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Session A: Productivity & sustainable management of ecosystems

VARIABILITY OF THE BASIC POSSESSION PARAMETERS IN PRIVATELY-OWNED FORESTS IN CROATIA

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Introduction

If we strive towards sustainable management, forest management is a challenge because it requires expertise, timeliness and moderation. It also means this type of management needs to be in line with the forest stand capability. In order to achieve influence and impact, these terms have to be implemented on the stand level, not the cadastral plot level that is the most prevalent management system in Croatian privately-owned forests. In this case, even if the landowner is well versed in proper management, he does not have influence towards sustainable management of the area if neighbouring landowner has different management vision and needs. This management system leads towards uneven quality and age, often resulting in stand degradation.

According to Čavlović (2004), the main challenge in private forests is small area management, heterogenity of the silvicultural forms, unsatisfactory cadastre, unmarked plot borders and forest degradation.

This is especially pronounced when there are many small plots/estates nearby, each with multiple owners. According to former studies, mean forest estate area is rather small (Posavec et al (2011) 0.51 ha; Gluck et al (2011) 0.96 ha; Paladinić et al (2008) i Posavec i Beljan (2012) 1.52 ha; Gluck et al (2009, 2011) 2.95 ha) although size is not consistent since studies have been done for different areas or specific smaller regions.

The goal of this paper is to signify the amplitude of above mentioned problems (size of the forest estate/cadastral plot, discrepancy in cadastral registry, number of landowners, etc.) for the whole area of the Croatia. Although this research is conducted on the sample, it shows representative overview in Croatia and represents one of the most extensive research in this theme field, comparing it with similar research found in literature (above mentioned).

Material and Methods

Data for processing is gathered during creation of the privately owned forest management plans (P-FMP) for the period from 2007 to 2013. It consists of the data on the cadastral municipalities (CMs) level (Table 1) analysed regarding their affiliation to specific region. P-FMP selection for the analysis is based on their spatial disposition to obtain relatively uniformly dispersed sample throughout Croatia (Picture 1).

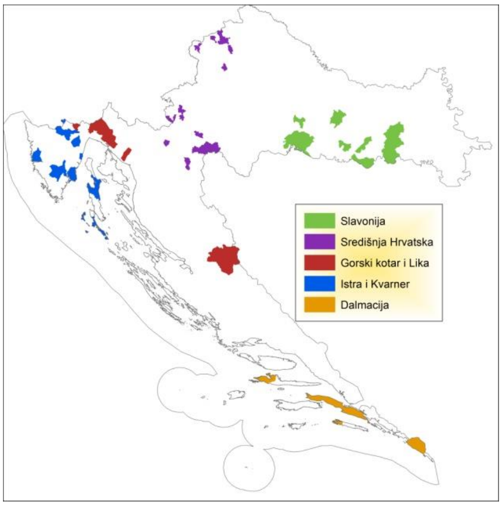

Figure 1. Spatial distribution of the processed CM across Croatian regions

Privately-owned forest area in those selected P-FMP is 91,348.10 ha (Table 1). This sample represents 15.69 % of the privately-owned forest in Croatia, comparing it to data from FMP for the whole area of Croatia (Croatian forest ltd, validity 2006-2015).

Table 1. Data overview on private forest management plan (P-FMP) level across Croatian regions

Region	**No. of CM**	**No. of cadastral plots in P-FMP**	**No. of landowners in P-FMP**	**No. of possession sheets in P-FMP**	**Area in P-FMP (ha)**	**Area of non-forest land inside P-FMP (ha)**
Dalmatia	68	58,178	12,557	8,957	21,716.54	2.097,09
Gorski kotar and Lika	40	38,175	14,560	10,206	13,117.36	4,291.30
Istria and Kvarner	56	113,867	28,475	17,932	31,053.97	11,315.40
Slavonia	104	17,002	11,823	9,088	6,679.26	2,161.81
Central Croatia	51	71,869	22,232	18,473	18,780.96	6,889.46
Total	**319**	**299,091**	**89,647**	**64,656**	**91,348.09**	**26,755.06**

Following variables was calculated based on the above mentioned data:

- Average area of the cadastral plots inside CMs included in P-FMP (regardless of the land use specified in the cadastre);
- Average area of the forest estates inside CMs included in P-FMP. Estate indicates all cadastral plots included in one possession sheet with one landowner or more co-owners;
- Average number of landowners in possession sheets by CMs included in P-FMP;
- Share of the privately-owned forests inside CMs;
- Share of the “non-forest” land according to cadastral land use data included in P-FMP, where “non-forest” land i.e. “non-forest” cadastral plots are considered to be plots that are by cadastral land use pasture, meadow, arable land, etc. but overgrown by forest vegetation. Plots that are included in P-FMP with mixed cadastral landuse and one of them is “forest” are not considered “non-forest” plots.

Data and variables used are identical as used in Berta et al. (2017) on which cluster analysis was performed. Cluster analysis of estate and area characteristics was performed in order to determine what clusters of similar characteristic are prevalent regarding regions. Here, this data is used to analyse its variability inside regions. Following statistical values are presented: mean, standard deviation (sd), minimum (0 %), values of the 1st quartile (25 %), median (50 %), value of the 3rd quartile (75 %) and maximum (100 %).

Results and discussion

In **Dalmatia**, forest estate area is the largest in comparison to other regions (median 2.75 ha, mean 14.54 ha, Table 3) which is the result of the large estates in South Dalmatia in the form of village property with area up to 600 ha. For more accurate results in this region, the sample should be increased, which is also visible in large discrepancy of the median and mean value. The same situation is with the size of the cadastral plots. Observing the number of landowners, this region has average value regarding other regions (median 1.51 landowner per possession sheet, Table 4) whilst the private forest share is almost the same as the other regions with high share of the private owned forests (Central Croatia; Istria and Kvarner). This region has the lowest share of the "non-forest" plots (Table 6) inside P-FMP (median 4.73 %, mean 9.81%) with maximum only up to 64.59 % which is the lowest maximum regarding other regions. This can be explained because the process of agricultural production abandonment on suitable areas was not so pronounced since these areas are sparse.

Gorski Kotar and Lika have mean values in comparison to other regions for the: forest plot area (median 0.3921 ha, Table 2), forest estate area (median 1.10 ha, Table 3), number of landowners (median 1.55 landowner per possession sheet, Table 4) and for the share of "non-forest" plots inside P-FMP (median of 27.09 %, Table 6). This region has lower share of private owned forests inside CMs unlike other regions (median 6.89 %, mean 13.44 %, Table 5). This variable is under the great influence of the CMs from Lika (21 CM's) where the shares of the private owned forest are up to 7 % of the CMs total size. Therefore, for more accurate result for this region, sample augmentation and possible diversification to 2 sub-regions should be considered.

As for **Istria and Kvarner**, this region has lower value for the forest plot area (average 0.2050 ha, Table 2) and average values for the: forest estate area (average of 1.3276 ha, Table 3), private owned forest share (median of 27.48 %, Table 5) and "non-forest" plot share inside P-FMP (median 32.64 %, Table 6). On the other hand, standard deviations are among the highest regarding other regions indicating great variability within the region. This region has the highest number of the landowners per possession sheets (average 2.08 landowners per possession sheet, Table 4), with the highest maximum of 4.44 landowners by estate.

Slavonia has the lowest and lower values regarding other regions for the: forest estate area (median of 0.5430 ha, Table 3), number of the landowners per possession sheets (median 1.38 landowners per possession sheet, Table 4) and private owned forest share (median of 2.83 %, Table 5). Accordingly, these variables have the lowest maximums when compared to other regions. Cadastral plot area is median (0.3410 ha, Table 2) and the share of "non-forest" plots inside P-FMP is the highest regarding other regions (median 38.81 %, Table 6). High share of "non-forest" plots with the lowest value for the private owned forests are showing that this is mainly agriculture region with notable agriculture production abandonment. This region has the lowest variability within region for the variables: forest estate area (sd 0.3296 ha, Table 3), number of landowners (sd 0.26, Table 4) and share of the private owned forests (sd 5.83 %, Table 5) whilst having the highest variability for the share of "non-forest" plots (sd 29.65 %, Table 6) regarding other regions.

Central Croatia has the lowest and lower values regarding other regions for the: cadastral plot area (median 0.2656 ha, Table 2), forest estate area (median 0.8444 ha, Table 3) and number of the landowners per possession sheets (average 1.34, Table 4). Private owned forest share (median 29.43 %, Table 6) and the share of "non-forest" plots inside P-FMP (median 28.72 %, Table 7) are of average value among regions. Nevertheless, standard deviation is among the highest in all regions, except for the forest plot size where variability is the lowest in all regions.

Table 2. Statistical data overview of the average size (ha) of the forest cadastral plots in CMs across Croatian regions

Region	mean	sd	0%	25%	50%	75%	100%	n
Dalmatia	0.6740	0.8375	0.0819	0.2132	0.2986	0.6896	4.0299	68
Gorski kotar and Lika	0.4688	0.2823	0.0909	0.2782	0.3921	0.6546	1.3297	40
Istria and Kvarner	0.3439	0.2021	0.0288	0.2050	0.3222	0.4400	1.2058	56
Slavonia	0.3767	0.1832	0.0000	0.2552	0.3410	0.4628	0.9465	104
Central Croatia	0.2763	0.1076	0.1323	0.1862	0.2656	0.3484	0.6229	51

Table 3. Statistical data overview of the average size (ha) of the forest estate in CMs across Croatian regions

Region	mean	sd	0%	25%	50%	75%	100%	n
Dalmatia	14.5392	71.8593	0.4074	1.3719	2.7453	6.5000	593.6302	68
Gorski kotar and Lika	1.2753	1.2647	0.1508	0.7821	1.1012	1.2902	7.7956	40
Istria and Kvarner	1.9013	2.2285	0.0561	0.8878	1.3276	2.1298	15.6924	56
Slavonia	0.6210	0.3296	0.0000	0.4126	0.5430	0.7628	1.9109	104
Central Croatia	1.1100	0.7154	0.3141	0.6195	0.8444	1.3713	3.0667	51

Table 4. Statistical data overview of the average number of landowners per possession sheet in CMs across Croatian regions

Region	mean	sd	0%	25%	50%	75%	100%	n
Dalmatia	1.64	0.39	1.08	1.40	1.51	1.84	2.74	68
Gorski kotar and Lika	1.58	0.44	0.00	1.36	1.55	1.69	2.83	40
Istria and Kvarner	2.12	0.58	1.39	1.65	2.08	2.43	4.44	56
Slavonia	1.34	0.26	0.00	1.32	1.38	1.43	1.81	104
Central Croatia.	1.49	0.56	1.07	1.17	1.34	1.54	3.84	51

Table 5. Statistical data overview of the privately-owned forest shares (%) in CMs across Croatian regions

Region	mean	sd	0%	25%	50%	75%	100%	n
Dalmatia	30.84	16.15	2.94	19.02	28.29	43.60	80.17	68
Gorski kotar and Lika	13.44	15.81	0.05	2.26	6.89	22.59	73.74	40
Istria and Kvarner	32.16	20.01	0.76	17.61	27.48	44.54	85.59	56
Slavonia	5.04	5.83	0.00	1.24	2.83	7.38	32.62	104
Central Croatia.	29.66	16.71	0.95	15.67	29.43	43.04	71.77	51

Table 6. Statistical data overview of the non-forest cadastral plot shares in P-FMPs across Croatian regions

Region	mean	sd	0%	25%	50%	75%	100%	n
Dalmatia	9.81	12.33	0.00	1.67	4.73	12.19	64.59	68
Gorski kotar and Lika	36.03	26.57	0.00	17.43	27.09	55.87	99.13	40
Istria and Kvarner	35.13	20.01	0.00	20.79	32.64	48.20	100.00	56
Slavonia	40.83	29.65	0.00	18.52	38.81	61.23	100.00	104
Central Croatia.	37.65	26.16	4.31	16.91	28.72	58.04	96.49	51

Conclusion

It is noted that specific regions are diversified from others by specific characteristics which is a consequence of the historical development of the cadastre, ownership and different intensity of the land use changes.

By analysing these results we can conclude that an average forest-landowner in Croatia possesses from 2 (Slavonia) to 9 (Dalmatia) forest plots, often having one more co-owner. The number of landowners by regions does not have great variability but by observing maximum values, we can conclude that it can present a significant obstacle in normal forest management especially when one of the co-owners is abroad (Canada, Australia, USA, etc) which is not a rare case.

Furthermore, every 3rd plot is overgrown agricultural land (in Dalmatia every 10th).

By observing share of the private owned forest it is noted that some CMs do not have any, whilst in some CMs private owned forests occupy up to 85 % of the CM's area.

This research also shows that sample augmentation should be considered for specific regions (Dalmatia; Gorski Kotar and Lika) since extreme values have notable influence on the end results.

Nevertheless, these results are valuable since they have, for the first time, displayed diversity by regions for the whole area of the Croatia, and are based on the 8.74 % of Croatian area i.e. 15.69 % of the private owned forest area in Croatia.

Bearing in mind all of the above stated variability and results as well as the fact that plots in landowner's possession are often far apart from each other (Gluck et al, 2009, 2011, Posavec 2012), it is obvious that sustainable forest management is hard to achieve and not feasible for the average private forest landowner in Croatia.

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BIG GAME POTENTIALS IN REGARDS TO MEAT PRODUCTION

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Introduction

Meat is used as common grocery worldwide and represents a significant source of animal proteins (1). According to Eurostat information in Republic of Croatia there is 2 200 t of sheep meat produced annually, 42 000 t of beef and 73 000 t of pork. Alongside cattle production meat as a grocery in one smaller part comes as a product of hunting management. Although wild game meat is relatively exotic and still unavailable grocery, there are numerous nutritional advantages of this product. Wild game meat compared to meat from domestic animals generally contains less fats (2; 3), it is darker (4; 3), more favorable fatty acid composition (5) and generally has bigger water content which depends on a larger number of factors (6; 7; 8; 9). Presence of fat in wild game is significantly lower than in domestic animals and amounts often under 1%. This is a consequence of partially genetically inheritance and the way of life which is subordinated to constant movement in quest for nutrients (10; 11; 12; 13; 14; 15; 4). Content of intramuscular fat in wild game meat is different in particular species and is at the level or below of the content in domestic ruminants or pigs (13). Cholesterol concentration in wild ruminants is different and tightly connected to their diet. In fallow deer there are recorded cases that in intensively bred animal's cholesterol levels where higher than in certain domestic ruminants (12). Cholesterol concentration research in red deer meat has shown significant differences in mature males (higher concentration) compared to sexually mature females (16). For the purpose of determining nutritional values of meat, apart from fat or cholesterol concentration, fatty acids composition is even more important. In wild game meat lower content of saturated fatty acids and higher content of unsaturated fatty acids is regularly noted, compared to domestic ruminants (17). This relationship differs by individual species and greatly depends on food that certain species prefers. It has been determined that species that are prone to pasture, compared to those that are prone to bud have larger content of unsaturated fatty acids (18; 19). Wild boars compared to domestic pigs or crossbreeds of domestic and wild pigs, have smaller fat content, smaller intramuscular fat content and somewhat more favorable composition of fatty acids (2; 3). Research of all qualitative parameters in wild boar compared to domestic animals in general shows the biggest variability, and there have been statistically significant differences noted within the same population caught in spring or summer compared to those that have been caught in winter period (5). This research will explore how big is the potential in meat production for three main species of big game (red deer, roe deer, wild boar) that make the framework of hunting management in Republic of Croatia.

Materials and methods

For the purpose of research, seven hunting grounds in total have been included in the research. These hunting grounds are located in the central and eastern part of Croatia and represent the cross section of habitat conditions from lowland to highland areas. On the territory of these seven hunting grounds total measured was 51 red deer, 52 roe deer and 55 wild boars. After slaughter processing weighing of bodies from three researched individuals has been conducted. Individuals have been sorted according to sex and grouped according to age in three age categories (juveniles, yearlings, adults). Theoretical parent found and cull in 100 necks of the found have been calculated by model from 20; 21.

Table 1: Cull structure PF = 100 (20; 21)

Cull (pcs.)			
Age	Red deer	Roe deer	Wild boar
Cubs	11	17	60
Yearlings	14	17	24
Adults	10	16	16
Total	35	50	100

Information on total cull and productive hunting areas for three researched species in Republic of Croatia has been taken from Central hunting records. Statistical analysis has been done in Statistica.

Results and discussion

Wild game meat exploitation in Croatia is similar to one in Europe. It amounts to less than one kilogram per capita annually. Wild game meat usage is largely oriented within families of hunters (22), while wild game meat market has been reduced to very limited and sporadic offer in retailers. Complex legislation does not allow preassembled sales to most of hunting rights holders, but the trade is done by selling whole bodies or halves in hide. Meat cutting facilities and preassembly and packaging are to big investment for most hunting rights holders. There are only a few of them in Croatia that surpass in their capacities seasonal loads, thereby questioning their own profitability of meat processing.

Table2: Average mass of carcasses

Game species/Age	Average carcasses (kg)					
	Cubs		Yearlings		Adults	
	Ar. mean	St.d.	Ar. Mean	St.d.	Ar. mean	St.d.
Red deer	28,50	4,55	43,58	12,74	93,08	28,42
Roe deer	9,37	1,84	14,29	1,93	14,50	3,32
Wild boar	19,31	6,11	27,45	8,47	48,85	11,03

Alongside parent found of 100 necks and met economic capacity in red deer, it is possible to produce in free outdoors total of 3085, 85 kg of meat, 1104,56 kg of roe deer meat and 5236,47 kg of wild boar meat. When looking at the three researched species of big game production amounts to 0,5 kg per capita on average. According to the number of registered hunters (65 000) it turns out that annual meat production from the three researched species amounts to 35kg per hunter, which is almost more than semiannual need of 60kg/capita in Croatia, and more than 1/3 of recommended annual need of 85kg/capita (23). Individually most meat is produced from wild boar (1 570 t), followed by roe meat (408 t) and red deer (335 t) (24). In Republic of Slovakia, which is in territory somewhat smaller than Croatia, for the period from 1999 until 2003 they are getting significantly smaller annual average values for roe deer (215 t) and wild boar (684 t), but also significantly more meat from red deer (804 t), which in the end would mean significantly smaller 1 703 t compared to 2 313 t in Croatia. However in the last years wild boar population is growing significantly, and in our country the focus of meat production is exactly on this species unlike common deer in Slovakia, therefore some newer information would probably be much closer considering that wild boar population is expanding throughout Europe (25; 26).

Table 3: Meat production per ha of productive hunting areas for three researched species

Game sp.	Meat production (kg)	
	Annual production	Production per ha
Red deer	335 024	0,73
Roe deer	408 688	0,31
Wild boar	1 570 939	1,28

Meat production by productive area for each individual species is on average the highest in wild boar and amounts to 1,28 kg/ha, followed by red deer 0,73 kg/ha and roe deer 0,31 kg/ha. Although individually red deer has the highest rate of body growth (27), the breeding potential that wild boar has per ha by far exceeds individual body growth. Although the numbers of wild boar are rising in the last years, and it has partially repressed other species especially small game in its habitat conquest, there is still the impression that red deer meat production is too low. For instance 28 show that red deer meat production in Scotland amounts to 1 kg/ha which is significantly higher than our amount. Roe deer meat production can generally be considered too low because of under use of habitat capacities and too low funds and cull.

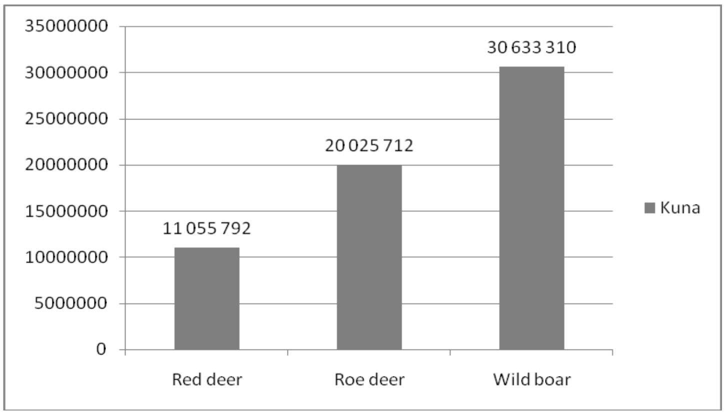

Figure 1: Meat production values in Croatian kuna for three researched species in Republic of Croatia

When taken into consideration that average selling price for 1 kg red deer meat is 33kn, roe deer 45kn and wild boar 20kn, annual production of over 60 mil Kuna is certainly a significant contributor to economy, however the problem is insignificantly spread market and still a closed circle of consumers and not only in Croatia but in most European countries. Market opening and more aggressive marketing would most likely lead to increase in meat value, especially of wild boar which is nutritionally as valuable as other two species, but undervalued by price of 20 kn/kg. On the other hand the legislation is quite complicated, where numerous certificates and analysis that are conducted are greatly slowing down meat placement on the market, and by this burdening the price of meat, making it even more unavailable to end consumers.

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ETHNOBOTANY: AN INNOVATIVE OPPORTUNITY OF VALORIZATION OF EDIBLE HERBS AS EXAMPLE OF GOOD PRACTICES FOR THE CIRCULAR ECONOMY AND SOCIAL WELL–BEING

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Abstract

The objectives for sustainable development for 2015-2030 give propulsion to the Age of Sustainable Development and strengthening of the Circular Economy. This paper focuses on the recovery of ancient customs and traditions of control, collection and utilization of food for traditional and/or healthful of wild plant species in Central Italy. The interest in *ethnobotany* has increased in recent years, saving these *knowledge* in fact, it also saves the cultural identity. Each farmer has a *complex knowledge* in respect of plants, knowing that is so important that some have called the farmers, who still retain them, *living treasures* or "*witnesses". The* paper analyzes more than ten years of experience in the Umbria Region(Italy) in which the research project, experiments and tests it is deployed by checking the organoleptic characteristics, nutritional and health benefits of the most widespread ecotypes. Configuring a wonderful example of fusion between tradition and innovation, organizational activities and *full circular character* life where the 3R (*reduce, reuse, recycle)* make possible the recovery of lifestyles especially in power *directed towards well-being*.

Key Words*: Biodiversity, Circular economy, Edible wild herbs, Ethnobotany, Sustainable Development, Territory Management*

Introduction

The objectives for sustainable development for 2015-2030 give propulsion to the Age of Sustainable Development and strengthening of the Circular Economy toward the IV industrial revolution. The sector of the edible wild herbs is concrete representation of the passage from the linear economy to the circular. The EU have recently promoted a strategy, for the future, with the *Package of Circular Economy* of 2nd December 2015. The man has lived for millennia in close relationship with the surrounding environment by learning to recognize, collect and use the fruits of the earth in a way that today would be called "sustainable." The use of the edible wild herbs, cross the history of man, from 10.000 year BC to nowadays as in ancient Rome even among the northern European popultaions. Wild herbs, however, also had a strong ritual value, as witnessed by *man of Tollund (300 BC),* found in a Danish bog in 1950 and perfectly preserved thanks to the particular anaerobic conditions. The rediscovery of spontaneous and used species related to them are being studied by a new discipline born in the late nineteenth century: the *Ethnobotany*. The English term of *Ethnobotany* was coined by the US botanical taxonomist John W. Harshberger, of the University of Pennsylvania who used the term for the first time *to define,* analyze, *the use of plants in primitive societies."* Botany, in fact, has always been intimately linked to medicine and *Ethnobotany* is a kind of return to the roots, and it's a bridge between *antiquity* and *modernity.*

Usefulness of edible species have extensive oral traditions and different written records that comes from the Florentine physician *Giovanni Targioni-Tozzetti* that, in 1767, published the book *"De foods urgentia*" with the subtitle *"Alimurgia".* He introduces for the first time the term "*alimurgia*" from which comes more late the term of *fitoalimurgia,* coined in the turn by the Piemonte Region botanist *Oreste Mattirolo* in his book *Phytoalimurgia pedemontana (1918).The* term fitoalimurgia comes from the Greek:*phyton = plant+, alimos=takes away hunger* and *+ergon = work). In this contribute the author present the results of researches concerning the use of the wild plants for the gastronomic purposes* After O.Mattirolo several researchers have studied *fitoalimurgia.* In the past century, and particularly in connection with the two world wars, it's necessary to mention specially R. Saccardo. (1921) and N.Arietti *(1941).* Following the socio-economic crisis linked to the II^ Second World War, Prof. A. Tukakov(1943)maked a *fitoalimurgia map* about Istria and Illyria to help local populations to overcome, with native species, the remarkable feeding difficulties mainly due to economic hardships. During the last world war, the US troops landed in Italy was hold of a *manual of fitoalimurgia*, prepared by a Committee of American botanists, to be used as handbook for survival. In the same period of hard existence, that touched many European including our local populations, they was collected the most unexpected wild species to prepare a frugal meal. Nothing was not wasted ;everything was *reduced* , *reused* and *recycled.* Food consumption was reduced at the use of local productions like the spontaneous wild herbs. It was realized a true circular economy(.K. Webster ,2015)) (see Fig 1 and Fig 2)that anticipated largely local sustainable development models nowadays very topical(.E.U. , 2014)

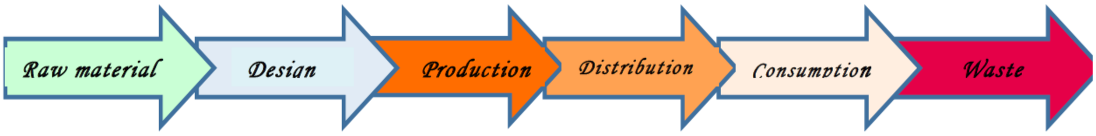

Fig 1-Theoretical linear economy scheme
Source:Our Elaboration

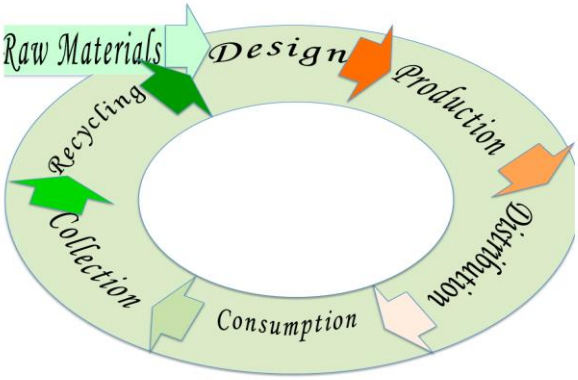

Fig 2- Theoretical circular economy scheme
Source:Our Elaboration

Material and Methods

This contribute is an exploratory and logical approach at the use of the edible wild herbs that as *ethnobotany* represent a practical contribute by the natural areas at the principles of the Circular Economy. The method is logical and inductive. It shown data that are the results of the research activity in this sector particularly for the part regarding ethnobotany. The materials used about the ethnobotany comes from deep bibliography studies and by the analysis of the results of researches in the field arranged during the last 10 years related to

Umbria Region located in Central Italy. About the part of the Circular Economy and Well Being the element of introduction, discussion and conclusions derive by the careful reading, analysis and interpretation of the most important and recent contributions, in these topics, in the recent years.

Results and discussion

In the general approach many recent studies have shown that the use of wild species for domestic use is widespread following traditions and customs associated with them, all over the world. The enhancement of the edible wild herbs and their uses in the kitchen is in the same time an action of biodiversity preservation. The Biodiversity, in fact, is not given only by the number of species present in an area, but also includes the ways in which plants are grown and are used in the kitchen. In the specific research arranged in Central Italy in 2009 the analysis of the questionnaires showed that the main species used and known belong to the family *Compositae* followed by *Cruciferae, Umbelliferae, Liliaceae,* and *Rosaceae* (Maurizi et al., 2015; Ranfa et al., 2015).This data is in line with the expectations of the national territory.The Compositae are the main edible wild specie of herbs which is part of the most famous chicory *(Cichorium intybus* L.*).*In the research was analyzed the different level of the average usage expressed in percentage . The main use for the 73% is as food followed, but in a small part, by the pharmaceutical and veterinary. These latter by the virtue of customs and traditions sometimes passed down in families between the different generations,(Fig. 3).

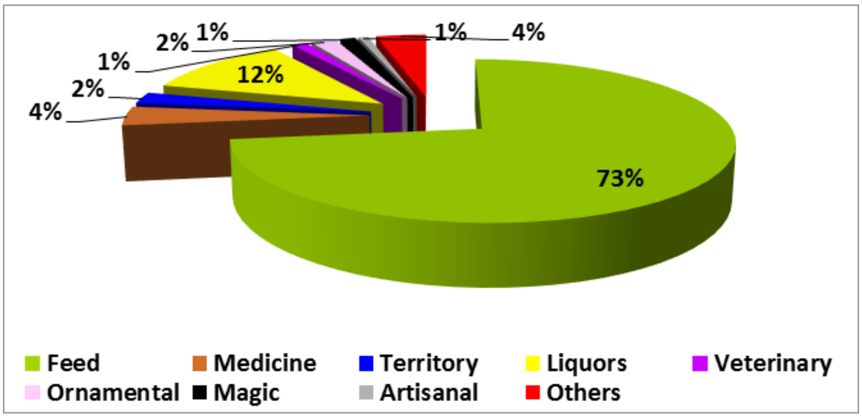

Fig 3- Average distribution of the edible wild herbs in Central Italy
Source: Own elaboration from 2009 Survey

Regarding the feed usage the main relevant is the gastronomic preparations Two most common ways of use of the species are or raw in salads or cooked as simple dish or for fillings some cakes or special pasta as ravioli. In all case is more frequently to mix several species and it’s rare to use only single one.

The research as permitted to verify that in the current analytical techniques it’s possible to determine, in addition to classical chemical composition percentage, the other components, so-called *"minor"*.About the main components,–the interest today is directed to the identification of the antioxidant properties .This relevant attention is related at the , currently, oxidative stress, of the people.This is considered responsible for the development of many degenerative diseases. All of this derive by the imbalance between the formation ,in the body ,of *reactive oxygen species* (ROS) and *nitrogen* (RNS) compared with the endogenous and exogenous antioxidants. The imbalance leads diseases of the cardiovascular

system (hypertension, atherosclerosis, heart attack and stroke),. certain forms of cancer, the autoimmune system, Alzheimer's dementia, Parkinson's disease, and acceleration of the physiological process of aging. The research have permitted to verify the relevance to determine the TAC(*Total Antioxidant Capac*ity) of these herbs. This verification was possible using the ORAC method (*Oxygen Radical Absorbance Capacity*) that is currently considered the most reliable analytical point of view. In general, by the previous work it's propose that the chemical composition of spontaneous species it's a pillar from the nutritional point of view(T. Jakovljevic,& Others,2011; Ł.Łuczaj & Oth., 2015.)This as for the *excellent contribution to offer in the water-salt balance maintenance,* as for *the intake in dietary fiber*, as well for the *really very low energy* (Maurizi et al., 2015; Ranfa et al., 2015).
In general, the ORAC results can be considered in itself interesting and higher are located, as well as in aromatic plants. In some wild species of great interest such as food *Sanguisorba minor, Bunias erucago*, *Chondrilla juncea* and *Bellis perennis* the results are very similar and sometimes even superior to those relating to certain cultivated species (Maurizi et al., 2015; Ranfa et al., 2015). The research have permitted also that the use of the edible wild herbs permit to implement in a concrete way the option of Circular Economy. Tis new model od development configuring a wonderful example of fusion between tradition and innovation, organizational activities and full circular character life where the 3R (reduce, reuse, recycle) make possible the recovery of lifestyles especially toward of Well Being option .These aspect confirm that the ethnobotany is in fact a best practice for the *affirmation of two (between 17)* of the main important SDG 2015-2030; the *number3- Ensure healthy lives and promote well-being for all at all ages* and the *number 12-Ensure sustainable consumption and production patterns* that are the pillar of the principles of the Well-being(A.Ciani&Oth, 2015)for all and for all age and for the implementation of the Circular Economy.

Conclusions

This paper has permit to demonstrate that the ethnobotany represent big role on the recovery of ancient customs and traditions of control, collection and utilization of food for traditional and/or healthful of *edible wild herbs species* in Central Italy.The interest in the *ethnobotany* has increased in recent years; saving these *knowledge* in fact, it also saves the cultural identity. Each farmer has a complex *knowledge* in respect of plants, *knowing* that is so important that some have called the farmers, who still retain them, *living treasures* or *witnesses.* More than ten years of research experiences in the Umbria Region(Italy) are shown about experiments and tests concerning the check of the organoleptic characteristics, nutritional and health benefits of the most widespread ecotypes. In current busy life, is not it time for the knowledge of the products of the earth that gives us the nature and the people, prefers to feed on the food of so-called fast food cuisine. The use of wild plants in the kitchen has always been deeply rooted in Italy especially in the inland areas and in rural areas, although in many cases are only the preserve by the only older people. In recent years, however, the situation is in a changing improvement because there is increasing interest for the consumption of different food plants, both in liquid preparations either salad or "green contours". The contribute of paper is also that *we can definitely say that the edible wild plants will have a role important feeding in the future front at the increasing of world population* .The research as demonstrate the updating linkage with the Sustainable Development Strategy stressed by the Sustainable Development Goals -SDG2015-2030 (UNGASS, 2015)that gives propulsion to the Age of Sustainable Development (J. Sachs, 2015))and strengthening of the Circular Economy(E.U.Commission, 2015) . The popular use of the discovery of a species is a stimulus for further study on the plants and to contribute to the recovery of the culinary traditions. Today there is a growing interest in all that is "natural"; the rediscovery of the kitchen made with foods of local tradition, it's symbol of healthy living.

The enhancement of the natural flora and its uses in the kitchen is also an action of biodiversity preservation understood in its broadest sense. The experience of the project has the character of *repeatability, traceability* and *transferability* and represents a way of further *transformation of the weaknesses* into *strengths* of the rural areas of each country including Croatia and an innovative *interlocking possible sustainable management and promotion of the territory.*

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TRUFFLE CULTIVATION: A MULTIFUNCTIONAL FORESTRY ACTIVITY AS CHALLENGE FOR THE SUSTAINABLE DEVELOPMENT OF THE RURAL AREAS IN TEMPERATE ZONE

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Introduction

Truffles are the finest and most expensive symbiotic fungi actively marketed all over the world (1). Spread throughout the Mediterranean basin and beyond, each truffle species has its own autecology as a particular growing niche that is currently threatened by the change in forest management, the abandonment of the countryside, the excessive truffle harvesting and the climate change.

