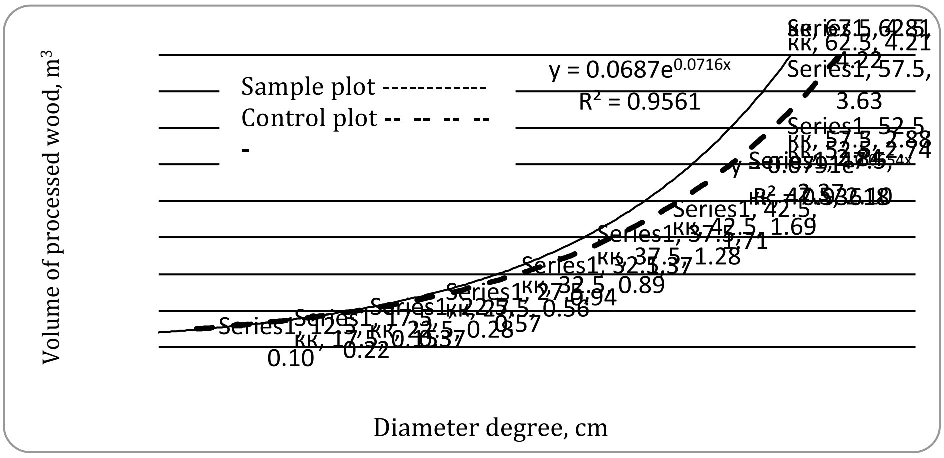

Figure 8. Correlation between tree diameter and the total volume of processed wood on the sample and control plots

The ratio of gross volume to net volume of processed wood is shown in Table 6.

Table 6. The ratio of gross to netwood volume of processed wood

Plot	Gross wood volume	Net wood volume	Net/Gross
	m^3	m^3	%
Sample	169,45	162,45	95,9
Control	120,61	109,37	90,7

The share of net volume of processed wood is by 5.2% larger on the sample plot. One of the reasons for that is that branches from 3 to 7 cm in diameter and large wood residue were not used when the assortment method was applied on the control plot. The wood assortments processed on the sample plot were transported to a skid trail by an adapted farm tractor with a double drum winch and then by the skid trail to a roadside landing.

The transport of wood on the sample plot was performed in 67 transport cycles, i.e. 190 winching operations. The average volume of a tour was 2.42 m^3 and the average volume of a winching operation 0.86 m^3. The average number of winching operations per tree was 2.14.

A total of 920 damages occurred on the regeneration, i.e. 4.8 damages per winching operation. These damages were manifested in the form of bark peeling, contusions, breakage or complete plant destruction. The average number of 7.8 damages to the regeneration was observed during the transport of processed parts of a single tree. In a case study from northern Iran, with winching and skidding operations 4.8% of regeneration was damaged [10]. The histogram of frequencies of skidding angles shows that the most common angle is between 50° and 60° (Figure 10).

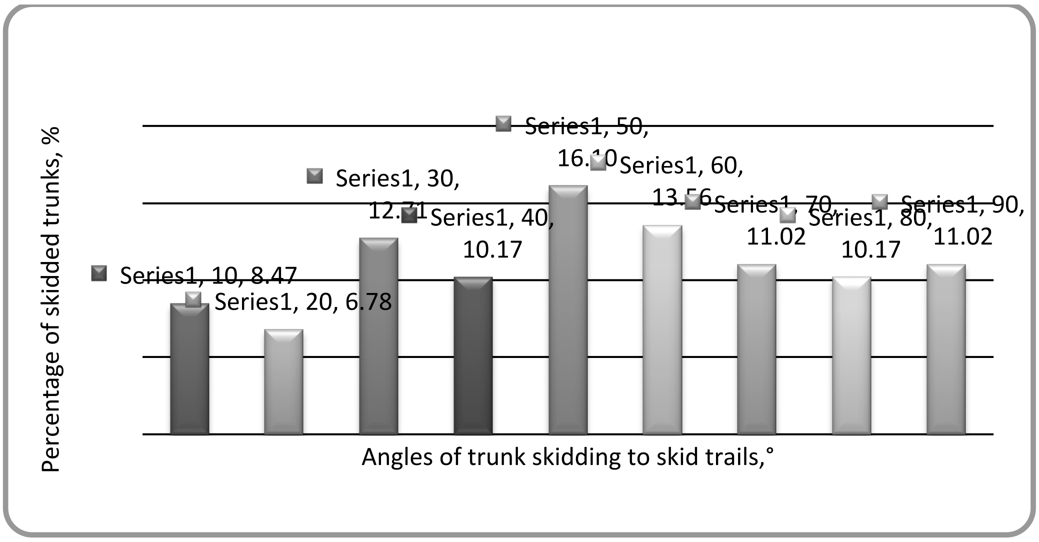

Figure 10. Histogaram of angles of trunk skidding to skid trails

Figure 9 shows the directions of felling and skidding to skid trails for 118 trunks.

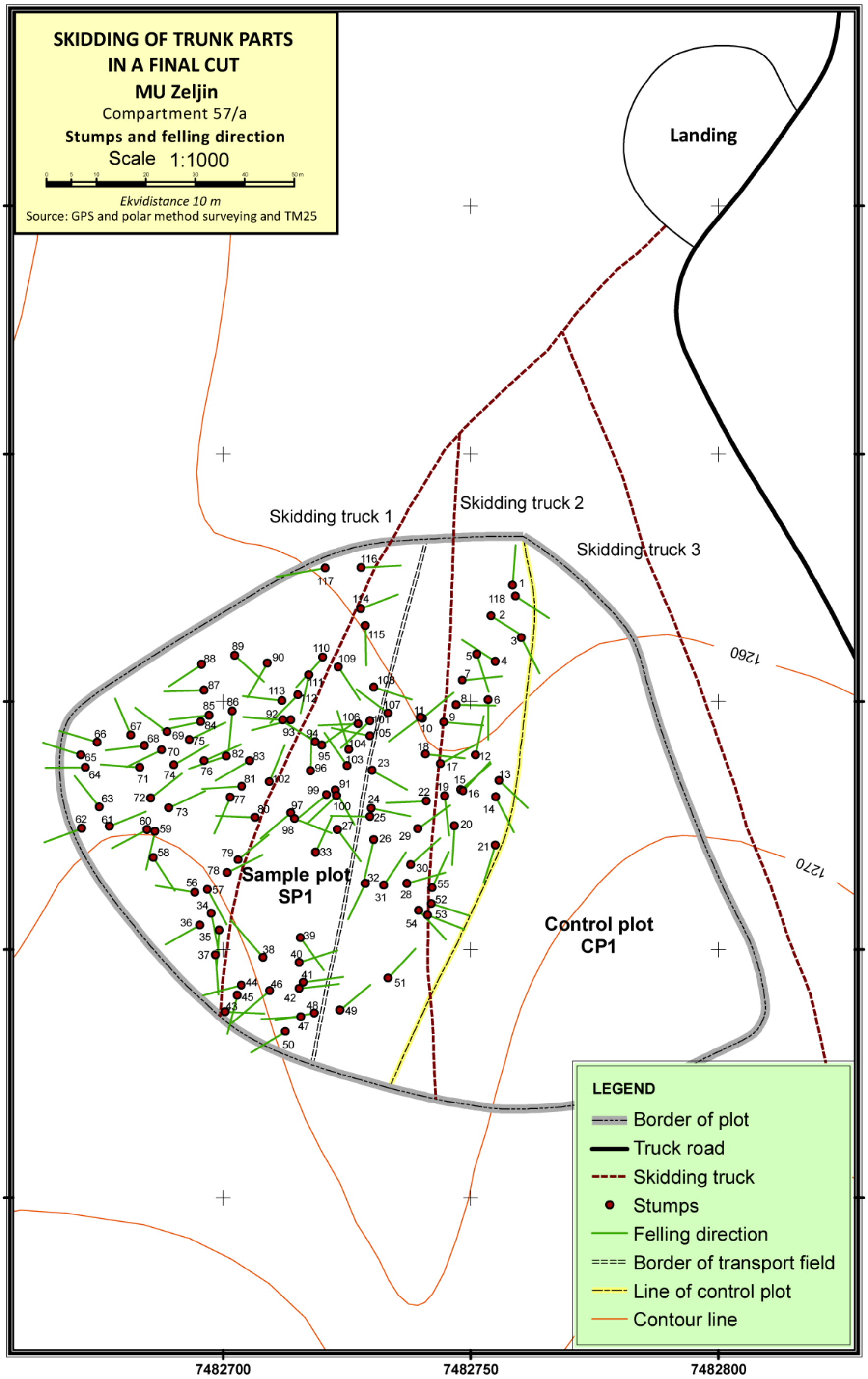

Figure 9. Felling directions on SP 1

The analysis of damage to the regeneration in phase I of transport was carried out on the control plot. The skidding of technical roundwood was performed using the same work tool as on the sample plot, whereas the stacked wood was transported with an adapted farm tractor IMT-558 on a single-axle trailer.

The roundwood was skidded to a roadside landing in 26 tours (cycles), and that required 76 winching operations. The average volume of a tour was 3.08 m^3. The average volume of a winching operation was 0.98 m^3. The maximum length of a transported assortment was 5.5 m and the minimum length amounted to 2.1 m. A total of 392 damages occurred on the regeneration. An average of 4.8 damages occurred per winching operation and the average number of damages per felled tree was 4.7. The stack wood was transported to a roadside landing in 14 tours. The average volume of a tour was 2.10 m^3 or 3.04 cbm. The reasons for a slightly smaller average volume of a tour are the short distance and the forming of tours with several standing points, designed to protect the regeneration. A total of 145 plants were damaged during transport, i.e. 2.01 damages per winching operation. A total of 1.7 damages occured per felled tree. The total number of damages on the control plot was 537, and the average number of damages per felled tree was 6.5.

The number of damages that occur is relatively small, if we take into account the fact that the operation performed was the final cut and considering the developmental stage of the regeneration. In addition, it is important to analyze the number of damages during felling and processing, given that the processing of assortments is performed at the stump, which significantly increases the number of damages in the regeneration.

Conclusions

The following conclusions can be drawn on the basis of the performed investigations:

- When the use of forest residues in the mountainous conditions of Serbia is concerned, the assortment method of processing is not acceptable from the technological and economic aspects.
- Larger quantity of wood from the felling site is used when the applied method is the Modified Half-tree Length Method of Broadleaves with Crown Parts.
- The efficacy of the studied method in a final cut depends on the openness of the area with a network of roads, i.e. less damage occurrs to the regeneration when the network of skid trails and tractor roads is dense.
- The size of regeneration affects the number of damages during a final cut.
- The share of wood residue and branches of 3 to 7 cm in diameter is 5.2% of the total amount of processed wood on the sample plot.
- The rise in tree diameter is correlated with an increase in the volumes of certain wood categories, in the first place technical roundwood and to the least extent in the case of branch wood of 3 to 7 cm in diameter.
- The number of damages that occur in phase I of transport is relatively small, considering that the examined felling is a final cut.
- The effects of the applied methods are significantly affected by the size of the roadside landing as the place of final assortment production.

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PRODUCTION OF WOOD BIOMASS IN CONIFEROUS FORESTS OF THE REPUBLIC OF SERBIA

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Introduction

Biomass is the greatest energy source after coal, oil and natural gas, and at the same time the most important renewable energy source [1]. In 2010, the share of wood biomass in the total global primary energy consumption accounted for about 9% and 65% of the primary energy consumption from renewable sources [2]. Despite the widespread use of biomass for energy, the current consumption is still far below the existing potential in terms of use of this resource [3]. Forest biomass, which includes parts of trees unsuitable for sale (small diameter trees, top ends, felling wedges, small branch wood and other parts made in the process of felling and processing of assortments) represents a potential raw material for energy production [4]. Interest in greater use of wood residues from forests appeared because of the declining reserves and rising prices of fossil fuels, increase in the emissions of greenhouse gases and the greenhouse effect, as well as threats of catastrophic fires [5]. The use of wood residue in the technological processes of felling and processing of assortments depends both on the tree species and stand or site conditions. The use of all tree parts is becoming more attractive in areas experiencing an expansion of energy production from forests. In this case, the biomass of all tree parts is equally important [6].

The part of the timber, such as thin branches with leaf mass, should be left in the forest for the return of part of nutrients to soil through the process of humification. From a biological point of view, other tree parts may be subject to use, although, in that case, there are technological limitations to its use. When conifers are concerned, wood mass obtained from branches and needles is recommended for use to prevent the spread of bark beetles, although in this case there are also some technological limitations.

In Serbia, as in most other countries, there are problems in the use of forest residues from forests in mountainous areas, and particularly forest residues from commercial forests. The material in them is usually small branch wood distributed across the entire surface and often on steep slopes. The use of this residue is not possible without the use of an appropriate technology, i.e. a technology that incurs small costs per unit of output.

In Austria, four possible models of production of raw materials for the market were analyzed. In that analysis it was found that the most economically acceptable model is the method of wood chipping directly at the terminal, and not at a roadside landing [7]. According to this study, forest residue was even defined as unsuitable for use in energy production due to its poor uneven quality.

Since the existing methods of processing of wood assortments do not enable an efficient use of biomass from spruce plantations in mountainous conditions of Serbia, we carried out a research of applicability of the modified assortment method. The specificity of the proposed method is that the overall wood mass is transported to a roadside landing, where pruning and chipping are performed. In this way, the amount of forest biomass (raw materials) from forests that can actually be used and offered on the market is significantly increased.

The aim of this study is to determine the amount of forest residue after a cut in a high even-aged stand of spruce, using the modified assortment method on short wood. In addition, the aim was to assess the efficacy of the applied method in terms of damage to the remaining trees and young stands on the one hand, and its economic applicability on the other.

Materials and Methods

The research was carried out in the PE "Srbijašume" Belgrade, FE "Stolovi" in Kraljevo and FMU "Radočelo-Crepuljnik".

The total area of the management unit is 1629.01 ha, of which 1426.71 ha or 87.5% are forest covered and 202.30 ha or 12.5% are barren.

The share of hardwoods in that volume is 27.4% and the portion of conifers 72.6%. Conifers in this management unit reach their natural optimum.

The most common species in this management unit is spruce, with a share of 48.6% in the total volume, followed by beech with a 27.4% share, fir 14.0%, white pine 9.8%, Douglas fir 0.2% and larch in small quantities [8]. The location where the research was carried out is a pure spruce stand in the FMU "Radočelo Crepuljnik" compartment 7/a.b., where the control sample plot was established.
The sample plot was established in a spruce stand in compartment 7/b. It is a high spruce stand that belongs to the cenoecological group (Piceion excelsae) on distric humus-siliceous brown soils.

A total of 84 trees were selected on the sample plot. The number of selected trees on the control plot was 72, and the trees were distributed per diameter classes.

The felling and processing of wood assortments were performed on the sample plot using the modified assortment method (marking and cutting of assortments was performed by the stump and pruning of branches at a roadside landing). This method of assortment production was applied in order to use the whole timber mass of the above-ground portion of a tree.

The felled trees were divided by bucking, abiding by the principle of maximum financial effect. After the production of wood assortments, forest residue (biomass) was measured. It included branches of up to 2 cm in diameter and top ends of below 5 cm in diameter without bark (Figure 1).

Figure 1. Measurement of branch wood

Figure 2. Skidding to a roadside landing

Figure 3. Pruning at a roadside landing

The branches of up to 2 cm in diameter with bark were measured, in most cases only the central ones, whereas in the case of lateral branches only the thicker ones (over 2 cm thick) were measured. The reason for such measurement was to determine the share of thick branches and top ends, and the portion of small branch wood and needles.

The felling of trees was performed with a Husqvarna 281 chainsaw in the organizational form of work 2MR. During felling, special attention was paid to the felling direction, as it significantly affects the occurrence of damages and safety at work.

The skidding of wood assortments was performed with an adapted farm tractor IMT-570 with a double drum winch "Bratstvo Travnik" - 6 tons (Figure 2).

In the skidding phase the task was to determine the number of damages on the remaining trees and the regeneration per winching operation and transport cycle.

During skidding, friction is considerably smaller in places of contact between standing trees and assortments without branches, due to the elasticity of spruce branches. The skidding of processed tree parts with reduced crowns was performed at a roadside landing, where also the pruning and stacking of branches were performed (Figure 3).

Pruning at the roadside landing was performed with a STHIL 341 chainsaw. Branches and wood residues were stacked in a pile for easy manipulation during chipping.

Figure 4. Mobile chipper

After pruning of the branches, the processed assortments were sorted at a roadside landing for an easier truck loading.

The chipping of the forest residue was performed with a Stump Heizohack-HM-KL 14-800 chipper mounted on a Mercedes-Benz 3344 truck (Figure 4).

The size of the roadside landing satisfied all the requirements necessary for undisturbed chipping of the forest residue. The chips were disposed into a special loading area and transported to a power plant as green biomass.

The technical roundwood and celulose wood on the control plot were produced using the assortment method. The felling processing and skidding of wood assortments were performed using the same work tools and organizational form as on the sample plot.

Results and discussion

A total of 84 trees were felled and processed on the sample plot. The average diameter of felled trees was 43.0 cm. The gross wood volume of felled timber on the sample plot was 122.11 m^3.

Table 1 shows the volumes of certain wood categories per diameter degrees. According to the SRPS standards large wood of conifers includes parts of trees with diameters of over 5 cm without bark. In this paper, parts of trees with from 2 to 5 cm in diameter with bark (branches and top ends) were also measured.

Table 1. Volumes of different wood categories on SP 2

Number of trees	Diameter degree, cm	Volume of technical roundwood, m³	Volume of cellulose wood of over 5 cm in diameter, m³	Volume of wood residue from 2 to 5 cm in diameter, m³	Total volume, m³
3	22.5	0.452	0.141	0.099	0.692
10	27.5	2.772	2.046	1.044	5.862
12	32.5	6.554	1.692	1.292	9.538
17	37.5	12.149	2.793	3.114	18.056
26	42.5	26.138	2.704	5.619	34.461
15	47.5	17.232	2.671	4.887	24.790
1	52.5	1.608	0.053	0.192	1.853
84	**-**	**66.905**	**12.100**	**16.247**	**95.252**

Figure 5 shows the shares of certain wood categories in the total volume. The share of technical roundwood (logs and mine timber) is 70.24%, the share of celulose wood with a diameter of over 5 cm without bark is 12.70% and forest residue (branches and top ends) with diameters from 2 to 5 cm with bark amounts to 17.06%.

Standish et al. [9] investigated the percentage shares of certain parts of coniferous trees by calculating the average value of 18 tree species with a 20 cm diameter at breast height. According to that survey, the share of needles in the total dry matter of a tree is 8.5%, the share of live branches with diameters of up to 0.5 cm in the volume is 3.1%, live branches with diameters from 0.5 - 2.5 cm constitute a share of 7.8% and the share of live branches with over 2.5 cm in diameter is 3.6%.

Hakkila [10] stated that after felling spruce trees aged 80 years, the share of needles in the total volume was 10.3%, of live branches 17.1% and of dead branches 1.1%, whereas the share of tree volume amounted to 71.5%. A total of 72 trees were felled on the sample plot. The average diameter of felled trees was 47.0 cm. Total gross volume of felled trees on the sample plot was 124.79 m^3. The volumes of certain wood categories by diameter degrees are shown in Table 2.