The gradual change of development strategies nowadays entrusts all of us a strong duty after the approved document, on 25th September 2015, by the UNGASS "Transforming our world: the 2030 Agenda for Sustainable Development" that deep involves, all the world, in the implementation of the seventeenth Sustainable Development Goals (SDG's) 2015-2030.

In recent years inside at two Industrial Revolutions (III^ and IV^) two possible pathways have become popular: the Green Economy and the Bio-Economy. The Bio-Economy introduces new paradigms in the production processes related to renewable natural resources.

Scientific breakthroughs and technological progress have paved the way for a larger bio-economy sector where fungi can play a significant role in the pursuit of sustainability (2; 3). Nevertheless, we have not to forget the great contribution from fungi in mycorrhizal symbiosis which allows the primary production of the food chain. Ectomycorrhizal associations include approximately 6000 fungal species and 20000-25000 green plants, and are globally recognized to have a key role in forest ecosystems (4; 5).

Truffles are underground fruiting bodies (sporophores) of certain Ascomycetous fungi and belong to the genus *Tuber. They* grow in ectomycorrhizal symbiosis with forest trees in Mediterranean conditions. Many of them have a thriving market and are sold worldwide for exorbitant prices, although the profit is strongly mediated by weather conditions and annual production levels during the season (6; 7). The white truffle (*Tuber magnatum* Pico) is the most expensive and can even reach the price of 5000 €/Kg. The cheapest truffle, whose trade and sale are prohibited in Italy, but not in France and Spain, is *T. indicum* Cooke & Massee, sold for about 10-50 €/kg in China. For the most marketed truffle species i.e., *T. magnatum*, *T. melanosporum* Vittad., *T. borchii* Vittad., *T. aestivum* Vittad., *T. brumale* Vittad., the amount of profit probably reaches hundreds of millions of euros per year (8). In Italy, approximately 80% of the national production of truffles goes to the food processing industry, of which about 65% is *T. aestivum* and 10% is *T. borchii* (6, 7).

Truffle harvesting is an enjoyable pastime for many cultures, age groups, and families, an activity that brings people to the forests (9).

During the first half of the 20th century natural truffle production declined dramatically. With the two world wars in Europe, the economic devastation and the slow migration of people to cities, truffle orchards were abandoned and production consequently collapsed. Woodcutters and grazing animals had kept the forests quite well maintained, but with the drift to urban areas large part of the producing sites became wild and overgrown (7). Moreover, changes in the management of forests has brought a substantial modification in the microclimate suitable for *T. melanosporum* in terms of forest coverage, competition between trees and consequent soil heating and water availability (9).

Nowadays truffle production is almost totally carried out in cultivated orchards, especially for *T. melanosporum* (10). Agro-forestry management is needed to renew the natural productive habitat. High quality plants produced with native material (i.e., truffles and host seeds) could be cultivated with the aim of maintaining the social and economic heritage provided by this niche product and preserve its tradition.

In view of the new trends requested by the sustainable development strategies in agriculture and forestry, the ecological requirements of the main species of truffles and their forest management in Mediterranean Areas, highlighting the legislative and professional requirements in order to facilitate the protection and conservation of this precious heritage.

Material and Methods

Based on the authors experiences and research and of the selected bibliography, we have analyzed the components of the Green Economy and the bio-economy and sustainable ecosystem management as a function of truffle resource, proposing measures to improve existing truffle grounds in the Mediterranean areas.

Results and discussion

The framework of reference

The world of truffles includes a very wide range of operators related to the management of this natural resource, from harvesting to trade and catering organized and expressed with the same Italian style recognized all over the world, especially for the agri-food testing, design and fashion.

In recent years two specific alternatives became popular: the Green Economy and the Bio-Economy. The Bio-Economy opens the perspective of introducing new production processes and new products related to renewable natural resources. This aspect, through the “Smart Grid”, has the main target to encourage the access to energy and water for all people in the world. The production of energy from biomass is a real possibility of extension of the functions that characterize the so-called "forestry production". The way indicated by the bio-economy is complicated and highlights the concrete technical processes of sustainable development, the opportunities offered by biodiversity and its enhancement with clean technologies, together with the use of advanced Innovation Communication Technologies (ICT) that promote the overall conservation and the development of the area.

The Sustainable Development Strategy

The Strategy of Sustainable Development dictates two new options for the model of development to prevent breaking of the equilibrium that sustains life on earth. The first is represented by the intra- and inter-generational vision of development, the second by the necessity of anchoring development indicators to not only an economic but, social, environmental, cultural and managerial aspect. Both of these options have undergone, since their direct enunciation in the official document “Our Common Future” in 1987, some very positive adjustments, such as the opportunity to measure the development of a country not more according to Gross Domestic Product but according to Happiness Gross National.

Bhutan is the first country which has seen its drafting national budget on the basis of this criterion.

In this context, the activities that emphasize the action of natural processes, such as truffle silviculture, reforestation and cultivation, have now values that goes beyond the simple and direct commercial interest, moving in the vast area of the environmentally sound management of the territory and to maintain equilibrium natural, present and future.

The protection of natural and cultivated truffle environments (particularly in suitable areas) is a moral and historic duty as well as scientific, in order to maintain over time the environmental integrity of these ecosystems.

The Value of Use versus the Total Economic Value of commodities

As traditionally accepted, the value of a commodity is given exclusively by the value of use or the marginal utility that from its use can be achieved. In today's terms of sustainable development, commodities and natural resources play an important role for present but also for future generations: in addition to the value of use natural assets assume, also in relation to the value which has their possible utilization for future generations, a non-use value. This non-use value in the last two or three decades was tested significantly and has now led to a more consistent realignment of the value of an asset. Thus, we no longer speak of simple use-value, but the total and economic value as well as clearly shown in **Table 1.**

Table 1. The Total Economic Value of commodities according to the vision of sustainable development.

Use-Value	Use-Value	Use-Value	Non-Use Value	Non-Use Value
Direct use	Indirect use	Optional value	Value of inheritance	Existence value
Directly available products	Functional benefits	Future direct and indirect value	Use value and not use value of environmental inheritance	Value derived from knowledge of the existence resource
Food Biomass	Flood control Protection from atmospherical events	Biodiversity Habitat conservation	Habitat Prevention from irreversible changes	Habitat Species
Recreation Health	Nutrient cycle			Genetic asset Ecosystems

Within this framework, it follows that some goods (e.g., environment, production processes, typical products), while maintaining their existence and bequest value must be able to change their legal status. Some natural resources or economic sectors can absolve a public function changing from only private goods they become common ones.

The Rural Development Program of the European Union for 2014-2020 describes the public function of the agriculture and forestry. In Italy and in other European countries exists the objective need to provide adequate financial support for farmers to avoid the gradual abandonment of the countryside and agricultural production.

To define agriculture as a sector with a public function means to safeguard its future and farmers will benefit from financial support planned by "WTO Green Box" especially for targets related to the so-called "no-trade concerns".

In this context also the forestry management of truffle natural productive sites as well as truffle cultivation should take on the role of public service linked to productivity but also to protect the bequest value and existence of truffle ecosystems.

Trufficulture: multifunctionality and total economic value of public forest commodities

In addition to the significant economic value, food and environmental sustainability of natural and cultivated truffles has a high added value. In particular, in **Table 2** is shown the multi-functionality of the truffle and therefore the size of its Total Economic Value.

Table 2. The Multi-functionality of forest management of natural truffle producing areas and truffle cultivation.

In the traditional approach	In the sustainable development approach
Production	Ecosystem
Rural Tourism	Biodiversity
Agritourism	Habitat
Food and Cooking	Landscape
Land Management	Biomass
	Renewable Energy
	Arts and Handcrafts
	Water Management
	Territory Promotion and Valorization
	Culture
	Tradition
	Innovation
	Territorial Marketing
	Carbon sequestration

Therefore, it is evident that an inadequate management of productive natural habitats in fact denies the perspective proposed by the document "The Future We Want" that in the point V concerning Action and Follow –up stresses the target of the Sustainable Agriculture and Forestry. It assigns a key role to sustainable management of mountain areas, soil conservation, promotion of the territory, protection of biodiversity and natural habitats, water regulation and the production capacity of the soil in order to promote food security, the development of disadvantaged economies and reduction of poverty.

In this sense, forest management of truffle productive sites, truffle cultivation and/or reforestation with truffle plants can give the truffle a real role to enhance and strengthen the common good, especially in suitable forest environments.

Forest management and conservation

Ecological factors and human management practices are known to directly influence the structure of the ectomycorrhizal communities in forest ecosystems (11) and, likewise, natural and cultivated truffle plantations too, where component species respond in different ways to them.

Thus, only when conditions are appropriate for mycelium development will truffles complete their life cycle and produce fruit bodies.

Conclusions

Truffles play a key role to sustainable management in rural areas, soil conservation, territory promotion, protection of biodiversity and natural habitats, water regulation and the production capacity of the soil in order to promote food security, development of disadvantaged economies and poverty reduction. Silvicultural interventions must take into account the truffle resource with the problems of forest management in order that appropriate action is taken for the conservation and enhancement of this important forest product. The truffle can be a concrete example where a coherent forest and land management, combining innovation with tradition, can achieve multiple objectives that the strategy of sustainable development requires us from the economic, social, ethical and moral point of view, to contribute at the end to the well-being and happiness of the society.

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ASSESSING RURAL AND AGRICULTURAL RIPARIAN AREAS OF GREECE WITH THE USE OF GIS AND SVAP

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Abstract

The designation of many riparian areas worldwide as protected indicates their importance. The need for their protection is the result of the many alterations induced by humans along with the many ecosystems services they offer. Riparian areas are adjacent to rivers and lakes and are considered corridors characterized by a very high level of fauna that is tolerant to flood conditions. While in most countries their importance has been a major priority, in Greece, only in the last decade riparian research has been brought to the forefront. An important aspect of riparian areas is their assessment in order to maintain their healthy functioning, structure and services. The purpose of this research was to evaluate tools for the quick assessment of riparian areas in order to help land managers better conserve and sustain them. More specifically, this study assessed a riparian area along a perennial stream reach adjacent to rural settlements and agricultural areas located in Drama Prefecture, in the Eastern Macedonia region of Greece. The selected stream reach has a typical riparian area of lowlands in the Mediterranean region. For the assessment, a field survey method along with Geographic Information Systems (GIS). GIS were used to evaluate the research area by integrating, storing, analyzing and presenting geographic correlated data. Using the buffer function in GIS we found that the riparian areas, in some cases even right along the stream bank, contain irrigated and non-irrigated agricultural crop fields, discontinuous urbanized areas, but also discontinuous broadleaved stands. For the field survey method, the Stream Visual Assessment Protocol (SVAP) protocol was chosen. It is a "rapid assessment protocol", since it provides a quick, visual and systematic assessment of these natural ecosystems. In addition, this protocol allows the collection of data without interfering and altering ecosystems, while it can be applicable to many different regions. Results based on the SVAP protocol indicate that the riparian area was highly degraded due to human interferences. Overall the combination of SVAP and GIS, provided tools to land managers to evaluate quickly and effectively riparian areas in rural and agricultural landscapes that will help them make management decisions in order to enhance and conserve riparian areas to maintain ecosystem services.

Keywords: *best practices, conservation, ecosystem services, new technologies, vegetation ecology*

Introduction

Riparian areas are unique transitional ecosystems between aquatic and terrestrial that exist in all biomes (Gregory et al. 1991; Malanson 1997; Ffolliott et al. 2004). The three main characteristics of riparian areas are the greater water availability (soils and flooding), young-undeveloped-rich in nutrient soils and unique dense with many canopy layers vegetative assemblages (National Research Council 2002). These characteristics lead to the many different ecosystem services that riparian areas offer (Malanson 1997).

The many ecosystem services of the riparian areas have led to their extensive utilization by humans for thousands of years (Naiman et al. 2005). This use has led many researchers to consider them as some of the most degraded and threatened ecosystems (National Research Council 2002). Two of main anthropogenic activities that have negatively impacted riparian areas are agriculture and human settlements. Agriculture activities have transformed many riparian areas into agricultural fields due to their fertile soils that can lead to high crop productions. Human settlements can lead to the fragmentation of riparian areas (Hutmacher et al. 2014; Iakovoglou et al. 2013). Connectivity is a key characteristic of riparian areas in order to maintain their ecosystem services (Zaimes and Emmanouloudis 2012).

One of the main reasons to protect and conserve riparian areas is that despite occupying only a small portion of the watershed, they offer disproportionately larger amount of ecosystem services (Patten 1998). By leaving or re-establishing the riparian vegetation along the water body, in agricultural areas or areas with human settlements, the riparian area can maintain their connectivity and most of their ecosystem services. The European Union (EU) has recognized their importance with the many riparian areas that have been designated as Natura 2000 sites (European Commission 2007) and because they are considered within the Water Framework Directive (2000/60/EC) as an essential feature in the hydromorphological assessment of freshwater bodies.

In Southern Europe the maintenance and re-establishment of riparian areas is a necessary task (Decamps et al. 1988; Corbacho et al. 2003). Most riparian areas have experienced extensive land-use changes and other human disturbances for hundreds of years. In Greece, in most lowland areas where agricultural activities are prevalent, riparian areas have been eliminated or have been severely degraded (Zaimes et al. 2010). Characteristic examples are the lowland areas of the tributaries of Nestos River that are, in most cases, cultivated up to the edge of the stream with the riparian vegetation completely eliminated (Zaimes et al. 2011a). Only the adjacent areas of the main reach of Nestos River are forested, belonging to the Natura 2000 network.

In Greece, in general, the study and conservation of riparian areas has been brought to the forefront only in the last decade (Zaimes et al. 2011b). The purpose of this study was to evaluate tools for the quick assessment of riparian areas in order to help land managers to better conserve and sustain them. This was done with a field survey visual assessment tool that was modified to the characteristics of the Euro-Mediterranean environment and with the use of the Geographic Information Systems (GIS).

Methods

The study was conducted in the region of Eastern Macedonia in northern Greece in the prefecture Drama (Figure 1). The lowlands of the prefecture are primarily used in agriculture production with an average yearly temperature and annual mean rainfall of 15 ^{O}C and 540 mm, respectively. To select the studied reach, a survey of the prefecture's riparian areas was conducted, initially through Google Earth and afterwards with actual field visits. The goal was to find a riparian area representative of Mediterranean lowlands that was heavily influenced by agricultural activities and rural settlements. A 12 km reach of Nikotsaras stream was selected (most northern reach 41°09'31.7"N 24°03'28.8"E and most southern reach 41°05'25.9"N 24°06'20.9"E) that is located 10 minutes east from the capital of the prefecture that is also called Drama (Figure 1). The surrounding topography is considered flat with elevation ranging from 55 to 110m. The adjacent areas to the study reach are primarily agricultural, although there are four rural settlements (Milopotamos, Nikotsaras, Kalos Agros and Koudounia) near the stream banks or in very close proximately to the studied reach. The agricultural activities and villages have major impacts on the stream and its riparian areas.

Initially the assessment of the riparian areas was done through the use of GIS. This allows the quick, 'from the office', assessment of large lengths of riparian areas and can provide a preliminary evaluation of their condition that should be further validated with field observations. For this part of the study the relevant map and images were initially geo-referenced to the Greek Geodetic Reference System GGRS (1987). Then the studied stream reach and its adjacent land-uses/cover were digitized. The final step was to estimate the area of the different land-uses/cover adjacent to the stream by using the GIS "buffer" function. The buffers' lengths used were of 20 and 50 m from the stream bank, that are typically the extent of riparian areas in such streams of the Mediterranean region.

For the field survey observation, the Stream Visual Assessment Protocol (SVAP) was selected. The SVAP provides information on the ecological status of streams and riparian areas and was developed in the United States (Bjorkland et al. 1998; 2001). In this study it was modified to the needs of Greek riparian areas (Zaimes et al. 2010), but could also be applied to other Euro-Mediterranean countries. For the assessment, initially the general information on the plot surveyed was collected. Afterwards, the specific stream and riparian characteristics were examined. For the modified version these characteristics were: a) Channel condition, b) Hydrologic alteration, c) Riparian zone condition, d) Bank stability, e) Water existence, f) Water appearance, g) Livestock shed presence, h) Instream fish cover, i) Pools, j) Insect/invertebrate habitat, k) Canopy cover, l) Manure presence, m) Biological wastewater treatment presence, and n) Garbage presence. The 14 characteristics were ranked with values of 0-10 depending on the actual conditions of the plot (with 0 the worst and 10 the best). The overall score for the plot was the average of the 14 characteristics. The categorization was as following:

- **Poor** with values lower than 6,
- **Moderate** with values from 6.1 to 7.4,
- **Good** with values from 7.5 to 8.9 and
- **Excellent** with values greater than 9.

Overall, 25 different point were assessed within the studied stream reach with the SVAP protocol. The exact location of the 25 points can be seen in Figure 1.

Figure 1. The location of the study area in Greece (bottom left corner) and a close up of the studied reach. In addition with red dots, the 25 points that were assessed using the Stream Visual Assessment Protocol (SVAP) are indicated.

Results and Discussion

The first step of the evaluation was to examine the type of land-uses/cover adjacent to the stream and in the riparian zone. This was done by using the buffer function in GIS and estimating the areas for a buffer of 20 and 50 m on both sides of the studied stream reach. This area within the two buffers was considered to be the natural riparian area. The detailed results with the areas of the land-uses/cover can be seen in Table 1.

Table 1. The land-uses/cover adjacent to the studied reach within a 20 and 50 m buffer.

Riparian land-uses	20 m buffer (ha)	50 m buffer (ha)
Non-irrigated farm land	20.01	29.18
Irrigated farm land	10.28	16.48
Discontinuous rural settlements	0.11	0.91
Broadleaved forest	14.52	20.66

Based on Table 1 the majority of the riparian area was categorized in agricultural land-uses, as expected, for both the 20 m (total 67.4%, non-irrigated 44.5% and irrigated 22.9%) and the 50 m (total 67.9%, non-irrigated 43.4% and irrigated 24.5%) buffer. The percentage of the irrigated, as well as of the total farm land increased slightly for the 50 m buffer. The discontinuous rural settlements increased the most for the 50 m buffer compared to the 20 m buffer (from 0.2% to 1.4%). Finally, for the broadleaved vegetation, it decreased slightly for the 50 m buffer (from 32.3% to 30.7%). This land-use/cover is of great importance since it represents the natural riparian vegetation. Overall, the natural vegetation covered less than the 1/3 for both buffers and this is alarming since it indicates fragmentation of the riparian area and decreased ecosystem services.

Average score values based on the SVAP assessment can be seen for all points in Figure 2. These scores indicated that only three points were categorized as Good (1, 5, 13), two as Moderate (3 and 12) and all the rest were categorized as Poor with values less than 6. This is also something that should be seriously considered because it indicates degraded and fragmented riparian areas that correlates well with the GIS analysis. The main culprits were the extensive agricultural activities in and adjacent to the riparian areas along with human rural settlements. In the past, researchers have shown that these practices can be detrimental to riparian areas (National Research Council 2002; Iakovoglou et al. 2013). Still, with proper management plans the anthropogenic activities can be maintained, while simultaneously enhancing riparian ecosystems as well as substantially improving their condition and services (National Research Council 2002; Naiman et al. 2005).

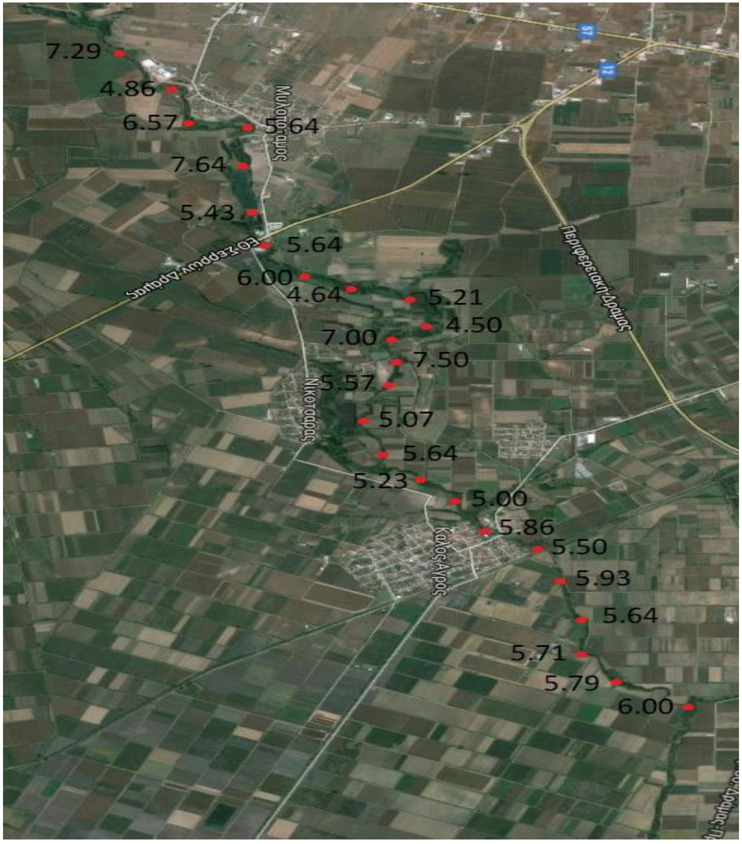

Figure 2. The SVAP values for each point that was assessed on the studied reach.

Conclusion

Overall, both assessment tools indicated that the riparian areas of Nikotsaras stream were degraded and need to be managed better. This is not surprising since other studies in the region found similar results for riparian areas in lowland areas of Eastern Macedonian and Thrace of Greece (Zaimes et al. 2011a). The main anthropogenic activities that negatively impacted the riparian areas were agriculture and rural settlements.

This clearly indicates that new management measures need to be taken and conservation practices need to be established. Some of the measures can include re-establishing the natural riparian vegetation at least along the 20 m buffer when possible. Another measure could be better management of the existing riparian vegetation to help improve their functionality and consequently their ecosystem services. Finally, since many banks were suffering from severe erosion, bio-engineering along with willow plantings are recommend to enhance bank stability that would also improve water quality.

Overall, both of these tools allow land mangers to quickly assess riparian area conditions in order to decide what and if any management actions should be taken to enhance the riparian areas of interest. These tools could also be adopted in other lowland riparian areas of the Balkans and the greater Euro-Mediterranean region. Nonetheless, for the final management and conservation practices to be implemented more detailed field measurements are required.

Acknowledgements

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Session B: Food as Natural Sources of Functional Ingredients

MINERAL COMPOSITION AND HEAVY METALS CONTENT OF SWEET CHESTNUT FRUITS IN FIVE NATURAL POPULATIONS FROM CENTRAL CROATIA

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Introduction

The sweet chestnut (*Castanea sativa* Mill.) is a noble hardwood providing multiple economic benefits (wood, fruit, honey, tannin, preservation of ecological and landscape values). It is distributed in the Mediterranean region, where chestnut forests spread over an area of 2,530,000 ha (Conedera *et al*. 2004). In Croatia, the sweet chestnut grows in the forests of the colline belt of the continental part, in Istria and on the islands of Krk and Cres. The largest part of the range of the sweet chestnut extends through central Croatia, from the Slovenian border to the border with Bosnia and Herzegovina (Medak *et al*. 2009).

Chestnut fruits are an important food in many European countries. In recent years, the consumers have been showing an increased interest in chestnut fruits because of their nutritional qualities and potential beneficial health effects (Barreira *et al*. 2012). Chestnuts are rich in carbohydrates and are a good source of essential fatty acids and minerals (Borges *et al*. 2008; Barreira *et al*. 2009; De Vasconcelos *et al*. 2010). Many studies about the nutritional composition of chestnut define them as fruit which, in contrast to the most other nuts, have high water content, contain small quantities of fat and oil and are cholesterol free (De La Montaña Míguelez 2004; Borges et al. 2007, 2008; Peña-Méndez *et al*. 2008; Barreira *et al*. 2009, 2012). They also contain high quality protein and are gluten free. Except of that, they are good source of vitamin C, B1, B2, and folates, as well as appreciable levels of fibre (De Vasconcelos *et al*. 2010). Breisch (1995) indicated that the main macroelements are potassium, phosphorus, calcium and magnesium.

Material and Methods

Sweet chestnut fruits were collected during October 2007 in five natural populations in Croatia, as shown in Figure 1. Fruits were collected from five different trees per each population. Directly after collection, the freshly picked fruits were peeled. The pericarp and seed coat were removed, after which only the edible part of the fruit remained, i.e. the kernel. The cleaned seeds were shredded using a shredding blender. From each tree, 100 g of shredded sample was placed in hermetically closed containers and stored in a freezer at -20 °C. Macro and micronutrients (K, Ca, Mg, Na, Fe and Zn) and toxic metals (Pb, Cd, Hg, As) were determined by atomic absorption spectrometry using a Varian SpectrAA 220 device.

For all of the studied variables, descriptive statistical parameters were calculated. Assumptions of normality were checked using the Shapiro-Wilk test, and the assumption of homogeneity of variance using Levene's test. Statistically significant differences between studied groups of trees were established using the analysis of variance (ANOVA). The differentiation among studied groups for variables (Hg, Cd) with a skewed and heteroscedastic distribution was verified using the Kruskal-Wallis ANOVA. The data used in the PC analysis were previously standardized using the z-score method. The specified statistical analyses were conducted using the STATISTICA 8.0 software.

Results and Discussion

The results of the descriptive statistical analysis are shown in Table 1. With regard to macronutrients, K (613.96 mg/100 g f.w.) was the most abundant, followed by Ca, 35.17 mg/100 g f.w.; Mg, 29.99 mg/100 g f.w.; and Na, 3.69 mg/100 g f.w. A similar pattern was observed by Bellini *et al*. (2007) and Poljak *et al*. (2016) for the Italian and Croatian chestnut cultivars. A smaller mass fraction of potassium and calcium was recorded in Spanish (Pereira-Lorenzo *et al*. 2006; Peña-Méndez *et al*. 2008) and Portuguese (Ferreira-Cardoso *et al*. 2005; Borges *et al*. 2008) chestnuts. Magnesium content on average is within the range reported by Pereira-Lorenzo *et al*. (2006) for Spanish chestnut cultivars. Smaller magnesium content on average is reported by Bellini *et al*. (2007) for the Italian chestnut cultivar ‘Marrone del Mugello' and higher by Borges *et al*. (2008) and Ferreira-Cardoso *et al*. (2005) for Portuguese chestnut cultivars, and Peña-Méndez *et al*. (2008) for Spanish chestnut cultivars. The sodium content of the chestnuts from natural populations is within the range reported by Pereira-Lorenzo *et al*. (2006) and Peña-Méndez *et al*. (2008) for Spanish chestnut cultivars. Smaller sodium content on average is reported by Borges *et al*. (2008), Ferreira-Cardoso *et al*. (2005), and Bellini *et al*. (2007) for the Portuguese and Italian chestnut cultivars. The average mass fraction of zinc in the chestnut fruits from the natural populations is in line with the data published for sweet chestnut cultivars (Ferreira-Cardoso *et al*. 2005; Pereira-Lorenzo *et al*. 2006; Peña-Méndez *et al*. 2008; Bellini *et al*. 2007; Borges *et al*. 2008; Poljak *et al*. 2016). Iron content ranged between 1.29 mg/100 g f.w. in population Gvozd and 1.56 mg/100 g f.w. in population Ozalj, with 1.37 mg/100 g f.w. as a mean value. Smaller mass fractions of iron was reported by Peña-Méndez *et al*. (2008) and Pereira-Lorenzo *et al*. (2006) in Spanish chestnut cultivars, and greater by Borges *et al*. (2008) and Ferreira-Cardoso *et al*. (2005) in Portuguese chestnut cultivars. This study confirmed that chestnuts from natural populations are a good source of macro- and micronutrients. The content of detected toxic heavy metals was lower in all samples than the maximum allowed amounts for the sweet chestnut fruits, which indicates an unpolluted environment. The cadmium and mercury level in approximately 25 % of the samples was below the limit of detection (< 0.01 mg/kg). According to the results of the ANOVA (Table 1), the studied populations differed in the mass fraction of potassium ($p<0.01$) and magnesium ($p<0.05$). The differentiation in the mass fraction of calcium, sodium, iron, zinc and toxic metals was not statistically significant. The PC analysis for the six studied macro and microelements established that the first two principal components account for 74.5 % of the total variability (Figures 2 and 3). The first principal component, which accounts for 54.0 % of variability, separates the populations with high potassium content, which is highly positively correlated with it, from the populations with high iron and zinc content, which is highly negatively correlated with the same principal component. The second principal component participates in the overall variance with 32.2 % and is in highly negative correlation with the content of magnesium. In other words, the bottom side of the diagram contains populations with a higher portion of the magnesium than those on the upper side. The differences found among the populations in mineral composition may reflect genetic factors and environmental conditions.

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Table 1. Descriptive statistical parameters, and level of significance.

Minerals and toxic metals	Population					P
	Ozalj	Moslavačka gora	Vojnić	Gvozd	Topusko	
K mg/100 g f.w.	602,58±36,67	579,18±20,54	632,07±23,74	629,95±10,05	626,02±21,01	< 0.01
Ca mg/100 g f.w.	37,04±5,68	39,21±3,74	32,42±5,15	34,67±4,73	32,49±2,63	ns
Mg mg/100 g f.w.	26,67±1,47	32,80±5,07	28,07±3,03	34,44±5,61	27,98±4,52	< 0.05
Na mg/100 g f.w.	4,70±2,80	3,92±1,60	3,96±1,99	3,90±2,37	1,98±0,35	ns
Fe mg/100 g f.w.	1,56±0,30	1,35±0,16	1,33±0,23	1,29±0,24	1,30±0,20	ns
Zn mg/100 g f.w.	0,87±0,24	0,69±0,12	0,67±0,21	0,59±0,08	0,69±0,19	ns
Pb mg/kg f.w.	0,048±0,016	0,044±0,032	0,053±0,029	0,039±0,016	0,037±0,009	ns
Cd mg/kg f.w.	0,018±0,006	0,014±0,004	0,019±0,003	0,017±0,008	0,022±0,008	ns
Hg mg/kg f.w.	0,013±0,005	0,034±0,018	0,040±0,008	0,010±0,000	0,016±0,005	ns
As mg/kg f.w.	0,034±0,005	0,034±0,009	0,032±0,015	0,018±0,008	0,032±0,004	ns

f.w. – fresh weight

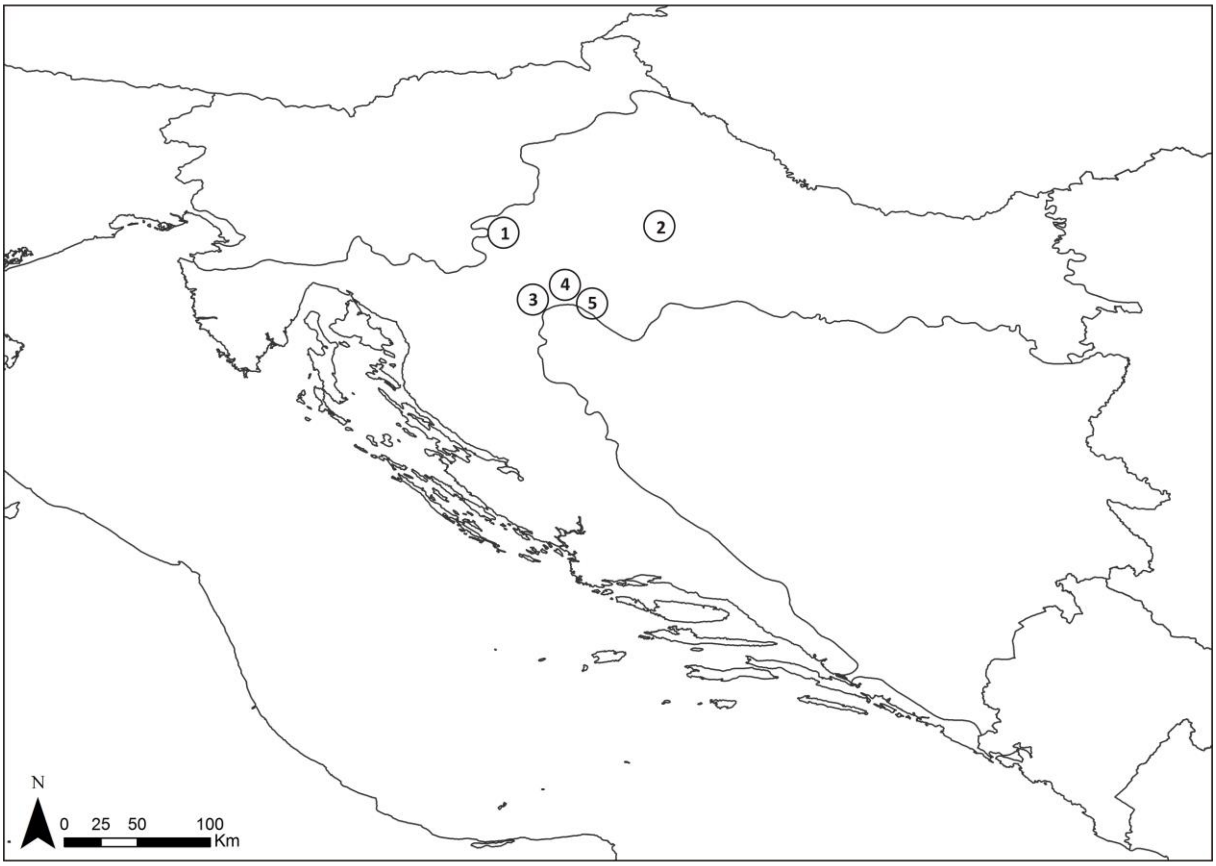

Figure 1. Sampled populations: (1) Ozalj; (2) Moslavačka gora; (3) Vojnić; (4) Gvozd; (5) Topusko.

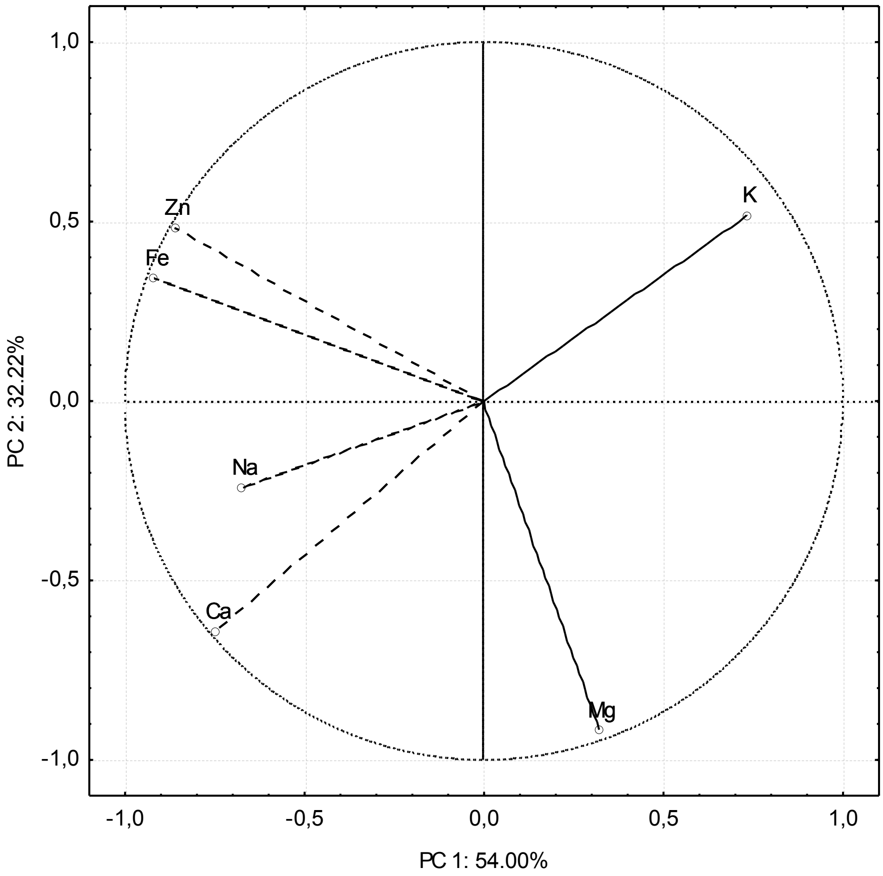

Figure 2. Score plot of the variables projected on the space of PC1 vs. PC2.

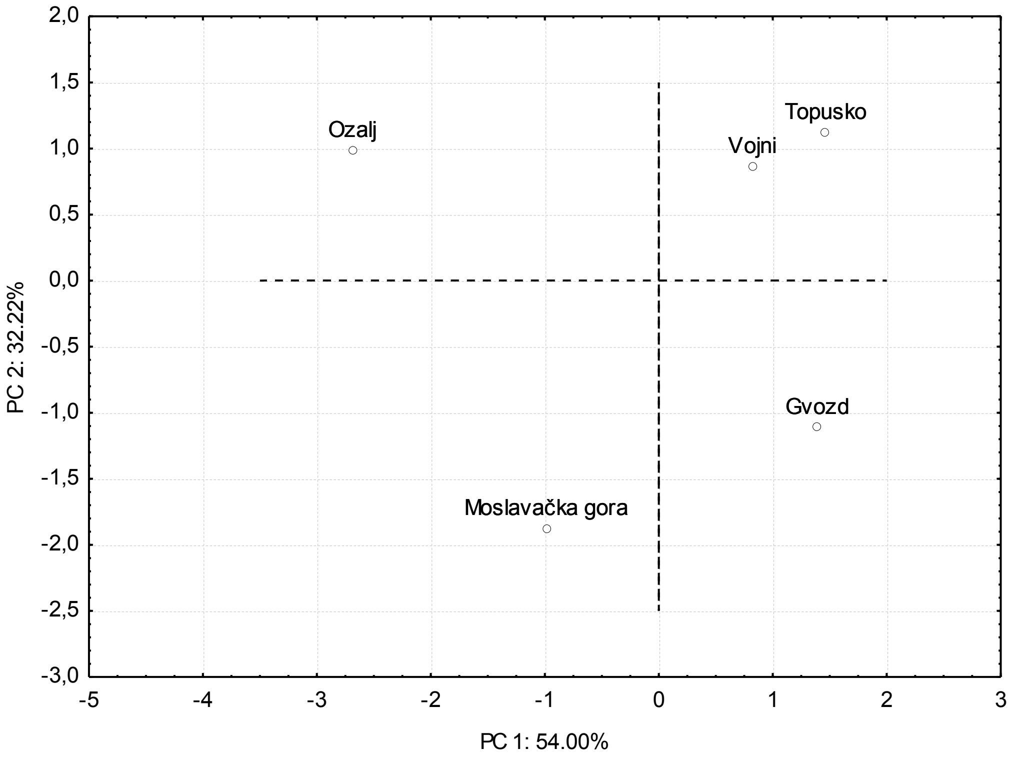

Figure 3. Score plot of the chestnut populations projected on the space of PC1 vs. PC2.

HEALTH PROMOTING AND SENSORY PROPERTIES OF CROATIAN MONOVARIETAL VIRGIN OLIVE OILS

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Introduction

Virgin olive oil (VOO) is the oldest of known oils, as well as one of the healthiest oils in human nutrition. It is a natural juice extracted directly from the fruit of *Olea europaea* L. VOO is a basic ingredient of Mediterranean diet, which many health experts recommend to maintain health due to its specific chemical composition (8, 15, 23). It is consumed unrefined and it is rich in important molecules, such as phenolic compounds (hydroxytyrosol, tyrosol, secoiridoids and flavonoids) and certain fatty acids (16, 20). These molecules not only influence the sensorial properties of both olives and VOO but they are also important markers for typicity, biodiversity and quality determination of this product (6, 7). VOO is characterized by the presence of biophenols, which are effective natural antioxidants that prevent the oxidation of LDL cholesterol (11). The health properties, sensory quality and oxidative stability of VOO stem from a prominent and particular chemical composition. It is composed of triacylglycerols (97-98%), minor compounds – partially glycerides, phospholipids and oxidized triacylglycerols, and around 1% of unsaponifiable constituents of varied structure and polarity (15). VOO is unique among other vegetable oils due to its high levels of monounsaturated fatty acids (MUFAs), mainly oleic acid, and also due to the presence of minor components, such as phenolic compounds, sterols, tocopherols, pigment compounds etc (1,2). Well-balanced fatty acid composition of VOO is one of the main reasons for its particular health protective role, primarily against cardiovascular diseases. VOO has low level of saturated fatty acids, high level of mono unsaturated oleic acid (18:1) and optimal level of essential fatty acids (linoleic and linolenic acid) that are responsible, along with presence of phenolic compounds, for prevention of different human diseases (coronary heart disease, breast and colon cancer, cognitive impairment and many others (5, 11, 21). The aim of this study was to evaluate the minor polar compound and fatty acid contents of monovarietal virgin olive oils, typical in Dalmatia olive region, in order to have better knowledge about the quali-quantitative profiles of these compounds in oils. Hereby we described the chemical composition of monovarietal Croatian VOO and the influence of specific chemical compounds or groups of compounds in investigated oils on their sensorial characteristics. The sensorial assessment was by trained professional panel performed on the investigated olive oil samples in order to define their sensorial profiles regarding cultivar.

Material and methods

Olive fruits from four autochthonous cultivars ‘Oblica’, Lastovka’, Levantinka’, and ‘Drobnica’ were hand-picked at the same ripening stage and processed in oil using laboratory system Abencor mc2, under equal processing conditions (malaxation for 40 min. at the 26^{o}C). After decantation, obtained oil was once again submitted to fine centrifugation in order to avoid sediment forming during storage. All samples were stored in dark amber glass bottles with headspace filled by nitrogen, at the +4 ^{o}C, until analyses were performed.

Basic qualitative parameters were evaluated in order to estimate the quality of obtained olive oils: free fatty acids (FFA), peroxide value (PV) and UV spectrophotometric indices K_{232} and K_{270}, according to the official analytical methods described in the EEC Regulation (9). All parameters were determined in triplicate for each sample.
Total phenolic content (TPC) was determined using the Folin–Ciocalteau method (10). The results for TPC are presented as mg of gallic acid per kg of oil. The spectrophotometric measurements were repeated three times for each extract.
Fatty acids methyl ester analysis (FAME) from the oil samples were obtained by alkaline treatment with 1M KOH in methanol, according to ISO method 5508 (13). The gas chromatographic analyses were carried out according to ISO method 5509 (14). The average was calculated from four replications for each sample.

Phenolic composition was determined by HPLC - DAD/MSD method (18). Measurement was carried out on the HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with two detectors (Diode-Array UV-VIS detector DAD and mass spectrometry detector MSDj). All analyzes were carried out at room temperature, at a wavelength of 280 nm. The average was calculated by four replications for each sample.

The sensory evaluation of olive oils was performed according IOC method (12). Sensory analyses of obtained olive oils were conducted by skilled panel group of sensory analysts for virgin olive oil. The results were calculated using computer program set in method for organoleptic assessment of VOO (12).

Results and discussion

Qualitative parameters and total phenol content in VOO samples presented in Table 1 show all values inside the range for extra virgin olive oil category.

Table 1. Basic quality parameters and total phenol content (TPC) for analysed monovarietal VOOs

VOOs	Basic quality parameters					TPC (mg/kg)
	FFA	PV	K232	K270	ΔK	
Oblica	0.16± 0.10	2.98 ±0.15	1.75 ±0.22	0.11± 0.05	0.005±0.001	315.57 ± 28.58
Lastovka	0.24 ± 0.15	2.25 ± 0.22	1.97± 0.35	0.13± 0.09	0.002±0.001	286.55 ± 42.86
Levantinka	0.18 ± 0.05	1.97± 0.32	2.15± 0.20	0.15± 0.05	-0.001±0.000	195.36 ± 56.88
Drobnica	0.26 ± 0.03	2.65 ± 0.15	2.18± 0.16	0.12± 0.07	0.001±0.000	354.72 ± 39.98

The total phenol content (TPC) measured in analysed oils varied depended on the cultivar. The lowest value was detected in ‘Levantinka’ oils (138.48 mg/kg), while ‘Drobnica’ oils showed the highest value for TPC (394.70 mg/kg).

Table 2. Fatty acid composition of analysed monovarietal VOOs

Fatty acid composition (% of total)	VOO			
	Oblica	**Lastovka**	**Levantinka**	**Drobnica**
18:1	75. 91 ± 1. 41	71. 81 ± 1.	76. 17 ± 0.	75. 14 ± 0. 34

		15	30	
18:2	9. 66 ± 0. 85	11. 63 ± 0. 29	6. 78 ± 0. 19	7. 93 ± 0. 04
18:3	0. 66 ± 0. 07	0. 66 ± 0. 04	0. 66 ± 0. 03	0. 55 ± 0. 35
18:1/18:2	7. 92	6. 18	11. 24	9. 47

The content of the most important fatty acids in VOO are presented in Table 2. ‘Oblica’ oils showed the highest content of oleic acid (up to 77.32% of total). All analyzed oil samples had oleic acid content (C18:1) higher than 70%. The monounsaturated fatty acids have great importance because of their nutritional implication and positive role on oxidative stability of oils (17). The content of linoleic acid (C18:2) had higher values (max. 11.92) in ‘Lastovka’ oils than in other samples. All cultivars showed a stable index expressed by the ratio of oleic acid to linoleic acid (C18:1/C18:2) with values near or higher than 7, with some exceptions in oils of ‘Oblica’. In particular, oils from ‘Levantinka’ cultivar had rather high C18:1/C18:2 ratios, with an average of 11.24 that implicates a good stability of those oils.

The composition of single phenol compounds is presented in Table 3. The highest level of hydroxytyrosol and oleuropein aglycon, two scpecies reponsable for bitter sensation, were detected in 'Lastovka' oil, which is in concordance with highest bitterness that is perceived in that monovarietal VOO.

Table 3. Single phenol composition in investigated monovarietal VOOs

VOO	**HYTY** (mg/kg)	**TY** (mg/kg)	**OA** (mg/kg)	**LA** (mg/kg)	**LU** (mg/kg)	**AP** (mg/kg)
Oblica	66.78±4.51	42.87±2.30	50.80±4.19	14.45±2.64	35.5±2.26	11.18±0.09
Lastovka	214.32±0.96	66.04±2.20	207.02±8.08	36.12±4.94	96.08±1.18	33.6±0.69
Levantinka	58.95±1.43	23.56±2.31	115.65±1.55	64.36±4.03	47.58±7.06	23.65±0.67
Drobnica	65.87±4.36	84.37±2.52	87.51±6.84	21.26±2.90	31.58±2.77	32.44±5.40

Symbols from table 3: HYTY – hydroxytyrosol, TY – tyrosol, OA – oleuropein aglycon, LA – ligstrozid aglycon, LU – Luteolin, AP - Apigenin

The sensory analyses of investigated oils showed specific characteristics of taste and aroma that is attributed exclusively to olive cultivar (19). Sensorial characteristics of the samples differ depending on the variety and have certain attributes for each monovarietal olive oil. Sensorial profile for two the most distinctive VOOs are presented in the form of spider net diagram (Figure 1) applying quantitative descriptive analyses, as a result of average value for each sensorial attribute.

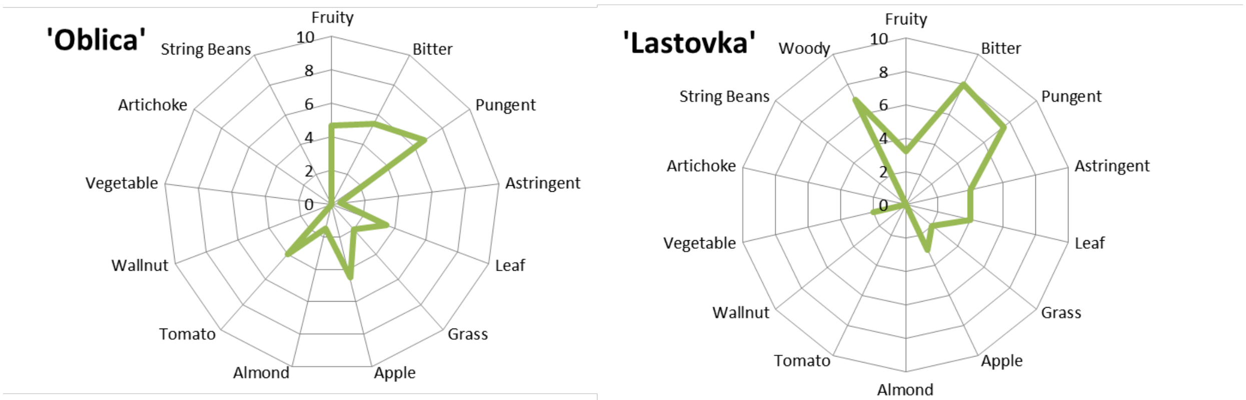

Figure 1. Quantitative descriptive analysis – sensorial profile of VOO from 'Oblica' and 'Lastovka'

Conclusions

The data reported in this paper represents the basis for creation of extra added value to Croatian monovarietal VOOs. The content of phenolic compounds is an important factor that influences the quality of VOO and also positive sensorial attributes (3, 4, 5). All samples in this study had very good phenol content and different phenol composition, depending on cultivar. These compounds are typical for unrefined oils and thus VOO has valuable functional, biological and nutritional function (21, 22). Sensorial characteristics differed from one variety to another and have particular attributes for each single oil. VOO from 'Drobnica' variety had the most intensive and the most balanced sensorial attributes, while olive oils form 'Oblica', the most common cultivar in Croatia, showed typical characteristics of mild, rather sweet oil. The highest intensity of bitter and pungent was detected in 'Lastovka' oils. Further studies are in progress to isolate the specific aroma compounds in order to investigate their impact to biological, nutritive and sensorial properties of these oils.

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KINETICS AND THERMODYNAMICS OF THE SOLID-LIQUID EXTRACTION PROCESS OF TOTAL POLYPHENOLS, ANTIOXIDANT CAPACITY AND EXTRACTION YIELD FROM NETTLE (*Urtica dioica* L.)

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Introduction

Medicinal plants are largely investigated nowadays, because they are natural products with biologically active organic compounds, which are usually non aggressive, and do not cause severe side effects [1]. Medicinal plants and extracts such as nettle (*Urtica dioica* L.) extracts are used for medical purposes and were applied in the folk therapy since centuries [2]-[3]. In order to separate bioactive components from plant materials, solid-liquid extraction is one of the simplest methods used in many food and pharmaceutical industries. To obtain the highest possible amount of bioactive compounds, detailed analysis of the extraction process dynamics is required, as well as the use of mathematical modelling in order to reduce energy, time and chemical reagents consumption. In this work, solid-liquid extraction of total polyphenols, antioxidant capacity and extraction yield from nettle in batch reactor with continuous stirring and controlled temperature were performed. Three kinetic models (Peleg's, Page's and Logarithmic model) were applied in order to describe the experimental results of nettle solid-liquid extraction. Initial extraction rate, extraction capacity, activation energy, Gibbs energy, enthalpy and entropy were determined to get a detailed insight into dynamics of the extraction process.

Materials and methods

Plant material: Dried plant material of nettle (leaves) was acquired from a specialized herbal store (Suban d.o.o., Zagreb, Croatia). Plant material was collected in the north-western part of Croatia during the flowering seasons of 2015., dried and properly stored until used. Dry matter content of plant material was determined by oven-drying at 105 °C, according to the standard AOAC method [4].

Chemicals and reagents: Folin-Ciocalteu's reagent and sodium carbonate were purchased from Kemika (Zagreb, Croatia). Trolox (6-hydroxy-2,5,7,8-tetra methylchromane-2-carboxylic acid) was obtained from Fluka (Buchs, Switzerland). DPPH (2,2-diphenyl-1-picrylhydrazyl) and gallic acid (3,4,5-trihydroxybenzoic acid) were purchased from Aldrich (Sigma–Aldrich Chemie, Steinheim, Germany). Methanol (HPLC grade) was obtained from J.T. Baker (Deventer, The Netherlands).

Extraction: An amount of 60 g of dry plant material was placed in a 2000 mL glass with 1500 mL of deionised water and heated to a specific temperature (T = 40, 60, 80 °C ± 0.5 °C) using Ika HBR4 digital oil-bath (IKA-Werk GmbH & Co.KG, Staufen, Germany). Experiment was performed with the plant material particle size range from 3000 – 4000 µm, rotational speed was set at 500 rpm and the extraction duration time was 90 minutes. Sampling was carried out at regular time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90 min.). Samples were immediately cooled in the water-ice mixture, filtered through a 100% cellulose paper filter (LLG Labware, Meckenheim, Germany) with 5 – 13 µm pore size and stored at 4 °C until analysed. All analyses were carried out in triplicate and the average values are reported (at 95% confidence interval results have no statistical difference).

Total polyphenols (TP) content: The total polyphenols (TP) content was determined spectrophotometrically by the Folin-Ciocalteu reagent, according to Lachman et al. [5].

Antioxidant capacity (AOX): Antioxidant capacity was determined spectrophotometrically by DPPH radical scavenging method according to Brand-Williams et al. [6].

Extraction yield: Extraction yield was expressed as the dry matter content of the liquid extracts. Dry matter was determined by a standard AOAC method [4].

Kinetics of solid-liquid extraction: Kinetic parameters of Peleg's, Page's and Logarithmic model, for total polyphenols, antioxidant capacity and extraction yield were estimated.

(a) Peleg's model (Eq.1.) where *c*(*t*) represents the concentration of total polyphenols/antioxidants/extraction yield at time *t*, K_1 is Peleg's rate constant K_2 is Peleg's capacity constant,:

$$c(t) = \frac{t}{K_1 + K_2 \cdot t} \qquad [1]$$

(b) Page's model (Eq.2.) where *k* and *n* are Page's model constants and *c*(*t*) represents the concentration of total polyphenols/antioxidants/extraction yield at time *t*,:

$$c(t) = e^{-k \cdot t^n} \qquad [2]$$

(c) Logarithmic model (Eq.3.) where *a* and *b* are Logarithmic model constants and *c*(*t*) represents the concentration of total polyphenols/antioxidants/extraction yield at time *t*:

$$c(t) = a \cdot \log t + b \qquad [3]$$

Kinetic parameters of Peleg's, Page's and Logarithmic model were estimated using Statistica 10.0 (Stat Soft Inc., SAD). The accordance of experimental data and model predicted results was established by a correlation coefficient (R^2).

Thermodynamic study: Influence of temperature on the initial extraction rate was assessed using Arrhenius equation. A plot of ln (*k*) versus *1/T* gives a straight slope where *–Ea/R* represents the activation energy of the extraction and the intercept represents the Arrhenius constant. The activation thermodynamic parameters were calculated using the following equations according to the transition state theory (Amin et al. 2010) (Eqs. 4.-6.):

$$A = \frac{R \cdot T}{N \cdot h} \cdot e^{\frac{\Delta S^{\#}}{R}} \qquad [4]$$

$$\Delta H^{\#} = Ea - R \cdot T \qquad [5]$$

$$\Delta G^{\#} = \Delta H^{\#} - T \cdot \Delta S^{\#} \qquad [6]$$

where *N* is the Avogadro's constant, *h* is the Planck's constant, $\Delta S^{\#}$ is the activation entropy, $\Delta H^{\#}$ is the activation enthalpy, and $\Delta G^{\#}$ is the activation free energy or Gibb's energy.

Results and discussion

The aim of this study was to examine the influence of different extraction temperatures (*T* = 40, 60 and 80 °C) on extractability of total polyphenols, antioxidantsand extraction yield from nettle, as well as to calculate the values of kinetic and thermodynamic parameters at given conditions. Profiles for polyphenols concentration change; antioxidant capacity change and extraction yield change over the extraction time at three analysed temperatures are presented at Fig.1. As seen in Fig1., maximum polyhenol concentration (1274.315 mg GAE g_{DM}^{-1}), antioxidant capacity (0.529 mmol g_{DM}^{-1}) and extraction yield (1.196 %) were obtained at *T* = 80 °C.

Peleg's model **Page's model** **Logarithmic model**

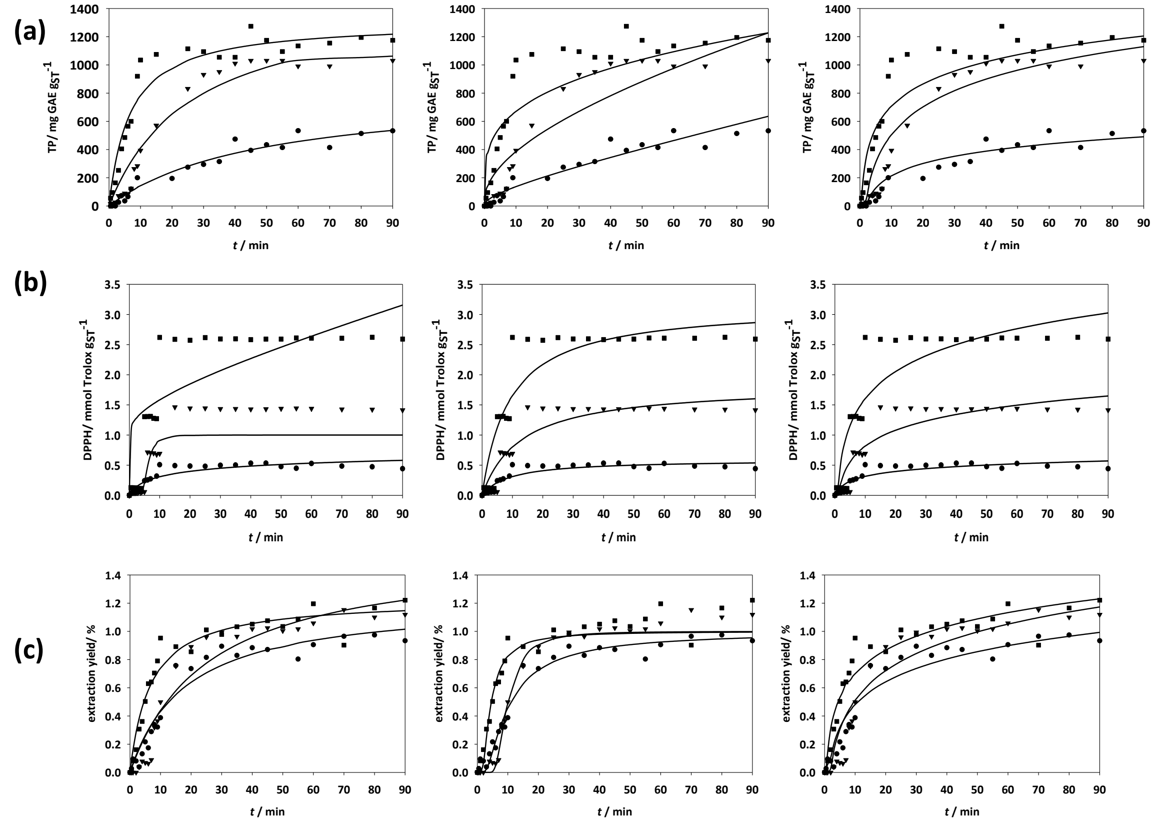

Figure 1. Comparison between experimental data and kinetic model simulations for solid-liquid extraction of (a) total polyphenols, (b) antioxidants and (c) extraction yield from nettle, at T = (•)40, (▽) 60 and (■) 80 °C.

By analysing the extraction curves for polyphenols, antioxidants and extraction yield, two periods of extraction were observed; (i) a rapid increase in at the early beginning of the extraction process, and (ii) a slow increase with further progress of the extraction. To get a detail insight in the extraction process, mathematical modelling of the extraction was applied. Peleg's, Page's and Logarithmic model were used for description of the extraction process. The list of estimated kinetic parameters and correlation coefficients for all three analysed temperatures are presented in Table 1.

Table 1. Values of estimated model parameters for solid/liquid extraction of total polyphenols (TP), antioxidant capacity (AOX) and extraction yield (EY)

		Peleg's model			**Page's model**			**Logarithmic model**		
	T/°C	**K₁**	**K₂**	**R²**	**k**	**n**	**R²**	**a**	**b**	**R²**
TP	**40**	0.056 ± 0.009	0.002 ± $1.000 \cdot 10^{-4}$	0.917	-15.128 ± 3.482	1.285 ± 0.097	0.992	0.233 ± 0.018	-0.056 ± 0.053	0.966
	60	0.017 ± 0.003	$6.650 \cdot 10^{-4}$ ± $7.900 \cdot 10^{-5}$	0.921	-468.438± 362.876	2.817 ± 0.351	0.994	0.301 ± 0.024	-0.179 ± 0.072	0.956
	80	0.005 ± $7.240 \cdot 10^{-4}$	$7.640 \cdot 10^{-4}$ ± $3.200 \cdot 10^{-5}$	0.933	-8.426 ± 3.724	1.636 ± 0.265	0.987	0.241± 0.014	0.143 ± 0.044	0.956
AOX	**40**	6.995 ± 1.306	0.547 ± 0.043	0.897	-2.635 ± 0.392	0.351 ± 0.047	0.958	0.116± 0.013	0.048 ± 0.037	0.958
	60	2.873 ± 0.317	0.551 ± 0.022	0.884	-1005.810 ± 4.025	4.025 ± 2.952	0.908	0.378± 0.035	-0.053 ± 0.010	0.955

	80	1.206 ± 3.742	1.691 ± 0.147	0.812	-0.176 ± 0.099	0.417 ± 0.140	0.897	0.646± 0.064	0.116 ± 0.019	0.958
EY	**40**	17.935 ± 1.855	0.818 ± 0.061	0.901	-15.128 ± 3.482	1.285 ± 0.097	0.991	0.233± 0.018	-0.056± 0.053	0.966
	60	16.370 ± 2.521	0.636 ± 0.061	0.939	-486.438 ± 362.786	2.817 ± 0.351	0.994	0.301± 0.024	-0.179± 0.072	0.956
	80	5.409 ± 0.595	0.812 ± 0.027	0.955	-8.426 ± 3.724	1.636 ± 0.265	0.987	0.242± 0.014	0.142± 0.045	0.987

Based on comparison of the obtained values for correlation coefficients, the Page's model gave the best description of the experimental data in case of total polyphenols and extraction yield, while Logarithmic model proved to be the most suitable for the description of the antioxidants extraction kinetics. The correlation coefficients between experimental data and Page's model simulation results for the total polyphenols extraction were in the range from 0.987 to 0.992, and for the extraction yield in the range from 0.987 to 0.984. On the other hand, the correlation coefficients between experimental data and Logarithmic model simulation results for the antioxidants extraction were in the range from 0.955 to 0.958. For all three analysed variables (total polyphenols, antioxidant capacity and extraction yield) the same trend was observed: the value of parameter K_1 decreased with higher extraction temperatures, thus indicating a higher initial extraction rate.

The influence of temperature on initial extraction rate of total polyphenols, antioxidants and extraction yield for nettle was analysed using the Arrhenius equations. Calculated values of activation thermodynamic parameters for solid-liquid extraction are given in Table 2. The calculated activation energies for total polyphenols extraction, antioxidants extraction and extraction yield were 470.097 kJ mol^{-1}, 510.015 kJ mol^{-1} and 26.694 kJ mol^{-1}. These results implied that the initial extraction rate was the most sensitive to temperature in case of extraction of antioxidants According to the obtained results, the positive values of the activation enthalpies indicated the process was endothermic and that an external source of energy was required to start the extraction process. Positive values of of $\Delta G^{\ddagger}$, indicated that the extraction processes at all analysed temperatures were endergonic and not spontaneous, while negative values of the activation entropy may arise as results of association mechanism.

Table 2. Activation thermodynamic parameters for analysed solid-liquid extractions

	Ea / kJ mol^{-1}	**T / K**	**$\Delta H^{\#}$ / kJ mol^{-1}**	**$\Delta S^{\#}$ / J K^{-1} mol^{-1}**	**$\Delta G^{\#}$ / kJ mol^{-1}**
TP	470.097	313.15	476.494	-155.883	516.309
		333.15	476.328	-156.397	519.412
		353.15	467.162	-156.882	522.565
AOX	510.015	313.15	507.411	-155.205	556.014
		333.15	507.245	-155.719	559.124
		353.15	507.079	-156.204	562.242
EY	26.694	313.15	24.095	-179.731	80.373
		333.15	23.924	-180.246	83.973
		353.15	23.758	-180.731	87.583

Conclusions

Based on comparison of the obtained values for correlation coefficients, the Page's model gave the best description of the experimental data in case of total polyphenols and extraction yield, while Logarithmic model proved to be the most suitable for the description of the antioxidants extraction kinetics. Obtained results for activation enthalpies indicated that the extraction processes were endothermic and Gibbs free energy of activation indicated that they were endergonic and not spontaneous.

Acknowledgements

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Session C: Green Technology for Sustainable Production

APPLICATION OF NANOSUSPENSION IN EXTRACTIVE DENITRIFICATION AND DESULFURIZATION OF FUELS

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Introduction

Considering the problems caused by sulfur and nitrogen compounds in liquid fuels, governments of many countries have adopted strict regulations for the production of fuels with very low sulfur and nitrogen compounds in order to protect the environment. Hydrodesulfurization (HDS) and hydrodenitrogenation (HDN) are widely used methods for the removal of mentioned compounds; these are commercial techniques that require considerable operating conditions such as high temperature and pressure, and large amounts of catalyst and hydrogen. However, this technology is not very effective in removing the cyclic S-/N- compounds such as thiophene, dibenzothiophene, carbazole and pyridine. [1,2]

In order to satisfy strict environmental regulations, it was necessary to develop new techniques to achieve ultra-low content of undesirable components in the fuels. Many desulfurization technologies have been developed such as biocatalysis, adsorption, bioadsorption, oxidative desulfurization, ultrasound desulfurization and extraction. Among all developed desulfurization technologies, extractive desulfurization should be easiest to implement in industry. Mild operation conditions, high desulfurization and denitrificaction efficiency and cheap equipments should be advantages of this technology. It should be emphasized that this method has no effect on the octane number of the fuel.[3] Commonly used solvents are mostly organic ones (alcohols, ethers, amines), which are volatile (VOC) and toxic so they caused serious environmental pollution.[1,3]

A class of solvents that is successful in the extraction processes and attractive due to their superior physical and chemical properties is known as deep eutectic solvents (DES). DESs can be easily prepared from cheap and nontoxic compounds which are biodegradable and biocompatible. [1,2,6]

DESs are formed from salt and hydrogen bond donor (HBD). Mixing of these two components for certain conditions and specific time results in a homogeneous colorless liquid whose melting point is lower than that of the individual constituent components. [1, 7]

This study compared the extraction efficiency of DES and nanosuspensions (NS) when used as solvent for extraction thiophene and pyridine from its mixture with hydrocarbons. Namely, in the last few years it is well-known that NSs possess enhanced thermal properties as a result of Brownian motion of well dispersed nanoparticles inside fluids. With respect to heat and mass transfer analogy improvement of mass transfer by diffusion and/or convection can be expected. Although the number of the scientific papers are increasing recently, the results are often contradictory so different influences on mass transfer are still not clearly defined.

The use of NS prepared from DES and nanoparticles in fuel purification unify two major requirements: the use of green solvents and increased extraction efficiency.

NSs prepared from three different types of nanoparticles and three DESs in order to investigate the improvement of mass transfer in the extractive desulfurization and denitrification.

Materials and Methods

Manufactured nanoparticles Al_2O_3 commercial name *AEROXIDE Alu C*, SiO_2 commercial name *Aerosil 90*, *Fumed Silica* manufacturer *Evonik Industries* and Carbon nanotube - CNT, multi-walled manufacturer *Sigma Aldrich* were used in preparation of NSs. Vacuum evaporator *IKA RV 10 Basic*, has been used for synthesis of DES. Nanoparticles were dispersed in prepared DESs using ultrasonic homogenizer device *Sonoplus, Bandelin HD 3200*. Extraction is carried out with a magnetic stirrer.

Preparation of DES

Quaternary ammonium salt, choline chloride was chosen as hydrogen bond acceptor (HBA) while organic compounds (glycerol, glucose and malonic acid) were chosen as hydrogen bond donor (HBD). Raw materials were dried in a vacuum dryer at 60˚C for 8 hours. Synthesis of DESs was performed by mixing different molar rations of salt (choline chloride-CH) with their corresponding HBD (glycerol-Gly, glucose-Glu and malic acid-Ma), CH:Gly-1:2, CH:Glu-2:1, CH:Ma-1:1. Synthesis was conducted in a vacuum evaporator at 60˚C for two hours i.e. until homogeneous transparent liquid was observed. After synthesis, 30 wt % of water was added to CH:Glu and CH:Ma unless their viscosities were too high for extraction process.