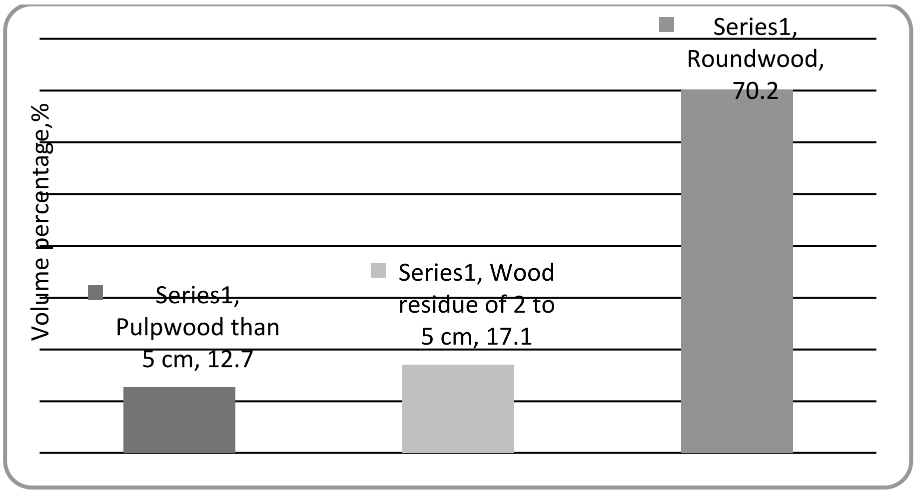

Figure 5. Shares of volumes of certain wood categories in the total volume on the sample plot

Table 2. Volumes of different wood categories on the control plot

Number of trees	Diameter degree, cm	Volume of technical roundwood, m³	Volume of cellulose wood of over 5 cm in diameter, m³	Total volume, m³
	[cm]	[m³]	[m³]	[m³]
1	17.5	-	0.123	0.123
1	22.5	-	0.352	0.352
5	27.5	0.845	0.934	1.779
8	32.5	2.131	2.178	4.309
13	37.5	6.225	3.985	10.210
19	42.5	15.119	5.488	20.607
16	47.5	17.235	2.445	19.680
6	52.5	8.661	1.807	10.468
3	57.5	6.263		6.263
72	**-**	**56.479**	**17.312**	**73.791**

Figure 6 shows the shares of different wood categories in the total volume on the control plot. The share of technical roundwood (logs and mine timber) is 76.54% and of celulose wood 23.46%.

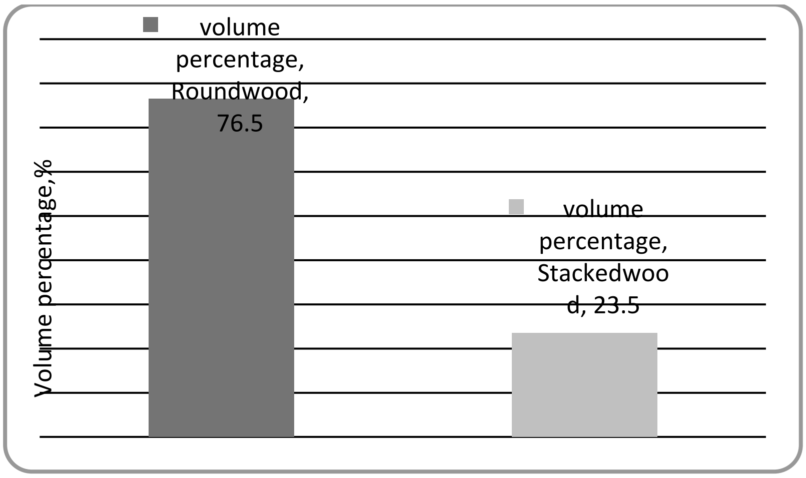

Figure 6. Shares of certain wood categories in the total volume on the control plot

The share of wood residue (branches and top ends with diameters of 2 to 5 cm) on the sample plot is 16.25m^3 i.e. 17.06% of the total volume of wood. Phase I of transport of assortments on the sample plot was performed with an adapted farm tractor IMT-570 with a double drum winch. The transport cycle was formed with multiple standing points. The assortments were transported with branches that served as good protection from damage to surrounding trees, as their elasticity alleviated direct contact with trees and regeneration, at the same time preparing the soil for natural regeneration. A total of 96 transport cycles were performed on the sample plot. The number of operations at the stage of skidding of wood assortments to the place where tours were formed was 255. The average volume of a tour was 0.99 m^3 and the average volume of a winching operation 0.37 m^3. The average number of winching operations per tree was 3.04. In order to form the smallest possible load width, the winches did not skid more than one crown at a time. The maximum length of assortments that were skidded to the landing was 8.0 m per winching operation.

The total number of damaged plants on the sample plot was 107, or an average of 0.42 per winching operation. The average number of damaged plants per skidded tree was 1.27. The average area of bark peeling per one processed tree was 17.52 cm ^{2}, or 5.77 cm ^{2} per winching operation. A total of 33 transport cycles were realized on the control plot, i.e. 86 winching operations. The average volume of a transport cycle was 2.22 m^3, and the average volume of a winching operation 0.86 m^3. The average number of winching operations per processed tree was 1.19. The maximum length of assortments that were skidded to the landing was 8.0 m per winching operation. The total number of damaged plants was 30, i.e. 0.35 per winching operation, or 0.42 per processed tree. The average area of bark peeling per one winching operation was 17.4 cm^2 and per single tree it amounted to 20.8 cm^2.

The chipping of forest residues at the roadside landing provided 90 nm^3 of chipwood of G 30 dimensions. The total weight of obtained chipwood was 33.5 t. If the total volume of wood chips is divided by the conversion coefficient of 2.43 that has been adopted, the obtained volume amounts to 37.04 m^3. The moisture content of wood chips was about 50%. If we assume that 1 m^3 of spruce wood with this moisture content weighs about 900 kg, it can be concluded that a volume of 37.22 m^3 can be obtained from 33.5 t of wood chips.

In this way is calculated the total volume of spruce wood residue on the sample plot.

The total wood volume on the sample plot is 116.13 m^3, which means that the share of forest biomass is 31.97% (Figure 7).

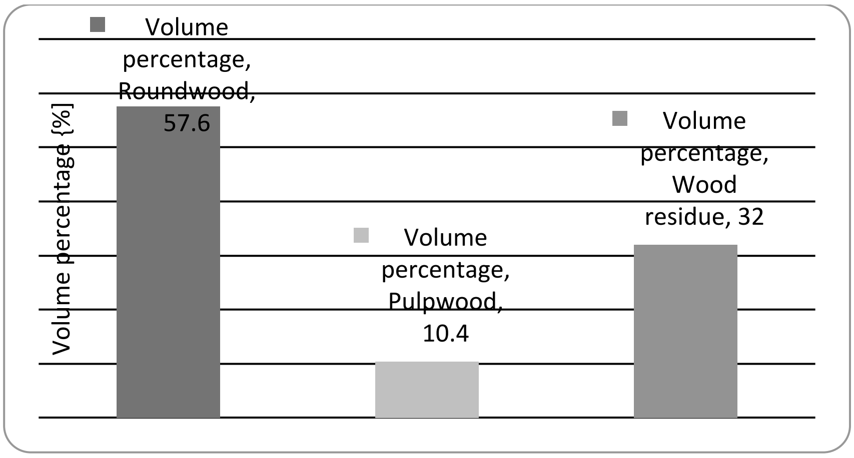

Figure 7. The share of volumes of certain wood categories with the total wood residue on the SP

The share of forest biomass is directly dependent on the degree of branching. The higher the degree of branching, the greater the share of residue that can be used for biomass. The ratio of volumes of gross and net wood masses on the sample and control plots is shown in Table 3.

Table 3. Ratio of gross and net wood volumes on SP2

Plot	Gross mass	Net mass	Net/ Gross
	[m^3]	[m^3]	[%]
Sample plot	122,11	116,13	95,11
Control plot	124,79	73,79	59,13

The resulting quotient of gross and net wood masses between the sample plot and the control plot is 35.98%. The share of wood residue on the sample plot was 31.97%, and similar data were obtained on the control plot, since a portion of wood mass was wasted during the processing of wood assortments.

Conclusions

- The assortment method is practically inapplicable in mountainous conditions from the technological and economic aspects, when the use of forest residues from spruce stands is concerned.
- In terms of the amount of residue that is skidded to a roadside landing together with the assortments, as well as the damage to the regeneration and the remaining trees, the modified assortment method is a logical technological solution.
- In applying this method, care should be taken that during load forming assortments with branches are combined with those without branches, which affects the size of the load.

- The efficiency of the method applied, among other things, depends on the size of the landing, as final assortment production and chipping are performed at a roadside landing.
- The chipping of forest residue at a roadside landing reduces transportation costs. In addition to that, the wood mass is homogenized from the aspect of quality of wood chips for use in power plants.
- The strength and capacity of the chipper should be adjusted to the structure and amount of timber that is subject to chipping.
- The share of forest residue is much greater in spruce plantations than in beech stands.
- The share of forest residue in spruce plantations is directly correlated with the degree of branching, i.e. the higher the degree of branching the greater that the share of residue that can be used for biomass.
- From the economic aspect, the modified assortment method is better than the standard assortment method.

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ENERGY BALANCE OF WOOD CHIPS PRODUCTION AND DELIVERY

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Introduction

Energy return on investment (EROI) in production of a specific energy product shows the ratio between obtained and consumed energy in the production process. Energy can not be produced without the energy consumed, and the proportions in which this occurs is a key indicator of the efficiency of the process in which the production takes place [1].

Proponents of EROI believes that analysis of net energy offers the possibility of real observation of the advantages and disadvantages of production of a certain type of fuel, and provides guidance for the possibilities for energy production and market in the future. Also we note that EROI itself is not necessarily sufficient criteria for judgment, although there favor of the majority, especially when one energy source has a much larger or smaller EROI compared to some others. In addition, it is important to take into account the current and future potential need for specific energy source and a possible change EROI in case of increased demand of an energy source.

EROI can be easily calculated using the following equation (1):

$$EROI = \frac{\text{Energy gained}}{\text{Energy required to get that energy}} \qquad (1)$$

Relationship between obtained and invested energy for the energy wood is 30: 1, which means that one liter of oil needed to obtain the amount of energy from energy wood (forest biomass) equivalent to the energy of 30 liters of oil [2]. But if we add CO_2 emission, where it is considered that emissions of CO_2during the combustion of biomass are zero because during growth of the biomass the same amount of CO_2, was used in the process of photosynthesis, the energy wood is in a more favorable relationship towards fossil fuels [3].

The aim of this study is to determine the energy balance of production and delivery of wood chips with a detailed elaboration of the parameters of energy consumption.

Materials and Methods

The paper shows the calculation of ratio between obtained and invested energy in the production of wood chips at the Forest Administration Vinkovci, „Croatian Forests“ Ltd. Zagreb. All presented data (data on productivity, number of machines, consumptions of fuel, lubricants and tyres as well as consumptions of chains, guidebars and sprockets of chainsaws and quantity of pesticides) were obtained (calculated) by the Department for Production of Forest Administration Vinkovci.

When calculating EROI it is important to include as many input parameters, or in this case the energy required to build all the machines and tools used in forest harvesting operations, construction and maintenance of forest roads, the energy of fuels and lubricants used by machines and vehicles, the energy required to build supplies, such as tires, chains, guidebars and sprockets of chainsaws, the energy required for the production of pesticides used in cases concerning the protection of forests, human energy, etc.

Output parameter in the calculation is the energy value of wood chips taken from the Manual of fuels from biomass [4] (hereinafter Manual). Energy value of the energy wood with a moisture content in the amount of 35% according to this Manual is 11.17 GJ/t, and calculated by the density of oak wood chips at the same moisture (852 kg/m^3) is 9.51 GJ/m^3.

Input parameters for the calculation EROI are distributed to the direct and indirect energy consumptions.

Indirect energy consumptions include:

- *Energy required for production of machines and vehicles*

In this energy is included the energy required for the production of materials for machinery, energy invested in manufacturing parts and transport of new machinery from place of production to the customer, and the energy required for the recycling of waste machines (after amortization period).

In calculation, the energy invested in machinery and vehicles is assumed to be 66 MJ/kg [5, 6].

The energy invested for production of motor cars is calculated according to [7] who used model from [8] and concluded that enrergy reqiured for production of motor cars is 33,4 MJ/kg.

Masses of machines/vehicles were taken from technical data of manufacturers (forwarders, dump trucks, graders, chainsaws, chippers, agricultural tractors) and drivers/owners of vehicles (trucks for transport of wood chips, motor cars), or were determined by direct measuring of axle loads (forwarders).

The productivity of each machine/vehicle is presented on an annual basis. Since all the input energy is reduced to unit MJ/m^3 (unit energy consumption) is necessary and energy invested in the production of machine/vehicle to express it that way. The total energy input for the production of material, construction and delivery of the machinery/vehicles divided into the depreciation of the machine/vehicle, and the result at the end, is also divided with an annual productivity of the machine/vehicle. For this reason it is necessary to know the depreciation life of any machine/vehicle, which is 7 years for forwarders and chippers, 10 years for agricultural tractors, graders, trucks, trucks with trailers and semi-trailers, 8 years for tractors with semi-trailers, 7 years for chainsaws and 5 years for motor cars.

Machines like grader and dump trucks for transportation of stone, and the farming tractors and motor cars indirectly linked to the productivity of Forest Administration Vinkovci for the year 2012 (414,714 m^3) is the annual productivity of each of these categories of vehicles identified with the total productivity of Forest Administration Vinkovci.

- *Energy required for production of pesticides*

Energy invested for production of pesticides was calculated according to [9] who estimated average energy consumption of 120 MJ for production of 1 kg of pesticide.

Total energy contained in pesticides was calculated on the basis of 4 different types of pesticides which were used for forest protection during 2012 on area of Forest Administration Vinkovci.

Direct energy consumptions include:

- *Fuel and lubricant consumptions*

The values for calculation of total energetic value of fuels and lubricants were taken from [5,10] who determined values of 55,3 MJ/kg for chainsaw fuel, 51,5 MJ/kg for diesel fuel and 83,7 MJ/kg for lubricants.

Since the energy content of the fuel mainly expressed in kg/m^3, and fuel and lubricant consumption is measured in L/m^3, all quantities of fuels and lubricants are calculated in kg/m^3 based on density fuel specified by [11]. Density of gasoline is 0.72 kg/L, diesel 0.875 kg/L, lubricant (oil) 0.832 kg/L at 80° C.

The concrete values of consumption in 2011 at the Forest Administration Vinkovci were taken to calculate the fuel and lubricants consumptions for the chainsaws. 59,404 L of fuel and 22,798 L of lubricant were spent for the production of technical roundwood and stacked wood in the amount of 282,772 m^3. This means that on the average value 21 L/m^3 (0.1512 kg/m^3) of fuel and 0.08 L/m^3 (0.06656 kg/m^3) lubricant calculated were spent

The total amount of spent fuel and lubricants was obtained from the database of the production department of Forest Administration Vinkovci for forwarders, farming tractors, graders, dump trucks and motor cars and chainsaws.

In the case of direct energy consumptions should be allocated per unit energy consumption of fuel, oil and tires of vehicles that indirectly affect the production. These are the following machines: farming tractors, graders, dump truck and motor cars.

Fuel consumption of the agricultural tractor that drives chippers was obtained by direct survey in the field, and the fuel consumption for truck for the transportation of wood chips was gained from conversation with the owner of the truck.

- *Consumptions of tires*

Quantity of energy invested in production of tires was calculated based on values described by [12] according to [13, 14] and the value is 94,448 MJ/kg of tyre. Mass of some tyre was measured by mobile scales while for the others the mass was taken for tehnical data of manufacturers.

Annual consumptions of tires for 2012. was taken for database of Production Department of Forest Administration Vinkovci for forwarders, agricultural tractors and motor cars. Durability of tires on trucks for wood chips transport is in averaga 80 000 km, while truck exceeds an average of 100 000 km per year.

- *Consumptions of spare parts of chainsaws (chain, guidebar, sprocket)*

Consumptions of spare parts of chainsaws were taken from Production Department of Forest Administration Vinkovci for 2012. All components are made from steel and energy required for production of steel is 19,742 MJ/kg [12, 13, 14]. It was assumed the mass of guidebar of chainsaw 1,1 kg, mass of chain 0,3 kg and mass of sprocket 0,1 kg in calcualtion of total invested energy.

Results and Discussion

Picture 1 shows the percentage proprtion of energy unit consumptions of each components used directly or indirectly in production at the Forest Adimistration Vinkovci in 2012. It could be pointed out that the most share of energy depends on eneregy of fuels for machines and vehicles (89 %). Lubricants have only 3 %, tires 1 % and production of spare parts of chainsaws less than 1 % of total energy consumption. Propotion of unit energy consumption for production of machines and vehicles is only 7 % of total energy consumption.

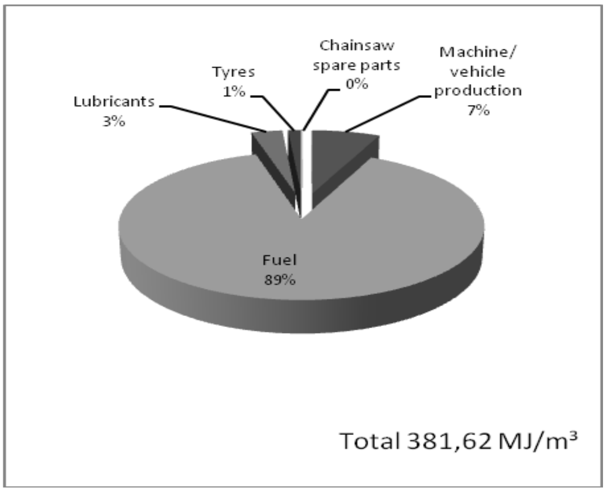

Figure 1. Propotion of unit energy consumption by components.

According to Figure 2, it can be concluded that the largest unit consumption in relation to the total unit energy consumption has an agricultural tractor with chipper in the amount of 46%. Behind chipper, about an equal share of unit energy consumption have forwarder and truck for transport of chips (each 17%). Further, the indirect energy consumption (protection and maintenance of forests and forest infrastructure, pesticides, personal vehicles) amounts to 16%. A chainsaw that was used for felling trees and processing energy wood for transport with forwarder involved in the amount of 4% of total energy consumption, which amounts to 381.62 MJ/^{3}.

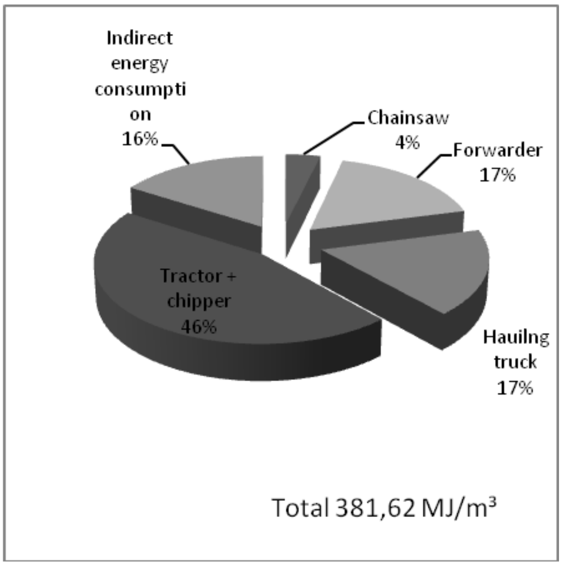

Figure 2. Propotion of unit energy consumption by each machine/vehicle included indirect energy consumption.

Table 1 shows values of energy return on investment (EROI) of production and transport of wood chips to the heating station on average distance of 50 km. The value was given on the basis of ratio between obtained and invested energy in the process.

Table 1. Energy return on investment (EROI) of production and transport of wood chips

Wood product	Energetic value			EROI
	Obtained		Invested	
	GJ/t [1]	GJ/m[3]	MJ/m[3]	
Wood chip (35 % moisture, ρ_{hrast} = 852 kg/m^3) [2]	11,17	**9,51**	**381,62**	**24,92**

[1,3] Manual of fuels from biomass (REGEA 2008).

[2] limit value of mositure in wood chip of 35 % which is demanded by market.

Calculated values of EROI in production process and transport of wood chips have amount of 24,92 which is close to the value which was determined by [2, 15]. According to [16] average value of EROI- for wood is 25.

Conclusions

The highest energy consumption in the production and delivery of wood chips is based on the consumption of fuels and in the amount of 89%. In doing so, the biggest consumer of energy (fuel) is an agricultural tractor which drives chipper. In the entire process represents a share of 46% of the total unit cost of energy (381.62 MJ/m^3). In the production of wood chips should strive to chippers with larger production capacity, and such chippers are generally self-propelled (eg. Silvator 2000), whose hourly fuel consumptions are slightly higher, but productivity is also at least more than twice higher in comparison with other chippers, which leads to a significantly smaller unit fuel consumption.

The next solution is transport of energy wood to the stationary chipper which mainly uses electricity as power and electricity is from the energy and economic point of view better fuel than diesel fuel. When using this method of production of wood chips problem was occurred with reduced utilization of cargo space of transport means (trucks) because the density of energy wood is very low. But there is a technical solution in the form of bandler machine which compress energy wood into a round bales. The use of mentioned machine will increase the mass yield of means of transport, but it also lead to increasing energy consumption in the whole process of production and delivery of wood chips.

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OPTIMIZATION OF ENERGY WOOD CHIPS QUALITY BY PROPER RAW MATERIAL MANIPULATION

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Introduction

World energy supplies have been dominated by fossil fuels for decades, but due to the increasing environmental concerns in recent years a shift towards sustainable sources has occurred, especially in industrialized countries. Among the available alternative energy sources (hydro, solar, wind , etc.) biomass is the only carbon-based sustainable option [1] and therefore can effectively be transformed into different energy carriers (heat, electricity and fuel for transportation). In comparison with coal, biomass generally contains less carbon, more oxygen, more potassium, less aluminium and iron, and has a lower calorific value and higher moisture content [1]. From a scientific and technical point of view biomass is a material of biological origin excluding material embedded in geological formations and/or transformed to fossil [2]. When subjected to different preparation processes it can be transformed to biofuel and finally converted to bioenergy. According to HRN EN 14588:2010 [2] sources of biofuels can be divided in four main groups: woody biomass, herbaceous biomass, fruit biomass and biomass blends and mixtures. Along with the firewood as the traditional form of forest fuel, with the increased mechanization of timber harvesting and introduction of modern and more efficient biofuel conversion facilities, wood chip production started in order to enable the easier handling of the forest fuel on the one side and to allow the greater use of the available biomass resource (logging residues, small-size trees, etc.) on the other. Croatian standard HRN EN 14961-1:2010 [3] specifies dimensions, moisture and ash content as normative characteristics of wood chips, nitrogen and chlorine content as normative for chemically treated biomass and informative for other biomass and net calorific value, bulk density and ash melting behavior as informative characteristics of wood chips. The wood chips for non-industrial (domestic, small commercial) use have more detailed requirements in terms of fuel quality specified in HRN EN 14961-4:2011 [4] which classifies wood chips for non-industrial use in four property classes (A1, A2, B1, B2). In order to guarantee the solid biofuel quality through the whole supply chain, from the origin to the delivery point and to provide adequate confidence that the specified quality requirements are fulfilled fuel quality assurance standards should be used both for wood chips [5] and for wood chips for non-industrial use [6]. Particle size distribution of wood chips is one of the most important parameters for efficient conversion into energy. When drying fresh wood chips a loss of wood substance occurs due to the growth of bacteria and fungi that break down cellulose in wood to carbon dioxide and water with the release of heat [7]. Increasing the size of the wood chips accelerates drying and reduces the health risks, the possibility of spontaneous combustion and loss of calorific value. On the other hand, oversize particles prevent the flow of wood chips by bridging and arching of automated supply units. The particle size of the wood chips depends on the raw material (the tree species and the part of a tree from which it is produced), chipper type and accompanying screen, and on the position and condition of the blade [8]. Unlike the other main features of the wood chips, the quality of the particle size distribution can be influenced by the selection of a proper comminution device and adjusting its work [9]. Khan et al. [1] distinguish physical (density, porosity, friability and internal surface area related to biomass species and bulk density, particle size, and shape distribution related to fuel preparation methods) and fuel (calorific value, moisture content, volatile matter and ash content) properties of biomass for energy production, and stress that of the fuel properties the biggest technical challenges that biomass today faces are all related to its ash content. Natural ash content of the forest fuel is a given value and varies mainly depending on the tree species and the tree part used as raw material, but polluting ash content as a consequence of (mis)handling in the supply chain can significantly deteriorate fuel quality [10]. Ash content in the fuel is crucial for the selection of appropriate combustion technologies and gas-cleaning technologies [11]. Ash as a non-combustible component of wood chips is either retained as bottom ash in the combustion chamber and interferes with the process of combustion, or in the form of fly ash causes corrosion of the heating circuits and flue pipes as well as the problems in gas emissions [12]. For ash disposal and eventual recycling its quantity and chemical composition is decisive. Mass content of the ash in debarked wood is relatively small (can be up to 0.5%). It grows to about 1.0% when wood with bark is combusted with a slight increase when needles are burned too, and a drastic increase, even at 5.0 to 10.0% if the wood chips are contaminated with soil, sand or stone [13]. This research was conducted in order to determine the impact of raw material preparation by different harvesting systems on the quality of produced wood chips in respect to particle size distribution and ash content.

Materials and Methods

Research was conducted on 21 wood chip samples (truck loads). All samples were collected while roadside chipping the material harvested during a first thinning of a 42 years old spruce forest plantation. Four harvesting systems were engaged in the investigated felling site. In partially mechanized harvesting systems (by assortment and full tree method) felling and processing of trees was done motor-manually by a chainsaw and skidder was used for extraction, and in mechanized harvesting systems (by assortment and load-length method) felling and processing was done by a harvester and extraction was performed by a forwarder (detailed description of the operational surrounding presented by Vusić [14]). All systems were complemented with a Jenz Hem 561 DQ chipper (360 kW; 10 standard blades; 35 × 35 mm sieve). Sampling was performed in regular intervals during the chipping process. In that way 4 samples of pulp wood chips (PM_PW) and 8 samples of whole tree chips (PM_WT) were gathered while chipping the material harvested by partly mechanized systems (by assortment and full tree method), and the rest of the samples were gathered while chipping the material harvested by mechanized systems; 3 samples of pulp wood chips harvested by assortment method (M_PW), and 4 samples of stem wood chips (M_SW) plus 2 samples of chips produced from the tops (M_TS) harvested by load-length method.

All the laboratory analyses of wood chips were conducted in the Forest Biomass Laboratory of the Forestry Faculty Zagreb. Particle size distribution was determined by oscillating screen method (on the Retsch AS 400; 250 min^{-1} for 5 min; round hole sieves 3.15 mm, 8 mm, 16 mm, 45 mm and 63 mm) according to the HRN EN 15149-1:2010 [15], and median value of the particle size distribution was calculated. Three particle size classes were formed; fine fraction (<3.15 mm), main fraction (3.15 – 63 mm), and coarse fraction (>63 mm). Sub-samples for ash content analysis were formed, and comminuted by a cutting mill Retsch SM 300 (1.500 min^{-1}) to the nominal top size of 1.0 mm. Ash content was determined according to the HRN EN 14775:2010 [16] using the Nabertherm L9/11 laboratory furnace.

Analysis of variance was performed on the median value of the particle size distribution of different raw material types and on the ash content of different raw material types. LSD post-hoc test was applied in order to determine which raw material type(s) statistically differ. Correlation between the quality parameters was tested.

Results and Discussion

Results of particle size distribution of all 21 samples show an average 12.11±10.35% of fine fraction content, average 87.80±10.39% of main fraction and an average of just 0.09±0.15% for coarse fraction content. The increase in the fine fraction content (Fig. 1 a) when chipping whole trees (PM_WT) and tops (M_TS) is expected considering the results of previous studies [8, 11, 17, 18, 19], and logical given the increased share of needles and twigs in raw material, particularly bearing in mind that unseasoned material was chipped. Particle size distribution of wood chips (Fig. 1) produced from pulpwood (PM_PW&M_PW) and stem wood (M_SW) is similar to particle size distribution of wood chips produced by chipping of roundwood by Jenz 560 D chipper reported in the research of the chipper impact on the particle size distribution of the wood chips in Italy [9]. In that study of nine chipper types of various manufacturers it has been found that Jenz 560 produces wood chips with the highest percentage of fine fraction (4.5%) and that most of the chippers produce wood chips of mixture fractions 3 – 16 mm and 16 – 45 mm with a small proportion of particles larger than 63 mm. It was concluded that even when chipping roundwood it is difficult to achieve a dominant share of a single fraction of wood chips with the expectation of deterioration of particle size distribution when chipping whole trees or brushwood.

Analysis of variance on the median value of the particle size distribution (Fig. 2 & Table 1) showed statistically significant impact of raw material type ($F = 4.2305$, $p = 0.0159$). Results of LSD post-hoc test (Table 2) indicate that there is statistically significant difference (at $p < 0.05$) between the pulpwood chips harvested by the partly mechanized system (PM_PW) and wood chips produced from the whole trees (PM_WT), stem wood (M_SW) and tops (M_TS) and between the pulpwood chips harvested by the mechanized system (M_PW) and chips produced from tops (M_TS).

Despite the obvious increase of ash content (Fig. 3) in whole tree chips (PM_WT) and chips produced from tops (M_TS), no statistically significant difference was found (Table 1), probably because of a large variation of ash content in those two raw material groups and just 2 samples for chips produced from tops. The increase of absolute value of the ash content as well as the increased variation can be explained by increase of the amount of needles (natural ash) in those two raw material types and increased possibility of contamination by dirt (polluting ash) especially when skidding whole trees.

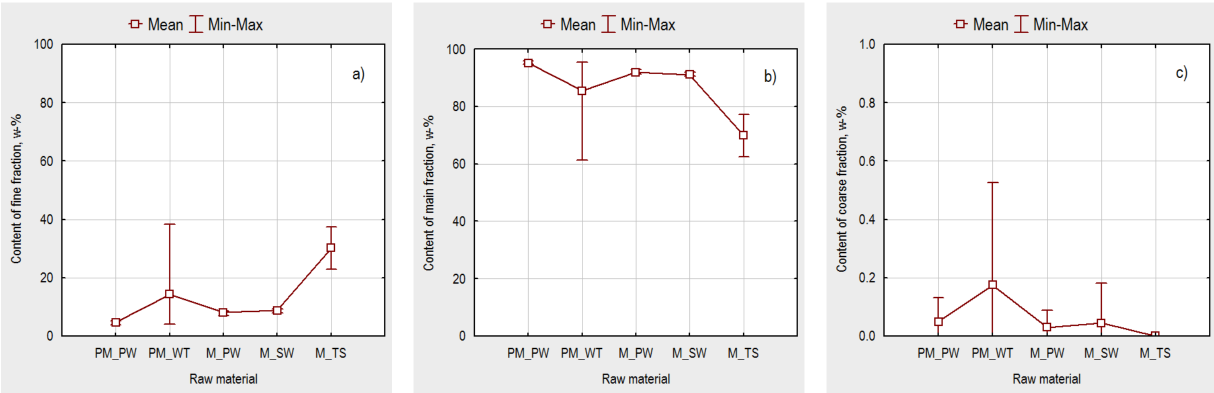

Figure 1. Mean plots of a) fine fraction content grouped by raw material; b) main fraction content grouped by raw material; c) coarse fraction content grouped by raw material.

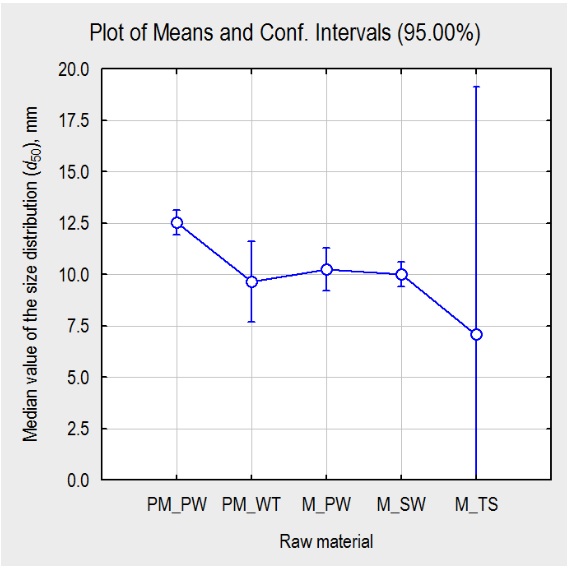

Figure 2. Median value of the size distribution vs. raw material type

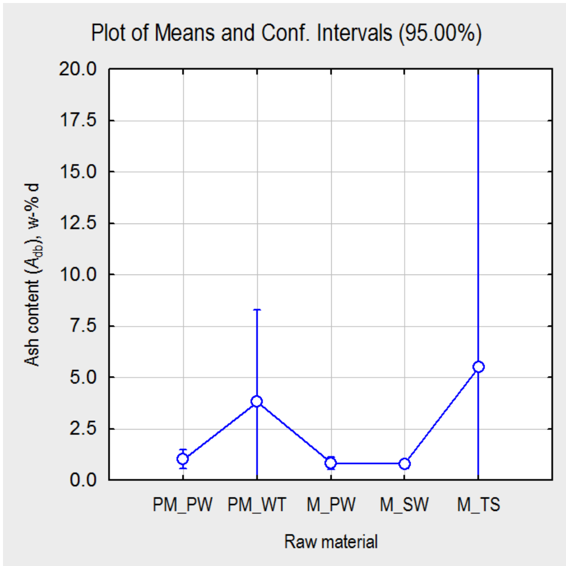

Figure 3. Ash content vs. raw material type

Table 1. Analysis of variance results (bolded effects are significant at $p < 0.05$)

Variable	SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
Median value of the size distribution, mm	**43.932**	**4**	**10.9829**	**41.538**	**16**	**2.5961**	**4.2305**	**0.0159**
Ash content, w-% d	60.583	4	15.1458	222.209	16	13.8881	1.0906	0.3944

Table 2. LSD post-hoc test results for median value of the particle size distribution (bolded differences are significant at $p < 0.05$)

Raw material	{1} M = 12.525	{2} M = 9.6375	{3} M = 10.233	{4} M = 10.000	{5} M = 7.0500
PM_PW {1}		**0.009882**	0.081041	**0.041518**	**0.001212**
PM_WT {2}	**0.009882**		0.592447	0.718141	0.059177
M_PW {3}	0.081041	0.592447		0.852001	**0.045913**
M_SW {4}	**0.041518**	0.718141	0.852001		0.050557
M_TS {5}	**0.001212**	0.059177	**0.045913**	0.050557	

Although no statistically significant differences of raw material type to ash content were found (Table 1), results of a correlation analysis (Table 3) are very indicative. Ash content is positively correlated with the amount of fine fraction, and negatively correlated with the amount of the main fraction (logical due to the minor amount of the coarse fraction). Negative correlation between the median value of the particle size distribution and ash content was also found.

Table 3. Correlation analysis results (bolded correlations are significant at $p < 0.05$)

Variable	Content of fine fraction, w-%	Content of main fraction, w-%	Content of coarse fraction, w-%	Median value of the size distribution, mm	Ash content, w-% d
Content of fine fraction, w-%	1.000000	**-0.999906**	0.234160	**-0.917934**	**0.667328**
Content of main fraction, w-%	**-0.999906**	1.000000	-0.247491	**0.917413**	**-0.666806**
Content of coarse fraction, w-%	0.234160	-0.247491	1.000000	-0.182880	0.124230
Median value of the size distribution, mm	**-0.917934**	**0.917413**	-0.182880	1.000000	**-0.616690**
Ash content, w-% d	**0.667328**	**-0.666806**	0.124230	**-0.616690**	1.000000

Conclusions

Forest fuel is by nature a very diverse source of energy. Most of its properties are given and limit the possibility of upgrading the fuel in the forest production process. Because of that, those that can be manipulated during the transformation from raw material source (tree biomass) to useful and easy to handle forest fuel form (wood chips) are of great importance. The impact of raw material for wood chips production, influenced by the harvesting method and the selection of primary transport means, has a substantial effect on the quality of produced wood chips. Wood chips produced from pulp wood (PW) and stem wood (SW) is of better quality in comparison to wood chips produced from whole trees (WT) and tops (TS) that show greater variability in particle size distribution and ash content. The increase of ash content is obvious in the case of raw material containing needles (whole trees and tops) as well as raw materials contaminated with inorganic substances (dirt). Depending on the composition of the available raw material, by using the appropriate and well maintained machinery in the comminution phase of production, it is possible to manipulate and directly improve the quality parameters of the particle size distribution. During all the phases of supply chain, in which forest fuel is handled, a special attention must be paid to minimizing the contact with polluting inorganic matter (dirt, sand, stone) in order to maintain the ash level close to natural one. Knowledge about previous experience in similar conditions and skills on the quality control of forest fuels are in this respect necessary in order to produce the forest fuel of the best possible quality even from the lower quality raw material and more important to avoid that from the high-quality raw materials a forest fuel of poor quality is produced. The results of ash content to median value of the particle size distribution (and fine/main fraction content) correlation indicate that ash content to particle size distribution relation should be investigated on a larger number of different samples. This could be done by testing the ash content of individual particle size classes and their differences in order to find new possibilities for upgrading the quality of the energy wood chips.

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Session E: Biodiversity and ecosystem service

THE ROLE OF FOREST VEGETATION IN KARST ON SOIL PROTECTION FROM EROSION

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Introduction

Land degradation has been recognized as a major environmental issue whose processes have been varying in places and time, within the Mediterranean basin. Forest management policies in this area have traditionally encouraged land cover changes, with the establishment of tree cover in natural or degraded ecosystems for soil conservation purposes: to reduce soil erosion and to increase the vegetation structure.

Forest systems have the lowest soil loss rates recorded, and they are most effective for preventing accelerated erosion, thus heavily contributing to soil protection [1]. In a well preserved forests, which has been maintained regularly, there is only normal erosion, where the quantity of soil erosion is under erosion tolerance.

Forests have highly performing regulatory functions on the water cycle among others especially in Mediterranean environments characterized by high temporal contrasts in weather conditions [2, 3, 4].

Additionally, the Mediterranean environments are subjected to strong seasonal climatic fluctuations which modify the soil conditions, and as consequence, hydrological processes [5]. On Mediterranean terrain slopes, either the static or the dynamic soil properties are not uniformly distributed [6] and many of them vary in patches related to micro-topography and vegetation [7, 8].

Soil water infiltration in these areas is highly dependent on the dynamic soil characteristics such as organic matter, root systems, macropores, vegetation cover, soil crust formation, soil aggregation [6], soil water content [9], rock fragment size, percentage and geometry [10].

Water erosion is a serious problem in the context of land degradation and desertification processes in the Mediterranean region of Croatia. Forest ecosystems in this region are highly degraded so their antierosional function has been reduced.

Of the total Mediterranean part of Croatia about 95 % of the area is subjected to different intensities of water-induced soil erosion, of which heavy erosion accounts for 40 %. Accordingly, the area is highly threatened by erosion, while some parts of area are completely degraded. The ecologically very sensitive area at high risk from erosion, soil degradation and vegetation devastation, contains 668 torrents with a total watershed area of 3,024 km^2, which makes it one of the largest torrent areas in Croatia [11, 12].

The aim of this study is to determine the effects of vegetation cover on watershed Suvava on soil protection from erosion in the period from 2003 to 2009. Data is compared with results from 1972 to 1975 year on the same watershed.

Materials and Methods

The experimental area is located in watershed of Suvava, 30 km N of Split, in central Dalmatia, south Croatia.

The watershed of Suvava takes the area of 1 823 ha, with the length of the main flow of 10.1 km and the entire length of greater and smaller tributary streams–torrents of 20.8 km. It extends itself between 460 and 961 m a.s.l. The climate of this area is submediterranean, with the average rainfall of 1094 mm. Vegetation cover in catchment Suvava, in 1975 and 2009 year is presented in table 1.

Table 1. Vegetation cover in catchment Suvava, 1975 and 2009 year

	1975		2009	
Vegetation cover	Area		Area	
	(ha)	%	(ha)	%
Black pine forests	194,7	10,7	773,4	42,4
Pubescent oak coppic forests	188,8	10,4	271,5	14,9
Hornbeam shrub	89,9	4,9	393,1	21,7
Black locust	-	-	5,4	0,3
Agricultural land	294,00	16,1	294,0	16,1
Bare	1 055,6	57,9	82,6	4,6
Sliv "Suvave"	1 823,0	100	1 823,0	100

In the mouth of torrent Suvava, on the catchment area, runoff and soil loss was investigated, during seven years (2003-2007) and it is compared with results from 1972-1975. Experimental plots are placed on the different inclination and have different/representative vegetation cover (Table 2).

Table 2. Characteristic of experimental plots

Plot	Slope (°)	Altitude (m)	Plot area (m^2)	Vegetation cover
BP_{10}	16	460	50	Black pine culture - 10 years old
BP_{80}	32	550	50	Black pine culture - 80 years old
HS	26	550	50	Hornbeam shrub
G	16	460	50	grass

The experimental plots were set parallel to the inclination. The dimensions of the plots were 20.0 x 2.5 m. They have the container for collecting alluvium and are fenced by cement enclosure. The enclosure is set so that it can not get water and soil suspension from side, nor can it uncontrollably lose it from area.