Preparation of nanosuspension

The nanoparticles Al_2O_3 and SiO_2 in a concentration of 0.3 vol % and CNT in a concentration of 0,1 vol % were added to the prepared DESs. An ultrasound probe type *TT 13* was used for dispersion of nanoparticles in DES. Nanoparticles were dispersed by ultrasound waves amplitude of 20% for a period of 10 minutes. During ultrasonification nanosuspension (NS) was cooled with water at 10˚C and the temperature of NS did not exceed 60˚C. The formed NSs were then kept at room temperature for one month to observe the settling rate.

Physical properties of DES and NS

The rheological properties of investigated DESs and NSs were determined using *Brookfield DV-III Ultra Programmable Rheometer*. The measurements were performed in the temperature range of 15 to 55 °C.

Extractive denitrification and desulfurization

Denitrification and desulfurization were conducted in a batch extractor. Pyridine was chosen as typical nitrogen and thiophene as typical sulfur compound. *N-hexane*, *n*-heptane and *i*-octane were chosen as typical hydrocarbons. Pyridine and thiophene were separately dissolved in those three solvents at concentrations of 6 wt %. Extraction processes were performed with a magnetic stirrer. The mixture was stirred for 30 min and 600 rpm at room temperature (25˚C), and the solvent ratio DES or NS to model oil were 1:1. After stirring, the mixture was left for 20 minutes to settle down. After extraction with NS refined phase is separated and centrifuged for 5 minutes at 5000 rpm, due to the potential transfer of nanoparticles from one phase (NS) to another (model oil). Concentrations of pyridine and thiophene were measured with refractometer (at 25˚C) and calculated using calibration curves.

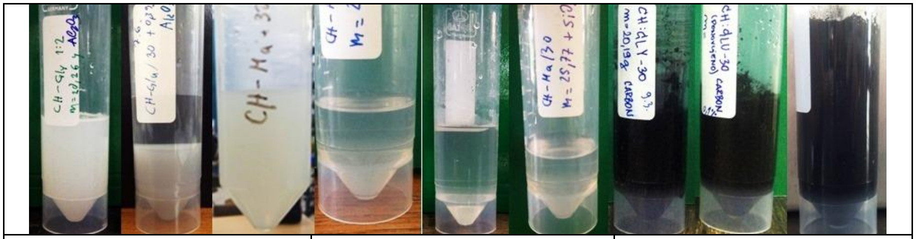
Results and discussion

Stability and viscosity of nanosuspension

A visual observation method was used to determine the stability of the NSs. Based on visual inception (Tab. 1) it can be concluded that NSs, except NS 2, are stable, as there is no precipitation or sediment that can be observed with the naked eye. NS 2 was stabile only for three to four days, after that the precipitation of nanoparticles begins. It is known that higher mole fractions of HBD results with lower stability of NS. With a higher content of the

HBD, the amount of hydroxyl groups also increases, which enhances the strength of hydrogen bonds between the molecules of DESs and nanoparticles[9]. In order to obtain stable NS with glucose for a longer period, NS 2 was additionally stabilized by sonification for 10 minutes.

Table 1. Visual observation of prepared nanosuspensions after three weeks



Al_2O_3			SiO_2			CNT		
CH-Gly	CH-Glu + H_2O	CH-Ma+ H_2O	CH-Gly	CH-Glu + H_2O	CH-Ma + H_2O	CH-Gly	CH-Glu + H_2O	CH-Ma + H_2O
NS 1	NS 2	NS 3	NS 4	NS 5	NS 6	NS 7	NS 8	NS 9

Extraction efficiency

Figure 1.a) reports viscosity of prepared DESs and NSs. DESs are Newtonian fluids as well as NSs prepared with Al_2O_3 and SiO_2. Most of the DESs exhibit a relatively high viscosity at room temperature (> 0,1 Pas). Since NSs with glycerol have been prepared without water they show the highest viscosity. Comparing two others DESs and the corresponding NSs, it can be seen the solutions with the biggest size of the ions (DES and NSs with glucose) have higher viscosity. It is due to dependency of viscosity on the size of the ions.[8] Fig. 2b). shows that the shear viscosity decreases with increasing shear rate, so NSs with CNT are non-Newtonian fluids.

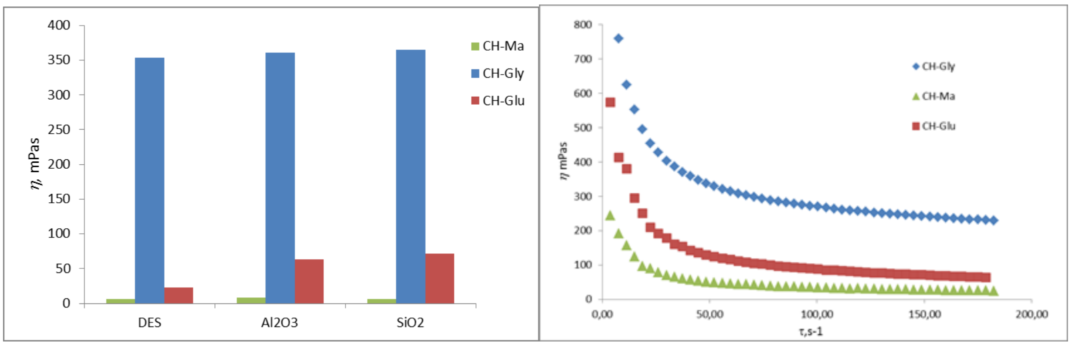
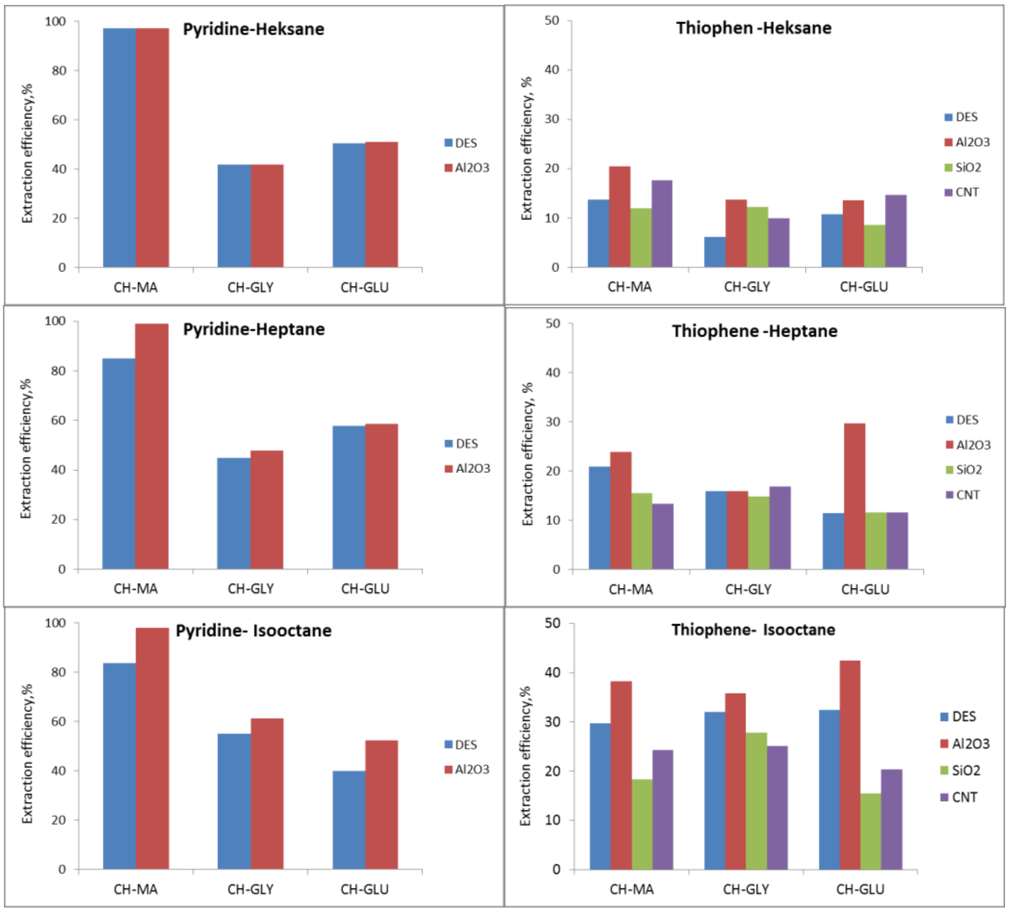


Figure 1. a) Viscosity of DESs and prepared NSs b) apparent shear viscosity of NSs with CNT.

The extractive denitrification and desulfurization were performed with DESs and NSs. The obtained extraction efficiencies are shown on six graphs in Fig.2. Extraction efficiency is higher for pyridine for all investigated DESs and NSs. Various efficiencies are obtained for different solvents but the highest efficiency was achieved by using CH-Ma. The reasons for such result are that CH-Ma has the lowest viscosity and because of that the stirring is better than with other DESs. Using NSs in the same system the improvement for most systems (1-20%) can be observed. Considering that obtained efficiency in system with CH-Ma is higher than 90%, experiments with other nanoparticles haven't been performed.



a) b)

Figure 2. Extraction efficiency of thiophene and pyridine from different fuels

In Fig. 2. b) results of extraction of thiophene from *n*-hexane, *n*-heptane and *i*-octane with three DESs and nine NSs are compared. Generally, it can be noted that the highest efficiency is obtained for the system with *i*-octane and the reason for that is higher solubility of thiophene in extractive phase. Extraction with NSs prepared with Al_2O_3 provides better efficiency, while with other suspensions improvements are not achieved. Enhance in mass transport depends on good dispersion, stability and stirring of system. NSs prepared with CNT show non-Newtonian fluid behavior which affects the mixing conditions and the extraction efficiency.

Conclusions

The prepared NSs show stability over three weeks. DESs and NSs prepared with Al_2O_3 and SiO_2 show Newtonian behavior while NS with CNT is pseudoplastic fluid.

The extraction efficiency is higher for pyridine for all investigated DESs or prepared NSs.

The most suitable DES for extractive desulfurization and denitrification is Choline Chloride - Malic acid.

Due to good stability and enhancement in mass transfer, extraction efficiencies with Al_2O_3 NSs show improvement of 10% for tiophene and over 15% for pyridine.

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COMPARATIVE STUDY OF BASIL OIL AND LINALOOL AS GREEN CORROSION INHIBITORS

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Introduction

An important and frequently used method for protecting metallic materials against deterioration due to corrosion is addition of corrosion inhibitors in aggressive environment. The selection of a suitable corrosion inhibitor is particularly based on the availability of electron-rich atoms, such as nitrogen, oxygen, sulphur and phosphor, in the inhibitor molecule, and especially the presence of multiple bonds or aromatic rings [1]. It has been found that most of the organic inhibitors act by adsorption on the metal surface, where electron-rich centres such as O, N, S atoms and multiple bonds in inhibitor molecule can serve as potential adsorption sites [2]. Adsorption depends not only on the nature and charge of the metal, the temperature and pH of the surrounding media, but also on the molecular structure of the inhibitor [3]. The other increasingly important aspect in the selection and design of organic corrosion inhibitors is their environmental acceptability, i.e. non-toxicity and biodegradability. Consequently, there exists the need to develop a new class of corrosion inhibitors with low toxicity and good efficiency, so-called green corrosion inhibitors. The essential oil isolated from different plant species comprise of mixtures of organic compounds and some have been reported as effective inhibitors of aluminium and aluminium alloy corrosion in different aggressive environments [4-6]. Aluminium is widely used in many industries, such as, the production of heat exchangers, pipes, filters, various containers, etc. It is well known, that the most important feature of aluminium is its corrosion resistance due to the formation of a thin, protective oxide film on its surface [7]. Despite of those advantages, when aluminium is exposed to aggressive environments such as acid pickling solutions, chemical and electrochemical etching, industrial cleaning or scale dissolving, these processes generally lead to significant mass loss of aluminium. One of the well-known and frequently used aromatic herbs in Mediterranean region is basil (*Ocimum basilicum* L.*)*. Essential oils isolated from *Ocimum basilicum* L., can be classified in four major basil essential oil chemotypes: methyl chavicol-rich, linalool-rich, methyleugenol-rich, methyl cinnamate-rich, and numerous subtypes. The aim of this work is to investigate the essential oil of basil (*Ocimum basilicum* L., chemotype linalool) and its main component, linalool as green inhibitors of the aluminium corrosion in acidic medium. The study was done by gravimetric measurements in a defined emperature range. Considering that inhibition efficiency of the plant extract vary with concentration, temperature, period of immersion and pH of corrosive medium, adsorption behavior of investigated compounds was obtained, and some thermodynamic and kinetic parameters were calculated, too.

Material and methods

Corrosion tests were performed using coupons prepared from 99.85% pure aluminium. The other components (wt%) were: Fe 0.08, Si 0.06 and Cu 0.001. Pure 100% *Ocimum basilicum* L., *ct.* linalool, essential oil was provided by "Oshadhi"and the main chemical composition of oil is linalool (65%), eugenol (4.5%), 1,8-cineole (4.4 %), geraniol (3.5%) and camphor

(0.25%). Linalool (97%, ρ= 0.870 g cm^{-3}) was purchased from Sigma-Aldrich. The stock solutions (30% (v/v)) of studied inhibitors were prepared by dissolving them in 96% ethanol. A specific volume of stock solution was added directly to 0.5 M HCl to prepare the desired concentrations in range from 200 ppm to 6000 ppm (v/v). All measurements were performed using aluminium coupons of dimension 2.0 cm × 2.0 cm × 0.2 cm. In each experiment, the cleaned aluminium coupon was weighed and suspended in a beaker containing 100 mL test solution. After 2 h of immersion time at 30 °C, 45 °C, 65 °C and 85 °C, the coupons were taken out, washed with distilled water, dried and re-weighed. The temperature was controlled using a thermostat. The corrosion rate (*W*) and surface coverage (ϑ) were calculated from equations (1) and (2):

$$W = \frac{\Delta m}{S \cdot t} \qquad (1)$$

$$\theta = \frac{W_{corr} - W_{corr}^{'}}{W_{corr}} \qquad (2)$$

where Δm is average weight loss of three parallel Al 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 Al coupons with and without inhibitor, respectively. While the inhibition efficiency η_w (%) can be expressed according to the following equation (3):

$$\eta_w(\%) = \theta \times 100 \qquad (3)$$

Results and discussion

The values of the corrosion rate, surface coverage and inhibition efficiency obtained for aluminium in 0.5 M HCl solution in absence and with addition of different concentrations of basil oil and linalool (200, 600, 1000, 2000, 4000 and 6000 ppm, respectively) are listed in Table 1. From given results is evident that the basil oil and its main component linalool drastically decreased the corrosion rates for aluminium in 0.5 M HCl solution at all concentrations used. Moreover, the inhibition efficiency of both investigated inhibitors is increased with their concentrations and reached the maximum value (68.6% for basil oil and 77.6% for linalool) in the presence of 6000 ppm. On the basis of present results we concluded that *Ocimum basilicum* L. oil and its main component linalool act as good corrosion inhibitors of aluminium in strong acid solution.

Table 1. Corrosion rate, surface coverage and inhibition efficiency values obtained for aluminium in 0.5 M HCl solution in the absence and presence of basil oil and linalool at 303 K, after 2 h immersion time.

c_{inh} (ppm)	***Ocimum basilicum* L.**				**Linalool**			
	c (g L^{-1})	**w (mg cm^{-2} h^{-1})**	**ϑ**	**η_{w} (%)**	**c (g L^{-1})**	**w (mg cm^{-2} h^{-1})**	**ϑ**	**η_{w} (%)**
0	-	1.027	-	-	-	1.027	-	-

200	0.19	0.474	0.538	53.8	0.17	0.573	0.443	44.3
600	0.57	0.438	0.573	57.3	0.52	0.510	0.503	50.3
1000	0.96	0.411	0.600	60.0	0.87	0.451	0.561	56.1
2000	1.91	0.384	0.626	62.6	1.74	0.346	0.663	66.3
4000	3.82	0.354	0.655	65.5	3.48	0.289	0.717	71.7
6000	5.74	0.322	0.686	68.6	5.22	0.235	0.776	77.6

Thermodynamic parameters are important to understand the adsorption of inhibitor on aluminium/solution interface. All thermodynamic parameters obtained for the adsorption of basil oil and linalool molecules on the aluminium surface in 0.5 M HCl solution are given in Table 2. It is well known that the most organic inhibitors can be adsorbed on the metal surface, where electron-rich centres in inhibitor molecule, serve as adsorption sites. Furthermore, when the fraction of the aluminium surface coverage with inhibitor molecules is determined as a function of the concentration at a constant temperature, adsorption isotherm could be evaluated at equilibrium conditions. Before giving the adsorption isotherm and calculating the parameters (*K_{ads}* and ΔG^0_{ads}**)**, inhibitors concentration (ppm) should be expressed as g L^{-1} [2]. From the obtained values of surface coverage (*ϑ*) for basil oil and linalool, adsorption isotherms were fitted and the Langmuir model is given by the following equation (4):

$$c/\theta = c + 1/K_{ads} \qquad (4)$$

where K_{ads} represents the equilibrium constant for the adsorption/desorption process of the inhibitor molecules [5]. K_{ads} value is related to the Gibbs free energy of adsorption (ΔG^0_{ads}):

$$K = \frac{1}{c_{H_2O}} \exp\left(\frac{-\Delta G^o_{ads}}{RT}\right) \qquad (5)$$

where c_{H_2O} is the concentration of water in solution expressed in g L^{-1}, *R* (kJ mol^{-1} K^{-1}) is the universal gas constant and *T* is temperature in K.

Generally, values of ΔG^0_{ads} up to 20 kJ mol^{-1} are consistent with physisorption, while those around 40 kJ mol^{-1} or higher are associated with chemisorption process [8]. In this work, the calculated ΔG^0_{ads} values were between -13.17 kJ mol^{-1} and -18.21 kJ mol^{-1}, indicating that the adsorption mechanism of basil oil and linalool on aluminium in 0.5 M HCl is generally physisorption.

One of the important thermodynamic parameter is the standard adsorption enthalpy (ΔH^0_{ads}), which can be calculated on the basis of Van't Hoff equation (6):

$$\frac{\mathrm{dln}K}{\mathrm{d}T} = \frac{\Delta H^0_{ads}}{RT^2} \qquad (6)$$

where *R* is the gas constant, *T* the absolute temperature (K), and K_{ads} is adsorptive equilibrium constant (L g^{-1}) [8]. The negative sign of ΔH^0_{ads} suggests that the adsorption of inhibitor is an exothermic process, which means that inhibition efficiency decreases with increasing temperature. This behavior can be interpreted on the fact that increasing temperature leads to desorption of some adsorbed inhibitor molecules from the aluminium surface. The heat of adsorption is low and therefore this type of adsorption is stable only at relatively low temperatures. For the chemisorption process, ΔH^0_{ads} approaches 100 kJ mol^{-1}, while for the physisorption, it is less than 40 kJ mol^{-1} [8]. In this study, the ΔH^0_{ads} value is smaller than the common physical adsorption enthalpy (-38.41 kJ mol^{-1} for basil oil and -22.05 kJ mol^{-1} for linalool), once again confirming that physical adsorption takes place. Finally, the standard adsorption entropy (ΔS^0_{ads}) can be calculated by the equation (7):

$$\Delta S^o_{ads} = \frac{\Delta H^o_{ads} - \Delta G^o_{ads}}{T} \qquad (7)$$

Table 2. Thermodinamic parameters for the adsorption of basil oil and linalool molecules on the aluminium surface in 0.5 M HCl solution at different temperatures.

Inhibitor	**Temperature (K)**	***K*ads (L g^{-1})**	ΔG^0_{ads} **(kJ mol^{-1})**	ΔH^0_{ads} **(kJ mol^{-1})**	ΔS^0_{ads} **(J mol^{-1} K^{-1})**
***Ocimum basilicum* L.**	303	0.875	-17.05	-38.41	-70.49
	318	0.408	-15.88		-70.84
	333	0.283	-15.61		-68.46
	358	0.084	-13.17		-70.50
Linalool	303	1.132	-17.05	-22.05	-16.50
	318	0.983	-18.21		-12.07
	333	0.654	-17.93		-12.37
	358	0.294	-16.91		-14.35

The ΔS^0_{ads} values obtained are negative, and this is in correlation to the usual expectation that the adsorption is an exothermic process and always accompanied by a decrease of entropy [9]. Before the adsorption of inhibitor on the metal surface, the inhibitor molecules moved chaotic in solution, but when inhibitor molecules started to adsorb, they are orderly adsorbed, leading to the decrease in entropy.

To evaluate the stability of adsorbed layer of inhibitor on aluminium surface as well as activation parameters of the corrosion process of aluminium in acidic medium, weight loss measurements were carried out in the range of temperature 303-358 K.

The temperature dependence of the corrosion rate is given by Arrhenius equation (8):

$$\log \frac{W_2}{W_1} = \frac{E_a}{2.303R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right) \qquad (8)$$

where E_a represent the activation energy, R is gas constant, while W_1 and W_2 are corrosion rates at temperatures T_1 and T_2, respectively.

E_a values are listed in Table 3, where can be seen that E_a values obtained for the solution with basil oil and linalool are higher than that for the uninhibited one. These higher values of E_a suggest that dissolution of aluminium in the presence of basil oil and linalool is inhibited.

Table 3. Calculated values of activation energy (E_a) for the corrosion of aluminium in 0.5 M HCl with basil oil and linalool in temperature range from 30 °C to 85 °C.

Inhibitor	***c* (ppm)**	**E_a (kJ mol^{-1})**
Blank	0	26.25
***Ocimum basilicum* L.**	200	35.86
	2000	42.35
Linalool	200	31.26
	2000	38.04

Conclusions

Thermodynamic and kinetic adsorption parameters obtained for inhibition of the corrosion process of Al in acidic solution using *Ocimum basilicum* L. oil and linalool as corrosion inhibitors showed, that both investigated compounds drastically inhibits the aluminium dissolution. The inhibition of corrosion process is due to the physical adsorption of inhibitors on aluminium surface. This can be explained with the fact that linalool molecules (the main component of basil oil) are easily protonated and exist in acidic medium in cationic form. Therefore, it is logical to assume that in this case the electrostatic adsorption is responsible for the good protective properties of basil oil and linalool molecules on aluminium corrosion in strong acidic medium.

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PERFORMANCE OF LABORATORY-SCALE CONSTRUCTED WETLAND FOR DEGRADATION OF SODIUM DODECYLBENZENESULFONATE

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1. Introduction

Linear alkylbenzene sulphonate (LAS) is an extensively used synthetic anionic surfactant, frequently used as the sodium salts. It represents more than 40% of all surfactants used, and about 82% - 87% of LAS produced is used for the production of household detergents and cleaning products [1]. LAS was introduced in 1964 as the readily biodegradable replacement for highly-branched alkylbenzene sulphonate (ABS). The LAS molecule contains an aromatic ring sulfonated at the para position and attached to a linear alkyl chain at any position except the terminal carbons. The alkyl carbon chain typically has 10 to 14 carbon atoms and the linearity of the alkyl chains ranges from 87 to 98%. The chemical structure of LAS is presented in Fig. 1. Commercial LAS is a compound of the various homologous and isomers, which differ in alkyl chain lengths and aromatic ring positions along the linear alkyl chain. Commercial sodium dodecylbenzenesulfonate consists of more than 20 individual components that feature variable alkyl chain lengths aside from C12, mainly ranging from C10-C16. Dodecylbenzenesulfonate is considered representative of the entire class of compounds, since the mean number of alkyl carbon atoms in the alkylbenzenesulfonates is 12. Studies demonstrate rapid and complete biodegradation of LAS in many of the available aerobic biodegradation tests, including soil and the aqueous environment. Half-life of anaerobic degradation of LAS is approximately 1–3 weeks [2]. The primary degradation intermediates are sulfophenyl carboxylates (SPCs), which further degrade to CO_2, SO_4^{2-}, and water, so intermediates are significantly less toxic than the parent LAS [3]. Under anaerobic conditions it degrades very slowly or not at all, causing it to exist in high concentrations [4, 5]. After use and disposal, LAS may enter the environment by one of several routes, including by direct discharge to surface water or discharge to water from sewage treatment plants. Although, in activated sludge wastewater treatment plants (WWTP) it is removed up to 95–99%, approximately a 20% loss over a 4.3 min residence time in stream surface water has been observed [3]. Sorption of LAS to solids and organic matter are other important parameters controlling the degradation of LAS in the soil compartment. LAS adsorbs quite strongly to solids, with partition coefficients for soils in the range of 120 l kgy^{-1}. The value of K_d appears to vary with the organic matter, carbon chain length, clay content, silt content, Ferro-oxides, pH, and $CaCO_3$ content [6, 7]. Existence of calcium in water prevents solubility of LAS, while binds to organic material in the humic litter may prevent degradation, respectively. Under anaerobic circumstances, aquatic organisms are exposed to considerable levels of surfactants, which exhibit relatively high toxicity. As for the fate of surfactants after discharge of untreated wastewater, the concentration in the water might be reduced by sorption onto riverine sediments, as well as by biodegradation through endogenous bacterial communities present in the stream. It has been reported that LAS and their degradation products can affect membrane permeability, enzyme and lysosomal activity.

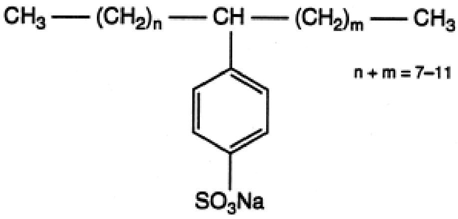

Fig.1. Chemical structure of LAS

The aim of this study is to present the potential of using aquatic plants for SDBS degradation. Phytoremediation has been highlighted as an effective way to reduce the content of organic matter and heavy metals in polluted waters and is commonly emphasized as a green technology.

2. Material and Methods

All reagents were purchased from Sigma–Aldrich and were of analytical grade or equivalent. In each set of experiments, working solutions were prepared from stock reagents immediately before use. Sodium dodecylbenzene sulphonate ($C_{18}H_{29}NaO_3S$, relative molar mass 348.48 g/mol, purity of 80%) was used as modeling solution (5 mg/dm^3). Hach- Lange kivete test LCK 332 for anionic surfactants and LCK 414 for COD measurements were used *Spirodela polyrhiza* L. collected from botanic garden TAL 2000, Ptuj, Slovenia was used as a model plant for carrying out the study. *Spirodela polyrhiza* L. plants were maintained indoor under environmental conditions. Before use, the *S. polyrhiza* L. plants were disinfected by immersing them briefly in ethanol 70% and then rinsing with distilled water for 5 min. The phytotoxicity assessment was carried out by measuring the changes in morphological parameters, average specific growth rate, frond doubling time and estimation of photosynthetic pigments. SDBS adsorption on sediment was taken into account.

The experiments were performed in the absence of nutrients. Glass aquarium tanks size 35x18x24 cm were filled with tap water (15 L). Sand (d=1-2 mm) was placed at the bottom of the aquarium. To test the effect of SDBS on plants, 100 fronds of *Spirodela polyrhiza* L. were put into system. After acclimatisation period of three days, experimental system was exposed to 5 mg/L concentration of SDBS. The experiments were performed in the absence of nutrients for 7 days. All experiments were performed in triplicate. Two different controls were carried out in parallel: (1) plants in a LAS free medium; (2) medium containing SDBS but free of plants. The aqueous culture media were sampled (15 mL) every 24 h for SDBS analysis and stored in glass vials with rubber stops in refrigerator at 5˚C. The concentration of SDBS was determined photometrically with a DR1900 Hach-Lange, with test kits; LCK 332 for anionic surfactants (λ= 653nm) and LCK 414 for COD measurements. SDBS adsorption process onto sand surface was taken into consideration.

The plant chlorophyll content was determined spectrophotometrically with UV/VIS spectrophotometer (Perkin-Elmer Lambda EZ 201). At the end of the experiments 0.5 g of fresh plants were placed in 25 ml flasks and 80% acetone was added to the mark of 25 ml. Three separate flasks were prepared for each treatment group. Flasks were all placed in the dark for 24 h. The absorption of light at 645 and 663 nm was measured in a cell of optical path length of 10 mm against 80% acetone as a blank. The amount of chlorophyll and chlorophyll b in plant leaves was calculated by the formula according to Maclachalam and Zalik (1963) [8]:

$$C_a = \frac{(12.3D_{663} - 0.86D_{645}) \cdot V}{d \cdot 1000 \cdot W},$$

$$C_b = \frac{(19.3D_{645} - 3.60D_{663}) \cdot V}{d \cdot 1000 \cdot W},$$

Where, Ca - concentration of chlorophyll a (mg g^{-1} FW), Cb – concentration of chlorophyll b (mg g^{-1}FW); D - optical density (OD) at wave length indicated, V - final volume (ml); -fresh weight of leaf materials (g), d - length of the light path in cm

The frond number (FN) was scored at the end of the experiments. All visible fronds were counted. Relative frond number (RFN) was calculated using the equation: RFN = (FN at day n – FN at day 0)/FN at day 0 [10].

3. Results and discussion

3.1. Potential of *S. polyrhiza* L. for the phytoremediation of SDBS

Great Duckweed (*Spirodela polyrhiza* L.) was selected as a model plant for evaluating the phytoremediation potential for SDBS removal. The values of SDBS removal from water is given in Fig. 2.

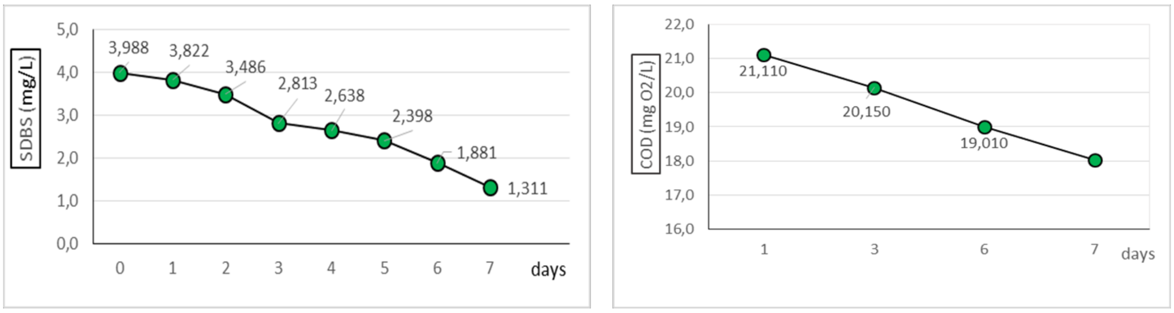

Figure 2. a) Phytoremediation data on SDBS degradation obtained in laboratory using *Spirodela polyrhiza* L. b) COD data during phytoremediation of SDBS

The amount of SDBS which was removed by *Spirodela polyrhiza* L. after seven days was 78.32%. LAS adsorption process onto sand surface was taken into consideration and is around 0.180 mg/kg. No SDBS was detected in control 1. The physico-chemical characteristics of the model solution were determined before and after the treatment and include temperature of water, Chemical Oxygen Demand (COD), conductivity, TDS and the pH [9, 11]. A decrease in value of pH (from 8.53 to 7.6) and COD indicated an improvement in water quality. TDS and conductivity of water was not affected. These results suggest that *Spirodela polyrhiza* L. plants were able to reduce COD to 52.98% in 7 days. No algal growth was detected in the aquarium tank.

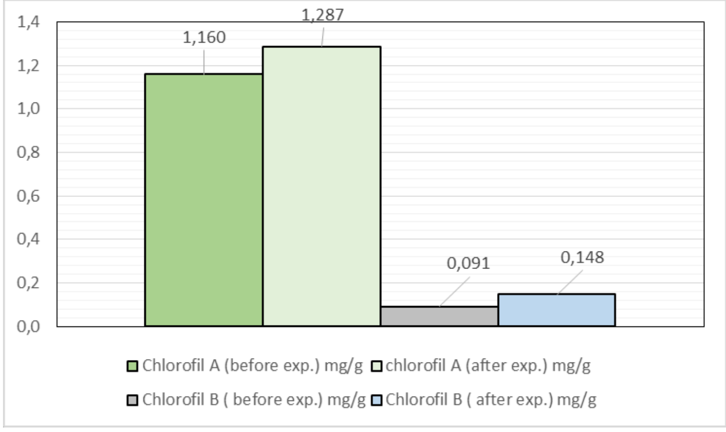

Figure 3. Chlorophyll concentration during phytoremediation of SDBS

At the end of experiment, there was no significant change in the chlorophyll content in analyzed tissue of treated plants compared to the control plant (figure 3). The results showed that under these experimental conditions (SDBS 5 mg/dm^3) there was no effect of SDBS on plants morphology [9, 12]. There were no visual changes in plant; yellowed or dehydrated leaves. Furthermore, an increase in the number of fronds in the system was recorded, from the initial 100 fronds to 176 fronds after seven days. The lower temperature (22◦C) during the experiment than optimal (26–28 °C) and cloudy weather affected rapid growth of plant.

4. Conclusions

This study demonstrated phytoremediation ability of duckweed (*Spirodela polyrhiza* L.) to effectively remove 78.32 % SDBS from water in seven days. Data obtained from this preliminary study demonstrated the phytoremediation potential of *Spirodela polyrhiza* L. for tackling the problem of environmental contamination by surfactants successfully. However, more intensive future research is required to explore various aspects of this approach.

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OPTIMIZING PROCESS PARAMETERS OF SWEET WHEY ULTRAFILTRATION

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Summary:

The aim of this work was to optimise whey ultrafiltration at two different temperatures, more precisely at 25°C and at 40°C. Thereat several factors which determine ultrafiltration efficiency were optimised and investigated with the optimal transmembrane pressure and the flow inlet of whey through the module being among the most important ones. After preliminary experiments, regardless of the process temperature two regimes of transmembrane pressure and flow inlet were chosen - 0.8 bar and 0.6 L/min and 1.2 bar and 1 L/min respectively. Subsequently, whey was subjected to ultrafiltration at those specific conditions and the obtained whey concentrate composition was analysed. The highest factor of concentration (5.73) was achieved at 40°C and at higher flow inlet and transmembrane pressure in comparison (0.6 L/min and 1.2 bar and 1 L/min) and was followed by the concentration factor (5.17) achieved at 25°C and the same processing conditions. Membrane fouling was significant at both temperatures and regimes, but was somewhat lower at milder processing conditions i.e. 0.8 bar and 0.6 L/min. Regarding all of the obtained results the most appropriate regime for ultrafiltration of sweet whey appeared to be at 40°C and 1.2 bar and 1 L/min.