In the lower part of the stream Suvava, limnigraph was set for monitoring water level and water flow. Samples (water and sediment) were collected for determining the production of erosion deposits at the basin and stream level, after each rainy day. Samples were taken, filtrated, dried and weighed. Weather station, placed on the experimental site, records meteorological data (precipitation, temperature).

Research was conducted in the period from 2003 to 2009. The values (flow off, soil loss, water levels and water flow) obtained seven-year study period and compared with the values from the period since 1972 to 1975 year.

Results and Discussion

Vegetation cover and rainfall characteristics determine runoff and soil erosion [5, 12, 13]. Many autors [12, 14, 15, 16, 17, 18, 19] have indicated protective effect of the vegetation cover in controling runoff and soil erosion. Vegetation cover diminishes the quantity of water that reaches the soil through the effect of interception [20], and also increases the heterogeneity of the spatial distribution of the rainfall toward the soil, through the stemflow and throughfall [13, 20].

According to the vegetation cover, some differences were expected in runoff and soil erosion between 10 and 80 years old Black pine culture, Hornbeam shrub and grass. During the study period were registered 758 rainy days, with an annual mean of 1094.2 mm.

Out of 758 rainy days 296 were erodible. Flow off and soil loss was caused by precipitation volume from 4.3 mm till 103.5 mm.

According to our results (Table 3), the runoff and soil loss, generated by vegetation cover type, were present by following order: HS < G < BP_{80} < BP_{10}.

Table 3. Annual runoff and soil loss on experimental plots by vegetation type cover

Vegetation		Black pine (10 year old)	Black pine (80 year old)	Hornbeam shrub	Grass
		Annual runoff (mm/m^2)			
Years	Rainfall (mm)	BP_{10}	BP_{80}	HS	G
2003	926.9	47.28	43.61	21.41	35.52
2004	1212.8	65.37	43.11	15.64	26.84
2005	1157.3	68.00	37.11	9.68	34.27
2006	828.3	16.88	18.04	12.02	16.21
2007	929.1	15.96	16.21	18.49	10.30
2008	1244.1	67.79	24.76	-	27.33
2009	1361.1	67.68	24.26	-	25.68
Mean	1094.2	49.85	29.59	12.87	25.16
		Annual soil loss (g/m^2)			
2003	926.9	1.767	1.777	0.376	1.185
2004	1212.8	3.317	2.336	0.224	1.295
2005	1157.3	2.155	2.180	0.754	1.307
2006	828.3	1.645	1.131	0.623	0.648
2007	929.1	1.721	1.093	0.376	1.085
2008	1244.1	3.289	1.531	-	1.399
2009	1361.1	1.742	2.373	-	0.785
Mean	1094.2	2.234	1.774	0.471	1.101

Runoff and soil loss differences on experimental plots with different vegetation cover could be influenced by the slopes (80 year old Black pine (32°) exceeds the slope in the 10 year old Black pine and grass plots (16°) and Hornbeam plots (26°) despite the importance of soil surface roughness [21].

Moreover, it was found that erosion on experimental plots under different vegetation covers do not exceed the erosion tolerance of 2 t/ha. Vegetation cover has very important influence on water erosion intensity. Permanent vegetation cover bind and stabilize the soil, ameliorate its structure, decrease the quickness of water infiltration, retains water and decrease runoff and soil loss [15].

Rainfall events lower than 10 mm, with an average intensity lower than 5 mm/h and short duration (less than 2 h) presents 70 % of total rainfall events.

These characteristics of precipitation and vegetation cover could explain the lower measured runoff and soil loss. It is relevant for this region that rainfall events are concentrated in a few very heavy rain days. The rainfall events with more than 10 mm (30%)

represent 75 % of accumulated annual precipitation volume with a mean maximum intensity (I_{15}) of 8.5 mm/h.

Precipitation events less than 10 mm (70%), generated between 17 % and 22 % of soil loss in all vegetation cover.

Runoff and soil loss caused by rainfall, reflect small values in both variables (vegetation cover and rain characteristics).

Comparing vegetation maps of the catchment of Suvava, from 1975 and 2009, changes in the vegetation cover were determined. After 34 years, the area covered by Black pine was increased by 32%, Pubescent oak coppice forests 4.5 % and Hornbeam shrubs by 17%. In the same period, bared areas were decreased by 53%.

Table 4. Annual water level, water flow and sediment in the flowing water of the stream Suvava

Year	Rainfall (mm)	Water level (cm)	Water flow (m^3)	Water flow in the stream (%)	Sediment in the flowing water (t)
1972	1452.5	1.3	1 764 029	6.6	40.90
1973	968.5	0.8	838 857	4.8	32.9
1974	1709.9	2.5	2 242 684	7.2	131.29
1975	1443.2	1.6	2 322 726	8.8	274.46
Mean	1393.5	1.6	1 792 074	7.0	119.89
2003	926.9	0.7	968 024	5.7	12.00
2004	1212.8	1.5	1 917 389	8.7	50.49
2005	1157.3	1.4	1 878 595	8.9	86.52
2006	828.3	1.0	1 350 604	8.9	23.29
2007	929.1	0.8	1 089 244	6.4	14.24
2008	1244.1	1.0	1 353 369	6.0	16.28
2009	1361.1	1.6	1 785 109	7.2	23.18
Mean	1094.2	1.1	1 477 476	7.4	32.28

Water flows in the stream of Suvava slightly increased while the annual sediment in the flowing water decreased by 3.7 times compared to the previous period, when more than

50% of the catchment area was without forest vegetation (Table 4). Percentage of annual runoff in the catchment is low, annually interception, evaporation and infiltration is 92.6%.

Conclusion

After seven years of research can be concluded that the flow off in the mouth of the torrent of Suvava slightly increased. Soil loss was 3.7 times lower than in the previous period, when more than 50 % of the catchment area was without forest vegetation. Protecting soil from erosion with natural vegetation and/or reforestation is the adequate method to prevent soil from water erosion. Annual coefficients of surface flow off at the basin and on the investigated types of vegetation cover are small and do not exceed 10 % of total annual rainfall. In line with this and annual soil loss are also low, and erosion is completely excluded. Forest vegetation in the mouth of the torrent of Suvava has a significant and very positive role in protecting soil from water erosion.

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THE STRUCTURE AND DYNAMICS OF MIXED-SPECIES STAND OF SCOTS PINE, NORWAY SPRUCE AND SILVER FIR ON MOUNTAIN KLEKOVAČA (BIH)

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Introduction

Forest stand dynamics is the study of changes in a forest stand structure over time, including stand behaviour during and after disturbances. Stand structure is the physical and temporal distribution of trees and other plants in a stand [1]. Understanding stand dynamics helps to focus management objectives by predicting future stand structures and development patterns, reducing silvicultural costs; increasing stand productivity, obtaining management information rapidly and inexpensively; obtaining desirable species compositions or physiognomies; developing diagnostic criteria for determining, prescribing, and making decisions for silvicultural operations; and enabling stands to be used as a general environmental "bioassay" for determining when stands are growing abnormally—perhaps as a result of pollution or other factors [2].

Materials and Methods

The research of the dynamics and structure was carried out in mixed stands of Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* Karst.) and Silver fir (*Abies alba* Mill.) on mountain Klekovača. Significant complexes of that forest type are located in the western part of Bosnia and Herzegovina. The researched stand is exempted from regular management (seed stand). Mixed stand of Scots pine, Norway spruce and Silver fir, Abieti Piceetum illyricum (Fuk.) Stef. 1962 subass. *pinetosum sylvestris*, is the successive stage of pine forests with spruce *Piceo-Pinetum illyricum* Stef. 1959, towards the permanent stage *Abieti Piceetum illyricum* (Fuk.) Stef. In 1962.

For the study of the dynamics and structure of these mixed stands in the area of mountain Klekovača, the site Ravna Glavica at an altitude of 958 to 1011 m was selected (forest management unit "Klekovača-Drinić"), where 5 sample circular plots (n) were set (radius of 30 m). In addition to surveying the main elements of the trees growth, those had diameter at breast height (dbh) larger than 5 cm were cored with increment borer at height of 0.5 m from forest floor, to estimate tree age. Corim maxi (instrument) was used to determine the tree-ring width from core samples. To estimate the stem volume of each tree volume tables of Špiranec [3] and Bezak [4] were used.

Results and Discussion

For the researched forest stand site classes were determined by comparing the tree-species height curves with standard site classes curves [5] as follows: for the Silver fir class I / II, for Norway spruce class III and for Scots pine site class I. The estimated average number of trees (having dbh greater than 5 cm) per hectare is 798. The variation of the number of trees on the sample plots, expressed by the coefficient of variation, is 14.8%. In the total number of trees the largest share has Silver fir with 46%, and the lowest Norway spruce with 19%. By observing species per sample plots, the largest variations are in the number of Silver fir trees (38.7%) and the lowest in the number of Norway spruce trees (15.7%). Estimated standing volume is 745.7 m^3 ha^{-1} with a variation of 9.2% in the sample plots. Contrary to the number of trees, the largest share in the standing volume has Scots pine with 69%. Volume increment is 11.54 m^3 ha^{-1} with a variation of 11.5% for all sample plots (Table 1).

Table 1. Basic stand structure elements (number of sample plots - n, number of trees – N, volume – V, current annual increment – Iv) and descriptive statistics (standard deviation - Std. dev., and coefficient of variation - CV)

Tree species	n	N			V			Iv		
		ha^{-1}	Std. dev. ha^{-1}	CV %	m^3 ha^{-1}	Std. dev. m^3 ha^{-1}	CV %	m^3 ha^{-1}	Std. dev. m^3 ha^{-1}	CV %
Silver fir	5	370	143	38.7	116.5	44.0	37.7	3.08	1.15	37.4
Norway spruce	5	154	25	15.7	108.1	49.4	45.7	2.63	1.01	38.4
Scots pine	5	271	68	25.1	518.0	112.7	21.7	5.83	1.21	20.8
Total	5	798	118	14.8	745.7	68.5	9.2	11.54	1.33	11.5

Diameter distribution of trees is shown in Figure 1. By using the appropriate statistical indicators, based on the measured trees diameter, the parameters that characterize the diameter distribution of the studied stand were obtained and presented in Table 2.

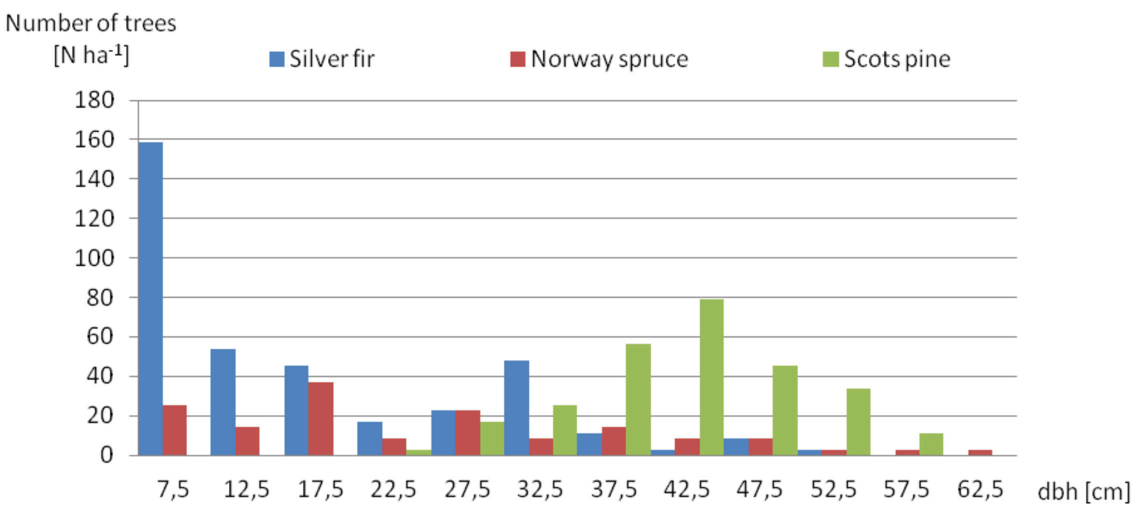

Figure 1. Diameter distribution of trees.

A significant difference is observed in the diameter distribution of those three species of trees. Arithmetic mean of the Silver fir trees dbh is 16.14 cm, for Norway spruce is 24.15 cm, and 41.57 cm for Scots pine. The largest absolute variation, in terms of the trees dbh, is observed for Norway spruce (14.33 cm), and largest relative variation for Silver fir trees (71.19%). Both absolute and relative variability are the lowest in Scots pine sample. Diameter distribution of Scots pine is characterized by a small negative asymmetry (Skewness = - 0.15) comparing to the distributions of Silver fir and Norway spruce trees which are characterized by strong positive asymmetry (Skewness = 1.00; 0.74; respectively). Kurtosis (factor) shows that the distribution of all three species is flattened compared to normal distribution. Testing the difference between the actual diameter distribution and normal distribution using the Shapiro-Wilk test (if the *p*-value is less than the chosen alpha level, then the null hypothesis is rejected and there is evidence that the data tested are not from a normally distributed population) shows that the actual frequencies of Scots pine trees match with the theoretical frequencies (p = 0.8178). Distributions of Norway spruce and Silver fir trees deviate significantly (p = 0.0022, p= 0.0000, respectively) relative to the normal distribution.

Table 2. Diameter distribution of trees - descriptive statistics

Tree species	dbh (cm)	Variance (cm)	Std. dev. (cm)	CV (%)	Skewness	Kurtosis	Shapiro-Wilk	
							W	*p*
Silver fir	16.14	132.04	11.49	71.19	1.00	-0.05	0.84992	0.0000
Norway spruce	24.15	205.35	14.33	59.34	0.74	-0.35	0.92540	0.0022
Scots pine	41.57	58.18	7.63	18.35	-0.15	-0.15	0.99168	0.8178
Total	26.36	248.96	15.78	59.86	0.14	-1.32	0.90947	0.0000

Height distribution of trees is shown in Figure 2. Estimated average height of Silver fir trees is 11.81 m, for Norway spruce is 17.80 m, for Scots pine is 29.25 m. The Scots pine height distribution has strong negative asymmetry (Skewness = -0.7486), and the distribution of Silver fir has strong positive asymmetry (Skewness = 0.6209). The observed Norway spruce height distribution is not asymmetric, actually it is flattened compared to normal distribution (Kurtosis = -1.2073). Testing the difference between the actual tree species distributions and normal distribution by using the Shapiro-Wilk test showed significant differences.

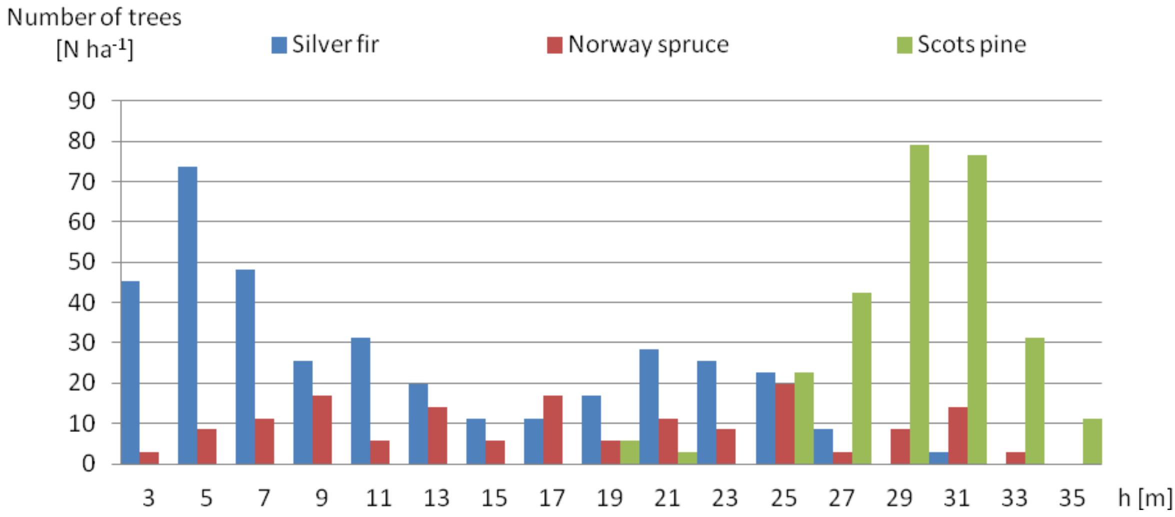

Figure 2. Height distribution of trees.

Homogeneity is defined with stand homogeneity index (H), according to De Camino [6], and it is graphically presented with the Lorenz's curve. According to Kramer [7], homogeneous stands have a higher stand homogeneity index. In even-aged stands with low thinnings, stand homogeneity indexes vary between 4 and 10, while in uneven-aged the indexes range from 1.3 and 2.8. The estimated stand homogeneity index ($H_{Silver\ fir}$ = 1.7, $H_{Norway\ spruce}$ = 2.1 and $H_{Scots\ pine}$ = 6.4) and the Lorenz's curve (where N% represent sum of stem percentages up to dbh class i, and V% is the sum of volume percentages up to dbh class i) indicate a significantly higher degree of homogeneity of the Scots pine component in the stand, in relation to Silver fir and Norway spruce (Figure 3). The stand homogeneity index of Scots pine component of stands tends to have the value typical for even-aged stands. On opposite, stand homogeneity index of Silver fir and Norway spruce components tend to the typical value for uneven-aged stands.

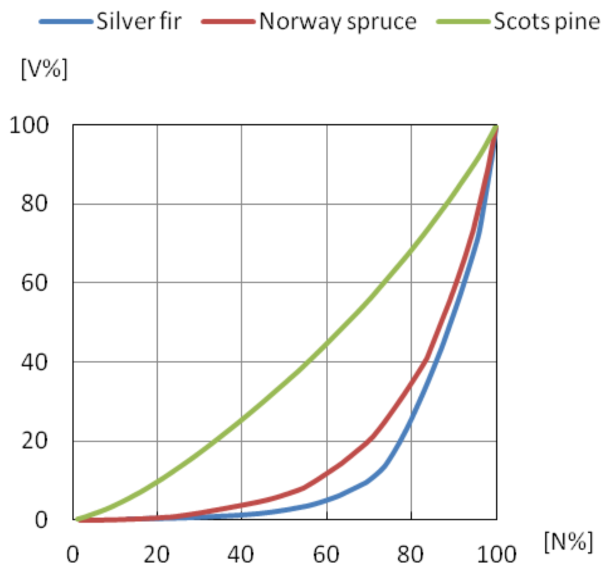

Figure 3. Lorenz's curve.

Figure 4 shows the distribution (relative frequency) of measured trees of tree species by age classes. The estimated average age of Silver fir trees is 65 years, for Norway spruce and Scots pine is 84 and 121 years respectively. The youngest Silver fir tree was 19 years old, while Norway spruce and Scots pine trees were 34 and 83 years old respectively. Accordingly, the youngest Scots pine tree reached breast height in 1930, Norway spruce in 1979 and Silver fir tree in 1994. Furthermore, in actual stand 24% of currently alive Silver fir trees, 15% of Norway spruce trees and 84% of Scots pine trees reached breast height until 1910.

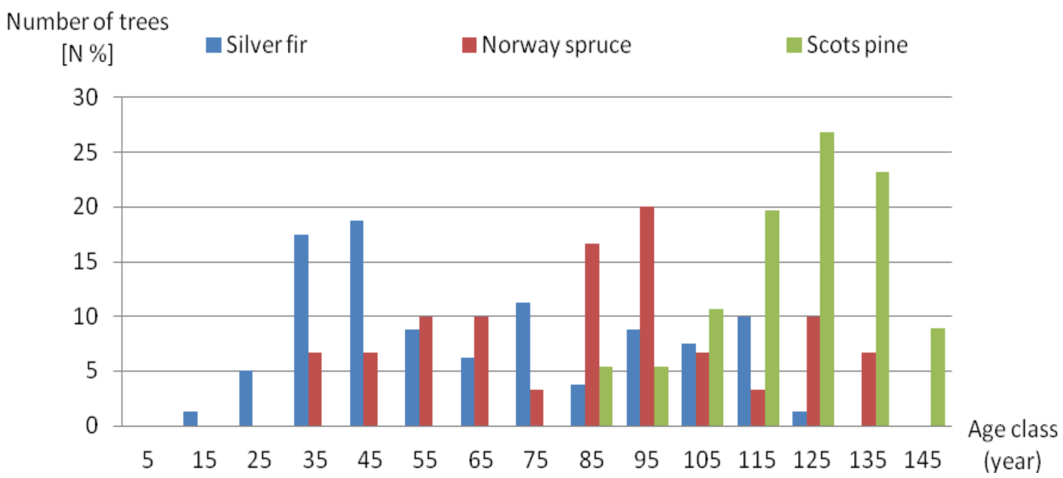

Figure 4. Age structure of living trees.

The dominant Scots pine trees had the largest diameter increment (I_d) until about 1945 and after then the smallest diameter increment comparing to other tree species. From 1945 until the last decade of twentieth Century, Norway spruce trees had the largest diameter increment, while in the first decades of 21st century, Silver fir and Norway spruce trees had similar diameter increment (Figure 5).

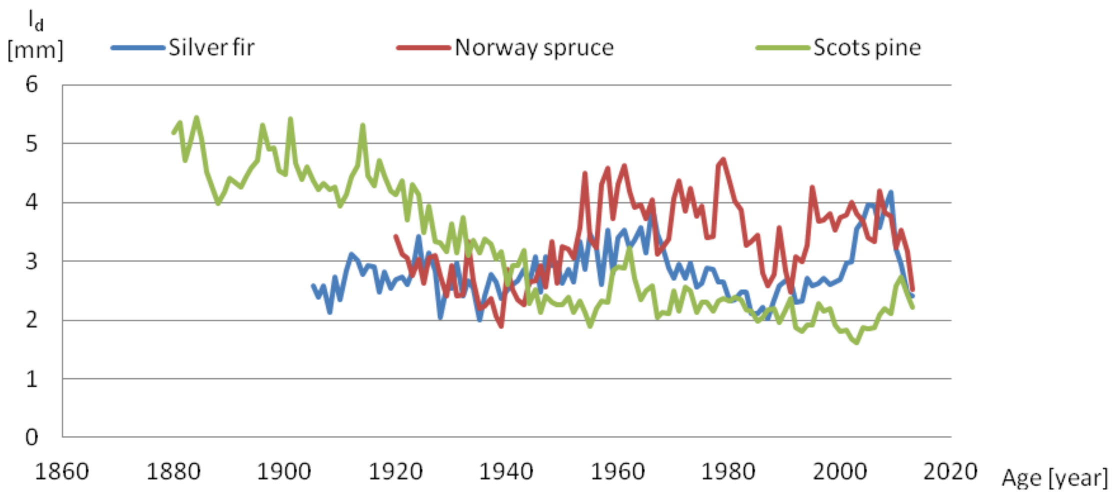

Figure 5. Average diameter increment (dominant trees, age > 100 years)

Conclusion

Performed analysis, primarily of diameter, height and age structure in the studied uneven-aged stand dominated by Silver fir, Norway spruce and Scots pine, indicated a very complex structure and dynamic change, especially in terms of the ratio of the tree species. The analysis of the age structure showed the dynamics of succession in the researched stand towards to a permanent state of *Abieti Piceetum illyricum* (Fuk.) Stef. 1962 and towards to the mixed Silver fir and Norway spruce forest. The youngest Silver fir tree was 19, Norway spruce is 34 and Scots pine is 83 years old, which means that the youngest tree of Scots pine reached breast height in 1930, Norway spruce in 1979 and silver fir in 1994. In the last two observed decades, no trees reached 5 cm at breast height, which is consequence of very high stand density currently. The estimated number of trees per hectare is 798 and standing volume is 745.7 m^3 ha^{-1}.

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NITROGEN DEPOSITION MEASUREMENT IN CROATIA AND SLOVENIA

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Introduction

Changes in forest condition are results of the multitude of stress factors including environmental pollution and rapidly changing climatic conditions, where air concentrations of nitrogen and acidifying compounds and their deposition play a predisposing, accompanying and locally, even a triggering role [1]. In order to detect the impact of air pollution, climate change and natural stress factors on forest condition, within the framework of the International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) and EU regulation, the continuous and intensive monitoring on plots throughout Europe is carried out. ICP Forests also tries to contribute to a better understanding of the casual relationship between changes in forest ecosystems and the factors influencing these changes, with particular focus on the atmospheric deposition (concentrations, amount of deposition, fluxes). Large-scale variations of forest condition over space and time in relation to natural and anthropogenic factors are assessed on approximately 760 permanent observation plots covering the most important forest ecosystems in Europe, located in 30 participating countries, including 500 plots with data on both atmospheric deposition and forests ecosystem impacts [2, 3]. Croatia is participating with 7 Level II plots and Slovenia with 11 Level II plots. With the intensification of agricultural and industrial activities over past several decades, such as combustion of fossil fuel, production and application of nitrogenous fertilizer and intensive stockbreeding, large amount of N compounds (mainly NO_x and NH_y) was released to the atmosphere, which subsequently resulted in elevated N deposition in terrestrial and aquatic ecosystems [4, 5]. A further deposition increase by a factor of 2.5 is projected at global level by the end of the century [6]. Concern about the actual and potential effects of high nitrogen deposition on forests embraces almost all forest ecosystem compartments, including vegetation, soil biota, soil, soil water and run-off [7, 8]. Negative impacts of high N deposition on the health of forest ecosystems include soil acidification, nutrient imbalance and forest decline. It is suggested that N deposition will be the third greatest driver of biodiversity loss on the global scale in this century, after land use and climate change [4]. A risk of atmospheric nitrogen deposition is assessed by means of calculations of critical loads and their exceedances [9]. Exceedance of nitrogen critical load describes the vulnerability of forests to environmental stress caused by anthropogenic impact. Since, critical loads for nitrogen is exceeded on many plots in Europe, this paper aims to present temporal (from 2008 to 2010) and spatial (two plots in Croatia and Slovenia) variation of nitrogen deposition and precipitation. Furthermore, the aim of this study is to evaluate and compare actual and critical loads of nitrogen on Level II plots in Croatia and Slovenia as well as to compare different samples typology.

Materials and Methods

Following the ICP Forests methodology bulk and throughfall deposition of nitrogen on Level II plots in Croatia and Slovenia is determinated and calculated as the means of the yearly sums (kg ha^{-1} y^{-1}) over the period 2008-2010. In order to evaluate actual loads and identify possible differences in nitrogen deposition and precipitation between the sites and across time, we compared monitoring data for two forest plots located in Croatia (Jastrebarski lugovi, 110) and Slovenia (Murska šuma, 11) both dominated by *Quercus robur* L. (Pendeculate oak) with similar age. Plot characteristics are shown in Table 1. The Croatian plot, Jastrebarski lugovi (110) is located in Pokupsko basin floodplain. Bulk open field depositions (BOF) in the open plot were sampled using continuously exposed collectors comprising a 1L polyethylene bottle, with a funnel of 19.5 cm in diameter. A polyethylene net in the funnel was used to prevent the collection of coarse debris, insects and leaves. The bottle was inserted into a PVC cylinder of a slightly larger diameter to leave an air jacket around the bottle and to shade the bottle. Three open field collectors were placed in the area to allow greater volume measurements and discarding of any samples which were clearly polluted without the loss of the whole sample for a specific sampling period. Throughfall deposition (THR) was sampled beneath the forest canopy, containing bulk+leached+dry deposition-absorbed ions. The collectors were the same as those described for open field sampling. Nine collectors were used for THR measurement, distributed evenly over the plot. The Slovenian plot, Murska šuma (11) is located in Pannonian floodplains of river Mura. For bulk deposition sampling, continuously exposed three (3) funnel-type samplers with diameter of 24 cm were used, at bottom connected with 8 L polyethylene bottles. The rims of the funnels were at 1.8 m height. For the chemical analysis the sample of each sampler was analyzed separately. For the throughfall the gutter-type samplers were installed. They have a slot-like opening with the length of 2 m and broad of 0.9 cm. They were slightly inclined towards one end and at the height of 40 to 80 cm. At the end of the lower part of each gutter PE tube leads to 5 L PE bottle, installed in the ground preventing the sunlight to reach the samples. Ten gutters were arranged in two lines by five. For the chemical analysis the samples from each five gutters were bulked into one sample. The precipitation samples were collected biweekly during one year and their volume was measured separately using a graduated cylinder and marked on the mailing form to be sent with the samples to the laboratory. The analyses were performed on filtered samples (0.45 µm) in the laboratory. The concentrations of nitrate (NO_3^-) and ammonium (NH_4^+) in collected samples were determinated using ion chromatography (Croatia-Dionex, USA and Slovenia-Metrohm, Swizzerland). Results were used for calculation of annual bulk and throughfall deposition of nitrogen. Analysis and data validation were in strict agreement with those adapted in the ICP Forests [10].

Table 1. Characteristics of plots in Croatia and Slovenia

Plot Code	Plot name	Age (y)	Longitude	Latitude	Main tree species	Tree density (n ha^{-1})
110 HR	Jastrebarski lugovi	136	+154134	+453842	*Quercus robur* L.	596
11 SLO	Murska šuma	130	+163100	+462900	*Quercus robur* L.	207

Results and Discussion

Measured average annual bulk deposition of nitrogen is plotted against the throughfall deposition for both plots (Figure 1) over the period 2008-2010. Measured average throughfall deposition of nitrogen declined from 14.28 kg ha^{-1} y^{-1} in 2008 to 3.39 kg ha^{-1} y^{-1} in 2010 on Croatian plot and from 18.59 kg ha^{-1} y^{-1} in 2009 to 9.75 kg ha^{-1} y^{-1} on Slovenian plot. Also, decline was measured for bulk deposition of nitrogen on Croatian plot but not for Slovenian plot where nitrogen deposition increased from 9.55 kg ha^{-1} y^{-1} in 2008 to 14.02 kg ha^{-1} y^{-1} in 2010. The annual amount of precipitation in bulk open field collectors was the highest in 2010 on both plots (1040 L/m^2 on plot 110 and 985 L/m^2 on plot 11) and in thoughfall collectors was the highest in 2010 on plot 110 (863 L/m^2) and in 2009 on plot 11 (716 L/m^2). The lowest amount of precipitation was recorded in 2008 on both plots (514 L/m^2 on plot 110 and 536 L/m^2 on plot 11). Smaller amount of precipitation was expected in THR samples due to the fact that these samples were sampled beneath the forest canopy and containing bulk+leached+dry deposition-absorbed ions. The critical load has been defined as „a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitivity elements of the enviroment do not occur according to present knowledge“. The loads above critical may have adverse impacts on tree health, such as nutrient imbalances and increased shoot-root ratios causing drought stress. On the other hand, input below 14 kg ha^{-1} y^{-1}, may hamper tree growth due to limited nitrogen availability [11]. Calculated annual average bulk open field and throughfall nitrogen deposition on Croatian plot are below the critical load for nitrogen (21-42 kg ha^{-1} y^{-1}) (Table 2). Nitrogen bulk and throughfall deposition were the highest in 2008 on plot 110. On plot 11 nitrogen bulk deposition was the highest in 2010 but throughfall deposition was the highest in 2009. The nitrogen deposition is higher on plot 11 than on plot 110. Results of this study are in agreement with results obtained in Jakovljević et al. [12] where atmospheric deposition measurements was performed in the lowland forest ecosystem of Pokupsko basin in Croatia. Although critical loads for nitrogen are exceeded on many plots in Europe, results of this study showed that nitrogen loads for bulk and throughfall deposition are not exceeded the critical load on both plots. Therefore, it`s still not influencing the stress on the *Quercus robur* L. forest ecosystem. For comparison, according to Ferretti et al. [13] average

N throughfall deposition in Italy over the period 2000-2009 ranged between 4 and 29 kg ha^{-1} y^{-1}, with critical loads exceeded at several sites.

Table 2. Actual and critical nitrogen loads for bulk open field (BOF) and throughfall (THR) on Level II plots in Croatia and Slovenia over 2008-2010

Year	Plot	Critical nitrogen loads (kg ha^{-1} y^{-1})	BOF (kg ha^{-1} y^{-1})	THR (kg ha^{-1} y^{-1})
2008	110	21-42	11.90	14.28
	11		9.55	13.43
2009	110		10.34	9.21
	11		8.57	18.59
2010	110		5.25	3.39
	11		14.02	9.75

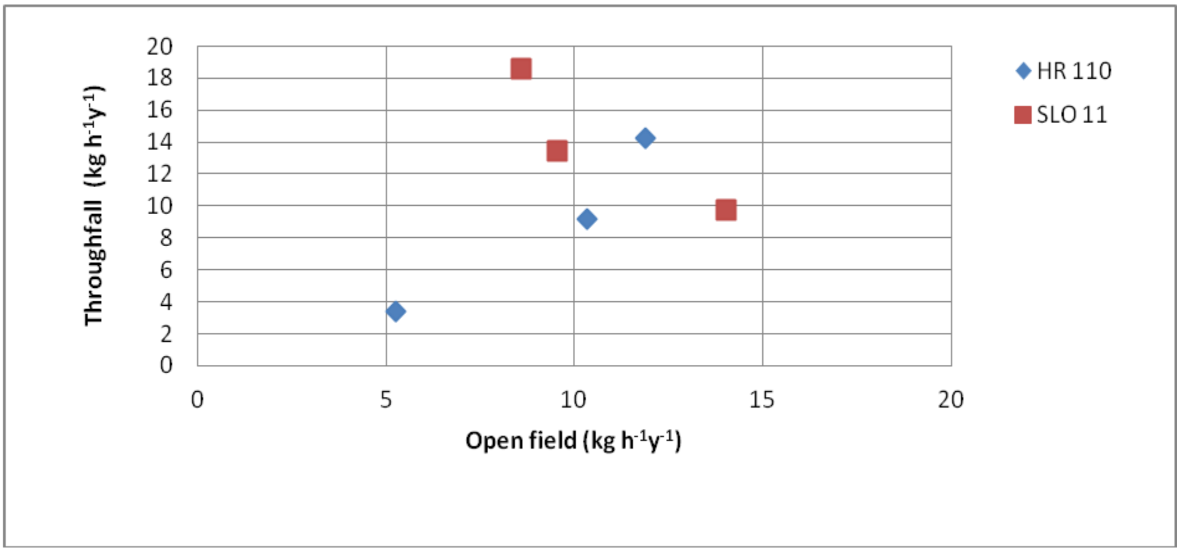

Figure 1. Bulk deposition of nitrogen plotted against the throughfall deposition for both plots

Conclusions

Unlike in some European countries, results of actual loads of nitrogen in the examined plots showed that their values were lower than the critical load for both type of samples. Therefore, it`s still not influencing the stress in the *Quercus robur* L. forest ecosystem. The average nitrogen deposition is higher on plot 11 (Slovenia) than on plot 110 (Croatia). For the assessment of ecosystem response to deposition it is crucial to know critical load for every plot under consideration. Future studies should therefore focus on calculation of critical load because it depends on biogeochemical processes in forest ecosystem. Calculated annual average bulk open field and throughfall nitrogen deposition on Croatian and Slovenian plot are below the critical load for nitrogen (21-42 kg ha^{-1} y^{-1}). The results also give an opportunity to extend the research on other permanent plots in Croatia and Slovenia.

Considerable reduction in industrial emissions and the resulting notable improvement of air quality in Europe were partly achieved due to the successful work of ICP Forests. This multifunctional monitoring programme offers a very good tool to record the extent and intensity of risk factors and to monitor and assess reactions of forest ecosystems. Such results contribute to the scientific basis for the development and reviews of the effectiveness of clean air politics.

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BOX TREE MOTH (*Cydalima perspectalis*), NEW INVASIVE INSECT PEST IN CROATIA

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Introduction

Alien species are considered as one of the major threats to biodiversity after habitat destruction (1, 2) causing enormous damage to ecosystems and economies (2, 3, 4). Hundreds of species are intentionally and unintentionally moved worldwide (5) and these introductions have been accelerated all over the world due to the increasing mobility of people and goods over the past decades (6). While a certain number of alien insect species have little impact and are thus rarely noticed, some cause substantial damage to plants and the environment, and may have catastrophic effects on biodiversity. Box tree moth (*Cydalima perspectalis* Walker, 1859; Lepidoptera; Crambidae) is one of the most recent introductions to Europe (7) as well as to Croatia (8) causing serious damage to ornamental box (*Buxus* sp.) shrubs and trees. This rapid spread and establishment in European countries can be attributed to the ornamental plant trade as in particular box plants (*Buxus semperivrens* L.) are very popular ornamental garden plants. It is thought that the species was originally introduced with imports from China (7). The larvae of the box tree moth are defoliating the plants posing a serious threat to these popular ornamentals especially in historical and formal gardens, hedging and topiary (7, 9).

Materials and Methods

Young larvae (first and second instars) of box tree moth were collected on various locations in Croatia, they were brought to entomological laboratory, Croatian Forest Research Institute and reared to pupae and moths. All developmental stages were photographed with Olympus camera E30 and Olympus stereo microscope SZ X7 (0,5x). The adults were identified according to Mally and Nuss 2010 (10).

Results and Discussion

The box tree moth was recorded for the first time in North Croatia in August 2013 when larvae were found defoliating box plants (*B. sempervirens*) in Arboretum Opeka, Vinica. The larvae and moths were identified as *C. prespectalis*. According to damages it can be assumed that the pest has been introduced to the region earlier (in 2011 or 2012) and that the primary infection has been undetected. In 2014 the pest has spread rapidly all over Croatia, in continental as well as mediterranean parts making serous damages and defoliating box plants. During this research we could not define the exact number of generations as first damages were visible in August but at least two generations per year could be assumed in Croatia in 2013 with possible three generations in 2014. The box tree moth has two to three generations per year in Europe, while in the native range up to 5 generations per year are possible (11). It overwinters as larva, spinning a cocoon between box leaves in autumn and completing its development the following spring. The damage caused on box tree plants at the locality of research was found to be serious. Young larvae feed in the lower surfaces of the leaves only and leave the upper epidermis intact, whereas older larval stages feed inside the webbing, leaving only the midribs intact, they also eat green bark of the young twigs. Younger larval instars feed sheltered between two spun leaves and later instars rest during the day in loosely spun webbing where they also overwinter. Webbing and larval excrement were found between leaves and twigs. After overwintering, the larvae continue feeding until the end of March and when fully grown, they pupate and the moths of first generation appear (5). The damaged box plants lose their amenity value as garden plant since defoliation is visible particularly on lower branches. Almost 90% of alien invertebrates in Europe were introduced unintentionally through human activities, mostly as contaminants of a commodity (12). The main pathway of introduction of alien and invasive insect species on trees and shrubs is trade of ornamental plants (13). There is a strong suspicion that ornamental plants are one of main pathways of introduction of alien insects to Croatia due to the increase of the imported volumes from year to year (14). Box tree hedges have an important value in historical gardens and are essential element of gardens and parks (15). It is likely that the box tree moth reached Europe on horticultural box tree plants imported from China (7). Eggs and small larvae are difficult to detect and are easily dispersed with contaminated plants. The box tree moth is a good flyer, so it can also disperse naturally (5 km/year) (16), with several generations per year and good flying abilities it has a relatively high self spread potential. It easily spreads from contaminated areas as its host plant is extensively traded all over Europe being one of the most popular and widely planted ornamental plants. The defoliation reduces the amenity value and repeated severe defoliation can result in the death of plants (7). If larvae, pupae or moths are found on box plants they cannot be misidentified for another species as this is the only one so far that makes such visible and characteristic damage. Ecological impact and damage may become particularly important when this pest reaches the main areas of natural distribution of Buxus spp. in Europe such as France, the Pyrenees, Montenegro and F.Y.R.O.M. where the European box tree is an essential component of unique forest ecosystems (7, 17). No natural enemies have been recorded in Europe so far (7) while it is neither attacked by predators (birds) because of the toxicity of the host plant (15).

Conclusion

Given all these circumstances this invasive pest has very favourable conditions (no natural enemies, favourable climate, widely available host plant) for spreading and establishing in new areas. Seven years after the first introduction, the pest has either naturally spread or been introduced multiple times and consequently it is now established widely across Europe (7).The pest is now recorded widely in Croatia and, based on its potential, it can be serious damages to box plants can be expected in the following years and thus becoming threat to gardens and parks in Croatia.

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RELATIONSHIP OF STAND STRUCTURE IN CROATIAN PEDUNCULATE OAK AND COMMON HORNBEAM FORESTS WITH GROWTH AND YIELD TABLES AS GUIDELINES FOR FOREST MANAGEMENT: CURRENT STATE AND CHALLENGES

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Introduction

Changes of climatic and other site factors, usually anthropogenic in origin, are exerting significant pressures on growth and development of managed forest stands over the last few decades (1,2). As a consequence of the changes, significant alteration of forest stand structures may arise. Given the extent and intensity of recent changes, as well as their complexity, challenges for forest management planning are growing.