Keywords: concentration factor, membrane, ultrafiltration, whey, whey proteins

Introduction

Whey is a valuable by product originating from cheese production. The high nutritional value of whey is mainly related to whey proteins. It has been proven that whey proteins, especially α-lactalbumin and ß-lactoglobulin are a source of bioactive peptides with several health effects, such as antimicrobial, antihypertensive or anticarcinogenic properties. Additionally, whey proteins have excellent functional properties why their concentrates are widely used in the food industry (Herceg and Režek, 2006; Madrueira et al., 2007, Hernandez-Ledesma et al, 2008). However, they start to unfold and precipitate during even the mildest thermal treatments which negatively influence the quality of whey products. The development of novel, non-thermal processing methods enables replacement of conventional technologies and overcoming of undesired changes such as sediment formation, protein denaturation, reduction in nutritional and sensory quality of whey, etc. (Jeličić et al., 2010).

Ultrafiltration (UF) belongs to the group of membrane techniques which enable new opportunities of whey utilization in human nutrition. The implementation of these processes ensures appropriate microbiological quality and the preservation of the nutritional value of the product. More precisely, ultrafiltration enables the concentration of whey without applying high processing temperatures, which in turn results in obtaining native whey protein concentrates with fully preserved nutritional and functional properties (Cancino et al., 2006, Baker, 2000). The main disadvantage of membrane processes including UF is the phenomenon of membrane fouling which largely influences process efficiency. The fouling intensity depends on processing parameters such as temperature, inlet and outlet pressure, transmembrane pressure, etc., but also on the characteristics of the fluid which is being filtered (Baker, 2000; Goulas and Grandison, 2008). Consequently, whey UF efficiency is largely limited by the deposition of remaining fat and casein particles, bacteria, salts and whey proteins (Barukčić et al., 2015).

Hence, the aim of this study was to optimise whey ultrafiltration at two different temperatures (25°C and 40°C). Thereby a special focus was put on the obtained whey protein concentration factor.

Materials and methods

Whey samples and ultrafiltration module

In all experiments fresh sweet whey obtained from the cheese production plant *Sirela dd* (Bjelovar, Croatia) was used.

For ultrafiltration of whey a *Pellicon 2* module (Millipore, USA) consisting of an easy load tubing pump, two digital pressure gauges, a mini membrane holder and a mini fitting holder for silicon tubes.

The membrane used for ultrafiltration was a combined synthetic *PLCGC* membrane (Millipore, USA) consisting of polyurethane (adhesive material) and of composite regenerated cellulose. The molecular weight cut off was 10 kDa, while the total filtration area was 0.1 m^2.

Filtration procedure and Calculations

In order to prevent severe fouling, fresh whey was filtered whereas residual fat and casein particles were removed. Subsequently, the filtered whey was preheated to 25°C or 40°C, respectively, and maintained constant during the entire UF process by using a water bath. The starting volume of fresh, filtered whey which was to be concentrated by UF was 5 L. During the UF process the obtained permeate was continuously removed in a separate container, while the obtained retentate was recirculated.

In the first stage of the research, different inlet flow rates (0.6 L min^{-1}, 0.8 L min^{-1}, 1.0 L min^{-1}) and the therefrom resulting TMP were tested at each process temperature (25°C, 40°C) in order find optimal UF parameters in terms of permeate flow rate and fouling intensity.

According to the results of preliminary investigations, filtration parameters were as follows – transmembrane pressure (TMP) 0.8 bar and inlet flow of 0.6 L min^{-1}and TMP of 1.2 bar and inlet flow of 1 L min^{-1}, respectively. Thereat at the beginning and after each 30 min the sampling of whey retentate was performed. Also, for each 250 mL of the removed permeate, a sample was also taken in order to analyse the electrical conductivity and pH value. After each UF experiment the membrane was flushed with demineralised water, cleaned with NaOH and NaOCl and preserved by cool storing in formaldehyde until the next use.

The membrane resistance R_M (1/m) was calculated using a linear regression method as Konieczny and Klomfas (2002) described in detail according to Equation 1:

$$J = \frac{TMP}{\eta \times R_m} \qquad (1)$$

with TMP (bar) being a transmembrane pressure, *J* being water flux ($Lm^{-2}h^{-1}$) prior to UF of whey and η ($kgm^{-2}s^{-1}$) representing water viscosity at 25°C or 45°C respectively.

Accordingly, irreversible (R_{ir}) and reversible (R_{rev}) resistance were calculated as follows:

$$J = \frac{TMP}{\eta \times (R_m + R_{ir})} \qquad (2)$$

where *J* represents water flux ($Lm^{-2}h^{-1}$) after UF of whey and η ($kgm^{-2}s^{-1}$) representing water viscosity at 25°C or 45°C respectively.

Reversible fouling resistance (R_{rev}) was claculted from Equation 3

$$Rr = \frac{TMP}{\eta \times J} - Rir - Rm \qquad (3)$$

where *J* represents whey flux ($Lm^{-2}h^{-1}$) at the end of filtration process and η ($kgm^{-2}s^{-1}$) representing whey viscosity at 25°C or 45°C respectively.

Analyses of fresh whey and UF retentates/permeates

In all samples of fresh whey and whey UF retentate pH, titratable acidity, electrical conductivity, total dry matter, the milk fat content, total protein content and particle size distribution were determined.

Electrical conductivity and pH value were determined by a combined pH meter Multi340i (WTW GmbH, Wellheim, Germany). Titratable acidity was determined according to the method of Soxhlet and Henkel and the milk fat content by a butyrometric method according to Gerber, while the total dry matter was determined by drying the samples at 102±2°C until reaching a constant mass (Božanić et al., 2010). The total protein content was determined according to the modified Kjeldahl method (AOAC 991.20; Persson et al., 2008) using a KjeltecTecator Digestion and Destillinig unit (Digestion system 6 1007 Digester, Destilling Unit 2100; Foss, Hilleroed, Denmark).

Statistical analysis

For each set of parameters 2 series of experiments were performed, while each filtration was conducted in a triplicate. The obtained results were statistically analysed in Microsoft Office 2007 software package.

Results & Discussion

As previously described, the aim of this study was to investigate and determine optimal parameters for UF of fresh sweet whey at 25°C and 40°C.

Figure 1 contains results related to specific water flux at different inlet flow rates and different transmembrane pressure (TMP). The maximum inlet flow rate for the used UF module was 1.2 L min^{-1} but at that flow rates TMP values could not be maintained constant, while TMP obtained at inlet flow rates of 0.8 and 1.0 L min^{-1} was almost the same. Accordingly, the optimal TMP and specific water flux through the membrane were achieved at inlet flow values of 0.6 L $min^{-1}$and 1 L min^{-1}, regardless of the process temperature. In that manner the following were chosen for UF of fresh sweet whey – TMP 0.8 bar with inlet flow of 0.6 L min^{-1} and TMP of 1.2 bar with inlet flow 1 L min^{-1}respectively.

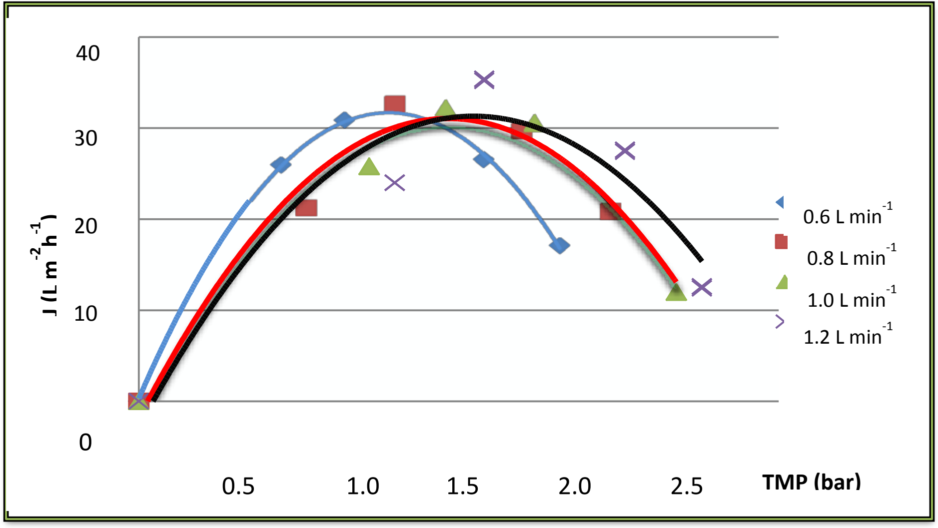

Figure 1. Specific water flux (J) in dependence on the transmembrane pressure (TMP) at different inlet flow rates (0.6; 0.8; 1.0 and 1.2 L min^{-1}) at 25°C

Permeate flow rates were monitored during filtrations. If the flux value did not change after three consecutive measurements, it was presumed that an equilibrium state entered. More precisely, once the permeate flux had reached a constant value, according to several models (Jonssson et al., 1996; Goulas and Grandison, 2008; Heino, 2009) the fouling also reached a maximum at which the transport through a membrane was controlled by the formed boundary layer rather than by the membrane itself. Thus, filtrations performed at milder conditions (TMP 0.8 bar with inlet flow of 0.6 L min^{-1}) lasted approximately 150 min, while filtrations performed at higher TMP (1.2 bar) and inlet flow rate (1.0 L min^{-1}) lasted about 120 min, regardless of the process temperature. Thereat, somewhat higher values of fouling resistances were obtained at lower process temperature and higher values of TMP and inlet flow rate, as can been seen from Table 1.

Table 1. Resistances of reversible (R_{rev}) and irreversible (R_{ir}) fouling during UF of whey at 25°C and 40°C

Inlet flow (L min^{-1})	***TMP(bar)***	***R_m (1/m) x 10^{12}***	***R_{ir} (1/m) x 10^{12}***		***R_r (1/m) x 10^{12}***	
			25°C	**40°C**	**25°C**	**40°C**
0.6	0.8	5.11	19.2	16.0	11.6	10.3
1	1.2	4.57	21.0	19.0	55.4	48.9

In general, viscosity of fluids is lower at higher temperatures, which implies that the mobility of present molecules is also higher and enables better and faster transport through the membrane. However, at higher TMP values higher forces are put upon whey constituents which in time results with thicker and more compact concentration polarization layer causing formation of higher filtration resistances (Vyas et al. 2000; Corbaton-Baguena et al. 2015). In that manner, more intense and faster formation of fouling layer was obtained at higher inlet flow and TMP values (Table 1). Further, similarly to findings of Barukčić et al. (2015), reversible type of fouling originating from deposition of the main whey constituents like whey proteins or milk fat residues also predominated also in the present study.

Table 2. Comparison of the final whey concentrates composition in relation to UF process conditions

	0.6 L min^{-1} and TMP 0.8 bar		***1.0 L min^{-1} and TMP 1.2 bar***		***Fresh whey prior to UF***
%	25°C	40°C	25°C	40°C	
Total dry matter	10.95 ± 1.94	12.18 ± 1.15	11.16 ± 1.13	12.9 ± 3.9	6.32 ± 0.00
Total proteins	4.41 ± 0.77	4.64 ± 0.78	3.93 ± 0.59	4.79 ± 1.1	0.76 ± 0.02

Milk fat	1.70 ± 0.22	1.90 ± 0.25	1.72 ± 0.32	2.10 ± 0.92	0.26 ± 0.05
Concentration factor for proteins	5.73	6.11	5.17	**6.3**	N/A

Table 2 contains the main results regarding whey concentration at different UF conditions. Regardless of the applied process parameters, the total dry matter was more or less two fold higher in all obtained concentrates in comparison to fresh whey. Thereat, proteins were concentrated up to 6.3 times while milk fat showed the highest concentration with up to 8.9 times higher contents in comparison to fresh whey (1.0 L min^{-1} and TMP 1.2 bar at 40°C).

Conclusion

According to all of the obtained results, the highest factor of concentration (5.73) was achieved at 40°C and at higher flow inlet and transmembrane pressure in comparison (0.6 L/min and 1.2 bar and 1 L/min) and was followed by the concentration factor (5.17) achieved at 25°C and the same processing conditions. Membrane fouling was significant at both temperatures and regimes, but was somewhat at milder processing conditions i.e. 0.8 bar and 0.6 L/min. The contents of components other than proteins (lactose, fats, dray matter and minerals) in the obtained concentrates were approximately the same regardless of the applied processing conditions.

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INFLUENCE OF HIGH POWER ULTRASOUND AS GREEN TECHNOLOGY ON WINE QUALITY

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Abstract

High power ultrasound (HPU) has entered the field of food science and technology as non-thermal technology offering new opportunities for managing food quality. However, very few researchers studied the possibilities of HPU in controlling biological stability of wine and benefits of its use as "green" alternative to reduce sulfites, as well as HPU impact to overall wine quality. In addition, more attention is needed in the selection of HPU process parameters that will be both effective and preserve sensorial characteristics of wine. The aim of this study was to investigate the effect of different HPU treatments on the sensory and chemical quality of wine and to select the HPU process parameters sustaining wine quality. Three different wines including Cabernet Sauvignon, Merlot and Plavac mali were treated with HPU were variations in amplitude (20, 30 and 40 %), treatment duration (2, 4 and 6 minutes) and probe tip size (12.7 and 19 mm) were applied. Effect of HPU treatment on wine quality was first evaluated by sensory analysis of trained panel group using 9-point hedonic scale. Chemical analysis was conducted on selected wine samples, after the sensory analysis was performed. Total polyphenols, anthocyanins and chromatic characteristic were analyzed by spectrophotometry, while free anthocyanins were analyzed by HPLC analysis. Wine aroma compounds were analyzed by GC analysis. The results obtained showed differences among treatment applied, where amplitude as well as probe tip size affected the overall characteristic and wine quality.

Keywords: aroma, high power ultrasound, phenolic compounds, red wine, sensory analysis

Introduction

High power ultrasound (HPU) is non-hazardous and environmental friendly technology, whose application has been evaluated in several food and beverage processes but has yet to be introduced to wine industry, since it offers potential application in several areas of winemaking [1, 2]. One of the key opportunities is use of HPU in modulating microbial activity, as green alternative or complement to SO_2 use, where HPU can be applied to inactivate microorganisms (MO) before fermentation, to control of spoilage or inoculated MO during fermentation or for barrel sanitation [2]. Moreover, some recent studies showed that HPU can successfully be applied as technique for extraction of polyphenols and aromas from grape to must, as well as the technique accelerating aging reactions in wine [1], by promoting the polymerization reactions of the phenolic compounds [3, 4]. Although, HPU can hardly affect wines basic physicochemical properties like pH, titratable and volatile acidity, SO_2, ethanol and sugars [5, 6], its effects still remains to be demonstrated. Particularly, more attention is needed in selection of HPU parameters preserving sensorial properties of wine since undesirable reactions or effects can also occur, like formation of negative oxidative smell (burn, smoke and oxidized aroma) [7], negative [5] or "scorched" flavor and loss of grape aroma in wine due to the degassing effect of ultrasound [6]. In addition, very little information is available about the effect of HPU on the phenolic and aroma compounds responsible for wine sensory characteristics, as color, flavor and taste [8, 9], and therefore overall wine quality. Hence, the aim of this work was to study effects of HPU treatments on Cabernet Sauvignon, Merlot and Plavac mali wine sensory and chemical (color, phenolic and aroma) characteristics. HPU processing parameters (diameter probe size, amplitude and time) sustaining wine quality were selected.

Material and Methods

Material and chemicals

Free anthocyanins (3-*O*-glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, 3-*O*-glucosides, 3-*O*-acetylglucosides and 3-(6-*O*-*p*-coumaroyl)glucosides of peonidin and malvidin) were purchased from Polyphenols (Sandnes, Norway), while wine aromas including ethyl esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, diethyl succinate, ethyl myristate and palmitate), acetate esters (*i*-butyl acetate, *i*-amyl acetate, hexyl acetate and 2-phenylethyl acetate), higher alcohols (*i*-butanol, *i*-amyl alcohol, 2,3-butanediol, 1-hexanol and 2-phenylethanol), volatile phenols (vanillin, 4-ethyl guaiacol and eugenol), volatile fatty acids (hexanoic and octanoic acid) and terpenes (linalool and α-terpineol) were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, USA).

Study was conducted on quality dry red wines of Cabernet Sauvignon-CS (Agrolaguna, Poreč, Croatia), Merlot-M (Agrolaguna, Poreč, Croatia) and Plavac mali-PL (Premium, Skaramuča, Pelješac, Croatia); all vintage 2014.

Methods

Ultrasound treatments

An ultrasonic processor (S-4000, Misonix Sonicators, Newtown, CT, USA) with 12.7 and 19 mm diameter sonotrode probe, set at 20 kHz was used for sonification. The ultrasound probe was submerged to the depth of 2 cm, and placed in the center of a 250 mL glass baker (treatment chamber) containing 100 mL of wine sample. The HPU treatment condition for each cultivar and single diameter probe included variation of amplitude levels, as well as processing time. Temperature of the samples during treatments was maintained at 25 °C by ice-water cooling of the treatment chamber. Each treatment was conducted in duplicate. Control sample was the wine sample not subjected to the treatment. All treated samples were coded, providing the information of (i) wine cultivar: CS, M and PL, (ii) diameter probe size 12.7 mm (1) and 19 mm (2), (iii) amplitude 20 % (A), 30 % (B) and 40 % (C) and (iv) processing time 2 min (a), 4 min (b) and 6 min (c). For instance, code CS_1Aa indicates the ultrasonification of Cabernet Sauvignon wine with 12.7 mm probe and amplitude of 20 % during 2 min.

Sensory and chemical analysis

The sensory analysis was conducted on 54 HPU treated wine samples by trained panel group (14 judges) from the Faculty of Food Technology and Biotechnology, University of Zagreb; in 6 individual sessions. Each session include the evaluation of 9 treated samples of single wine cultivar and diameter probe with variations of amplitude and time. Overall impact of HPU treatment on color, aroma and taste was evaluated using verbal 9-point hedonic scale (1=dislike extremely, 2=dislike very much, 3=dislike moderately, 4=dislike slightly, 5=neither like or dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely).

The chemical analysis was conducted on selected wine samples, based on the results of sensory analysis. Samples scored with 5 (neither like nor dislike) were selected after each sensory session since they represented lower limit of treatment acceptability. In addition, the single lowest-rated sample in the group was also selected, in order to gain the information of final possible negative outcomes. Selected wine samples included (i) CS_1Ac, CS_1Bb, CS_1Cb and CS_1Cc, (ii) CS_2Aa, CS_2Ab and CS_2Cc, (iii) M_1Cb, M_1Bb and M_1Cc, (iv) M_2Aa, M_2Ab and CS_2Cb, (v) PL_1Aa, PL_1Ba and PL_1Cc, (vi) PL_2Aa, PL_2Ba, PL_2Cb and PL_2Cc. Spectrophotometric analyses were performed on double-beam Specord 50 Plus spectrophotometer (AnalytikJena, Jena, Germany) and included the analysis of total phenol content determined by Folin-Ciocalteu method [10], analysis of total anthocyanins by bisulfite bleaching method [11] and analysis of wine chromatic characteristics [L (lightness), a (red/green color component), b (blue/yellow color component), C (chroma) and h (hue angle)] according to the CIELab [12]. Analysis of 9 free anthocyanins (see *Material and chemicals*) were performed on Agilent 1200 Series HPLC (Agilent Technologies, Santa Clara, CA, SAD) with DAD detection [8], while analysis of wine aromas (see *Material and chemicals*) were performed on Agilent Gas Chromatograph 6890 series coupled with an Agilent 5973 Inert mass-selective detector (Agilent Technologies, Santa Clara, CA, USA) [9]. All chemical analyses were performed in duplicate.

Statistical analysis

The statistical data analysis was carried out using Statistica V.7 software (Statsoft Inc., Tulsa, OK, USA). The principal component analysis (PCA) was used to examine possible grouping of control and HPU treated wine samples of each cultivar studied.

Results and discussion

High power ultrasound (HPU) induced different sensory and chemical changes in treated wines of all three cultivars (Cabernet Sauvignon, Merlot and Plavac mali). In accordance to earlier studies [3, 4, 6], all analyzed treated wines showed changes in phenolic composition, chromatic characteristics as well as the aroma composition. However, these chemical changes showed to be treatment and cultivar dependent. The effects of HPU are presented in Fig. 1, where overall chemical data (phenolic composition, color parameters and aroma composition) of control and treated wine samples were processed by principal component analysis (PCA). The first two PC in the case of Cabernet Sauvignon wines accounted for 70.74 % of total variance. Displacement of the control sample on the left side of PC1 compared to HPU treated wine indicates that application of HPU caused the decrease in L, a, b, C and H values, total phenols, total and free anthocyanins, ethyl esters (ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate) and acetate esters (*i*-butyl acetate and *i*-amyl acetate), since these variables were highly negatively correlated with PC1 (Fig. 1A). HPU promoted polymerization of phenolic compounds could be responsible for their decrease and color changes [1, 4, 6], while HPU degassing effect could be related to the loss of volatile compounds [6]. On the other hand, all treated samples showed higher content of wine aging aromas diethyl succinate and 2-phenylethanol (variables that were strongly positively correlated with PC1), probably due to the accelerated chemical reactions responsible for shortening wine aging process [6]. Moreover, afore mentioned changes showed to be treatment depended, and less pronounced in the 1Ac, 1Bb and 1Cb samples (treated with 12.7 mm probe size) placed on the left side of PC1. These results showed that dimeter probe size is the important factor affecting the wine quality, where application of 12.7 mm probe size on Cabernet Sauvignon wine showed to be more effective in preserving wine chemical and sensory characteristics than to 19 mm probe size. In addition, the control, 1Ac, 2Ab and 2Aa samples were placed above the PC2 due to the lower content of octanoic acid and ethyl myristate (variables that highly negatively correlated with PC2). This finding suggests that amplitude is another important factor affecting the wine quality, where amplitude levels of 20 % showed lesser effect than levels of 30 and 40 %.

Distribution of Merlot wines obtained after HPU treatments defined by PC1 and PC2, that explained 68.97 % of the total variance, is shown in Fig. 1B. Displacement of control sample on the left side if first factorial plane showed similar trends earlier noticed in Cabernet Sauvignon. In fact, HPU treatment of Merlot wine, as previously reported [3, 4, 6], induced decrease in content of total phenols, sum of free anthocyanins, H value, ethyl esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl decanoate), acetate esters (*i*- butyl acetate, *i*-amyl acetate and hexyl acetate) and higher alcohol *i*-butanol (variables highly positively correlated with PC1); as well as the increase in the content of diethyl succinate, 2-phenylethanol and ethyl myristate (variables highly negatively correlated with PC1). Also, grouping of the control, 1Bb, 1Cb samples below the PC2 confirms the effect of diameter probe size. Namely, application of 12.7 mm probe size in Merlot wines preserved higher content of total anthocyanins, higher L, a, b, C values, as well as the content of hexanoic acid and ethyl palmitate; but simultaneously slightly decreased the content of *i*-amyl alcohol, 1-hexanol and 2,3-butanediol.

Plavac mali wine, among all three tested cultivars, showed to be the most susceptible to sensory changes caused by HPU treatments, where application of only 2 min caused the significant sensory changes. Prior studies have also noted the importance of selection of HPU parameters preserving sensorial properties, due to the possible formation of negative oxidized or “scorched” flavor [5, 7]. Therefore, in the case of Plavac mali wine, 2 min treatments like 1Aa and 1Ba were selected. Projection of control and HPU treated wine of Plavac mali on fist two PC that accounted 77.33 % of total variance (Fig. 1C). As shown, control wine was placed in third quadrant, since HPU caused decrease of total phenols, total and free anthocyanins, ethyl esters (ethyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate), acetate esters (*i*-amyl acetate), higher alcohol (*i*-amyl alcohol and *i*-butanol). Nevertheless, 2 min treatments 1Aa and 1Ba since placed on the left side of PC1 were less affected by these changes. Also, grouping of the 2Aa, 2Ba, 2Cb and 2Cb samples on the left side of PC1 indicates that probe size diameter is the most important factor in HPU treatment of wine. In addition, treated samples demonstrated higher content of ethyl esters (diethyl succinate, ethyl myristate and palmitate), higher alcohols (1-hexanol), fatty acids (hexanoic and octanoic acid) and terpene linalool.

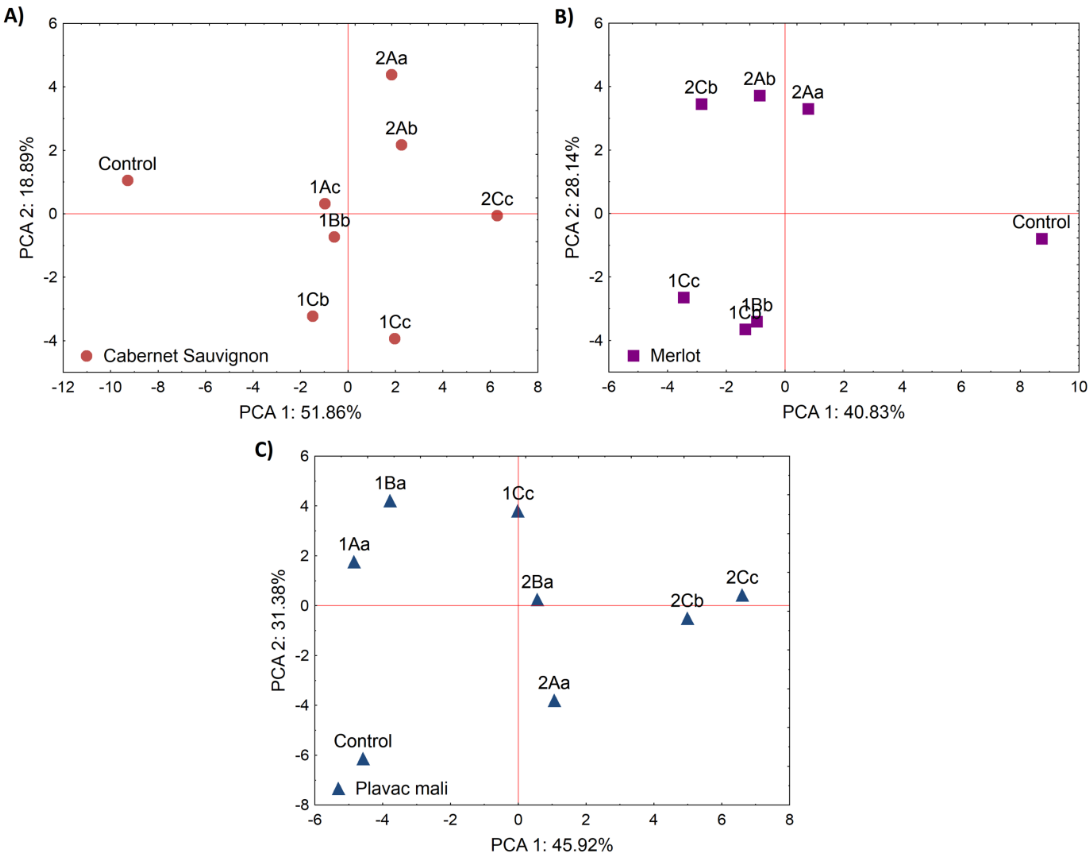

Figure 1. PCA of the phenolic, color and aroma data in Cabernet Sauvignon (A), Merlot (B) and Plavac mali (C) wine: projection of the control and HPU treated wines on first two principal components.

Conclusions

Application of HPU as green technology on red wine resulted in sensory and chemical changes (decrease in phenols, color parameters and aroma), that were both treatment and cultivar dependent. Probe diameter sizes was the most discriminatory parameter influencing the outcome of HPU treatment, where 12.7 mm probe was more effective in preserving wine chemical and sensory characteristics than to 19 mm size. Plavac mali, showed to be more susceptible to HPU treatment than Cabernet Sauvignon and Merlot wine.

Acknowledgments

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Session D: Biomass and sustainability

THE RUB RESISTANCE OF PRINTED PAPERS WITH VARIABLE CONTENT OF WHEAT PULP

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Introduction

Printing is widely used in our society to pass on information especially by newspaper publications on a daily basis. The main printing process for printing newspapers in western Europe is coldest web offset at a very fast rate. The most important pigment in coldest printing is black colour, which is drying by absorption (penetration into paper substrate i.e. newsprint). Because newsprint is not heated, that allows little time for the ink to air-dry. The ink is absorbed by the inner fibers of the sheet of paper and remains there a bit damp during most of the transient life of the paper (the vehicle doesn't completely evaporate) [1]. So, it is very common for newspaper readers to have black fingers by the time they reading the newspaper. Namely, it is a consequence of the black ink rubbed off the newsprint and the amount depends on how fresh the newspapers are. Undoubtedly on the newspaper print quality influence the paper substrate as well. The printing substrate with a low abrasion resistance may suffer a significant decrease in product appearance and readability of printed information. Newsprint intended for the printing of newspapers is a relatively inexpensive uncoated low-grade paper. It could be made of different pulps, especially deinked pulp or virgin mechanical pulps. Newsprint made from mechanical pulp have high lignin content, while newsprint samples made from recycled paper have low values of lignin because during recycling they had been mixed with fibres from higher-grade paper to increase the strength of the paper. This addition of longer fibres during recycling process is necessary because after five to seven cycles of recycling process the fibres become too short for producing new paper. Regardless of the origin of the fibres and pulp in the composition of newsprint its characteristic is a low brightness. The aim of this research was to explore the reproduction quality of printed laboratory papers made of variable content of wheat pulp. Wheat straw is chosen as agricultural residue and renewable natural resources of virgin cellulose. The reproduction quality of printed laboratory papers was observed through rub resistance analysis. The rub resistance of printed laboratory papers was determined 6 and 24h after printing.

Materials and Methods

In this research laboratory made straw-containing papers were used. For that purpose winter types of wheat straw was converted to a semi chemical pulp according to the Soda method. The conversion of straw into pulp was done by pulping process involving chemical treatment to remove part of the lignocelluloses fiber-bonding material and mechanical refining to complete the pulping action [2, 3]. Pulping conditions are presented in Table 1.

Table 1: Extraction Conditions during Pulping

Types of straw	Pulping method	Extraction conditions
Wheat	Soda pulping	Temperature of 120 °C, alkali level of 16% for 60 min, and a 10:1 liquid to biomass ratio

After thermal treatment under controlled and defined extraction conditions, the pulp slurry was removed from the black process liquor by decantation and rinsed with water. In a Valley beater (Techlab Systems (TLS), Spain), pulp was diluted with tap water to maintain the pulp suspension at a 1.5% consistency and fiberized at pH 9, 24 °C, and 500 rpm for 40 min. Finally, the pulp was drained by Manual Sheet Former TAPPI (Techlab Systems (TLS), Spain) and allowed to dry to a moisture content of ca. 7% at room temperature. Obtained unbleached wheat pulp was mixed with recycled newsprint in different weight ratios (0:100, 10:90, 20:80 and 30:70) for forming laboratory papers using a Rapid Köthen Sheet Machine (Frank–PTI GmbH, Birkenau, Germany) needed for this research (Fig.1). Commercial UPM News C paper was used as a basic component to which was added wheat pulp [3, 4]. Altogether, three paper sheets (42.5 g/m^2, 20 cm diameter) with variable content of wheat pulp and a control sheet were formed.

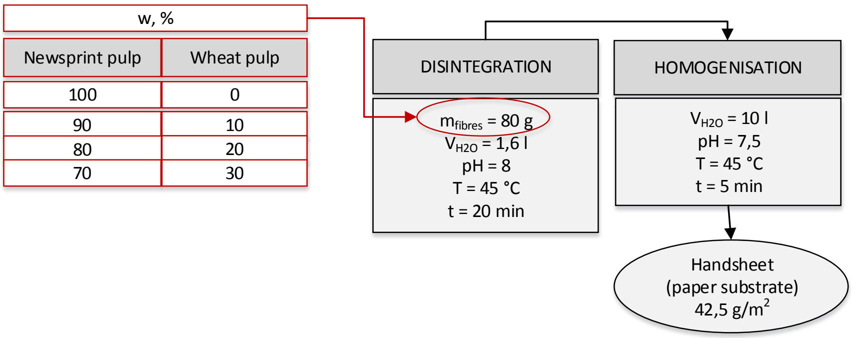

Figure 1: Workflow of laboratory paper production

In Table 2 some characteristic of formed laboratory papers are presented [3].

Table 2. Unprinted Laboratory Papers Properties

Laboratory Papers		Thickness (μm)	Brightness (%)	Ash (%)	Bekk smoothness (sek)	
					felt side	wire side
straw pulp	0	94.0 ± 2.79	58.59 ± 0.43	4.73 ± 0.22	16.70 ± 2.67	8.86 ± 0.51
wheat	10	95.3 ± 2.83	53.36 ± 0.50	4.14 ± 0.43	15.06 ± 1.94	8.78 ± 0.68
	20	98.1 ± 5.28	49.62 ± 0.38	3.77 ± 0.31	14.86 ± 1.35	8.54 ± 0.78
	30	101.5 ± 5.32	45.81 ± 0.42	3.64 ± 0.07	14.30 ± 1.22	9.34 ± 0.58

The laboratory printing was carried out on Prufbau Printability Testing Machine (Fig.2).

Figure 2: Prüfbau laboratory printing process, which simulates offset printing process

In the printings, a carbon black ink, which is generally used for newsprint in a coldset, offset process was used. The ink dosage of 2.00 cm^3 was distributed evenly over the printing cylinder and the ink was transferred to the laboratory paper samples with a width of 40 mm, an applied load of 150 N cm^{-3} and a speed of 1 m/s. In this way, all samples were printed in full-tone black and dried naturally. To observe the reproduction quality of the printed papers the rub resistance was determined. Since the time interval between the printing process and the testing of resistance to rubbing strongly affect the results, testing was performed 6 and 24 hours after printing. Drying time of 24 hours is in accordance with the recommendations of the World Association of Newspaper Publishers (WAN-IFRA), while the time of 6 hours was selected with regard to newspapers distribution to market. The rub resistance of prints was provided in accordance with BS 3110:1959 standard [5] by Rub and Abrasion tester (Hanatek, UK). Entire contact surface of prints were evenly rubbed on the principle of circular movement with white uncoated paper (Arcoprint 110 g m^{-2}, manufactured by Fedrigoni, UK) at a pressure of 107.15 Pa, which is determined by the mass and number of 10 rpm. Rub resistance of the printed papers was defined based on optical properties of the prints (L*, C* and h* colour parameters), which were computed in accordance with the TAPPI Standard (T524-om-02). Where L* represented the lightness (from 0 to 100), C* represented chroma or relative saturation and h* represented hue angle of the colours in the CIELab colour space. The Euclidean colour difference (ΔE_{00}*) of all analysed prints were calculated with the following equation [6], using the corresponding starting material (unrubbed print) as reference:

$$\Delta E^{*}_{00} = \left(\frac{\Delta L'}{k_L S_L}\right)^2 + \left(\frac{\Delta C'}{k_C S_C}\right)^2 + \left(\frac{\Delta H'}{k_H S_H}\right)^2 + R_T \frac{\Delta C'}{k_C S_C}\frac{\Delta H'}{k_H S_H} \tag{1}$$

Results and discussion

At Figure 3 colour differences of prints affected by rub resistance test provided 6 hours after printing (Fig 3a) and 24 hours after printing ((Fig 3b) are presented.