In Croatia, similar to many countries in the region and Central Europe, growth and yield tables are still used as major guidelines for forest planning and management. Foundations of these tables are usually mathematical models which are static in nature and are developed on the data collected long before the onset of the climate change.

Intention of our contribution is to relate the actual state of the structural elements of mixed pedunculate oak and hornbeam forests in Croatia with the values presented in the growth and yield tables of domestic authors currently in everyday use. Our goal is to assess the rate of (diss)agreement between the actual condition of these stands and the theoretical models used as the guidelines for their management. Comparison is based on stand basal area, given the fact that this stand structural element is used as the basis for determination of stocking and normality of the stand. All prescriptions for future management are than derived from these values.

Materials and Methods

For our research we have utilized database of "Croatian forests" Ltd. ("HS Fond"), from which we extracted data on 5,060 subcompartments with total area of 76,000 ha through seven filtering steps. Methodology we applied allowed us to analyze 77% of the total area of pedunculate oak and common hornbeam stands in Croatia. Prescribed rotation period for the observed forest stands is 140 years. Stand structure was analyzed on stands from site class I, due to the fact that the majority of these forests in Croatia (68% by number and 71% by area) grow on site class I (3).

Development of stand density and total stand volume during the stand rotation was related to the corresponding values given by the growth and yield tables (G&YT) of domestic authors. For the purpose of comparison we have used three most common G&YT in Croatia: Špiranec G&YT for pure oak stands on I. site class (4), Meštrović G&YT for mixed pedunculate oak and common hornbeam stands on I. site class (5) and EGT II-G-10 G&YT for mixed pedunculate oak and common hornbeam stands (6). Furthermore, we made a graphical comparison of stand basal area development for actual stands with the values given in three G&YT, and the analysis of stand stocking by basal area. Distribution of the grade of agreement with the G&YT was also made.

Results and Discussion

Development of the stand density and total stand volume through the stand age was compared with the values given in three G&YT of domestic authors (Figure 1 and 2, respectively).

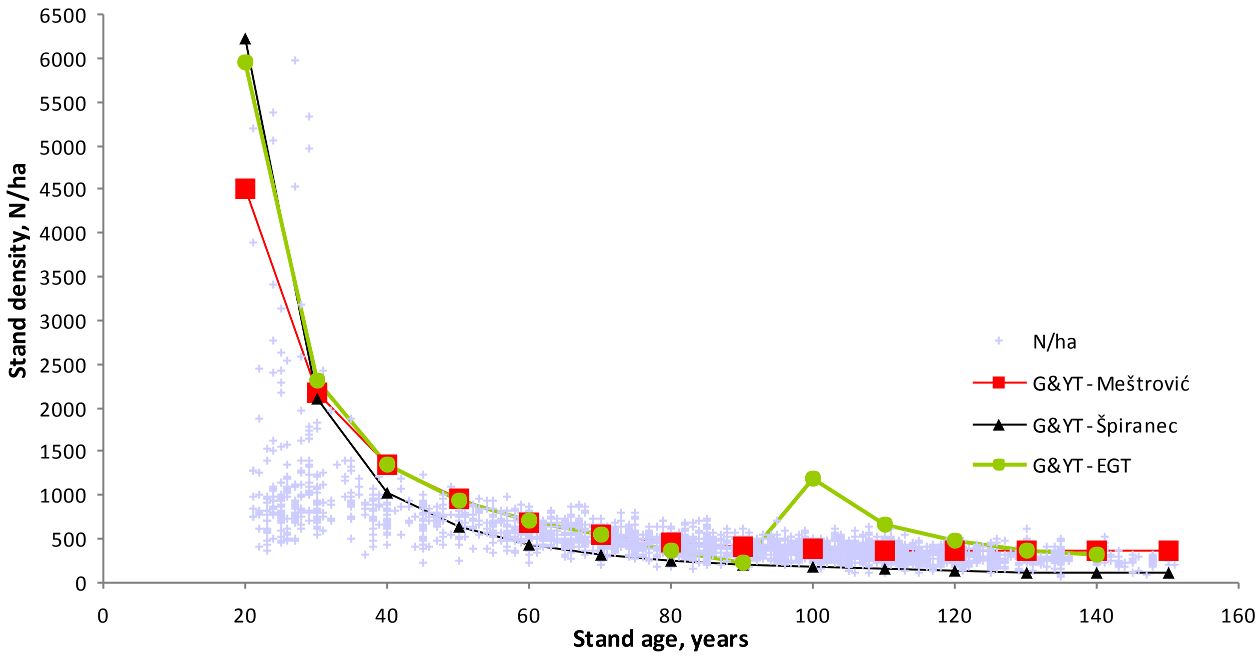

Figure 1. Comparison of actual stand density and the values from three G&Y tables (I. site class)

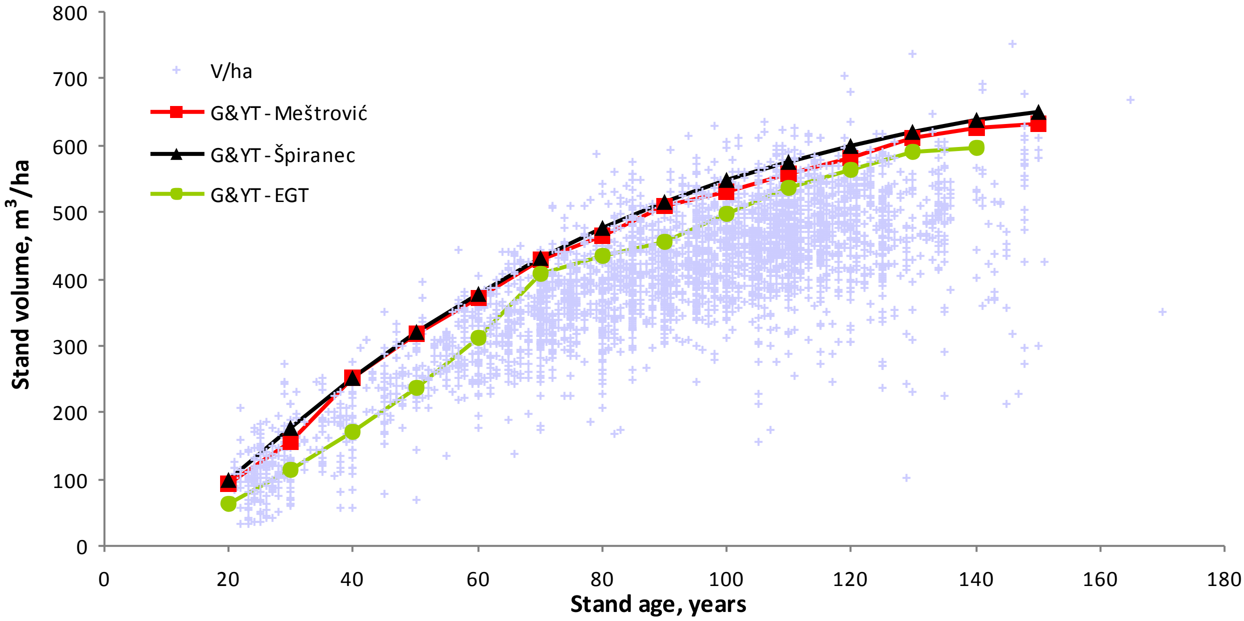

Figure 2. Comparison of actual total stand volume and the values from three G&Y tables (for the I. site class)

Presented values of stand density and total stand volume by stand age indicate high variability of those structural elements. It is easy to notice that values given by three G&YT account for only a minor share of analyzed stands.

Stand basal areas of actual stands are graphically compared with values given by G&YT in Figure 3. Normality of the stands, i.e. their stocking according to each of the three G&YT, is calculated as a ratio of actual stand basal area and the basal area given in G&YT for corresponding stand age. The results are given graphically in Figures 4, 5 and 6.

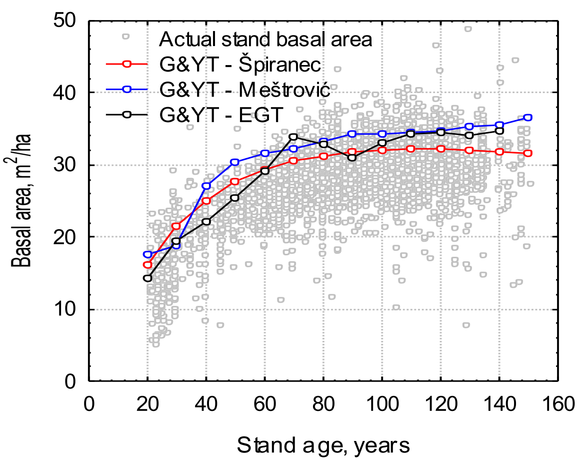

Figure 3. Comparison of the observed total stand basal areas and the values from three G&Y tables for the I. site class

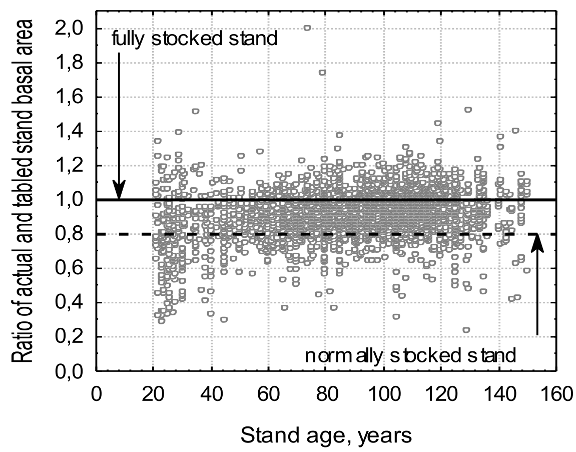

Figure 4. Ratio of observed total stand basal area and the values given in the Špiranec G&Y tables over stand age (I. site class)

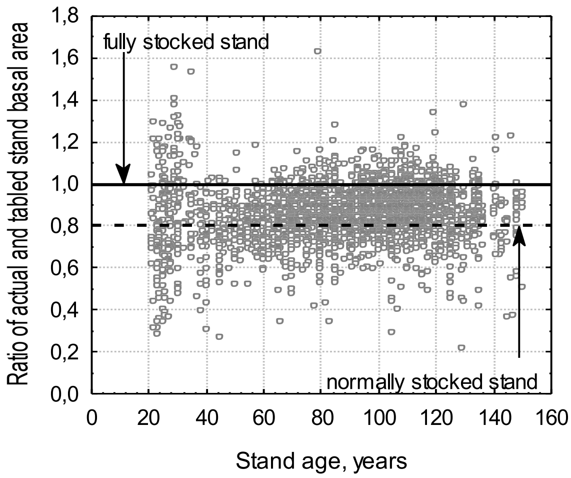

Figure 5. Ratio of observed total stand basal area and the values given in the Meštrović G&Y tables over stand age (I. site class)

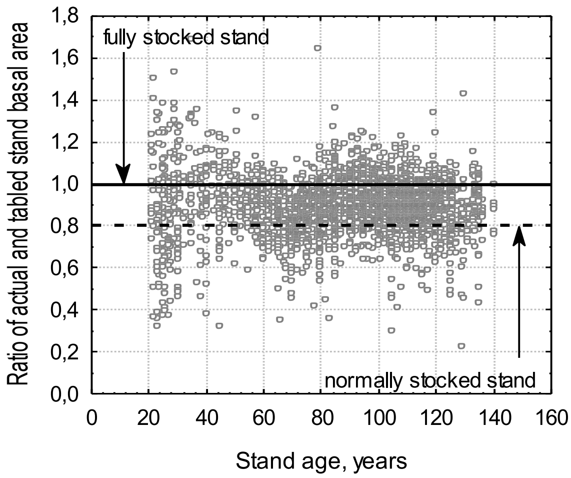

Figure 6. Ratio of observed total stand basal area and the values given in the EGT G&Y tables over stand age (I. site class)

Guideline values of stand basal area as proposed by the G&YT correspond only to a minor share of actual forest stands, as presented in Figure 3. With respect to stand stockings given in Figures 4, 5 and 6, it can be concluded that significant share of analyzed stands corresponds to "normal" stocking, i.e. with stocking values between 0.8 and 1.0. However, there is still a large share of stands that can be classified as under-stocked (stocking < 0.8) and over-stocked (stocking > 1.0) (7).

Share of stands with respect to stocking degree is further elucidated according to stand age classes in Tables 1 and 2 for G&YT of Meštrović and EGT II-G-10, respectively. Results indicate somewhat better agreement in stockings for EGT II-G-10 G&YT, both by stand age classes and in total.

Table 1. Number of stands by stocking degree according to Meštrović G&YT and age classes

Stocking	Age class – AC (width of AC in years)							Total		Total without VIII AC	
	II (0-20)	III (21-40)	IV (21-40)	V (21-40)	VI (21-40)	VII (21-40)	VIII (141-160)	n	%	n	%
>1,0	65	39	14	98	55	14	4	289	8,37	285	8,40
0,8-1,0	133	159	356	672	834	248	34	2436	70,55	2402	70,77
0,5-0,8	76	37	222	97	162	78	19	691	20,01	672	19,80
0,5<	17	1	4	2	5	6	2	37	1,07	35	1,03
Total	291	236	596	869	1056	346	59	3453	100,00	3394	100,00
% of stocking >1,0	68,04	83,90	62,08	88,61	84,19	75,72	64,41	78,92	78,92	79,17	79,17
% of stocking between 0,8 and 1,0	45,70	67,37	59,73	77,33	78,98	71,68	57,63	70,55	70,55	70,77	70,77

Table 2. Number of stands by stocking degree according to EGT- II-G-10 G&YT and age classes

Stocking	Age class – AC (width of AC in years)							Total		Total without VIII AC	
	II (0-20)	III (21-40)	IV (21-40)	V (21-40)	VI (21-40)	VII (21-40)	VIII (141-160)	n	%	n	%
>1,0	45	3	14	28	45	8	4	147	4,26	143	4,21
0,8-1,0	114	125	381	622	819	234	33	2328	67,42	2295	67,62
0,5-0,8	110	107	196	217	187	97	20	934	27,05	914	26,93
0,5<	22	1	5	2	5	7	2	44	1,27	42	1,24
Total	291	236	596	869	1056	346	59	3453	100,00	3394	100,00
% of stocking >1,0	54,64	54,24	66,28	74,80	81,82	69,94	62,71	71,68	71,68	71,83	71,83
% of stocking between 0,8 and 1,0	39,18	52,97	63,93	71,58	77,56	67,63	55,93	67,42	67,42	67,62	67,62

Conclusions

Current condition of managed stands of pedunculate oak and common hornbeam is characterized by the large variability of stand structural elements within the same age class, in all stages of stand development.

Comparison of basal area of investigated stands with values given by three G&YT of domestic authors revealed that approximately 20% (according to EGT II-G-10 G&YT) up to 30% (Meštorvić G&YT) of the stands have stocking less than normal (i.e. under 0.8). With respect to age classes, share of stands with stocking under 0.8 is even larger (35-45%) in young and middle-aged stands (II, III and IV age class).

Forest stands included in this research are managed for several decades according to the domestic G&Y tables. Apparently, goals set in these tables were not reached, therefore the question arises on the underlying causes of the established discrepancy between the actual forest stand and the values of G&Y tables used as management guidelines.

Raised question demands a concrete answer in order to facilitate successful and sustainable management of these highly valuable forest stands in Croatian forestry during the next decades. To develop a proper answer, forest science and forestry practice will have to address a series of further questions and serious challenges, of which we name only the few most important:

- ✓ What is the actual practical value of stocking expressed as a ratio of actual stand basal area and the basal area for a corresponding stand age from G&YT, given that the G&Y tables are simple mathematical models static in nature, developed on data collected before the onset of observed site and climate changes?
- ✓ Is stocking expressed as a ratio of actual stand basal area and the basal area for a corresponding stand age given by the G&Y tables enough for forest practitioner to assess all segments of forest stand structure that are important for forest

management, furthermore, is this ratio good enough basis for planning of future forest management?

- ✓ What would be the optimal way to integrate climatic and anthropogenic changes into predictions of future stand development, given the fact that G&YT are not dynamic models, i.e. they do not posses mechanisms of adaptation to the changes of basic conditions for growth and development of forest stands?

In the times of climate change it is a significant challenge to manage stable and healthy pedunculate oak and common hornbeam forest stands that would simultaneously provide for ecological, sociological and economical needs of the society. Only with right and timely management actions based on precise analyzes of past and current state of forest stands and with accurate prognosis of future stand development can this challenge be surmounted. The analyzes needed are only obtainable with the help of advanced computer simulators which use adequate number of key variables to describe the complexity and dynamics of forest stand development while taking into account constantly changing site and climate factors.

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THE EVALUATION OF GROUND VEGETATION STRUCTURE BY THE TIME SERIES ANALYSES IN TURKISH RED PINE (*Pinus brutia*) PLANTATIONS IN THE WESTERN BLACK SEA REGION IN TURKEY

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Introduction

There are various vegetation types along the climatic and topographic gradient in Turkey [1]. Especially in the Western Black Sea Region of Turkey, they contain a number of differing biotopes such as grassland above the timber line, evergreen coniferous and deciduous broad-leaved forests, scrubland, river beds, coastal woodland, and sand dunes. And, the cold climate in the north and semi-arid zones in the south, help to enhance the ecological diversity of the Anatolian Peninsula and there is a considerable diversity of flora in the mountainous region [2]. If global warming occurred, it will most likely affect the vertical distribution of the vegetation of this region through changing complexly the distribution of each species [3].

Material and Methods

The field surveys of vegetation distribution and land use were done on the basin of the Filyos and Bartın rivers from 2000 to 2014, and 50 experimental plots were set to investigate the stand structure of main forests in the research area. Coniferous forest dominated by *Pinus brutia* Ten. is to be found up to an altitude of 550-1000m and is replaced by *Abies nordmanniana* subsp. *Bornmülleriana* Mattf., *Platanus orientalis* L. and *Carpinus betulus* L. dominates in the gallery forests (near the Bartın and Filyos rivers). Furthermore, up to an altitude of 550m, a dense, scrubland, dominates and ground vegetation of *Pinus brutia* Ten. plantation forest becomes rich. The ground vegetation mainly consists of several species of evergreen shrub.

In this study, the changes in structure of forest vegetation in *P. brutia* plantation area in Bartın-Gözpınarı region have been investigated in detail. Within the scope of these investigations, both the annual and perennial plants constituting the vegetation have been determined, and also the changes in these species in 14 years between 2000 and 2014 have been examined. For this purpose, the number and kind of species have been determined by sampling randomly from 30 study areas in 550-1000m altitude zones, and by taking photos. Also, in mentioned 50 study areas, the density, distribution and frequency of the species have been determined via Braun-Blanquet method [4]. These values have been used as dependent and independent variables in determining the changes in-time in population dynamics of the species located in *P. brutia* plantation areas and constituting the vegetation.

In this research, 2 statistical methods, the principal component analysis and time series analysis, were used. SPSS package statistics software was used in implementation of these methods. Also, while implementing the time series method, non-linear time series models have been used because of continuously varying structures and composition rates of the plants. For this purpose, Kolmogorov-Simirnov test has been used to all the frequency values determined in 30 study areas where the plants have been collected.

In this study, Principal components analysis (PCA) is a technique used to reduce multidimensional data sets to lower dimensions for analysis. PCA is mathematically defined as an orthogonal linear transformation that transforms data to a new coordinate system such that the projection of data on the first latent coordinate (called the first principal component) has the highest variance; projection to the second on the second principle component has the second greatest variance, and so on. A time series is a set of observations where each observation is recorded at a specific time. In discrete time series, the observations are made as a discrete set. In this case the observations are made at fixed time intervals [5]. Most of discrete time series are constructed by sampling of continuous time series. The time series is said to be deterministic, if the future values of a time series are exactly determined by some mathematical function, while statistical time series are results of stochastic processes and governed by an underlying probabilistic mechanism [6]. Stationary processes are a very special class of stochastic processes and based on the assumption that the process is in a particular state of statistical equilibrium [7]. Stochastic processes are called “strictly stationary processes” if the probability distribution does not change with respect to time. A process is “weakly stationary” if the mean (μ) and variance (σ) are constant. In this study, ARIMA (3,1,2), ARMA (3,1) and ARIMA (3,1,1) models are frequently encountered [5].

Results and Discussion

Above the timberline, which is at about 1000m above sea level, sub-alpine grasslands, characterized by thorn-cushion formations of *Cyclamen sp., Lysimachia vulgaris L., Primula vulgaris Huds., Ranunculaceae, Ranunculus constantinopolitanus (DC.) Urv., Ranunculus repens L., Ranunculus ficaria L., Delphinum sp., Helleborus orientalis Lam., Hypericaceae (Guttiferae), Hypericum perforatum L., Fabaceae (Leguminosae), Galega officinalis L., Genista sp., Lathyrus aphaca L., Lotus corniculatus L.,Melilotus officinalis L., Ononis spinosa L., Sophora jaubertii Spach., Trifolium medium L.,Trifolium repensL.,Trifolium pratense L. RosaceaeAgrimonia eupatoria L.Filipendula vulgarisMoench , Apiaceae (Umbelliferae), Angelica sylvestris L., Daucus carotaL., Pimpinella saxifragaL., Asteraceae (Compositae), Bellis perennis L., Centaureasp.Gundelia tournefortii L., Jurinea sp., Lapsana communis L., Matricaria chamomilla L.,Petasites sp.,Pilosella hoppeana (Schultes) C.H. & F.W. Schultz., Pilosella auriciloides(A.F. Lang) Sell & West, Senecio vulgaris L., Taraxacum officinale Weber, Tussilago farfara L., Dipsacaceae, Dipsacus laciniatus L., Campanulaceae, Campanula* rapunculus (L.) var. lambertiana (A.DC.) Boiss., dominate.

Between 700-1200m and 2000m, *Castanea sativa* Mill*., Pinus pinea L.,* and *Pinus nigra* Arnold. form a montane forest type. Various species of deciduous oak, such as *Quercus cerris* L., and others like *Ostrya carpinifolia* Scop*., Carpinus orientalis* L*, Fraxinus sp*. and *Sorbus sp*. mix with conifers or dominate. *Fagus orientalis* Lipsky. and *Quercus robur* L. appear partly at the middle and lower parts of this range. *Styrax officinalis*, *Daphne oleoides* Schreb*, Sambucus nigra* L. etc. are seen at lower tree layer and the shrub layer, too [2].

Around the river estuaries, salt marshes cover large areas on the hydromorphic alluvial soils. This type of biotope has an almost uniform vegetation aspect, consisting of halophytes such as; *Pilosella auriciloides(A.F. Lang) Sell & West,Senecio vulgaris L., Taraxacum officinale Weber, Tussilago farfara L., Dipsacaceae, Dipsacus laciniatus L., Campanulaceae, Campanula rapunculus (L.) var. lambertiana (A.DC.) Boiss., Campanulasp., Gentianaceae, Centaurium erythraeaRafn., Geraniaceae, Geraniumsp., Geranium pyrenaicum Burm., Brassicaceae (Cruciferae), Arabis sp., Capsella bursa-pastoris (L.) Medik, Convolvulaceae, Convolvulus arvensis L., Boraginaceae, Echium vulgareL., Verbenaceae,Verbena officinalis L., Lamiaceae (Labiatae),Lamium purpureum L.,Mentha piperita L., Marrubium vulgareL.,Nepeta catariaL.,Prunella vulgarisL.,Prunella laciniata (L.) L.,Salvia tomentosa Mill., Salvia verticillataL., Salvia forskahleiL. Lythraceae, Lythrum salicariaL., Plantaginaceae, Plantago lanceolata L.,Plantago majorL., Euphorbiaceae,Euphorbia helioscopiaL., Rubiaceae,Galium palustreL., Galium verum L.,Sherardia arvensis L., Urticaceae,Urtica dioica L., Violaceae,Viola sp., Scrophulariaceae, Veronica sp., Liliopsida (Monocotyledoneae), Amaryllidaceae,Galanthus plicatus Bieb. subsp. Byzantinus (Baker) D.A. Webb., Juncaceae, Juncus effususL., Typhaceae,Typha latifolia L., Liliaceae,Muscari sp* [8]. On the other hand, thus, the lower area on the basin of the Kozcağız and Bartın rivers is already considered to be close to the lower limit of the forest and to be very sensitive to environmental change.

As a result of time series models implemented according to non-linear ARIMA models, the changes during 14 years in P. *brutia* plantation zones located in riverside zone up to 550 m of altitude, in forest and woodland zone between altitudes of 550m and 1000m, and in grassland zone over altitude of 1000m are presented in Figures 1, 2, and 3.

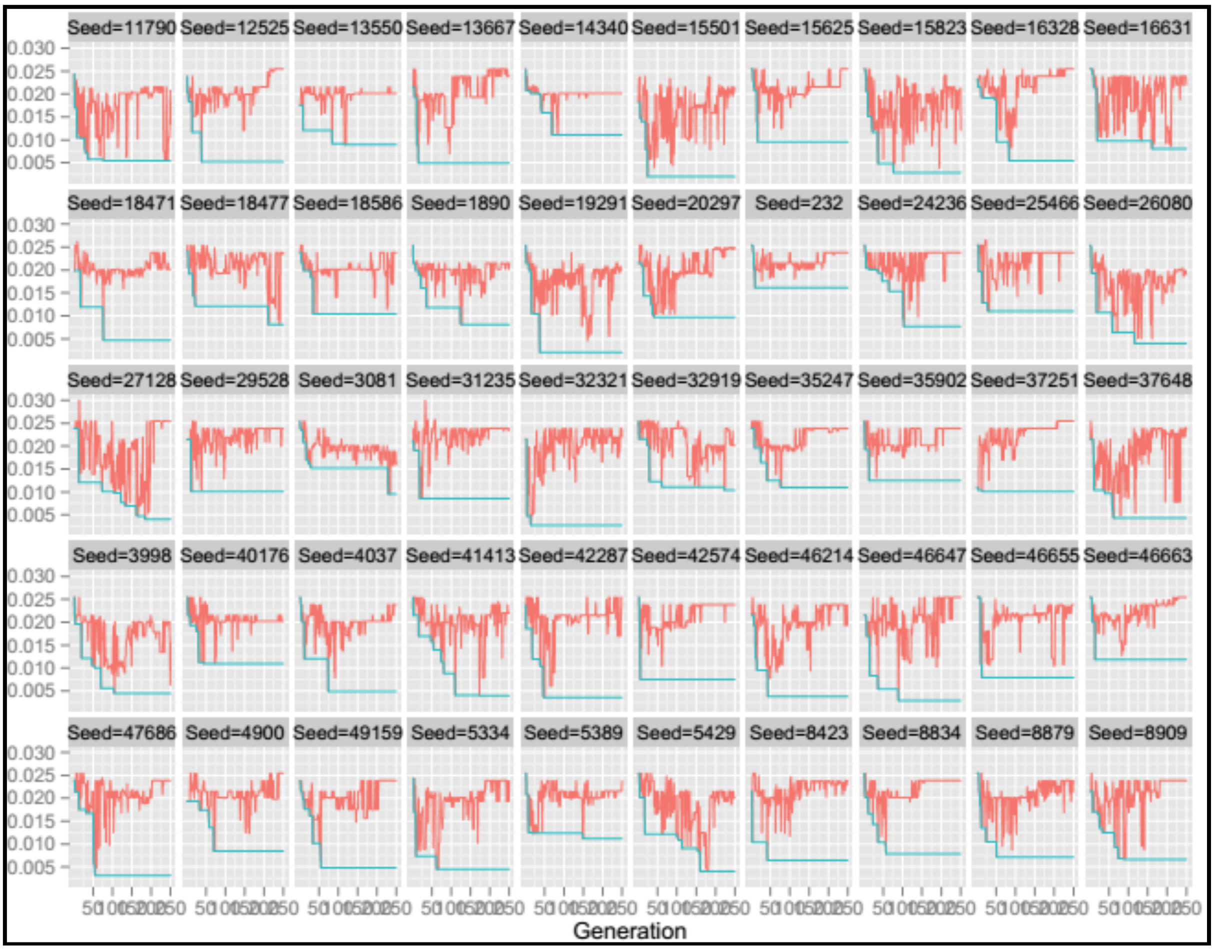

Figure 1. Change in structure of vegetation in study areas in riverside zone up to 550m during 14 years

Ehen Figure 1 is evaluated; it can be seen particularly in riverside zone that significant decreases occurred in 26 families and 34 taxons in 14 years due to pasturage, agriculture and residence activities. Thus, the results of a study of Yılmaz [2] carried out in Bartın region corroborate this situation. In Figure 2, the changes in species constituting the vegetation in forest and woodland zone in altitudes of 550-1000m during 14 years are presented.

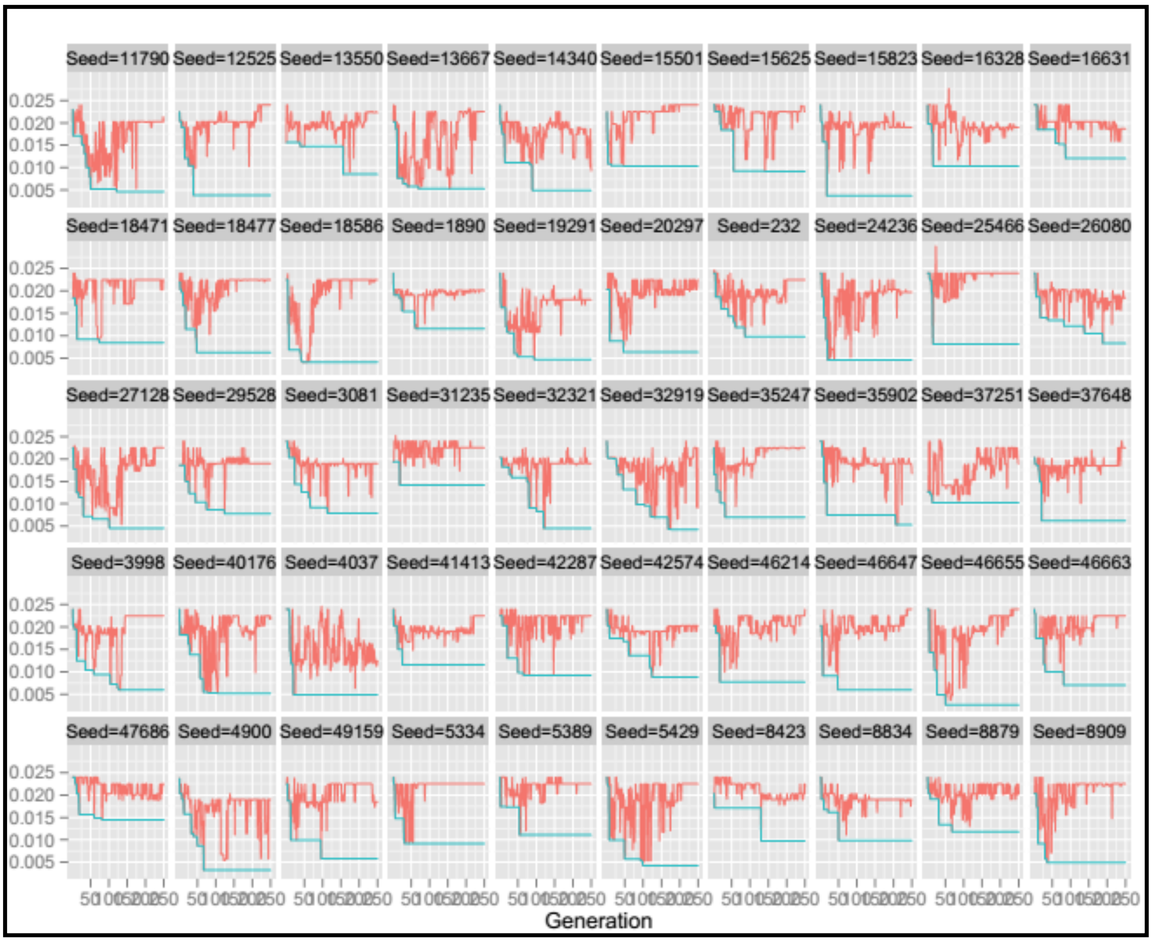

Figure 2. Change by study areas during 14 years in structure of vegetation in study areas in forest and woodland zone between altitudes of 550m and 1000m

When Figure 2 has been evaluated, it has been determined that the forest vegetation located in altitude level of 550-1000m has been dramatically demolished in first years, and this vegetation structure has recovered itself with success in *Pinus brutia* plantation efforts in region, and the species diversity has increased. Also in this duration of 14 years, significant decreases occurred in 17 families and 25 taxons at this altitude level. In another study on this topic, similar results have been obtained [8]. The changes in plant cover in natural grassland zones over altitude of 1000m are presented in Figure 3.

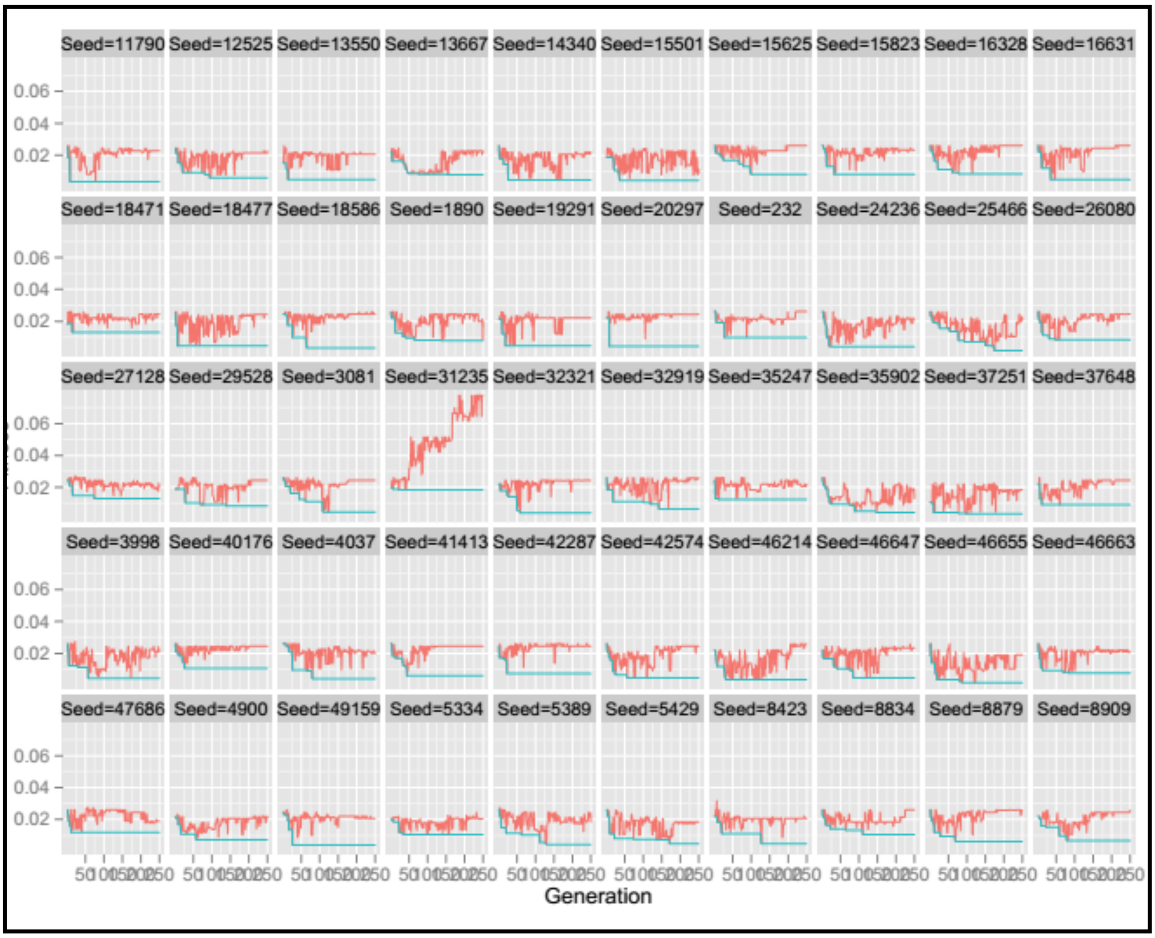

Figure 3. Change by study areas during 14 years in structure of vegetation in study areas in natural grassland zone over altitude of 1000m

When Figure 3 was evaluated, it has been determined that the plant diversity in natural grassland zones 0ver altitude of 1000m in Gözpınarı region, where the study has been carried out, has been significantly damaged as a result of wrong pasturage activities in years 2000, 2001, and 2002. But, as a natural result of plantation efforts with *Pinus brutia* in forest zone, this pastureland has been protected, and significant improvements occurred both in diversity and species composition dynamics. Particularly in recent period covering the years 2011, 2012, 2013, and 2014, it has been observed that species diversity dynamism has followed a stable course. In performed investigations identification of the plants, it has been determined that some decreases have been observed in 11 families and 23 taxons at mentioned altitude level during 14 years. Thus, in another study carried out in natural grassland in region, it has been determined that significant decreased occurred in approximately 20-65 taxons [9].

Under the light of the findings obtained, making unproductive forest lands become productive again by carrying out plantation activities with appropriate species and origins in medium altitude belts in order to protect both the riverside zone and the natural grassland zone in higher altitudes in study area and similar regions is of great importance. Moreover, the forest structures established via successful foresting activities provide important benefits from the aspect of ensuring the sustainability of existing natural plant composition and protecting the plant species located within the established forest structures and facing with extinction threat.

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Juniperus oxycedrus


Quercus cerris


Ostrya carpinifolia


Carpinus orientalis


Fagus orientalis


Styrax officinalis


Daphne oleoides


Sambucus nigra


Pinus brutia


Platanus orientalis


Tamarix smyrnensis


Nerium oleander


Cotinus coggyria


Quercus coccifera


Phillyrea latifolia


Myrtus communis


Arbutus andrachne


Erica manipuliflora


Pistacia terebinthus


Pistacia lentiscus


Cercis siliquastrum


Cistus sp.


Lithodora hispidula


Rubus sanctus


Calicotome villosa


Capparis spinosa


Paliurus spina-christi


Quercus ithaburensis


Prosopis farcta


Phragmites australis


Arthrocnemum fruticosum


Salicornia europaea


Atriplex portulacoides


Pinus halepensis


Juncus maritimus


Vitex agnus-castus


Imperata cylindrica


Cionura erecta


Cakile maritima


Salsola kali


Saccharum ravennae


Ipomoea stolonifera


Pancratium maritimum


Euphorbia peplis


Medicago marina


Zygophyllum album


Echinops ritro


Eryngium maritimum

NUTRITIONAL STATUS AND STRESS TOLERANCE INDEX IN EFFECTIVE SELECTION OF POPLAR CLONES

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Introduction

The assessment of poplar clone productivity, with the goal of selection of elite genotypes for diverse ecological conditions, is the main pillar of tree breeding. The significance of GEI (genotype x environment interaction) in the volume stock production is the consequence of differences among genotypes on different sites or the changes in the relative ranking of genotypes [1].

Biotope-related stress is mostly linked to the low availability of elementary plant requirements, such as water and nutrients. There are three main factors that determine a success in poplar production: well aerated soil, favourable water supply and adequate mineral nutrition [2]. In the last decade the problems in management of poplar plantations and cultures (low increment, dieback of trees, difficult reforestation) in eastern Slavonia are becoming more common. As these are concurrent with the significant changes in the regime of floods and groundwater table, it is logical to assume that these phenomena are related. The stress caused by drought is characterized by loss of water, reduced water potential and leaf turgor loss with stomatal closure, which leads to a halt photosynthesis, disruption of metabolism and ultimately to the death of plants [3]. The growth and development of fast-growing trees such as poplars is dependent on the significant water consumption – for poplar plantations about 500 mm for a vegetation season [4], significantly more than the autochtonous forest vegetation. The understanding of morphological-anatomical and physiological changes caused by the lack of water can be used as a tool in tree breeding selection [5].

Optimum nutrition of fast-growing trees – given the adequate climatic conditions, water supply and soils free from toxic substances – is the most important prerequisite for high and lasting yields [6]. One of the most important goals of poplar selection is finding the genotypes that can produce high volume stock with a lower nutrient consumption. While for cultivation on most productive and optimal sites the genotypes with high demand for nutrients and water are most interesting, but selection of genotypes with smaller requirements will benefit the efficiency of poplar cultures on less productive sites [2]. The selection of poplar genotypes with a high adaptation value (high stress tolerance and potential production) in various stand conditions is the main goal of selection of poplar clones in eastern Slavonia.