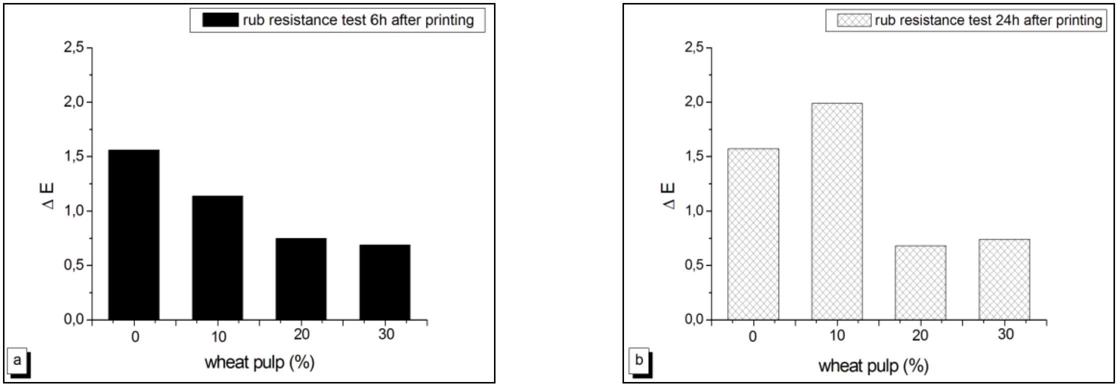

Figure 3: The Euclidean colour difference (ΔE_{00}) of prints affected by rub resistance test: a) 6 hours after printing; b) 24 hours after printing*

Results of this analysis simulate some of the stresses that a printed substrate is subject to during its life cycle (printing process, transport, distribution, storage, handling and end-use) [7]. During the rub resistance analysis ink could be rubbed off in higher or smaller share from the treated printed substrate. The Euclidean colour difference (ΔE_{00}*) refers to the distance between two colours in the CIELab colour space. Therefore, from its value it can be concluded how the colour is different from the original sample (unrubbed printed substrate). According to tolerance definition $\Delta E_{00}^* \leq 2$ is classified as very small noticeable difference for standard observer, while $\Delta E_{00}^* = 5$ is defined like big noticeable difference in the colour whose standard observer can recognized. From results of measurements presented at Figure 3a, it is clearly notable that with increasing the share of wheat pulps in paper substrate the rub resistance of prints is increasing (ΔE_{00}* is decreasing) when the analysis were done 6 hours after printing. By extension drying time of the ink to 24 hours, the worse rub results were achieved (Figure 3b). It is important to point out that the use of straw pulp in the production of paper for newsprint provides acceptable print quality considering the rub resistance of prints.

Conclusion

Wheat pulp in newsprint provides good rub resistance of prints, which means that wheat pulp can be used as a raw material for this grade of paper in commercial newsprint sector. Namely, with increasing the share of wheat pulp in paper, the rub resistance of the prints is better.

Acknowledgement

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BIOSORPTION OF Cu^{2+} FROM AQUEOUS SOLUTION BY HYDROCHAR OF GRAPE POMACE

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1. Introduction

Copper is one of the toxic metals commonly found in industrial effluents [1, 2]. Although low concentrations of copper are essential, its excessive levels in waters can be detrimental [3]. Conventional wastewater treatments such as chemical precipitation and reduction, filtration, flocculation, ion exchange, electrochemical treatment, are insufficiently selective, oftentimes polluting, expensive, and have various technical limitations especially at low concentrations of contaminants [4].

In recent years, increase of environmental awareness has resulted in the development of new, eco-friendly technologies for the removal of wastewater contaminants. One approach has been the use of different biological materials as adsorbents of pollutants from aqueous solutions [4-6]. Compared to the conventional methods, biosorption has numerous advantages: selectivity, low-price, high efficiency and sustainability [4]. Expended biomass can be regenerated, or subsequently reused in different ways. Also, the adsorption efficiency of biomass can be increased by physical, chemical, thermal or combined modifications [7-9].

Along with the biosorption, development of new methods for conversion of waste biomass, focused on design of novel multifunctional materials, become very attractive. One of such technologies is hydrothermal carbonization (HTC) [10-12]. HTC is a thermochemical process for converting of lignocellulosic feedstock into lignite-like product at elevated temperatures (180-280°C) and high pressure (2-10 MPa) in the presence of water [13]. The process converts all the carbon from biomass to the coal, with no methane or CO_2 release into the atmosphere [11]. Unlike gasification or pyrolysis, HTC does not require intensive and expensive pre-drying of raw material. The resulting product-hydrochar, created via reactions between subcritical water and lignocellulosic residues, is hydrophobic, porous, solid material with high carbon content [14]. So far, HTC has been utilized on a wide range of different feedstock [13-16].

One of raw materials that is highly suitable for HTC is grape pomace (GP), produced in the course of winemaking [13, 17]. According to the statistic, grape production in Serbia takes place on about 58,000 hectares, with a total annual production of about 430,000 tons of fruit [18]. However, up to 25% of the used amount of raw material, in form of pomace, remains after grape processing stored on permanent or makeshift dumps [18]. This waste biomass with a high water content (>60%), composed of grape seeds, skins, and stems, due to degradation of organic matter, drainage and evaporation, has a negative impact on the environment. Therefore, appropriate utilization of GP is necessary.

The aim of this research was to investigate the ability of GP hydrochar (HC-GP) to remove copper ions (Cu^{2+}) from aqueous solution, simulating wastewater with low toxic metal concentrations. Particular interest of this study was to evaluate the suitability of GP as a precursor for preparation of adsorbent, because this type of biomass is largely available in Serbia. The effect of sample dose on Cu^{2+} sorption for the adsorbent prepared from the GP using HTC was determined using different initial metal concentrations. Equilibrium sorption isotherms were assessed together with the modeling of the obtained experimental results. Binding mechanism of copper for HC-GP surface was implied using FTIR spectroscopy.

2. Material and Methods

2.1 Biomass

The used GP was randomly collected from landfill sites, which have left behind after processing of red grape grown on a test plot Radmilovac, property of the Faculty of Agriculture in Belgrade, Serbia. Biomass was air-dried to constant weight and grinded in order to obtain homogeneity of samples. Grinded material was sieved and fraction size of 0.5 mm was used in further HTC experiments.

2.2 HTC experiment

Preparation of HC-GP at 220°C we have previously described elsewhere [13]. Briefly, 250 g of selected GP was carbonized in 2000 mL autoclave (Model 10253, Deutsch & Neumann), at 220°C within one hour. After cooling of the reactor to room temperature, HTC process streams (solid HC and process water) were divided by filtration. Obtained HC-GP was rinsed several times with distilled water and dried at 105°C in the oven. Sorption experiments were performed with HC-GP particle size between 0.1 and 0.5 mm.

2.3 Chemicals

Copper solutions and standards were prepared using analytical grade copper nitrate salt ($Cu(NO_3)_2$, Sigma-Aldrich). A stock copper nitrate solution (1mM $Cu(NO_3)_2 \times 3H_2O$) was prepared with deionized water. The copper (II) solutions of varying concentrations were prepared by diluting the stock solution with deionized water.

2.4 Batch adsorption experiments

The sorption tests were conducted by contacting weighed amounts of HC-GP samples (0.01-0.05 g) and 50 ml of copper ion solutions of different initial concentrations (0.1, 0.5 and 1mM) placed in 100 ml glass flasks. Erlenmeyer flasks were then located on orbital shaker (Heidolph, model Unimax 1010) and shaken at room temperature (25±0.5 °C) for 180 min at 250 rpm. The pH value of all solutions was adjusted to pH 5, using 0.1 M KOH and/or 0.1 M HNO_3 solutions. The pH value was selected based on reported results indicating that the highest sorption of copper ions has been attained at pH 5 [4]. The pH was monitored using SensION MM340, Hach pH meter, with integrated magnetic stirrer. All experiments were performed in duplicates and average values of obtained results were shown. The concentration of copper ions was determined using atomic adsorption spectrometry (AAS) (Perkin Elmer Analyst 300).

Equilibrium isotherms for Cu^{2+} were obtained using sample dose of 0.5 g L^{-1} HC-GP in a range of initial metal concentrations between 0.001-1 mM. The general procedure depicted above was followed, applying the same experimental conditions. The suspensions were stirred for 120 min to attain equilibrium.

The amount of metal adsorbed by the HC-GP was calculated using Eq. (1):

$$q = \frac{V(C_i - C_e)}{m} \tag{1}$$

where sorption capacity, q, is the amount of metal adsorbed by adsorbent (mmol g^{-1}), C_i and C_e are the initial and equilibrium metal concentrations (mmol L^{-1}), V is the volume of metal solution (L) and m is the mass of the adsorbent (g).

Metal removal efficiency (%) was calculated from Eq. (2):

$$R = \frac{C_i - C_e}{C_i} x100 \tag{2}$$

Non-linear fitting method using Origin 9.0 software was employed to investigate applied isotherm models.

2.5 FTIR spectroscopy

FTIR analysis of samples before and after adsorption was performed in transmission mode using Thermo Scientific Nicolet iS50. The spectra were obtained in the spectral range of 4000-400 cm^{-1}.

3. Results and discussion

3.1 Effect of hydrochar dose on biosorption

The dose response for Cu^{2+} was studied by varying amount of HC-GP from 0.2 to 1 g L^{-1} at different initial metal concentrations (0.1, 0.5 and 1 mM) (Fig. 1). Sorption experiment was performed at constant pH 5, since at pH values higher than 6.1, precipitations of metal hydroxides occurs [19]. Also, the pH value of the zero-point charge for HC-GP of 4.5 (data not shown), indicate that the repulsion of positively charged metal ions at lower pH values might take place together with a possible competition with hydrogen ions towards active sites [19].

Fig. 1 clearly shows that regardless of the initial metal concentration, HC-GP removes up to 85% of Cu^{2+} from the solution. As it was expected, at constant initial concentration of Cu^{2+}, increasing of the sample dose up to 0.5 g L^{-1} provides a greater surface area and number of available sorption sites for metal ions uptake. Increase of dose above this threshold did not increase the percentage of Cu^{2+} removal, and thus dose of 0.5 g L^{-1} of adsorbent was chosen for further experiments.

<Figure 1>

3.2 Effect of contact time

The effect of contact time was studied using selected dose of HC-GP (0.5 g L^{-1}) and initial Cu^{2+} concentration of 1 mmol L^{-1} at constant pH 5.0 and contact time up to 180 min (Fig. 2). Contact time of 90 min was enough to reach equilibrium. Therefore, in order to ensure sorption equilibrium, the contact time of 120 min was chosen in further experiments. Copper adsorption capacity of 0.206 mmol g^{-1}, indicated efficient removal of Cu^{2+} from water solution by HC-GP.

<Figure 2>

3.3 Equilibrium isotherm models

In this study, Langmuir and Freudlich isotherm models were applied for analyzing the Cu^{2+} adsorption process on HC-GP surface in accordance to the obtained experimental data.

The Langmuir isotherm model, based on the assumption of a monolayer adsorption process onto a homogeneous surface, is represented by the following equation:

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e} \tag{3}$$

where the C_e is the equilibrium concentration (mmol L^{-1}), q_m is the maximum amounts of Cu^{2+} ion adsorbed on the HC (mmol g^{-1}) and K_L is the Langmuir constant (L $mmol^{-1}$), related to the affinity of the binding sites [20].

The Freundlich isotherm model that assumes a heterogeneous sorption surface with different sites energies can be expressed in non-linear form as:

$$q_e = K_F C_e^{1/n} \quad (4)$$

where q_e, represents the amount of the adsorbate per unit weight of the adsorbent (mmol g^{-1}), K_F the Freundlich constant, ($L^{1/n}$ $mmol^{1-1/n}$ g^{-1}), C_e the equilibrium Cu^{2+} concentration in the solution (mmol L^{-1}) and 1/n, the adsorption intensity (Freundlich parameter) [21]. Both applied isotherm curves are shown in Fig. 3.

< Figure 3 >

According to the values of the resulted correlation coefficients R^2, the Langmuir isotherm (R^2=0.999) gave slightly better description of the experimental data than the Freundlich isotherm model (R^2 = 0.998), with a maximum adsorption capacity for Cu^{2+} of 0.211 mmol g^{-1}. Adsorption isotherm parameters for Cu^{2+} are given in Table 1.

<Table 1>

3.4 FTIR analysis

The FTIR spectra of analyzed HC-GP samples before and after adsorption of copper ions are presented in relevant spectral regions (3700-2800 cm^{-1} and 1850-500 cm^{-1}) in Fig. 4. Detailed spectroscopic analysis of the used HC-GP was given elsewhere [13].

Binding of copper for HC surface resulted in a reduction of the O-H stretching band intensity (3346 cm^{-1}) and its shift to higher wavenumbers. This may be ascribed to the complexation between the oxygen-containing functional groups on HC surface and metal ions [22]. Also, notable weakening of aromatic C-H band at 783 cm^{-1} and slightly decrease of peaks at 1613 cm^{-1} and 1515 cm^{-1} (attributed to C=C stretching of aromatic rings) after interaction with Cu^{2+}, may indicate the formation of coordination bonds between the d-electron of copper and π -electron of C=C bond of aromatic rings [22, 23].

<Figure 4>

4. Conclusion

Hydrochar produced by HTC from grape pomace have been studied as an adsorbent of Cu^{2+} from aqueous solutions. Resulted copper adsorption capacity of 0.206 mmol g^{-1}, indicated efficient copper removal using HC-GP. The sorption isotherms of Cu^{2+} onto HC-GP were well described by the Langmuir model, with a maximum theoretical adsorption capacity for Cu^{2+} of 0.211 mmol g^{-1}. The differences in FTIR spectra of HC-GP samples before and after adsorption of copper ions seem to correlate well with the proposed mechanisms of metal binding for the oxygen-containing functional groups present on HC surface. Our findings suggest that the GP is a promising precursor for production of quality adsorbents for copper removal from wastewaters using HTC.

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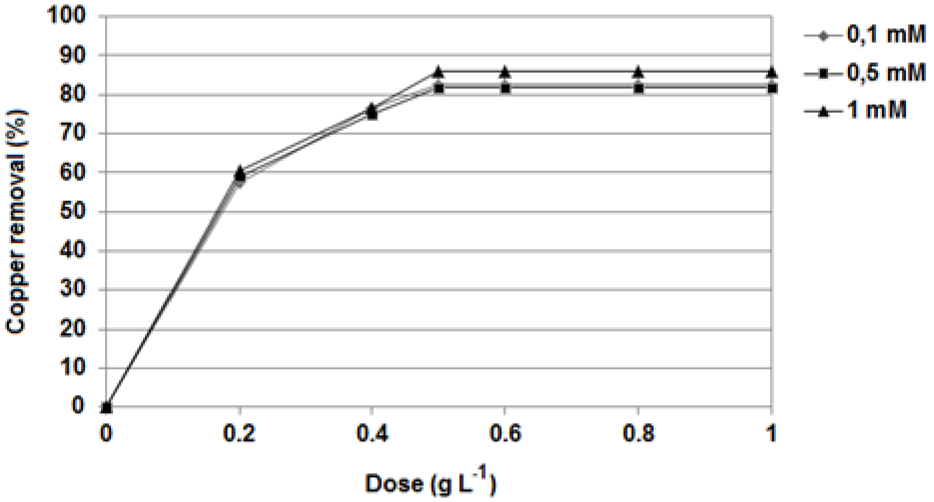

Figure 1. Effect of the HC-GP dose on the equilibrium sorption of copper at different initial metal concentrations (pH=5.0, T=25°C, t=180 min).

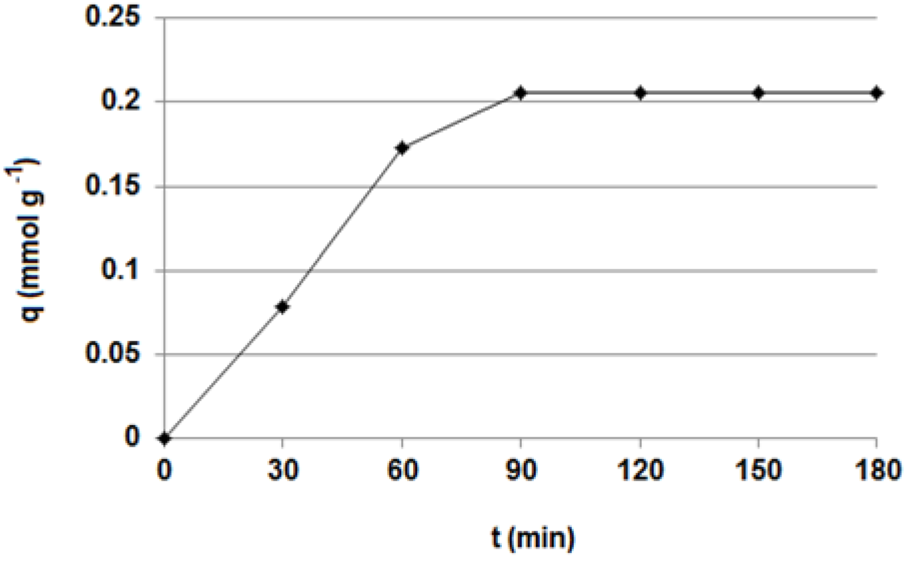

Figure 2. Effect of contact time on adsorption of copper by HC-GP (pH=5.0, sorbent dose 0.5 g L^{-1}, initial metal concentration 1mM, T=25°C).

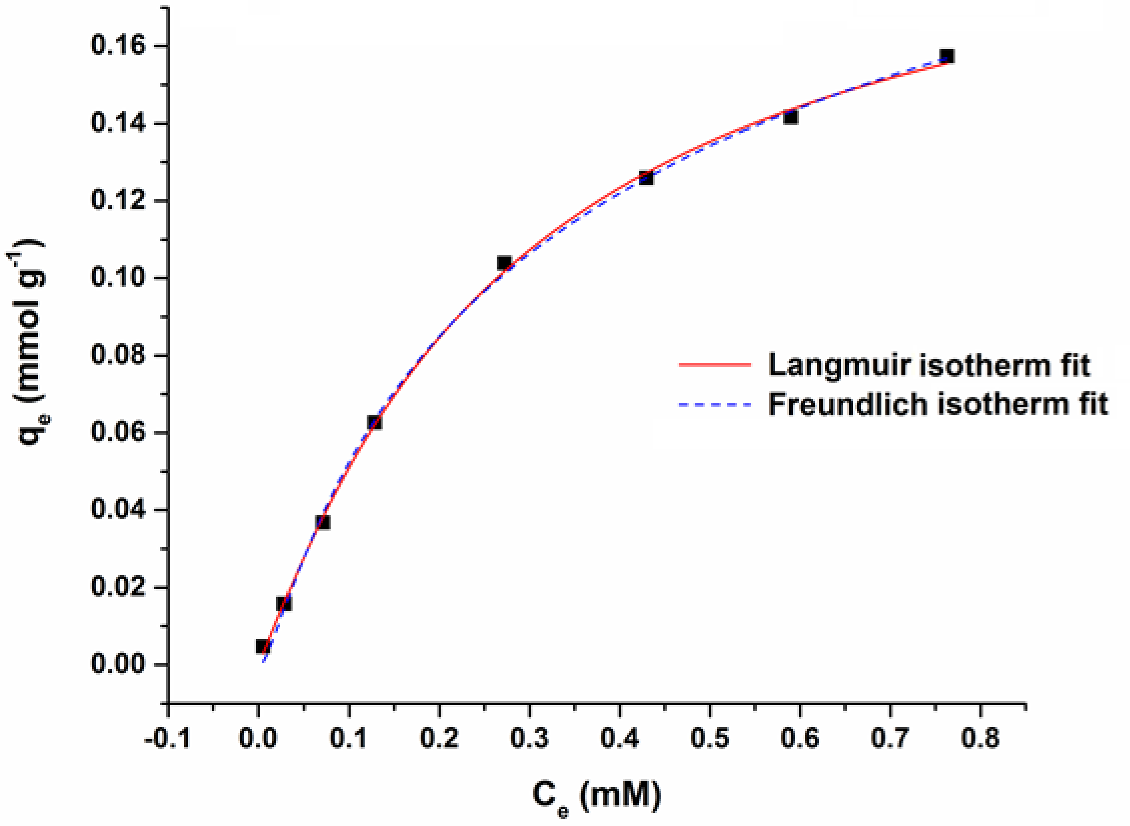

Figure 3. Adsorption isotherms of Cu^{2+} onto HC-GP (pH=5.0, sorbent dose 0.5 g L^{-1} T=25°C, t=120 min)

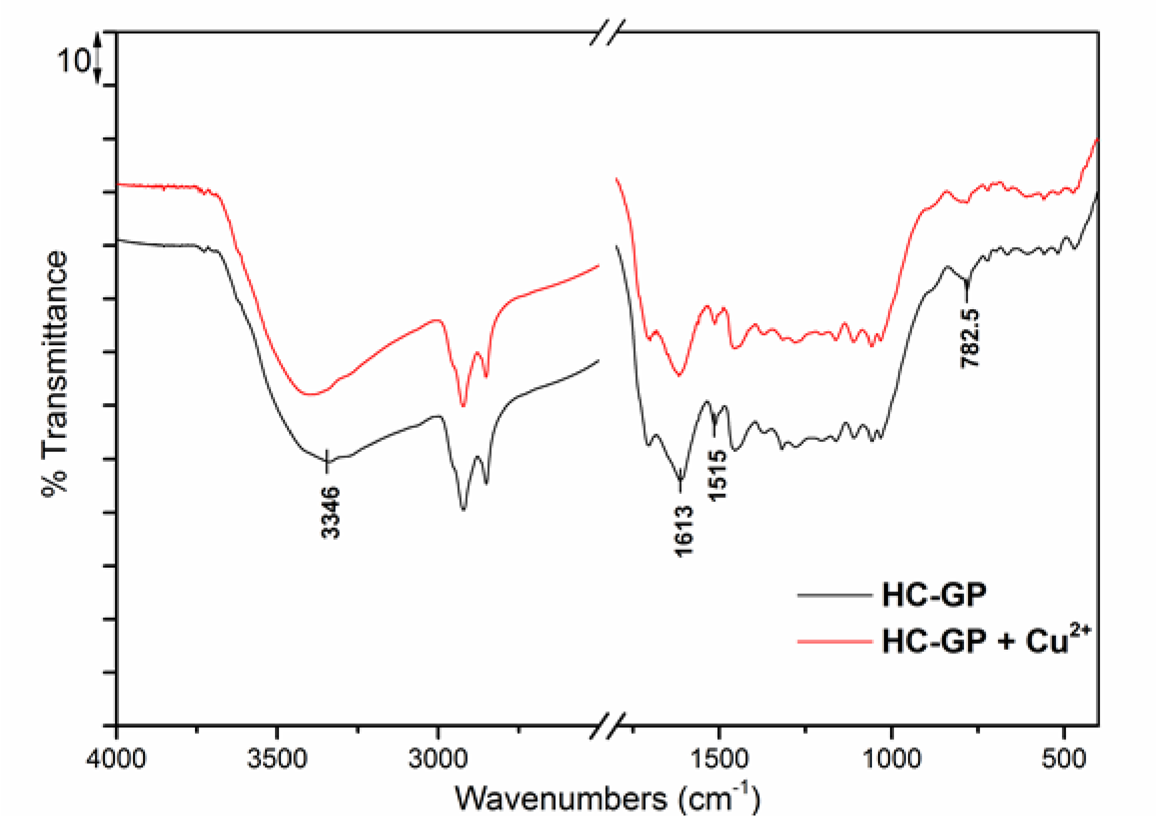

Figure 4. FTIR spectra of HC-GP before and after adsorption of copper (pH=5.0, sorbent dose 0.5 g L^{-1}*,* C_e*= 0.206 mM, T=25°C, t=120 min)*

Table 1. Calculated parameters of applied isotherm models for copper biosorption by HC-GP

Langmuir	*Freundlich*
$q_{\max}$ = 0.211 mmol g^{-1}	K_F = 0.171 $L^{1/n}$ $mmol^{1-1/n}$ g^{-1}
K_L = 3.77 L $mmol^{-1}$	1/n = 0.294
R^2 = 0.999	R^2 = 0.998

THE USE OF BEVERAGE STORAGE POCKETS (TETRA PAK™) WASTE IN WOOD-PLASTIC COMPOSITES

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Introduction

Considering the conducted researches in Europe, it has been found that a substantial amount of municipal solid wastes is originated from the waste of liquid storage package such as fruit juices and milk which has been estimated to be over 313,000 tons per year.[1] Therefore, it causes environmental pollution resulted from high volume of these materials in municipal solid waste due to lack of recyclability mechanism of these materials in lignocellulosic industry.[2] As these packages contain Non-woody elements in their structures, these materials recycling in the pulp and paper industry would be time consuming and costly, therefore, leading to its ineffectiveness and elimination.

Applying these materials as fillers or reinforcement in polymers is a prospective attitude in industrial application, which will flourish the potentialities of such materials, and will create non-food markets for liquid storage packages. Beverage storage pockets, which are referred to as Tetra Pak™ (TP) in Iran, are used for packaging milk and juices and are made of different materials including Kraft paper (75%), low density polyethylene (LDPE) (20%), and aluminum (5%). Generally, the TP are made of six different layers including: 1-PE used as waterproof and making the contents antibacterial, 2-layers of Kraft paper for increasing durability, 3-PEas binding layer, 4-Aluminum to create resistance against oxygen permeation, 5-LDPE as a binding layer and 6-PE to seal off the liquid contents[3].

A review of the related literature shows that few studies were performed on the application of these wastes in the composites industry. For example: Hidalgo (2011) developed a rough panel board composite from TP wastes. The results showed better composite tensile strength and low water absorption compared with the control samples.[4] Ayrilmis et al (2013) also made lignocellulosic composite materials from wasted materials and TP packages. Their results suggested that an increase in lignocellulosic material in TP composite would increase tensile and flexural modulus. By adding lignocellulosic materials as much as 50 percent, the tensile strength would be increased while bending strength would witness a decrease. They estimated that this increase in elasticity modulus was due to uniform distribution of aluminum composite in the matrix.[5]

The purpose of this research is to build a wood-plastic composite using waste TP. Thus, wood flour:TP proportions were as follows: 40:0, 30:10, 20:20, 10:30% and maleic anhydride grafted polyethylene (MAPE) coordinated (0, 3%) to investigate their physical and mechanical properties.

Material and Methods

Low Density Polyethylene (LDPE) with melting flow index of 0.43-0.57 g/10 min and a density of 0.925 g/cm3 was purchased from Bandar Imam Petro Chemical Company and was selected as the matrix. The MAPE from Kimia Javid Sepahan, commercially named as I3OS, with the melting flow index of 23 g in 10 minutes was used as a coupling agent. Pockets of waste recycled juice from waste collection centres in Sari, north of Iran, were used to make the samples. After preparing the juice pockets, in order to prepare the pockets for cutting, juice and milk pockets were treated with hot water. The applied condition for treating procedure in hot water was conducted at 50 °C for 48 hours. Afterward, the juice packets were grinded. Poplar wood flour (WF) was taken from wood factory in northern Iran, being passed through the 60 mesh and the remaining was passed through 80 meshes, and was used as fibrous filler. The Poplar wood flour was dried at 100 °C with oven for 24 hours to reach the moisture level of below 1 percent.

Sample preparation

The composite formulas are provided in Table 1. The proportion for poplar flour:TP waste were 40:0, 30:10, 20:20, 10:30% and the MAPE was chosen in two levels of 0 and 3%. The mixing materials stage operation was done in HAAKE mixer at 160 °C with the speed of 60 per minute. At first, LDPE was added to the mixer, after being melted, the natural fibers, TP and the coupling agent were added. Thus, the entire preparation operation took 7 minutes. After mixing the compounds, the prepared mixture was grinded by semi-industrial WIESER grinder and was transferred to the injection-molding machine and the test samples were prepared under 10 MPa nozzle pressure at a temperature of 180 °C. Finally, in order to acclimatize the samples, they were exposed to 23 °C and 50% relative humidity for at least 40 hours based on ASTM D99-618 standard.

Table 1- Composition of evaluated formulations

Number	Composite Formula	LDPE (wt%)	Wood flour (wt%)	Tetra Pak (wt%)	MAPE (wt%)
1	60LDPE/40WF/0TP/0M	60	40	0	0
2	60LDPE/30WF/10TP/0M	60	30	10	0
3	60LDPE/20WF/20TP/0M	60	20	20	0
4	60LDPE/10WF/30TP/0M	60	10	30	0
5	57LDPE/40WF/0TP/3M	57	40	0	3
6	57LDPE/30WF/10TP/3M	57	30	10	3
7	57LDPE/20WF/20TP/3M	57	20	20	3
8	57LDPE/10WF/30TP/3M	57	10	30	3

Mechanical Test

In this research, the tensile strength was measured by mechanical testing machines, Instron Model 1186, based on the ASTM D638 Standard. The test was done according to the loading speed of 5 minutes per mm. The German Zwick pendulum instrument, 5102 model, was used to test the composite strength to Izod notched impact. All the samples were tested according to ASTM D256 standard.

Morphology

To study the morphology of the structure, the scanning electron microscope (SEM), KYKY-EM3200 model from China, was used. The fractured surfaces were covered with a thin layer of gold in ion coating machine. Then, the SEM pictures of the compounds using voltage of 26 kV were prepared.

Results and Discussion

The effect of filler percentage and the amount of MAPE as coupling agent on the tensile strength and modulus of composites made from TP and LDPE are given in Figure 1 and 2, respectively. As can be witnessed, adding TP resulted in an increase in tensile strength. Such increase for adding 10% of TP to the samples, compared with control samples, was 20.21%. By adding 20% and 30% of TP to the samples, the tensile strength will be increased to 26% and 24.4%, respectively, indicating an improvement in the tension transfer to the interface area. This increase in tensile strength is due to the presence of the aluminum sheets, allowing a uniform tension to be transferred in the matrix to the fiber phase.[2, 4, 6]

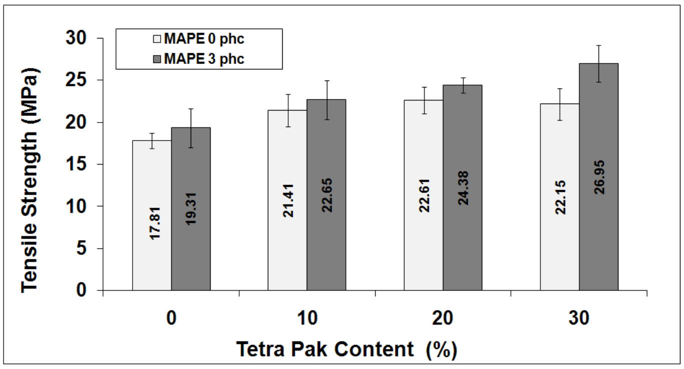

Figure 1. Tensile strength of the WF/LDPE composites as a function of TP flour content and MAPE

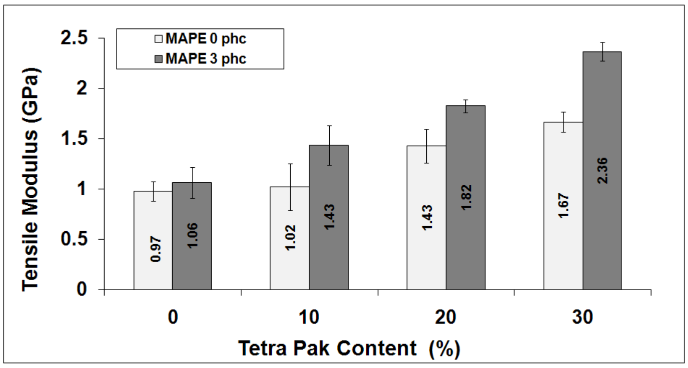

Figure 2. Tensile module of the WF/LDPE composites as a function of TP flour content and MAPE

In addition, the results showed that the addition of MAPE significantly increased the tensile strength of the composites containing TP. This increase is due to the improvement in the connection in the interfacial area between TP and the polymeric matrix[7].

Tensile modulus of the samples increased by increasing TP from 0 to 30% (fig 2). In a way that the tensile modulus of the samples containing 30% of TP without MAPE (2.36 GPa) was 143% higher than the samples without TP. Mainly, the composites tensile modulus was a part of its components elasticity modulus. These results showed that the aluminum foils were more rigid in composite than the polymer. Thus, the elasticity modulus of the composite contain TP was more than the samples without ones. The increase in tensile modulus of the composite originating from TP represented a good distribution of TP and appropriate tension transmission between the polymer and the filler[5]. In addition, the tensile modulus increased by the MAPE addition.

Figure 3 shows the results of TP filled composites' Izod notched impact strength . The samples without TP in comparison with samples containing 10 to 30% TP had a higher impact resistance (40.67J/M). It is expected because the presence of TP in the polymer matrix created stress concentration points which are prone to develop cracks. Another reason for decreasing the Izod notched impact strength may be due to the hardening polymer chains because of the link between the matrix and the TP.[7] The addition of 3% MAPE had a positive effect on the samples Izod notched impact strength. By adding 3% of MAPE, the impact resistance of the samples containing 10, 20 and 30% TP increased to 3.9%, 6.23% and 5.1%, respectively.

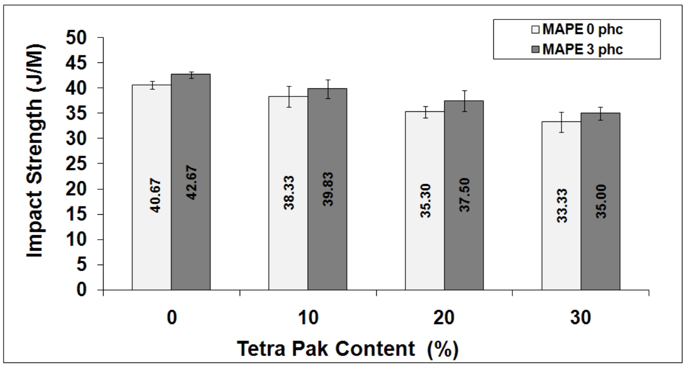

Figure 3. Impact strength of the WF/LDPE composites as a function of TP flour content and MAPE

Morphology of the samples surface factures

There is a relationship between the composites properties and their internal structure. These figures can be very promising instruments to explain the physical and mechanical properties of composites.

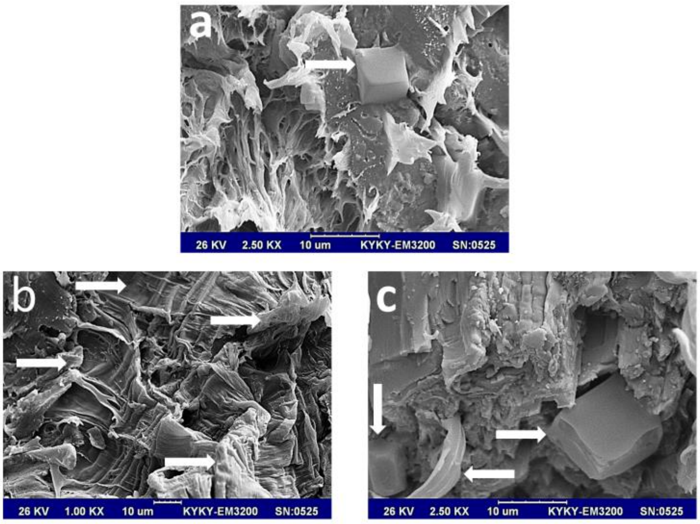

Figure 4. SEM micrographs of fractured samples of composites: a) 20%TP+20% flour+0% MAPE b) 10%TP+30% flour+3%MAPE with 2500X magnification c) 10%TP+30 % flour+3% MAPE with 1000X magnification

In Figure 4a shows the image of a sample containing 20% of TP without MAPE shown with 2500X magnification. The Aluminum in TP is shown with arrows in the matrix. It is also seen in the strength and tensile modulus diagram that by increasing TP, the mechanical strength is increased due to the presence of aluminum particles in these areas. Due to the absence of MAPE, many holes (cavities) have been seen in some areas in the matrix instead of fibers.

These indicate weak bond between the matrix and the lignocellulosic fibers. Figure 4c shows the composite containing 10% TP and 3% MAPE with 2500X magnification. As seen, the presence of TP and polymer in the composite indicates the particle dispersion in the matrix. According to Izod notched impact strength diagram, it can be seen that TP, prone to cracks, reduces the impact resistance. Thus, as the TP amount increases, the amount of impact strength decreases. The presence of MAPE as a coupling agent leads to cohesion of the surface and prevents the development of cracks in the matrix. This phenomenon can be seen in Figure 4b with 1000X magnification. Due to activating effect of fiber surface, the compound created a bond between LDPE and wood fiber encapsulating the TP particles, as well.

Conclusion

Based on the findings of the study, the following conclusion can be proposed:

1. By increasing the TP content up to 30%, the composite strength increased.
2. The MAPE compatibilizer had a positive effect on the composites' mechanical strength by improving the bond in the interface area and wetting the fiber surface.
3. The composites' Izod notched Impact strength witnessed a decrease as the TP content increased, however, the MAPE addition improved the bond.
4. The SEM images results indicated the appropriate TP presence and distribution up to 30% in the sample. However, the images showed that because of the TP aluminum sheets presence in the matrix, the composites' impact resistance reduced. Besides, the addition of 3% MAPE caused a better dispersion of the wood fibers, TP and matrix causing the matrix to cover the fillers better.
5. The highest amount of tensile strength and modulus of elasticity belonged to composites containing 30% TP and 3% MAPE. In addition, the highest amount of Izod notched impact strength belonged to the samples containing 3% of MAPE with no TP.

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REMOVAL OF CONGO RED FROM AQUEOUS SOLUTION USING LIGNOCELLULOSIC BIOSORBENT AND SOLID STATE FERMENTATION OF DYE-ADSORBED BIOSORBENT

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Introduction

Discharge of highly coloured industrial wastewaters, containing synthetic dyes, is considered to be an important source of water contamination. The common characteristics of synthetic dyes are physicochemical, thermal and optical stability, which enable their prolonged persistence in the environment (Li et al., 2014). Furthermore, they exhibit recalcitrance towards biodegradation, thus making their removal by conventional biological treatment technologies inefficient. Congo red (CR) is a diazo dye derived from benzidine, widely used in textile and paper industries. Wastewater containing CR is not highly biodegradable, since this dye is toxic to many organisms (Li et al., 2014).

Among the methods employed for dye removal from wastewaters, adsorption has been found to be superior to other methods because of its capability to efficiently adsorb a whole range of structurally different dyes, low operational cost, ease of design and insensitivity to toxic substances (Li et al., 2014). Activated carbon is the most commonly used adsorbent. However, its use in wastewater treatment is somewhat limited due to the high price and therefore the need for regeneration and reuse (Rafatullah et al., 2010).

In recent years, there has been an increasing amount of literature on the use of low-cost lignocellulosic materials, arising from agricultural or industrial residues as biosorbents for dye removal (Velić et al., 2015; Li et al., 2014). In order to be considered “low-cost”, the adsorbent has to be abundant in nature or has to be an industrial by-product or waste material that requires little or no processing (Rafatullah et al., 2010). The brewers’ spent grain meets all the above-mentioned criteria. Once the waste material has been used for adsorptive removal of dyes, dye-loaded material should not be directly discarded to the environment. Subsequent treatment using microorganisms that can degrade or mineralize both dye and lignocellulosic material would represent an efficient solution to the problem.

White-rot fungi are a group of fungi that degrade lignin and lignin-like substances. In addition, the specific extracellular enzyme systems of white-rot fungi enable them to degrade or mineralize a broad spectrum of different environmental pollutants, including many synthetic dyes (Jayasinghe et al., 2008).

The objectives of this study were: a) to use the brewers’ spent grain as a biosorbent for the CR removal from aqueous solutions and to evaluate its adsorption properties by batch adsorption experiments; b) to screen different white-rot fungi for their CR decolourisation ability; c) to evaluate the CR decolourization ability of the selected white-rot fungus cultivated under solid-state conditions using dye-adsorbed brewers' spent grain as a substrate.

Material and Methods

Biosorbent. Brewers' spent grain (BSG), kindly donated by "Osječka pivovara d.d." brewery was dried (oven dried at 60 °C for 48 h) and milled using standard laboratory knife mill with 1 mm screen (MF10 basic, IKA Labortechnik, Germany), sieved and 100-500 µm fraction was used for the experiments. No other chemical or physical treatments were applied prior to adsorption experiments.

Adsorption experiments. Batch adsorption experiments were carried out by adding a fixed amount (5, 10 and 15 g L^{-1}) of adsorbent to 100 mL CR (Kemika d.d., Zagreb, Croatia) solution (30, 50, 100 and 150 mg L^{-1}) taken in Erlenmeyer flask. The pH was adjusted to 7 (or higher for pH dependency experiments) and measured using MP230 pH-meter (Mettler Toledo, Switzerland). The flasks were placed in the incubator shaker (INNOVA 4340, New Brunswick Scientific, New Jersey, USA) at 25 °C and 150 rpm for 240 min. The samples were taken at different intervals (5, 10, 20, 30, 60, 90, 120, 150 and 240 min) for spectrophotometric determination of colour removal. Dye solution samples taken at various intervals were centrifuged for 5 min at 10 000 rpm (Tehtnica Centric 322A, Domel d.o.o., Slovenia). The dye concentrations in clarified supernatants were determined at 498 nm (Lambda 25, Perkin Elmer, USA). The percentage removal of dye was calculated by equation:

$$\textbf{\% dye removal} = 100\ (\gamma_0 - \gamma)\ /\ \gamma_0$$

where γ_0 and γ are the initial dye concentration and dye concentration after certain contact time, respectively.

Screening fungi for CR decolourisation ability. Prior to solid-state fermentation experiment, four white rot fungi strains, *Phanerochaete chrysosporium* CCBAS 570, *Trametes versicolor* CCBAS AG613 (both The Culture Collection of Basidiomycetes, Prague, Czech Republic), *T. versicolor* TV6 and *Ceriporiopsis subvermispora* (both The Microbial Culture Collection of the National Institute of Chemistry, Ljubljana, Slovenia), were screened for their ability to decolourise CR during cultivation (9 days at 27 °C) on agar plates containing CR at final concentrations of 50, 100 and 150 mg L^{-1}. Radial growth and the zone of colour change on agar plates were measured (at two perpendicular directions) every three days. The results were expressed as decolourisation index (decolourization diameter/mycelial diameter) (Jayasinghe et al., 2008).

Biological treatment of dye-adsorbed biosorbent. White-rot fungus *T. versicolor* CCBAS AG613 (The Culture Collection of Basidiomycetes, Prague, Czech Republic) was cultivated on potato dextrose agar (Liofilchem, Italy) in Petri dishes for 7 days at 28 °C. Mycelial discs (diameter of 6 mm) were used as inoculum for solid-state cultivation that was carried out in a horizontal cylindrical glass jar (bioreactor, volume 4.25 L) equipped with appropriate sensors. Aeration was carried out continuously (air flow rate was set at 30 L h^{-1}) using sterile air filter and air compressor, while the mixing was intermittent and manual (every 24 h for 5 minutes). The bioreactor was filled with 250 g BSG and 350 mL dye solution (150 mg L^{-1}), mixed and sterilized. After cooling, the bioreactor was inoculated with 25 mycelial discs. The experiment was conducted at ambient temperature for 21 days. Abiotic control containing only dye-adsorbed BSG and biotic control containing BSG and fungus were run for comparison. The samples were taken every seven days and analysed for proteins and colour. The temperature, relative humidity and mass loss in the bioreactor were monitored on-line. The temperature was measured at different points (headspace, inlet, outlet and bioreactor surroundings) and in the substrate bed (bottom) using stainless steel thermocouple penetration probes (type T), connected to the 8-channels Pico A/D converter and a PC application PicoLog (Pico Technology Limited, England) for the data acquisition. Testo 635 and 350 devices (Testo Inc., Sparta, New Jersey, USA) were used for additional temperature and relative humidity monitoring. The bioreactor was placed on the balance (EOD120, OHAUS, Switzerland) for continuous mass loss monitoring. Sample *water content determination* was conducted thermogravimetrically using Halogen Moisture Analyzer HR73 (Mettler Toledo, Switzerland). The *colour* of samples was measured using chromameter (Konica Minolta CR 400) in *Lab* colour space system. Colour changes of samples during the experiment were expressed as total colour changes (ΔE) and the colour intensity change (chroma) (C*ab). The samples' *protein content* was determined by standard Kjeldahl procedure.

All analytical results were expressed as means of three replicates.

Results and Discussion

The adsorption experiments

The adsorptive efficiency of BSG was investigated and the results are presented in Figure 1. The effect of contact time and initial dye concentration are given in Figure 1 a. It can be seen that CR adsorption on BSG (i.e. CR concentration decrease) followed three-step process, a rapid initial adsorption within the first 60 min of the experiment, followed by a period of slower uptake and no significant uptake at the end of experiment. This is consistent with Ahmad and Kumar (2010) using low-cost adsorbent bael shell carbon for CR removal. The initial dye concentration has a significant effect on the adsorptive removal of dyes from aqueous solutions (Ahmad and Kumar, 2010). The percentage adsorption of CR decreased with the increase in the initial dye concentration, which is expected taken into account that at constant adsorbent concentration at higher initial dye concentration there are fewer available adsorption sites. As shown in Figure 1b the percentage removal of CR increased with the increasing adsorbent concentrations, which is in accordance with the results reported by Salleh et al (2011). However, at later stages of the experiment (contact time 120 min) the removal was similar for all runs. The effect of pH on adsorption was studied in the range of 6.5-10 (data not shown), which is just slightly above the usual wastewater pH range (6.5-9). It was found that the removal efficiency increased from 69.03 % to 93.88 % due to change in pH from 10 to 6.5, which is consistent with literature data (Dawood and Sen, 2012).

Screening fungi for CR decolourisation ability

The results of screening the four fungal strains for CR decolourisation ability on agar plates revealed that only three strains were able to decolourise CR and CR did not strongly inhibit the mycelial growth of the investigated fungi (data not shown). *P. chrysosporium* CCBAS 570 did not exhibit CR decolourisation ability, while both *T. versicolor* strains, as well as *C. subvermispora* were able to decolorize CR to some extent. However, based on the highest decolourisation index ranging from 1.05 to 1.13 for CR concentrations ranging from 50 to 150 mg L^{-1}, *T. versicolor* CCBAS AG613 was chosen for further SSF experiments.

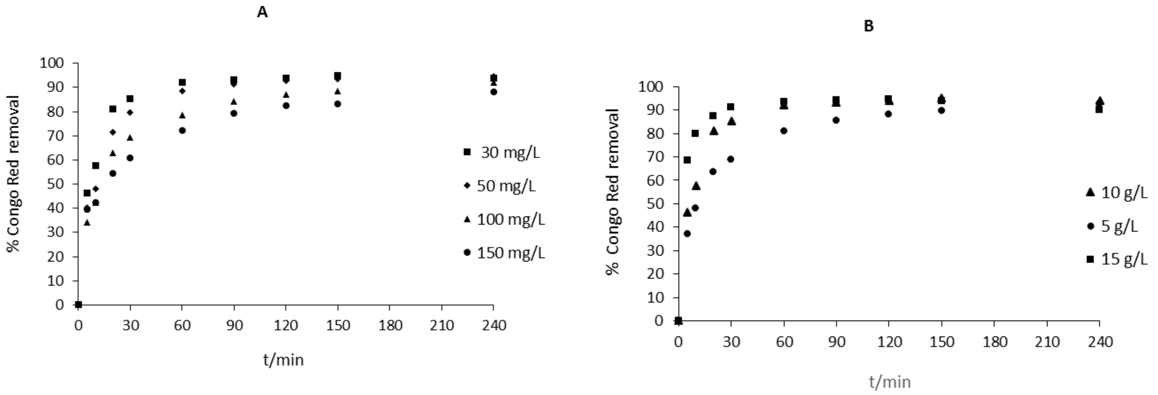

Figure 1 A) the effect of contact time and the initial dye concentration on the CR adsorption on BSG (t = 25 °C, 150 rpm, pH = 7, V= 100 mL, $\gamma_{adsorbent}$ = 10 g L^{-1}), B) the effect of adsorbent concentration on the CR adsorption on BSG (t = 25 °C, 150 rpm, pH = 7, V= 100 mL, γ_{dye} = 30 mg L^{-1})

Biological treatment of dye-adsorbed biosorbent

T. versicolor CCBAS AG613 grew readily under solid-state conditions using dye-loaded BSG as a substrate and carrier. Apart from visible confirmation of fungal colonisation of the substrate, the increase in substrate bed temperature, compared to ambient temperature, also indicated actively growing culture (Figure 2). No significant changes in relative humidity were observed during the cultivation.

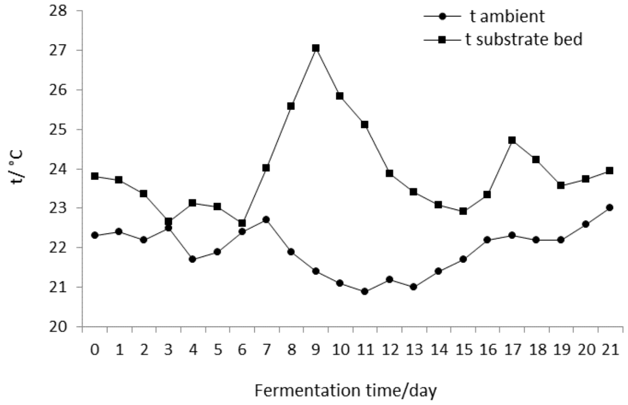

Figure 2. Temperature profiles during fermentation

Furthermore, the protein content results (data not shown) for biotic control and bioreactor sample show continuous increase (from 23.02 to 26.00 and from 23.02 to 24.90, respectively) after 7, 14 and 21 days of cultivation, which is in accordance with the literature (Peksen et al., 2011). However, the higher increase for biotic control is probably the result of slight inhibition of fungal growth by CR. The protein content of abiotic control remained the same throughout the experiment. Even though the substrate was not completely decolourised, CR decolourisation (expressed as total colour change and colour intensity change of fermented dye-adsorbed BSG samples compared to their abiotic controls after 7, 14 and 21 days) increased with the extension of cultivation time (Figure 3a). The same was observed for mass loss (Figure 3b).

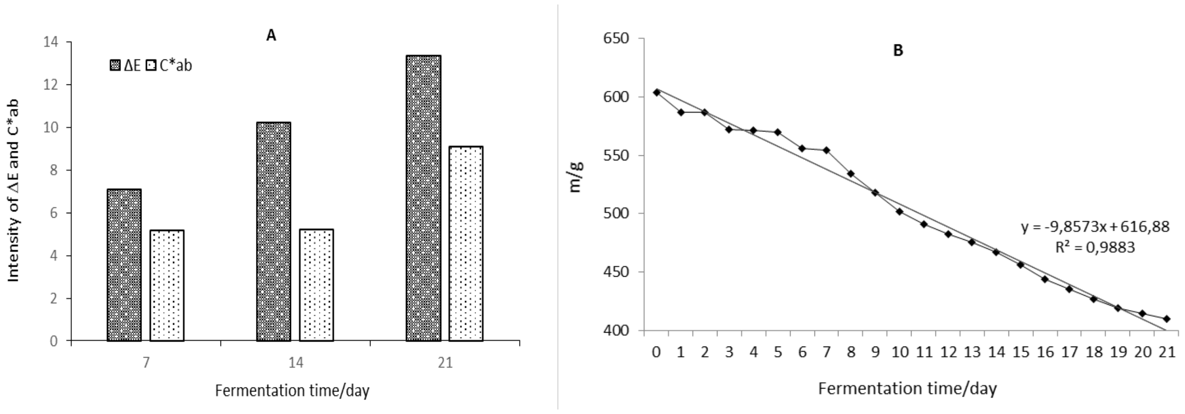

Figure 3 a) total colour change and colour intensity change of fermented samples compared to their abiotic controls after 7, 14 and 21 days of fermentation, b) time course of substrate mass loss

Conclusion

Brewers' spent grains proved to be highly efficient biosorbent for Congo red removal from aqueous solutions. The resulting dye-adsorbed BSG was successfully used as substrate for *T. versicolor* CCBAS AG613 cultivation under solid-state conditions. These findings provide the following insights for future research: detailed characterization of BSG as possible commercial adsorbent and elucidation of the mechanisms involved in the decolourisation of dye-adsorbed biosorbents by *T. versicolor* CCBAS AG613.

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Session E: Biodiversity and Ecosystem Services

NEW EMERGING POLLUTANTS IN THE SOIL

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1.1 Introduction

"Emerging pollutants" can be defined as pollutants that are currently not included in routine monitoring programs at the European level. They may be candidates for future regulation, depending on results of their (eco)toxicity, potential health effects and public perception as well as on monitoring data regarding their occurrence in the various environmental compartments. Norman list of emerging substances includes the following substances: surfactants, flame retardants, pharmaceuticals and personal care products, gasoline additives and their degradation products, biocides, polar pesticides and their degradation products, and various proven or suspected endocrine disrupting compounds (NORMAN list, 2016).

Flame retardants (FR) is a group of chemicals which are added to manufactured materials that are commonly used in electronic equipment, textiles, furniture and insulation materials in order to reduce the degree of flammability of these materials. There are several groups of FR depending on their chemical contents: brominated, phosphorus, nitrogen, chlorinated and inorganic. Brominated flame retardants (BFR) prevent the occurrence of fire and its spreading. Because they are not chemically bound to the material, they are mobile (Dodder, et al. 2011, Dickhut, et al., 2012), persistent and have a high bio-accumulation potential. Therefore, BFRs represent a potential danger to the environment (Hu, et al., 2010). In this group of compounds polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBPA) and polybrominated biphenyls (PBBs) are most harmful to the human health and the environment (Wang, et al., 2015).

PBDEs are industrial chemicals that are used more than 40 years. They are usually released in the environment during their production process and use as well as during the process of servicing appliances containing PBDE, their improper recycling and disposal in landfills. Also a source of PBDEs is old, discarded furniture that falls apart under the influence of atmospheric conditions (sun, wind, rain, extreme temperatures) in tiny particles that become part of the soil, air and groundwater. Human uptake is thought to be through inhalation, dermal absorption and consumption of contaminated food (Trudel et al., 2011). These chemicals are among the most toxic chemicals ever synthesized. There are evidence that they cause cancer (Wenning et al., 2002), also they cause damage to the central and peripheral nervous systems (Banasik et al., 2011), diseases of the immune system (Fernie et al., 2005), reproductive function disorders (Kodavanti et al., 2010), functional disorders of the endocrine system (Yu et al., 2011), and most importantly they affect neurological development and growth (Dingemans et al., 2011).

Due to the potential danger resulting from the presence of PBDEs in the environment, the aim of this paper was to detect the potentially contaminated areas and to determine the concentrations of PBDEs..

1.2 Matherials and methods

In order to investigate the presence of the PBDE congeners, twenty four samples of surface soil were collected in the period from May to September 2015 from five regions of Vojvodina Region. Samples were taken according to the template method (IAEA, 2004).

Chemical reagents and standard solutions which were used during the analysis of the soil samples were: hexane and dichloromethane for UV, IR, HPLC, ACS, puchased from Panreac (AppliChem, Germany), silica gel 60 (Davisil grade 636, 35-60 mesh) and alumina (activated, neutral, Brockmann I, 58 Å) from Sigma Aldrich and SPE columns (54222-U) from Supelco. Silica gel was activated at 190°C for 24 h. Acid silica gel was prepared by combining concentrated sulfuric acid (30 ml) with activated neutral silica gel (50 g). PBDEs standard solution (8 congeners of primary interest) was purchased from Accu Standard (USA). For the analysis the following equipment was used: a rotary vacuum evaporator (Buchi), analytical balance (Kern ABJ 120), vacuum manifold (Stock No. 57030-U, Supelco), gas chromatograph Agilent 7890 B with CTCPAL sampler for liquid and gaseous samples with EC detector, capillary column HP-5 (Agilent J&W GC Columns, 30 m x 0,30 mm x 0,25 µm).

After air drying at room temperature the samples were pulverized and sieved through a 250 µm sieve in order to obtain a homogeneous matrix. For recovery testing, ten grams of blank soil matrix was spiked with 50 µl PBDE standard solution with concentration of 2 µgml^{-1}. USEPA Soxhlet extraction method (Method 1614, EPA 2007) was used for PBDEs extraction from spiked soil. The volume ratio of solvents was Hexane/DCM 1:1 with extraction time duration of 12 hours. After the end of extraction the volume of the extract was reduced down to 2 ml by rotary evaporation in the presence of steam of nitrogen at a temperature of 45°C. Purification was carried out by two columns using the procedure explained in the literature (Covaci et al., 2002). The final determination was carried out with gas chromatography with electron capture detector (Agilent 7890B). Working conditions of the GC system were set as follows: injector at 300°C, detector at 310°C, initial oven temperature at 90°C, for 2.0 min, heating to 220 °C at rate 50°C/min, then to 300°C at rate 5°C/min, with a final 20 min hold. Splitless injection with flow of 60 ml/min was used. Chromatogram obtained under these conditions is shown on Fig. 1.

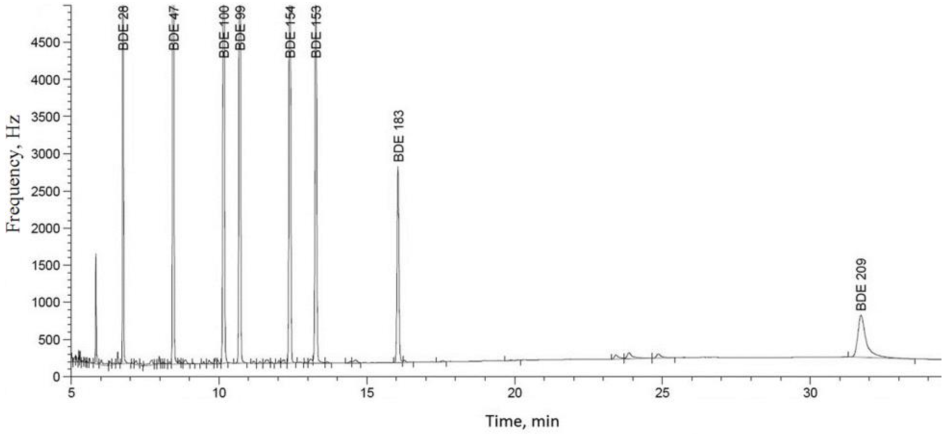

Figure 1. GC-ECD chromatogram of spiked blank soil matrix with mix of eight PBDE congeners (BDE 28, BDE 47, BDE 100, BDE 99, BDE 154, BDE 153, BDE 183 and BDE 209)

Basic chemical properties of the soil samples like pH, the percentage of potassium, carbonates, humus, phosphates and total nitrogen were analysed using methods described in literature (M. Belić et al., 2014).

1.3 Results and discussion

The PBDEs contents in 24 soil samples are shown in Table 1.

Table 1. Concentrations of PBDEs in soil samples

	Concentration (µgkg^{-1})								
Sample/Congener	BDE 28	BDE 47	BDE 100	BDE 99	BDE 154	BDE 153	BDE 183	BDE 209	total
1 landfill	0.48	0.01	0.05	0.15	0.21	0.28	0.46	65.79	67.43
2 landfill	0.03	0,09	0.00	0,.09	0.07	0.10	0.09	7.50	7.97
3 landfill	0.07	0.06	< LOD	0.07	0.06	0.07	0.04	6.59	6.96
4 ex marshalling yard	0.32	0.78	< LOD	61.59	0.35	0.06	0.03	15.72	78.84
5 ex marshalling yard	3.04	0.38	< LOD	0.84	0.07	0.04	0.51	11.59	16.48
6 landfill	0.47	0.35	< LOD	0.16	0.03	0.03	0.06	6.18	7.28
7 car waste	0.28	1.20	0.84	3.71	0.52	0.58	0.22	13.32	20.69
8 landfill	1.06	0.42	0.07	0.25	0.02	0.11	0.13	4.53	6.59
9 landfill	6.82	1.19	0.36	0.33	0.29	0.20	0.48	7.42	17.08
10 landfill	1.00	0.38	0.00	0.07	0.04	0.05	0.19	5.36	7.09
11 landfill	0.16	0.15	< LOD	0.20	0.03	0.04	0.06	5.77	6.41
12 landfill	3.86	0.57	0.10	0.10	0.05	0.04	0.13	1.65	6.51
13 landfill	1.44	2.09	0.75	0.23	0.16	0.09	0.55	6.18	11.50
14 landfill	0.64	24.70	< LOD	1.21	0.04	0.05	< LOD	5.77	32.40
15 landfill	2.69	2.42	0.44	0.06	0.24	0.16	0.39	7.01	13.40
16 landfill	5.09	6.41	1.72	6.04	0.26	0.59	1.00	12.90	34.1
17 landfill	0.44	0.14	0.01	0.04	0.05	0.07	0.08	7.63	8.46
18 ilegal landfill	0.52	0.40	0.06	0.04	0.03	0.10	0.15	3.71	5.02
19 landfill	0.43	0.26	< LOD	0.12	0.06	0.23	0.28	12.10	13.40
20 landfill	2.85	6.51	2.94	0.27	1.01	2.05	0.41	6.18	22.20
21 landfill	0.23	0.33	0.03	0.16	0.08	0.08	0.09	4.12	5.12
22 landfill	0.45	0.40	0.20	0.18	0.06	0.08	0.23	2.83	4.43
23 landfill	1.25	1.18	0.17	0.20	0.07	0.12	0.13	10.78	13.90
24 secondary raw materials dump	237.45	277.96	8.60	32.79	0.27	6.64	4.55	160.8	729.1
MIN	0.03	0.01	0.03	0.04	0.02	0.03	0.03	1.65	4.43
MAX	237.45	277.96	8.60	61.59	1.01	6.64	4.55	160.8	729.13
average	1.46	2.19	0.48	3.31	0.17	0.23	0.26	10.03	17.97
LOD*	0.003	0.003	0.003	0.003	0.003	0.003	0.003	1.500	

***LOD = Limit of Detection (calculated as 3 times the standard deviation of the baseline noise).**

Recovery values of BDE congeners ranged between 80% and 115%. All samples contained PBDEs and total amount for eight congeners varied between 4.4 and 723 µgkg^{-1} with a median concentration of 11.48 µgkg^{-1}. The highest values of PBDEs concentrations were detected in a sample taken at the outer edge of the land plot used for the purchase and storage of recyclable waste materials. The company that owns the mentioned land plot deals with the purchase of iron, batteries, aluminum, stainless steel, brass, copper and electronic waste. The total content of PBDEs in sample 25, taken from mentioned plot, was 723 µgkg^{-1}. High concentrations of total PBDEs were also found in a sample taken from ex marshalling yard located in a residential part of Novi Sad. Today, this location is used as a repair yard for trains. Two samples were taken from this location. Sample 4 was taken close to the railway line where since 2006 are parked an old unusable rolling stocks (manufactured in the seventies of the last century). Railway wagons, which were in vicinity of the second sample (sample 5) were recently , placed there (in 2012).

The obtained data were analyzed using the Pearson's chi-squared test which showed that the hypothesis of independence of concentrations of PBDE congeners and the percentage of organic carbon, and humus in soil can be rejected. In contrast to dependence of concentrations of PBDE congeners and the mechanical properties of soil, hypothesis of independence can not be rejected. Humus and total nitrogen showed strong positive correlation with six BDE congeners, due to the characteristics of humus for absorption and retention of pollutants as reported by Andreux et al. (1991).

1.4 Conclusion

According to the examination of 24 soil samples collected from landfils, ex marshalling yard, dump of secondary raw materials and automotive waste, using the described experimental procedure, the presence of eight BDE congeners was confirmed. Total PBDEs concentrations ranged from 4.,4 to 729 µgkg^{-1} of a.d.s. which is in the range detected near a typical e-waste recycling sites in China (Y. Wang et al., 2011). The highest contributions to the total sum had BDE 99, BDE 47 and BDE 209. The largest concentration of total amounts of PBDEs were measured in samples from the landfill receiving zone in Novi Sad, ex marshalling yard and at secondary raw materials dump.

From the presented results of measured PBDEs in the soil, negative impact of bad waste management on the environment was seen.

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VARIABILITY OF MORPHOLOGICAL TRAITS OF EIGHT YEARS OLD PLANTS OF PEDUNCULATE OAK IN PROVENANCE TEST ŽEPČE, BOSNIA AND HERZEGOVINA

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Summary

Natural oak populations in Bosnia and Herzegovina represent specific communities in disappearance, which differ from those of its optimum in central Europe, and are very important for maintaining the diversity of pedunculate oak in Europe.

The task of research is to determine variability within and between provenances of pedunculate oak through the measurement of morphological characteristics: height and root collar diameter on eight years old plants.

Survey was conducted in Bosnian provenance test of pedunculate oak in Žepče. The test was established in spring of 2009, by planting one year old seedlings. The test consists of 28 provenances and each was initially represented with 108 plants (36 plants per block in three blocks).

Height and root collar diameter was measured on eight years old plants. The resulting data were entered into a computer and statistically analyzed by statistical package SPSS 20.0.

The highest mean value of plant heights had provenance Jelah (219,03 cm), and the lowest Visoko Muhašinovići (123,55 cm).

The lowest mean value od root collar diameter had provenance Visoko Muhašinovići (33,0 mm), and the highest provenance Jelah (53,32 mm).

Introduction

Pedunculate oak (*Quercus robur*, L.) was once economically important species of forests in Bosnia and Herzegovina, but due to excessive exploitation first during Ottoman and then Austro-Hungarian rule, it almost completely disappeared. According to the State Forest Inventory in large areas of Bosnia and Herzegovina (6) only about 10,000 ha belong to pedunculate oak forests.

Natural populations of pedunculate oak in Bosnia and Herzegovina represent a specific community in extinction, which differs from its optimum in Central Europe and is very important for maintaining the diversity of pedunculate oak in Europe.

The aim of this study is to determine the variability within and between provenances of pedunculate oak through measurements of morphological characteristics: height and root collar diameter on eight-year old plants.

In Bosnia and Herzegovina only a few studies on pedunculate oak have been done so far. Probable reason for this is the fact that pedunculate oak has almost completely disappeared due to excessive logging. Since this is economically very valuable species, it is necessary to make its reintroduction, where we must take into account the genetic characteristics and origin of seeds.

In the study of leaf material (2, 9) identified significant intrapopulation and interpopulation differences. Differences at intrapopulation level were higher than at interpopulation level.

In the research of survival of plants (1) in the Bosnia and Herzegovina pedunculate oak provenance test Žepče results showed that provenances react differently to transplantation and physiological stress. Average percentage of survival by provenance ranged from 61.10% in Bugojno provenance to 89.81% in Kiseljak provenance.

According to (7, 8), who explored plant heights and root collar diameters in a provenance test in Žepče, statistically significant differences between provenances has been found.

In neighbouring Croatia, many studies of pedunculate oak morphological trait were conducted. According to (11) there is very strong intrapopulation variability in plant height, weight of dry matter produced by plants and number of root apexes, when plants from soil with excess water and from drained soil are tested under intense moisturizing.

In the research of (4), who investigated pedunculate oak plants in the first experiment with 16 provenances of *Quercus robur*, L from Croatia, established in the nursery of the Forest Research Institute in Jastrebarsko on two localities (Slavir and Gajno) in the spring of 1986, the average height of all 16 provenances at Gajno in 1990 was 65,7 cm, while at Slavir it was 38,7 cm.

According to (3), multivariate and univariate analysis of morphological traits of pedunculate oak leaves showed significant differences on interpopulation and intrapopulation level. Differences between individual plants within populations are also in this study greater than the difference between the populations.

In the research of (12), plant heights and root collar diameters in nursery test of pedunculate oak at the age of 1 + 0, 2 + 0 and 3 + 0 years were studied. Research of height and their increments indicates statistically significant differences between samples from different seed zones and districts of pedunculate oak in Croatia, while the results of research in root collar diameter suggest some less visible differences between observed samples.

(13) conducted study of pedunculate oak plant growth in different seed zones and regions of Croatia at two sites (nursery Institute Jastrebarsko and Sedlarac site), and noted the differences between different seed units and zones, as well as between the same zones at different locations.