Materials and Methods

Analyses and measurements included two poplar clones: 'M1' (*P. xcanadensis*) and 'S 1-8' (*Populus deltoides*) in flooded and non-flooded sites on the territory of Forest office Osijek and Forest office Valpovo. Flooded localities were Erdutske podravske šume 18a (EPŠ 18a, both clones sampled), Valpovačke podravske šume 1a (VPŠ 1a, clone 'M1') and 13c (VPŠ 13c, clone 'S 1-8'), and non-flooded localities were Osječke podravske šume 13a and Valpovačke podravske šume 25h (OPŠ 13a and VPŠ 25h with both clones). Foliage for determination of nutritional status was sampled from 5 trees per location, soil samples taken by drill as a composite sample per location from depths 0-30 and 30-60 cm, and samples for radial increment sampled by the use of Pressler auger at breast height. Each tree was cored on the west side of the trunk, and increment cores were collected in repetitions. The cores were scanned in high resolution (600 dpi. min.), annual increment detected by Cdendro programme, and arithmetic mean calculated for year and clone. For further analysis we used the Stress tolerance index – STI [7]. In our research $STI = \frac{YpxYs}{(\overline{Yp})^2}$, where Yp = radial increment on flooded locality, Ys = radial increment on non-flooded locality, and $\overline{Yp}$ = mean radial increment on flooded locality. As the STI value increases, so does the stress tolerance and the potential production in stress site conditions.

Foliar and soil analyses were performed in the Physical-chemical laboratory of the Department for forest ecology of Croatian Forest Research Institute and the laboratory of the department for Plant nutrition, Faculty of Agronomy, University in Zagreb. Foliar samples were dryed at 105°C until constant mass and weighed, then analysed for N concentration on elemental analyser LECO CNS 2000. For analyses of other elements the ground samples were wet ashed by using the concentrated sulphuric acid with perchloric acid as a catalyst. Phosphorus was determined colorimetrically on UV/VIS spectrophotometer Perkin-Elmer Lambda 1A. Concentrations of potassium, calcium and magnesium were determined directly from filtrate on AAS PE 3110.

The pH value of soil samples were determined in H_2O and 1M KCl on pH-meter HACH EC 30, total carbonate by calcimeter according to Scheibler, total nitrogen on elemental analyser LECO CNS 2000, humus according to Tjurin, and plant available phosphorus and potassium by AL method according to Egnér- Riehm-Domingo. Phosphorus was determined on UV/VIS spectrophotometer Perkin-Elmer Lambda 1A using the molibdate blue method, and potassium directly from filtrate on flame photometer Eppendorf. A detailed description of methods is given by Škorić [8].

Results and Discussion

Values of radial increment and STI derived from it are very similar between clones, depending mostly on the location. The plantations in the non-flooded area of Forest office Osijek obviously belong to the higher stress environment in comparison with non- flooded area of Forest office Valpovo. Flooded areas do not always have larger increment. Both clones in the non-flooded area of Forest office Valpovo have a higher radial increment than in the flooded area, showing that the adequate water supply is just one of the conditions for good growth of poplars. Inconsistent results revealed in the analysis of STI based on water availability focused in additional research on the nutrition of poplar clones to more precise source of the stress.

Table 1 Mean values of radial increment (i_r) and STI for 'M1' clone

Radial increment, mm		STI
Non-flooded OPŠ 13a	Flooded EPŠ 18a	
7,34	10,10	0,7267
Non-flooded VPŠ 25h	Flooded VPŠ 1a	
8,99	6,96	1,2917

Table 2 Mean values of radial increment (i_r) and STI for clone 'S 1-8'

Radial increment, mm		STI
Non-flooded OPŠ 13a	Flooded EPŠ 18a	
7,36	10,40	0,7077
Non-flooded VPŠ 25h	Flooded VPŠ 13c	
8,70	7,64	1,1387

All analysed soil samples are rich in calcium carbonate. Soils in the flooded area have (with the exception of locality EPŠ) higher concentrations of humus and nitrogen in the surface layer in comparison with non-flooded locations. The supply of plant available P and K is low, especially in the deeper layer, with somewhat higher concentrations in EPŠ 18a (flooded) and VPŠ 25h (non-flooded), and where we also determined higher values of radial increment for both clones. Peterson et al. [9] state that even in stands with high yield, insufficient P and K nutrition limits poplar growth. On sandy soils poor in potassium, yield declines from one year to another with the potassium reserves diminishing. That effect is often difficult to distinguish from yield changes caused by other environmental factors [10].

Table 3 Concentrations of nitrogen, phosphorus, potassium, calcium and magnesium and dry leaf mass in foliar samples from flooded and non-flooded localities

Locality	Flooded / non-flooded	Dry leaf mass (100 leaves, g)	N (mg/g)	P (mg/g)	K (mg/g)	Ca (mg/g)	Mg (mg/g)
EPŠ 18a 'M1'	flooded	34,89	22,4	1,66	9,40	11,2	4,11
EPŠ 18a 'S1-8'	flooded	108,67	21,5	2,02	8,96	11,12	4,13
OPŠ 13a 'M1'	non-flooded	25,03	23,5	1,89	9,87	11,35	4,2
OPŠ 13a 'S1-8'	non-flooded	133,51	18,8	1,92	9,15	11,13	4,18
VPŠ 1a 'M1'	flooded	32,98	20,4	1,66	10,05	11,39	4,24
VPŠ 13c 'S1-8'	flooded	89,67	22,1	1,26	10,35	11,46	4,26
VPŠ 25h 'M1'	non-flooded	45,32	16,5	1,33	10,55	11,44	4,26
VPŠ 25h 'S1-8'	non-flooded	44,49	16,1	1,06	9,73	11,29	4,18

Lodhiyal and Lodhiyal [11] determined that a plantation of *P. deltoides* clones at age 4, and with planting distance of 3 x 5 m takes away 176 kg of nitrogen, 12-19 kg of phosphorus, and 49-94 kg potassium annually. From this we can deduct that insufficient quatities of nutrients in the soil can have an effect on the sustainability of forest management, especially where the frequency of floods was reduced and nutrients are not regularly replenished.

'M1' clone did not acquire sufficient nitrogen concentrations in leaves at any location, while 'S 1-8' had normal N concentrations on flooded and insufficient on non-flooded localities. 'M1' acquired the highest values of N and P in OPŠ 13a, and the lowest in VPŠ 25h, although at the highest leaf mass (both localities are non-flooded). 'S 1-8' had the lowest N and P concentrations also in VPŠ 25h, the highest N concentrations in VPŠ 13c, and phosphorus in EPŠ 18a (both flooded) and in OPŠ 13a acquired a high phosphorus concentration along with an insufficient nitrogen concentration. The reason for that is the dillution effect, because in this locality 'S 1-8' clone had the highest leaf mass. Potassium concentrations are insufficient on all localities, calcium is at the lower limit of sufficiency or insufficient, and magnesium normal to optimal in comparison with nutritional ranges given by Van den Burg [12]. While chemical soil composition is difficult to relate to 'M1' nutrition, for 'S 1-8' the sites can be roughly divided according to soil nutritional potential on flooded and non-flooded localities. And while low potassium concentrations in foliage can be attributed to low supply of potassium in soil, foliar Ca concentrations depend mostly on water availability, therefore low foliar Ca on these Ca-rich soils is the result of insufficient water supply to the roots of poplar.

Conclusions

The reasons of recent poor performance of poplar clones in eastern Slavonia cannot be attributed to only one factor, but to various interactions of water availability and nutrients to plants that are specific for different siites.

To better determine the causes for low increment, tree dieback and problematic reforestation of poplar cultures in eastern Slavonia this research should be directed towards a more detaild determination of water availability to the roots of a larger number of poplar clones in comparison with their nutritional status, preferably through several vegetational seasons, so as to encompass the dynamics of floods, soil water table and the influence of meteorological parameters. Since 'M1' and 'S 1-8' genotypes have modest yields both in high- and low-stress environments, future research should be based on a larger number of poplar clones.

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STUDIES ON MORPHOGENETIC CHARACTERISTICS OF JUVENILE AND ONE YEAR OLD SEEDLINGS OF SOME REGISTERED BRUTIAN PINE (*Pinus brutia* Ten.) SEED STANDS IN LAKES DISTRICT

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Introduction

Brutian pine (*Pinus brutia* Ten.) has a natural distribution of about 5.9 million ha which represents 41% of coniferous forests and 27% of the total forest areas in Turkey [1]. It is one of the major forest tree species of the Mediterranean region as it is a drought resistant pine that withstands more aridity and poor soils than most timber species growing in Mediterranean climate [2]. Besides its good performance on poor sites, as a hardy species, it also has considerably faster growth rate on good sites (up to 12 cu. meters/ha. per anum) as compared to the other coniferous species in Turkey. In addition to wide-range utilization of its wood in varios wood-based industries, it also provides the total resin production of the country [3].

The factors negatively affecting its forests are various and extensive in Turkey. The ways of forest destruction range from illegal cutting to entomological factors, and the role of fire and grazing in diminishing the forests are extensive. On the other hand, the planned and unplanned interventions against pure and mixed stands of the species have been realized for centuries. Because of all these problems many parts of the species forests are far from providing expected economical, ecological, and collective-cultural functions.

It is vital to afforest such kind of degraded forest lands by using seeds of suitable origin which have adaptability to climate conditions, soil properties and physiographic characteristics of those lands. In order to realize that, breeding activities of species should be prioritised. Actually, up to now although many researchers have studied the species there is few researches on its seed stands. In this study, the variation and the relations between some morphological characteristics of juvenile and one year old seedlings of four registered Brutian pine seed stands of the lakes district in Turkey were investigated.

Material and Methods

Cones were collected from the middle part of the crown of 40 open-pollinated parent trees of four populations sampled from Isparta Regional Directorate of Forestry in the Lakes District of Turkey during March of 2004 (Table 1). In order to represent gene poll of the populations, the parent trees were sampled at random with the following restrictions: 1) they were to be separated by at least 50 m, 2) the range of elevation of the parent trees had to be not greater than 100 m within any population, 3) they were to be approximately of the same age (± 10 years).

Table 1. The characteristics of seed stands

Forest District	Location	Altitude (m)	Exposure	Latitude (°)	Longitude (°)	Area (ha)
Bucak	Merkez	805	N	37° 30′ 30′′	30° 41′ 15′′	164
Bucak	Pamucak	820	NW	37° 24′ 45′′	30° 37′ 20′′	221
Gölhisar	Gölhisar	850	NE	37° 04′ 30′′	29° 32′ 40′′	45.5
Sütçüler	Karadağ	752	N	37° 30′ 50′′	30° 52′ 10′′	316

Cones were desiccated on the great sacks under outdoor conditions, and also sprinkled water 2 to 3 times day. Seeds were extracted manually and cleaned. The seeds were sown by family in a randomised complete block design with three replications in the beginning of May, 2004. Each family (tree) was represented by 150 seeds. Seeds were not pretreated except soaking in warm water for 48 hours prior to sowing. Sowing depth was 15 mm. The distance between two rows was constant (20 cm). After sowing, mulching was applied on the sowing lines by means of the mixture containing 50% of fine river sand and forest soil. Cultivation activities such as irrigation (twice a day by sprinkling) and weed control (manually once a month) were regularly performed in the nursery during the growing season of 2004.

The study was conducted in the Eğirdir Forest Nursery-Turkey (37°53’ N, 30°52’ E, 926 m asl). The nursery soil was sandy clay loam with a soil pH of 6.8-7.8 in the rooting zone [4]. Annual precipitation average is about 76.3 cm with an average temperature of 13°C [5]. The growing season is about 210-220 days long.

In November of 2004, a total of 300 juvenile seedlings that are seventy days old (30 for each tree) and 150 seedlings that are one year old (15 for each tree) for each population were uprooted without harming the roots. The hand-lifted seedlings were examined in terms of morphological characteristics [CN: Cotyledon number, EL: Epycotyl length, RL: Rootlet lenght and JSW: Juvenile seedling weight of juvenile seedlings; SH: seedling height, RCD: Root collar diameter, LRL: The longest root lenght and SRR: Oven dry shoot/root ratio of one year old seedlings].

Statistical analysis was carried out by using SPSS [6]. An ANOVA was performed to determine whether or not the means of seed stands were all equal. The means were compared by using the adjusted Duncan’s multiple range test ($p < 0.05$). In addition, simple and multiple regression analyses were used for estimating the relationships among the measured characteristics (variables) of seed stands.

Results and Discussion

Mean values of juvenile and 1 + 0 year old seedling characteristics, their standard deviations, ranges, F ratios, significance levels and homogen groups were given in Table 2. The analysis of variance showed significant differences between populations at the 0.001 probability level for only epicotyl lenght and juvenile seedling weight. Juvenile seedling weight (JSW) contributed a significant portion to the differentiation among populations ($F_{jsw} = 81.57$; $P < 0.001$). On the other hand, there were differences at the 0.05 level among the populations from stand point of the cotyledon number, rootlet length, and oven dry shoot/root ratio. Population Gölhisar-Gölhisar showed the lowest performance for the majority of the morphological characteristics. The mean cotyledon number (7.70) was within the range (7.70-9.28) reported by Aslan and Uğurlu [7]. While the mean epycotyl length (1.45 cm) found in this study was different to that (3.39 cm) reported by Gülcü [8] it was similar to that (1.74 cm) reached from Aslan and Uğurlu [7].

All juvenile seedling characteristics showed the differences among populations but the other characteristics of one year seedling with the exception of oven dry shoot/root ratio have no difference. While differences in juvenile seedling characteristics among populations were affected by the parental environments in the natural populations, young (1 + 0 year old) seedling characteristics were assessed in a common garden experiment minimizing the differences among populations that are due to environmental effects. For cotyledon number, rootlet length and juvenile seedling weight Gölhisar-Gölhisar and Sütçüler-Karadağ populations were within the same homogen group. Bucak-Merkez, Gölhisar-Gölhisar and Sütçüler-Karadağ populations were the transition populations when Bucak-Pamucak population replaced individually in one group (Table 2).

Table 2. Mean ± standard deviation, F ratio and significance (P) for the studied characters

	Juvenile Seedling Characters				1+0 year old Seedlings Characters			
Pop. Name	**CN**	**EL (cm)**	**RL (cm)**	**JSW (g)**	**SH (cm)**	**RCD (mm)**	**LRL (cm)**	**SRR**
Bucak-Merkez	7.78 ±0.06b	1.31 ±0.03a	17.20 ±0.19ab	0.38 ±0.01b	38.79 ±0.34a	2.47 ±0.03a	26.56 ±0.30a	1.90 ±0.07a
Bucak-Pamucak	7.88 ±0.06b	1.56 ±0.02b	17.66 ±0.22b	0.51 ±0.01c	38.78 ±0.32a	2.44 ±0.03a	26.31 ±0.27a	1.70 ±0.03b
Gölhisar-Gölhisar	7.41 ±0.07ab	0.92 ±0.20c	16.94 ±0.21a	0.35 ±0.01a	38.79 ±0.35a	2.44 ±0.03a	26.16 ±0.28a	1.79 ±0.05ab
Sütçüler-Karadağ	7.71 ±0.06a	2.00 ±0.03d	16.95± 0.19a	0.33 ±0.01a	38.79 ±0.32a	2.42 ±0.03a	26.27 ±0.25a	1.72 ±0.02a
Mean	7.70 ±0.14	1.45 ±0.33	17.19 ±0.24	0.39 ±0.06	38.79 ±0.00	2.44 ±0.01	26.33 ±0.12	1.78 ±0.07
Range	7.41 - 7.88	0.92 - 2.00	16.94 - 17.66	0.33 - 0.51	38.78- 38.79	2.42- 2.47	26.16- 26.56	1.70- 1.90
F ratio	2.97	78.46	2.71	81.57	0.61	1.61	1.23	3.41
P	0.031	0.000	0.044	0.000	0.611	0.203	0.315	0.017

CN: Cotyledon number, EL: Epycotyl length, RL: Rootlet lenght, JSW: Juvenile seedling weight, SH: seedling height, RCD: Root collar diameter, LRL: The longest root lenght, SRR: Oven dry shoot/root ratio

In only Gölhisar-Gölhisar population, it was determined a strong relationship at 0.01 level between cotyledon number (x) and epycotyl length (y) (Table 3). Thus Gülcü [8] and Yahyaoğlu [9] have found the similar positive relation for juvenile seedlings of Brutian pine.

Table 3. Cotyledon number (x) influence on epycotyl length (y) in Gölhisar-Gölhisar population

Population	**Equation**	***N***	***r^2***	***P≤***
Gölhisar-Gölhisar	y = 0.07x + 0.42	300	0.03	0.01

In Bucak-Pamucak, Gölhisar-Gölhisar and Sütçüler-Karadağ populations a significant positive relationship between cotyledon number and juvenile seedling weight occurred (r^2=0.11, 0.07, and 0.05, respectively; P<0.001 for all populations) such that shifts in cotyledon number was linked with the increased juvenile seedling weight (Table 4).

Table 4. Cotyledon number (x) influence on Juvenile seedling weight (y) in three populations

Population	Equation	*N*	r^2	*P≤*
Bucak-Pamucak	y = 0.05x + 0.09	300	0.11	0.001
Gölhisar-Gölhisar	y = 0.03x + 0.16	300	0.07	0.001
Sütçüler-Karadağ	y = 0.03x + 0.11	300	0.05	0.001

It was found out that root collar diameter (97%) explained the variation of the seedling height more than the longest root length (81%) in Gölhisar-Gölhisar population (Table 5). Namely, root collar diameter compared to the longest root length was more effective on seedling height.

Table 5. The longest root length (x) and root collar diameter (x) influences on seedling height (y) in Gölhisar-Gölhisar population

Population	Equation	N	r^2	P≤
Gölhisar-Gölhisar	y = 0.02x (LRL) + 39.26	150	0.81	0.001
Gölhisar-Gölhisar	y = 3.24x (RCD) - 123.13	150	0.97	0.001

Seedling height, root collar diameter and the longest root length collectively have a strong influence on oven dry shoot/root ratio (r^2=0.85, P<0.001; Table 6) in Gölhisar-Gölhisar population. However, seedling height influence was non significant. On the other hand, the influence of the longest root length (P<0.001) on seedling height was more than that of root collar diameter (P<0.05).

Table 6. Associate influence of seedling height (x_1), root collar diameter (x_2) and the longest root length (x_3) on oven dry shoot/root ratio (y) in Gölhisar-Gölhisar population

Population	Equation	N	r^2	P≤
Gölhisar-Gölhisar	$y = -6.34x_1 + 10.26x_2 + 0.65x_3 + 205.72$	150	0.85	x_1: ns* x_2: 0.05 x_3: 0.001

*: Seedling height influence on oven dry shoot/root ratio is non-significant

Conclusions

There were important differences among the populations from stand point of the characteristics of their juvenile and one year old seedlings. Bucak-Merkez, Gölhisar-Gölhisar and Sütçüler-Karadağ populations were the transition populations when Bucak-Pamucak population replaced individually in one group. This result showed that seeds from any of Bucak-Merkez, Gölhisar-Gölhisar and Sütçüler-Karadağ populations could be used as a seed source instead of the other one for the similar afforestation area, when any of populations did not have enough seeds. However, seeds of other three populations should not be used for Bucak-Pamucak population.

There were significant relations at 0.05-0.001 levels between cotyledon number - juvenile seedling weight in Bucak-Pamucak, Gölhisar-Gölhisar and Sütçüler-Karadağ populations; between cotyledon number - epycotyl length, the longest root length - seedling height, root collar diameter - seedling height, the longest root length and root collar diameter - oven dry shoot/root ratio in Gölhisar-Gölhisar population. Interestingly, all relationships among pairs of the studied characteristics were determined in Gölhisar-Gölhisar population. This result might be resulted from the decrease in the number of samples or measurement mistakes. Essentially, capillary root system was more important than the longest root length for afforestation activities. So in future studies this character should be taken into consideration. Besides, oven dry shoot/root ratio was so important for the afforestations of arid and semi arid areas. The smaller oven dry shoot/root ratio was the more successful afforestation activities were. Finally, by means of this study, although it was reached some main biosystematic data, it could be also indicated here that future studies are necessary to provide deeper insights in to the subject.

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