In investigation of morphometric characteristics of acorns and height of annual seedlings of pedunculate oak from seventeen seed stands in Croatia (5), sampled stands are significantly different in all investigated properties of seeds, but did not match the current seed division, i.e. did not confirm geographical pattern of population differentiation. The same applies to seedling height.

For the four-year-old plants in “Jastrebarski Lugovi” test plantation, (10) determined average height of 162,3 cm, in the interval between 154,8 and 179,3 cm. Average height of five-year-old plants was 182,7 cm while maximum height reached 204,5 cm.

Material and methods

Survey was conducted in Bosnian provenance test of pedunculate oak in Žepče. The test was established in spring of 2009, by planting one year old seedlings. The test consists of 28 provenances and each was initially represented with 108 plants (36 plants per block in three blocks).

Heights and root collar diameters of surviving plants were measured in the spring of 2016. The data was entered into a computer and statistically analysed using statistical software package SPSS 20.0. The following statistical analyses were made: descriptive analysis and analysis of variance.

Table 1. List of investigated provenances

No.	Provenance	Latitude	Longitude	Altitude	No.	Provenance	Latitude	Longitude	Altitude
1	Bijeljina	44º43'50''	19º13'30''	93	15	Lukavica	43º49'26''	18º21'58''	552
2	B. Dubica	45º06'24''	16º40'32''	145	16	Miljevina	43º31'06''	18º38'56''	627
3	B. Gradiška	45º06'64''	17º18'63''	91	17	Mrk. Grad	44º27'04''	16º58'42''	753
4	B. Brod	45º05'27''	18º00'38''	84	18	Cazin	44º58'55''	15º50'54''	270
5	B. Grahovo	44º01'05''	16º38'24''	703	19	Nević p.	44º11'46''	17º42' 1''	476
6	Bugojno	44º06'00''	17º26'31''	537	20	Olovo	44º07'44''	18º36' 1''	542
7	Drvar	44º23'39''	16º21'54''	462	21	Sokolac	43º55'17''	18º48' 3''	866
8	Srebrenik	44º49'06''	18º34'11''	133	22	Ilidža	43º48'40''	18º17' 5''	506
9	Jelah	44º39'09''	17º56'46''	181	23	Vinac	44º15' 8''	17º17'08''	408
10	Kaćuni	44º03'59''	17º56'13''	443	24	Visoko	44º00'38''	18º08' 5''	413
11	Kiseljak	43º56'30''	18º04'56''	477	25	Zavidovići	44º26' 7''	18º07'49''	210
12	Ključ	44º30'56''	16º48'42''	260	26	Zvornik	44º25'01''	19º07' 2''	141
13	Knežina	44º01'40''	18º44'53''	759	27	Žepče	44º25'35''	18º03'10''	224
14	K. Varoš	44º39'07''	17º21'35''	252	28	Živinice	44º27'58''	18º41'09''	216

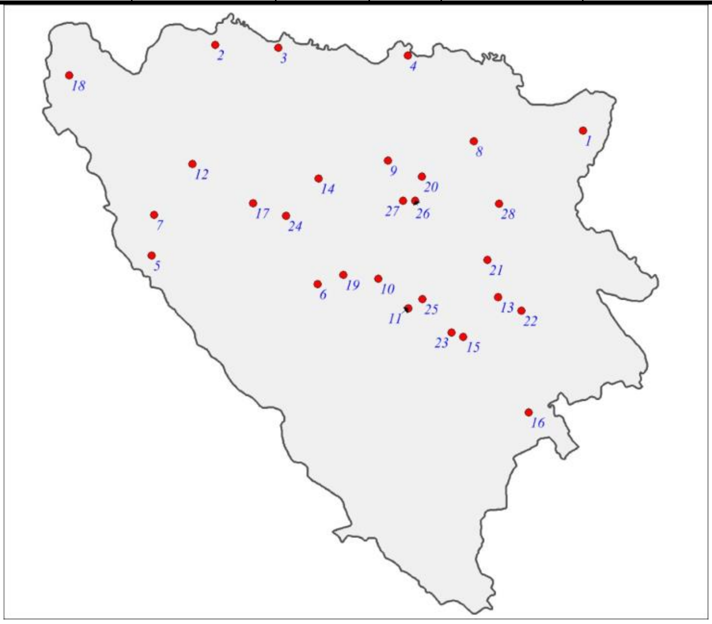

Figure 1. Map of investigated provenances

Results and discussion

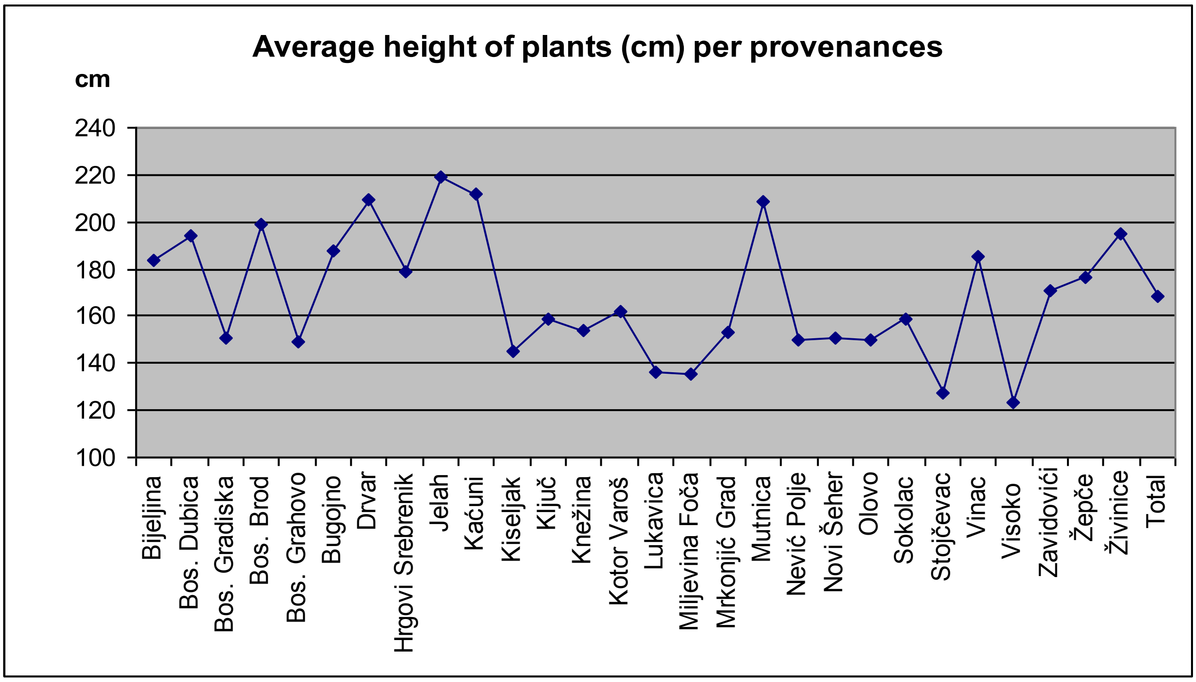

Figure 2. Average height of plants per provenances

The lowest average height of eight-year-old plants (figure 2) is detected in Visoko provenance (123,5 cm). According to the researches of (7, 8), the lowest average height of four-year-old plants in the same provenance test is detected in Miljevina provenance while the lovest average height of five and six-year-old plants is found in Stojčevac provenance. The highest average height of eight-year-old plants (figure 2) is found in Jelah provenance (219 cm), as was the case with all previous measurements (8).

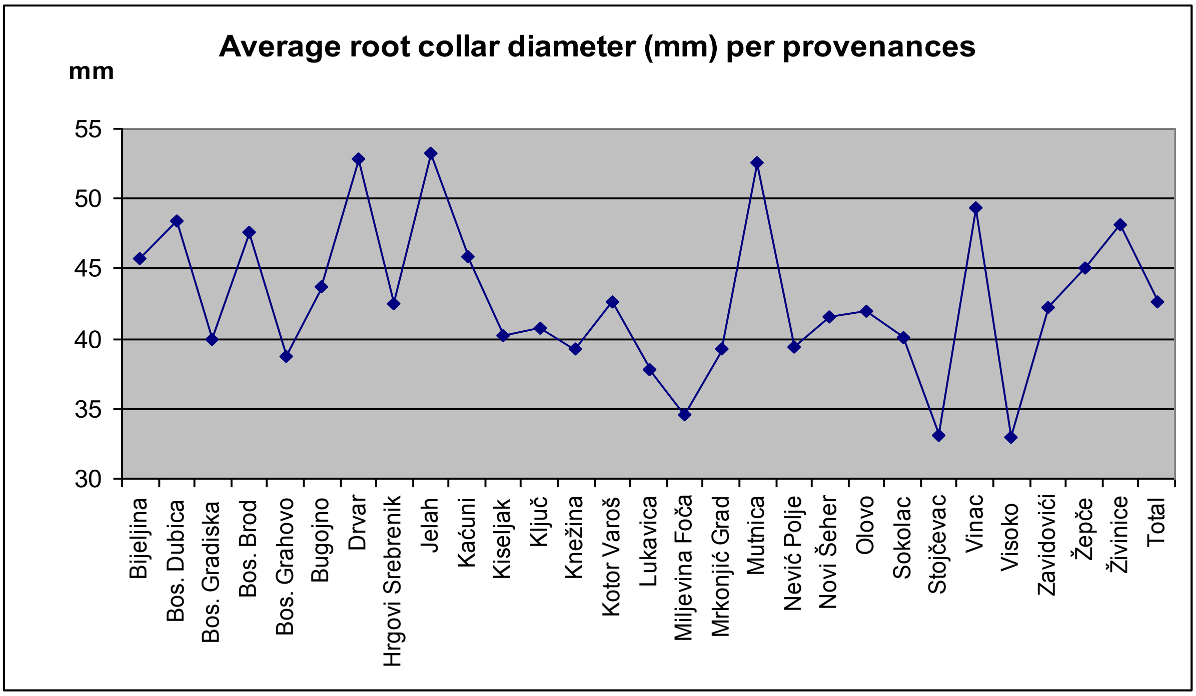

Figure 3. Average root collar diameter of plants per provenances

The smallest average root collar diameter (figure 3) is found in Visoko provenance (33,0 mm), in contrast to the previous years (7, 8), when the smallest average diameter of root collar was detected in Vinac provenance (4-year-old plants) and Stojčevac (5 and 6-year-old plants). The largest average root collar diameter (figure 3) reaches 53,3 mm in Jelah provenance, as was the case with the four and five-year-old plants (7), while the largest average root collar diameter of six-year-old plants is detected in Mutnica provenance.

In a survey conducted by (13), the average height of plants of the age 3 + 2 ranged from 496,7 to 606,7 mm in field test in nursery of Jastrebarsko Institute, and 607,8 to 720,5 mm at Sedlarac site. Average root collar diameter of plants of the age 3 + 2 ranged from 8,87 to 11,28 mm in Jastrebarsko nursery, and from 12,69 to 13,65 mm at Sedlarac site.

For four-year-old plants in „Jastrebarski Lugovi“ test plantation, (10) determined the average height of 162,3 cm, in the interval between 154,8 and 179,3 cm. The average height of five-year-old plants was 182,7 cm, while maximum height reached 204,5 cm.

Table 2. Variance analysis (p<0,05)

Trait	**Source of variation**	**Sum of Squares**	**df**	**Mean Square**	**F**	**Sig.**
Plant height	Between Groups	1507268,114	27	55824,745	12,584	0,000
	Within Groups	9502380,784	2142	4436,219		
	Total	11009648,898	2169			
Root collar diameter	Between Groups	60506,389	27	2240,977	7,799	0,000
	Within Groups	615503,412	2142	287,350		
	Total	676009,801	2169			

Analysis of variance showed that there are statistically significant differences between provenances.

Conclusions

The lowest average height of eight-year-old plants per provenance is detected in Visoko provenance, reacheing 123,5 cm.

The highest average height of eight-year-old plant per provenance is detected in Jelah provenance, reaching 219,0 cm.

The smallest average root collar diameter of eight-year-old plants per provenance is detected in Visoko provenance, amounting to 33,0 mm.

The largest average root collar diameter reaches 53,3 mm (Jelah provenance).

In this study we were unable to confirm earlier assertion that southern provenances grow faster than northern provenances, due to the specific relief in Bosnia and Herzegovina, and probably juvelity of test material. Thus, Jelah provenance, which northern provenance, grows faster than other provenances, but it is located at the edge of the Pannonian Basin.

Both properties tested in variance analysis showed statistically significant differences between provenances.

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RUNOFF AND SEDIMENT TRANSPORT ON MEDITERRANEAN MICRO-PLOTS SCALE WITH DIFFERENT VEGETATION COVER

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Introduction

Land degradation has been recognized as a major environmental issue whose processes have been varying in space and time. Soil erosion exacerbates the loss of soil nutrients and water, pollutes surface waterways, constitutes the prime cause of deforestation, contributes to global change and reduces environmental productivity (27). Each year, about 75 billion tons of soil is eroded from the world`s terrestrial ecosystems (16). Because soil is formed very slowly, this means that soil is being lost 13 - 40 times faster than the rate of renewal and rate of sustainability. Rainfall energy is the prime cause of erosion from tilled or bare land, occurring when the soil lacks protective vegetation cover.

According to Naylor et al. (2002) the effects of vegetation on soil can be divided into two major related categories: bioprotection and bioconstruction. Vegetation cover protects soil against erosion by reducing water runoff (11) and by increasing water infiltration into the soil matrix (26). Vegetation shelter and fix the soil with their roots (10) reduce the energy of raindrops with their canopy (11). Also, vegetation can act as a physical barrier, altering sediment flow at the soil surface (27).

The importance of vegetation cover in controlling water erosion is widely accepted. In the short term, vegetation influences erosion mainly by intercepting rainfall drops, and by intercepting runoff. In the long term, vegetation influences the fluxes of water and sediments by increasing the soil-aggregate stability and cohesion as well as by improving water infiltration (27).

Mediterranean area is highly susceptible to the soil erosion because of its climatic characteristics. In this area precipitation is characterized by scarcity, torrential storms and extreme variability in space and time (18). Mediterranean environments are subjected to strong seasonal climatic fluctuations which modify the soil conditions, and as consequence, hydrological processes (7). On Mediterranean terrain slopes, either the static or the dynamic soil properties are not uniformly distributed (4) and many of them vary in patches related to micro-topography and vegetation (5, 13). 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 (4), soil water content (19), rock fragment size, percentage and geometry (17).

Water erosion is a serious problem in the context of land degradation and desertification processes in the Mediterranean region of Croatia. 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 (21, 22). The annual soil loss, or the quantity of soil that is irreversibly carried into the Adriatic Sea, has been estimated at 1,140 hectares with a depth layer of 20 cm. Since the soil formation process on karst, as an irreplaceable natural resource is very slow, the issue of its protection is of outmost importance. With these reasons, the Department of Forestry of the Institute for Adriatic Crops and Karst Reclamation in Split launched scientific-research activities dealing with erosion and soil protection on karst. Research results to date have been published in several papers (21, 22, 24). In recent years, more and more research in our country and in the world is dedicated to the role of vegetation cover, soil protection from erosion and the protection of quality of water resources (2, 6, 23, 25).

Therefore, the main objective of this study was to assess the effects of vegetation cover (grass, Spanish broom - *Spartium junceum* L.) on runoff and protection of soil from erosion. The data was compared with erosion processes on bare ground, obtained on experimental plot.

Material and methods

The study area was in the “Suvava” torrent watershed in central Dalmatia, south Croatia. According to Köppen´s climate classification, using the data of Seletković and Katušin (1992) the climate is classified as Cfsax˝ type with annual rainfall of 1200 mm and annual temperature of 13.1°C. It is moderately warm, rainy climate with hot and dry summers. The largest amount of annual precipitation falls in spring, autumn and winter. The soil is classified as marl limestone, shallow and very skeletal soil (calci-mollic cambisol).

Experimental plots were set parallel to the inclination of 16°, one plot on the bare ground, one plot on the area covered by grass and one by the Spanish broom. The dimensions of the plots were 20.0 x 2.5 m. They have the tank for collecting alluvium and are fenced by concrete enclosure. The enclosure is set so that it cannot get water and soil suspension from side, nor can it uncontrollably lose it from area (14). The plots were equipped with rainfall gauge from which the data were collected every day at 7 AM.

Runoff quantity was determined by measuring the water level in the tanks after every rainy day during the eight years of research. Sediment concentrations in the runoff samples, from each plot, were determined by collecting 1 l water from the tank (the tank was previously vigorously shaken) and then filtering it through filter paper. The filters were dried at 105 °C for 24 h.

The runoff coefficient correlates the volume of runoff and precipitation, specifying the percentage of rainfall that appears as runoff from specific surface area.

Pearson Correlations were done using statistical software package STATISTICA 7.1.

Results and discussion

Many authors have studied the relationship between rainfall, runoff and sediment transport on areas with different vegetation cover (1, 8, 12). According to Chirino (2006) this relationship is a function of several factors: rainfall, soil parameters (physics and hydro-physics); and also vegetation structure. All the research results indicate that the protective effect of the vegetation cover controls runoff and sediment transport.

According to the vegetation cover, some differences were expected in runoff and sediment transport between experimental plots with different vegetation cover (bare ground, grass and Spanish broom). During the study period of eight years were registered 870 rainy days, with an annual mean of 1469 mm.

The average monthly runoff was highly variable, ranging between 0.10 to 4.40 mm m^{-2} on the plot covered by Spanish broom; between 0.30 to 9.86 mm m^{-2} on the plots with grass cover and between 1.10 to 21.70 mm m^{-2} on the bare ground. The monthly sediment transport also was highly variable. It was ranging between 0.04 to 0.71 kg ha^{-1} on the plots covered by Spanish broom; grass cover between 0.06 to 11.68 kg ha^{-1} and on bare ground between 0.4 to 558.1 kg ha^{-1}.

Maximal runoff coefficient on plot under Spanish broom amounted to 0.019, under grass cover 0.044 and on bare ground 0.132.

Accordingly, the greatest annual runoff and sediment transport was on bare ground experimental plot, then on plot covered by grass and minimal was on plot covered by Spanish broom. Their relations are shown in Table 1.

Table 1: Relation of greatest annual runoff and sediment transport on experimental plots

Plant cover		Bare ground	Grass cover	Spanish broom
Runoff mm.m^{-2}		112	42	11
Sediment transport kg.ha^{-1}		2	41	3
Relation	Runoff	10	4	1
	Sediment transport	690	13	1

It is clear that vegetation cover and rainfall made important impact on runoff. Even drizzle (from 2.5 mm) on bare ground caused runoff, what was not a case on the plots covered by vegetation. This also happens when all other conditions, on which the runoff depends, have maximum value. Soil is completely saturated by water and it is not able to accept any other quantity of water.

An example of above conclusion is illustrated in a way that the 2.5 mm rainfall on plot without cover (bare ground), caused runoff 2.2 mm m^{-2} and sediment transport 39.9 kg ha^{-1}. This was preceded by 114.3 mm rainfall, which caused 11.4 mm m^{-2} and sediment transport 46.4 kg ha^{-1}.

According to Pearson correlation matrix between rainfall and runoff and rainfall and sediment transport (table 2) the relation between rainfall and runoff on experimental plot covered by Spanish broom is medium high, covered by grass high and on bare ground very high. Relation between rainfall quantity and sediment transport is weak on plot covered by Spanish broom and bare ground and medium high on plot covered by grass.

Table 2: Pearson correlation matrix between rainfall and runoff and rainfall and sediment transport on experimental plots

	Rainfall - Runoff	Rainfall - Sediment transport
Bare ground	0,803	0,344
Grass cover	0,752	0,444
Spanish broom	0,443	0,261

Many studies have reported that the vegetation cover reduces the water quantity that reaches the soil through the effect of interception, and also increases the heterogenity of the spatial distribution of the rainwater toward the soil, through stemflow and throughfall (3, 4, 17, 23). Permanent vegetation cover binds and stabilizes the soil, ameliorates its structure, engrosses the quickness of water infiltration, retains water and inhibits its quick runoff and so diminishes soil loss.

Conclusion

This study provided conclusion that vegetation cover have very positive antierosive and hydrologic role. Runoff on the area covered by vegetation is small and the erosion is excluded. Soil loss on bare ground plot is 690 times bigger and on plot covered by grass 13 times bigger than on plot covered by Spanish broom.

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DETERMINATION OF CARBON AND NITROGEN IN REFERENCE SOIL SAMPLE

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Introduction

Within the project "Changes of organic carbon and nitrogen storage capacity in soils and their trends in Croatia" funded by the Croatian Nature and Environment Protection Agency (HAOP), research and chemical analyses of soil has been carried out in different Croatian ecosystems. In order to monitor the reliability of methods of laboratory analyses, as well as to ensure operational quality of analytical methods that can be applied in soil analysis, Inter-laboratory compare on reference sample in laboratory of Croatian Forest Research Institute and Croatian Geological Survey has been organized. In this work, the interlaboratory measurements and repeatability and reproducibility were tested between results of determination total nitrogen and carbon in the reference sample on elemental analyzer CNS 2000 (Method A) in the laboratory of Croatian Forest Research Institute and on the elemental analyzer Flash 2000 (Method B) in the laboratory of Croatian Geological Survey. Statistical analysis included comparative description of results for determination of total nitrogen and carbon, descriptive statistics for 2 methods, Boxplot analysis and Regression analyses. Chemical analyses of soil are reduced for choice of instruments and methods for determination of elements because of specificity and heterogeneity of samples [1]. Therefore this kind of testing will confirm applicability of different element analyzer for determination of total nitrogen and carbon.

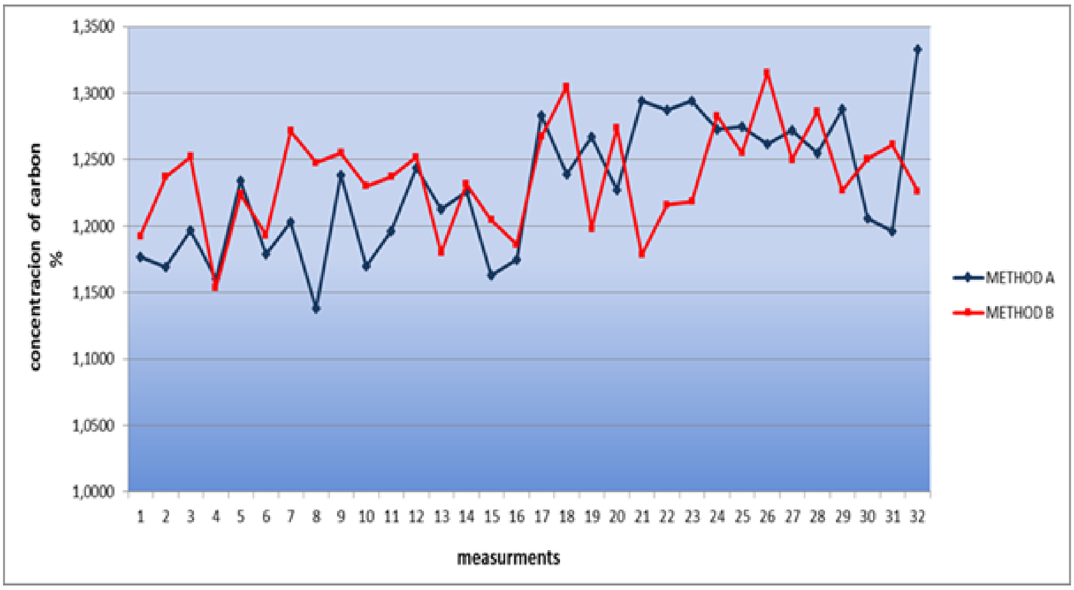
Material and Methods

Reference soil sample was the sample from ICP Forests Soil Interlaboratory Test Programme - 8th Test 2015. The 20 kg of soil was taken at a depth of 10 to 30 cm in Sweet chestnut forest ecosystem in Istria, Croatia. The type of soil was *terra rossa*. Soil sample was air dried at 40°C and subsequently milled above a 2 mm sieve [1]. After that they have been homogenized by riffling and divided over 100 subsamples. There were two riffling cycles. From sample A we have randomly selected 8 subsamples [2]. Only a sample representative of the initial material can provide meaningful analysis results so we used the sample divider PT 100 (Retch, Germany) which divides the sample that the composition of each fraction of the sample corresponds exactly to that of the original bulk sample [3]. Variation between subsamples and within subsamples was compared using ANOVA and Tukey's test. The variation between the subsamples was smaller than the variation within the sample and therefore they were considered to be homogeneous. These samples were used for the determation of total nitrogen and carbon by two elemental analyzers CNS 2000 (Leco, USA) (Method A) in the laboratory of Croatian Forest Research Institute and on the elemental analyzer Flash 2000 (Thermo Fisher scientific, USA) (Method B) in the laboratory of Croatian Geological Survey. Determination was done by standard ISO method [4].Two instruments are in general very close to each other. The major difference between them is the amount of the sample; CNS 2000 analyses ca 200 g sample and Flash 2000 analyses ca 50 mg [5,6].The results of all determinations variables were statistically analyzed so that we could determine what is the connection between these two method. The initial hypothesis is that measuring results Methods A corresponding measurement results methods B. The input data included: specification limits for observed parameters taken from report of soil Interlaboratory Test Programme – 8th Test 0, 07667 % – 0, 12733 % for nitrogen and 1,136 % – 1,337 % for carbon [7]**.** RSD measurements of observed parameters is between 1.5% and 2 %, the level of significance of statistical test is 95% ($p < 0.05$) and 95% error limits are ± 3SD.

Steps of analysis included: determining sample size (number of needed measurements) for statistical testing, a comparative display of measurement results between elemental analyzers; descriptive statistics for both method, Boxplot, F test for testing differences between variances of both methods, T test for dependent samples and regression analyses. For all statistical analyses a significance level of 5% was considered statistically significant [8]. All parameters of the analysis and graphical representations are made using Excel.

Results and discussion

Two methods are in general very similar. They both represent elemental analysis. After determining the sample size N=32 there were made comparative views from which was evident that there were certain oscillations between the results of Method A and Method B for carbon, but what is important that values does not exceed specification limits. For nitrogen, Method A shows significantly less oscillations in results in relation to method B and also here value does not exceed the specification limits (Figure 1).



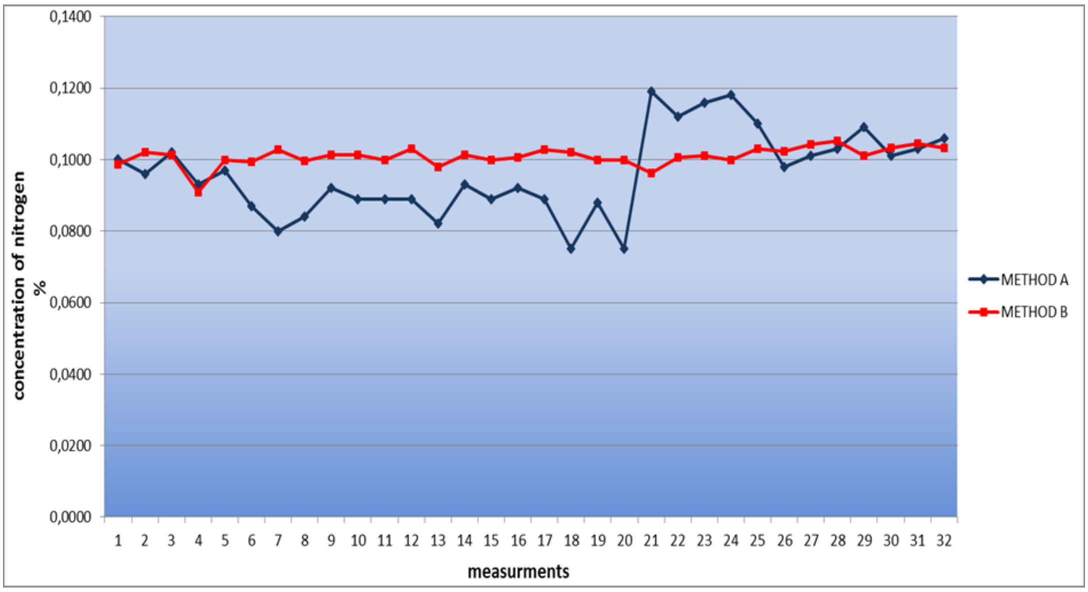


Figure 1 Comparation of results for determination of total nitrogen and carbon by Method A and Method B

In next step descriptive statistic was made (middle value, standard deviation, number discussed results, minimum, maximum and median) for each method (Table 2)

Table 2 Descripive statistic for metods of determining total nitrogen in laboratory of Croatian Forest Research Institute (Method A) and Croatian Geological Survey (Method B)

Determination of nitrogen	*Method A* (%)	*Method B (%)*
Mean	0,0962	0,1009
Standard Error	0,0021	0,0005
Median	0,0945	0,1012
Standard Deviation	0,0117	0,0027
Minimum	0,075	0,0909
Maximum	0,119	0,1051
Sum	3,077	3,2287
Count	32	32
Confidence Level (95 %)	0,0042	0,0010

Table 3 Descriptive statistic for methods of determining total carbon in laboratory of Croatian Forest Research Institute (Method A) and Croatian Geological Survey (Method B)

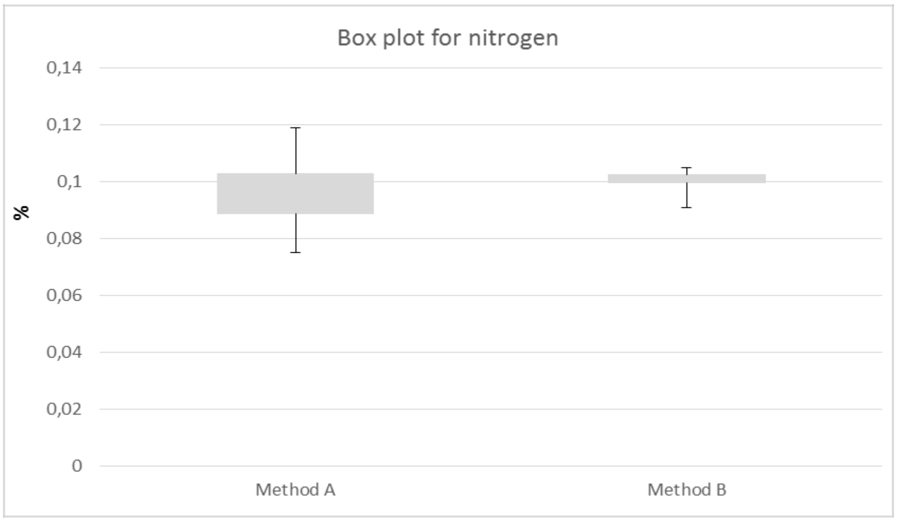
Determination of carbon	*Method A* (%)	*Method B (%)*
Mean	1,2292	1,2362
Standard Error	0,0087	0,0067
Median	1,2305	1,2370
Standard Deviation	0,0495	0,0381
Minimum	1,138	1,1535
Maximum	1,333	1,3155
Sum	39,333	39,5597
Count	32	32
Confidence Level (95 %)	0,0178	0,0137

Since the regression analysis obtained correlation coefficient r (Table 5), for the methods of determining nitrogen, we have a very weak correlation (0.00 - 0.20), while for the methods of determining carbon we have a weak correlation (0.20-0.40) between these two methods [8]. Furthermore, we have considered whether the correlation coefficient r is really significant. The significance of the correlation was tested by t-test (Table 6) which confirmed that the results of these methods, for the determination of total nitrogen and carbon with a confidence level of 95%, are significantly different. It was found that the value of t is greater than the critical value of p = 0.005 and therefore the null hypothesis that methods are same is discarded.

Table 5 Regression analysis for methods of determination nitrogen and carbon

Analysis	nitrogen	carbon
r	0,0654	0,2302
R^2	0,0043	0,0530
Standard Error	0,0119	0,0489
Sample number	32	32

Boxplots shows us the symmetry of data and distribution of repeated measurements (Figure 2). In determining of nitrogen we could see that the results of Method B show very little dispersion of data in regard of method A. From box plot of carbon we see that the method A shows a slightly greater dispersion of data in relation to the method B but all the results are within specification limits.



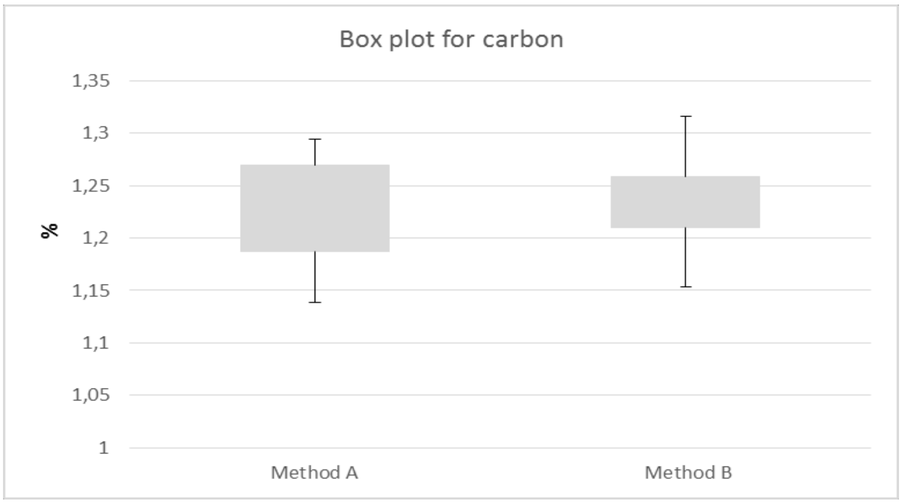


Figure 2 Box plot graphs for determination of nitrogen and carbon by Method A and Method B

Conclusions

All results of the method A and B for determine the total nitrogen and carbon are within the specification limits of the reference material. From comparative graphs, A method for nitrogen shows very small variations in the results as opposed to the B method in which the oscillations are higher. For carbon there are no large deviations of results between A and B method. From box plots we could conclude that method A have bigger dispersion of data that method B which is refer with the weighing of reference samples. Small sample quantity of reference material for instrument calibration in compere whit weighs of the sample, effect negatively on process of calibration. Chemical analyzes of forest soil are limited to the choice of instruments and methods for the determination of certain elements, so tests like these confirm the applicability of elemental analyzer such as CNS 2000 for the determination of nitrogen and carbon due to the specific of sample.

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